JOÃO ANTONIO BATISTA DE SIQUEIRA

THE TICK TOCK OF BIOLOGICAL CLOCKS ON CROP DOMESTICATION AND PLANT ENVIRONMENTAL RESPONSES

Thesis submitted to the Plant Physiology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Orientador: Wagner L. Araújo

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ABSTRACT

SIQUEIRA, João Antonio Batista de, D.Sc., Universidade Federal de Viçosa, February, 2022. **The Tick Tock of Biological Clocks on Crop Domestication and Plant Environmental Responses**. Advisor: Wagner Luiz Araújo.

Timing governs practically all processes documented, and thus since is extremely complex to alter temporal dynamics. Meanwhile, precise mechanisms to monitor the time are fundamental to ensure life on Earth. Organisms naturally develop differential abilities to monitor the time, wherein the biological chronometers regulate spatiotemporal dynamics, ensuring the emergence of new cells and allowing the organism can anticipate unfavorable environmental conditions. In this context, the biological clocks are highlighted due to their ability to timekeeping the circadian rhythms, development, and metabolism. This thesis is largely focused on the investigation of the functions of biological clocks in the context of plant growth and stress response as well as on investigating the functional role of the photoperiod to mediate aluminum (Al) tolerance. To this end, several complementary approaches were undertaken to understand: (i) the significance of biological clocks on crop domestication; (ii) the diel regulation of the Al tolerance in plants; and (iii) the specific behavior of root clock and its implication on plant yield. Firstly, it was demonstrated that tomato domestication apparently synchronized the distinct biological clocks of this species. Notably, the results demonstrate how this synchronization has contributed to the increased yield of cultivated tomatoes yet reduced their ability to tolerate abiotic stresses in comparison with wild tomatoes. In an attempt to demonstrate this, it was investigated the significance of water supply at different periods of the day in tomatoes. To this end, *Solanum pennellii* and *S. lycopersicum* (cv. M82) plants were exposed to different watering regimes: at dawn, dawn/dusk, and dusk. I was noted that the dusk watering treatment promoted a significant reduction in the number of leaves only in M82, whereas the other watering treatments did not alter the plant height in both genotypes. By analyzing the height and number of leaves in the different genotypes (MT, SFT^{ox}, and $SP5G^{pen}$), it was observed an overall trend, revealing that dusk watering treatment resulted in smaller plants with fewer leaves than dawn and dawn/dusk watered plants. In conclusion, the analyses of the impacts from different periods of watering during the day suggested that watering tomatoes near dusk can significantly improve crop yield. Next, and mostly based on recent evidence, it was discussed that modifications of the DNA and metabolic checkpoints can mediate Al tolerance. Accordingly, mitochondrial organic acid metabolism and the genetic manipulation of DNA checkpoints were demonstrated to be not sufficient to promote plant survival under high Al concentrations. Compelling evidence showed that DNA checkpoint alterations are coupled with significant changes in mitochondrial metabolism. Thus, it was suggested that interactions between both mechanisms can occur in plants with elevated Al tolerance, supporting their growth even in soils with excessive Al levels. Following, it was demonstrated that photoperiod is closely associated with Al responses, wherein short-days (SD) favor the Al tolerance and long-days (LD) are related to Al sensitiveness. Genes involved in DNA checkpoints are induced specifically under LD conditions, arresting cell divisions and root elongation. Likewise, it was described how the photoperiodic regulation shapes Al tolerance in plants, which can contribute to developing a stable tolerance to Al in crops. Furthermore, the evidence suggested the occurrence of unique developmental phases for roots, in which these would be uncoupled from shoot phases. Altogether, it was indicated new frontiers to be pursued in plant biology, which have enormous potential to enhance nutrient use efficiency and reduce the use of chemical fertilizers in the next generation of crops.

Keywords: Domestication. Biological clocks. Energetic stress. Metabolism.

RESUMO

SIQUEIRA, João Antonio Batista de, D.Sc., Universidade Federal de Viçosa, fevereiro de 2022. **O Tic Tac dos relógios biológicos sobre a domesticação dos cultivos e na respostas ao ambiente**. Orientador: Wagner Luiz Araújo

A temporização governa praticamente todos os processos já documentados, onde é extremamente complexo alterar as dinâmicas temporais, e mecanismos processos para monitorar o tempo são fundamentais para garantir a vida na Terra. Os organismos naturalmente desenvolvem habilidades de monitorar o tempo, em que seus cronômetros regulam as dinâmicas espaço-tempo, garantindo a emergência de novas células, permitindo assim aos que o organismo possa antecipar condições ambientais desfavoráveis. Neste contexto, dentre os relógios biológicos destacam-se os mecanismos de cronometragem associados aos ritmos circadianos, desenvolviemnto e metabolismo. Esta tese está focada amplamente na investigação das funções dos relógios biológicos sobre o crescimento de plantas e respostas a estresses, bem como na investigação de um papel funcional do fotoperíodo em mediar a tolerância ao alumínio (Al). Para este fim, diversas e complementares abordagens foram usadas para entender: (i) a significância de relógios biológicos na domesticação de plantas; (ii) a regulação periódica da tolerância ao Al em plantas; e (iii) o comportamente específico do relógio da raiz e sua implicação do desempenho das plantas. Primeiramente, foi demonstrado que a domesticação do tomateiro aparentemente sincronizou os distintos relógios na espécie emergente. Notavelmente, os resultados demonstraram como essa sincronização pode ter contribuído para aumentar a produtividade do tomateiro cultivado e, ainda, reduzir sua habilidade em tolerar estresses abióticos quando comparado aos parentais silvestres. Na tentativa de demonstrar esse aspecto, tentou se entender a significância do suprimento de água em diferentes períodos do dia para tomateiro. Assim sendo, plantas de *Solanum pennellii* e *S. lycopersicum* (cv. M82) foram submetidas a três regimes de rega: pela manhã, manhã/anoitecer ou ao anoitecer. As plantas regadas ao anoitecer apresentaram significativa redução no número de folhas, especificamente M82, não sendo observadas variações na altura das plantas em nenhuma das espécies. Analisando a altura e o número de folhas em diferentes genótipos (MT, *SFT*^{ox}, e

$SP5G^{pen}$), foi observado que a rega ao anoitecer resultou em plantas com baixa estatura e com menos folhas que as regadas ao manhã/anoitecer e anoitecer. Em conclusão, as análises do período de rega das plantas revelaram que essa prática ao anoitecer favorece a produtividade da espécie-alvo. Subsequentemente, discutiu-se como modificações em pontos de checagem do DNA e metabólicos podem mediar a tolerância ao Al. Assim sendo, o metabolismo mitocondrial de ácidos orgânicos e manipulações genéticas dos pontos de checagem do DNA foram insuficientes em permitir a sobrevivêencia de plantas sob altas concentrações de Al. Foi sugerido que modificações nos pontos de checagem do DNA podem estar associadas à significativas mudanças no metabolismo mitocondrial. Desta forma, as interações entre esses mecanismos podem resultar em plantas com tolerância ao Al, suportando o crescimento mesmo sob altas concentrações do metal. Na sequência, foi demonstrado que o fotoperíodo é intimamente associado com as respostas ao Al, onde dias curtos (DC) favorecem a tolerância ao Al e dias longos (DL) são associados à sensibilidade ao metal. Desta forma, genes associados aos pontos de checagem do DNA são induzidos especificamente sob condições de DL, bloqueando as divisões celulares e o alongamento radicular. Em conclusão, foi descrito que a regulação fotoperiódica da tolerância ao Al em plantas pode ser fundamental para o desenvolvimento de uma tolerância estável ao Al em plantas cultivadas. Além disso, foi sugerido a ocorrência de fases do desenvolvimento específicas para raízes, as quais são independentes das fases parte área. Com isso, foram indicadas novas fronteiras a serem buscadas no tocante à biologia vegetal, sendo que as mesmas apresentam um enorme potencial para aumentar a eficiência no uso dos nutrientes e reduzir o uso de fertilizantes químicos na próxima geração de plantas cultivadas.

Palavras-chave: Domesticação. Relógios biológicos. Estresse energético. Metabolismo.

TABLE OF CONTENT

General Introduction

Time (*t*) is a variable that has been receiving great attention from humanity, and over centuries scientists have been trying to understand its relevance in most scientific fields. *t* describes the relationships between space and velocity, reflecting the record of coincidences, which will re-occur through phenomena and processes. The exact sciences use temporal variables since almost 3500 BC, which was made by Babylonian astronomers (Aaboe, 2009). Meanwhile, biological sciences began to pay more attention to variables related to *t* only recently. In the mid-seventies, the genetic basis for diurnal oscillations of *Drosophila melanogaster* started to be elucidated, emerging the term circadian clock from these observations, which have direct relationships with *t* measured by this organism. Briefly, it was postulated that overall living beings display an oscillatory system synchronizing their physiology with "solar time", which comprises oscillating rhythms according to a period of 24 hours, being this system currently termed the circadian clock. Notably, Kloppstech (1985) revealed the association between oscillations at photosynthesis-related transcripts and circadian rhythms of *Pisum sativum*, which on the sequence was extended for wheat (Nagy et al., 1988). A large set of genes was demonstrated to have the transcription rate and mRNA accumulation resembling circadian rhythm dynamics (Millar and Kay, 1991; McClung and Kay, 1994). Currently, it is widely accepted that the circadian clock control at least 30% of *Arabidopsis thaliana* transcripts under constant light conditions, whereas approximately 90% of transcripts show oscillations under a diel period, which comprises variations of either light/dark cycles or temperature (Millar et al., 2016). Intriguingly, the circadian clock is the most characterized biological clock in regulating temporal variations of the organism, wherein a single organism may have contrasting circadian rhythms for its organs.

Practically all organism biological patterns are affected by *t*, while traits related to development, physiology, and metabolism may influence *t* responsiveness. In other words, an organism can establish temporal patterns beyond the circadian clock, characterizing other biological clocks. Accordingly, the developmental clock of an organism describes its set of chronometers that define the emergence of cells, tissues, and organs in consonance with time, defining the unique genetic, biochemical, and morphological features of the organism (Siqueira et al., 2022a). For instance, the process of cell aging might arrest the maximal cell division rates in determined periods, which can be subsequently restored in shorter periods. This characterizes a disruption of temporal dynamics by the developmental clock and makes the *t* pass differently for organisms from the same species or among different species. Remarkably, the metabolic program also follows autonomous oscillations varying independently from environmental conditions (Papagiannakis et al. 2017; Siqueira et al., 2018), similar to the situation observed for the circadian clock. Furthermore, these autonomous metabolic oscillations in some moments appear to operate in coordination with cell cycle progression, supporting cell division and differentiation (Papagiannakis et al. 2017). Collectively, the *t* for complete oscillations seems to be different for the most diverse biological clocks of living organisms. As aforementioned, not only the circadian clock but also metabolic oscillations are required to adjust the development of plants. These oscillations seem also to connect plant growth and stress responses yet our understanding of the molecular and metabolic basis of these phenomena remains poorly elucidated. Thus, this thesis is largely focused on the investigation of the functions of the circadian clock in the context of plant growth and stress response as well as investigating the functional role of the photoperiod in mediating this response. To reach this goal, a range of complementary approaches were used and thus this thesis is organized as a compilation of six independent chapters. Within the first section, we aimed to demonstrate how tomato domestication apparently synchronized distinct biological clocks of this specie. Briefly, we demonstrate how this synchronization has contributed to the increased yield of cultivated tomato yet reduced their ability to tolerate abiotic stresses in comparison with wild tomatoes. Following this, we were interested in revealing whether the tomato responsiveness is differentially modulated by watering during different periods of the day. Our findings revealed that watering near from evening can accelerate the tomato developmental program improving fruit production without any yield penalty, demonstrating that this period is better than morning watering. Within the second section, we attempted to reveal how diel variations of light/dark cycles have been selecting aluminum (Al) tolerance across the plant kingdom. In the last section, we posit the existence of particular developmental phases from roots, phases that are independent of shoot phases.

Section 1: Plant Domestication and Biological Clocks synchronization

By domesticating wild plants, our ancestors successfully modified traits related to the developmental and circadian clocks of these plants. From these changes emerged the modern crops with higher productivity and ability to be spread in highly contrasting environments of the origin center of wild species. Notably, the emergent domesticated species turned out to be slower to respond to the environment and began to harbor a reduced genetic variability. Modern crops have been allowing the growth of the human population over years, improving both availabilities of calories and food quality. To this end, genetic selection on wild plants occurred mainly in regulatory regions of target genes that regulate developmental, circadian, and metabolic traits (Doebley et al. 2006; Müller et al., 2016). These traits are regulated according to *t*, which is a variable closely associated with biological clocks regulation. Similarly, compelling evidence has been suggesting that the limited genetic variability and reduced ability to respond to environmental changes are the major barriers to improving the yield of modern crops beyond the values known nowadays.

Alterations in the developmental clock were demonstrated to be shaped by the domestication of crops, revealing that distinct interfaces were reconfigured. It is notorious that wild species display higher branching patterns than cultivated plants, being this trait is easily observed to have been selected through crop domestication. Maize (*Zea mays*) plants exhibit alterations in cis-elements of *teosinte branched1* (*tb1*) gene comparatively to their wild relative, teosinte, which contributed to altering architecture and development as well as to domesticating the maize as we know (Doebley, et al., 1997). Likewise, the meristem maturation clock shows a more advanced maturation status for domesticated tomatoes (*Solanum lycopersicum*) than in tomato wild species, resulting in a fast transition from vegetative meristem to floral meristem (Park et al., 2012). Following, flowering induction is widely dependent on photoperiod cues, in which domestication altered day-length sensitivity in most crop species. For instance, the flowering repressor *Self-Pruning 5G* (*SP5G*) is induced on wild tomatoes growing under long-days (LD), a fact that is not observed for tomato cultivated varieties (Soyk et al. 2017). Therefore, these varieties are characterized by variations in cis-regulatory regions of the gene *SP5G* that reduce its expression allowing the meristem maturation in plants growing under LD conditions, making the cultivated tomato a neutral-day specie (Soyk et al. 2017). It has been demonstrated that photoperiod is a pivotal environmental factor regulating developmental, circadian, and metabolic clocks (Minow et al. 2018; Adole et al., 2019). Thus, selection pressure on photoperiod and circadian clock genes was reported to be involved in the domestication processes of barley (*Hordeum vulgare*), pigeonpea (*Cajanus cajan*), and maize (Faure et al. 2012, Varshney et al. 2017; Navarro et al. 2017). Intriguingly, another factor related to this intricate network covering photoperiod sensitiveness is the circadian metabolism, in which crop domestication is assumed to have promoted selections of genes associated with sucrose and starch metabolism (Sauvage et al., 2017). Chronic metabolic alterations seem to have played a crucial role in plant domestication. Indeed, during the domestication of adzuki bean (*Vigna angularis*) and barley (*Hordeum vulgare*), traits related to starch metabolism were selected, boosting the correlations between genes related to photoperiod-responses and circadian clocks were achieved (Yang et al., 2015; Pankin et al., 2018). On the other hand, the QTL harboring *SP5G* gene in wild tomatoes also harbors genes involved in the regulation of primary metabolism, and in the transition from vegetative to reproductive phase (Schauer et al., 2006). This indicates a likely parallel selection in these traits during tomato domestication. In agreement with this assumption, one of the pivotal proteins in maize domestication, Tassels Replace Upper Ears (TRU1), affects SAM maturation and apparently can also control the flux of diffusible substances into this region (Dong et al., 2017), wherein can occur a large number of primary metabolites like sugars. Both the transport and the accumulation of carbohydrates were suggested to be important factors mediating phenotypic evolution during rice domestication (Zheng et al., 2019). Accordingly, SAM regulation is directly altered by sugars, whereas arabinose metabolism is fundamental for the meristem cell growth (Xu et al., 2015). This mechanism was suggested to control stem cell proliferation during tomato domestication, enhancing fruit size (Xu et al., 2015). Therefore, it seems tempting to suggest that the fundamental core of biological clocks could module the life cycle and environmental responses of plants, contributing to local adaptations.

During the last years, the domestication process for diverse cultivated plants has attracted great attention from plant biologists, wherein diverse strategies are described as able to ensure food safety for humanity. Accordingly, it is not surprising that cultivated plants are more sensitive to adverse environmental conditions than their wild relatives. Thus, it is mandatory the identification of regulatory mechanisms associated with circadian and developmental clocks that were lost during domestication, which in turn could contribute to improving plant performance under environmental fluctuations. To this end, this section is based on three independent but complementary chapters.

Chapter 1. Plant Domestication: Setting Biological Clocks

Over the last decades, plant breeders have been facing the genetic erosion of crop species. Diverse breeding strategies are proposed to overcome this problem that causes the loss of genes, yet most strategies are focused on genetic selection and genome engineering. Within this chapter, we present novel visions of how crop domestication promoted the adjustment for development, circadian clock, and physiology across a single plant, characterizing its biological clocks. By contrast, the wild relatives of crop species seem to operate these clocks independently across the plant segments. Altogether, this independence observed in wild species culminates in a higher resilience under biotic and abiotic stresses, whereas the connection among the plant segments/organs in domesticated species may boost the production of fruits and seeds. In summary, by revisiting crop domestication we posit that the identification of new players modulating these processes may help us to generate resilient crops with significant improvements in yield.

Chapter 2. The Synchronized Biological Clocks of Domesticated Tomato

Circadian clocks can be heterogeneous across organs of animals and wild plants, exhibiting a reduced coherence between physiology and development. The major contrast emanating from the circadian clocks of animals and plants resides in the existence of a central clock setting peripheral clocks. Tomato domestication reduced the circadian period in the domesticated species *Solanum lycopersicum*, whereas wild tomato species are characterized by faster circadian rhythms. We hypothesized that beyond decelerating circadian rhythms of *S. lycopersicum*, the domestication of this species also synchronized circadian rhythms within distinct leaves. Our results indicated that circadian rhythms are similar in the cotyledons of *S. lycopersicum* while these rhythms are rather different in *S. pimpinellifolium*.

Accordingly, leaf development obeys a physiological coherence only in the domesticated species, since wild tomatoes seem to not have clear source/sink relationships between young and mature leaves. Furthermore, gene edition on *S. pimpinellifolium* at target genes related to tomato domestication promotes the acquisition of developmental coherence in those edited lines, which is comparable to *S. lycopersicum*. Collectively, our findings indicate a key process associated with tomato domestication, which can be fundamental to paving the way to improve tomato yield regardless of stressful conditions.

Chapter 3. Dusk watering accelerates tomato development without yield penalty

Global climate changes have been rising drought episodes worldwide, and additionally, water evaporation from soil to the atmosphere is rather variable over the day. Leaf physiology also manifests differences over morning and evening, as a consequence Chronoculture has been suggested to improve water use efficiency (WUE) by supplying water during different periods of the day. Previous efforts have suggested that dusk watering improved significantly the total fruit production of domesticated tomato (*Solanum lycopersicum*), whereas the pathways ensuring this phenomenon remained unknown. Here, we found not only compelling evidence supporting previous studies but also indicating that *SINGLE FLOWER TRUSS* (*SFT*) mediates improvements in *S. lycopersicum* yield following dusk watering. Net photosynthesis and stomatal conductance were elevated in dusk-watered plants, contributing to reduced leaf number and specific leaf area in wild-type plants, whereas this pattern was not observed for *sft* mutant plants. Altogether, these changes culminated in higher WUE based on total fruit amount for wild-type plants that received water at dusk, revealing a notion that in fact, evening watering is a promising strategy to increase tomato productivity without altering neither the levels of water supplementation nor the yield.

Section 2: “The interfaces between Aluminum tolerance and photoperiod sensitiveness”

Acidic soils (pH ≤ 5.5) are spread worldwide impairing root development of many important crops, which constraints food safety in many developing countries. The major factor reducing root *fitness* at low soil pH is the availability of aluminum (Al) cations that bind to diverse biomolecules (e.g. DNA, RNA, proteins, and lipids) arresting root cell divisions (Kochian et al., 2015). Over the last decades, several mechanisms have been elucidated revealing how plants cope with Al stress, highlighting manipulations on organic acids (OA) metabolism and DNA checkpoints modifications (de la Fuente et al., 1997; Nunes-Nesi et al., 2014; Kochian et al., 2015; Eekhout et al., 2017). However, when considered individually, both approaches were shown to be insufficient to overcome Al toxicity. OA exudation, which was reported to neutralize Al inside roots and around the rhizosphere, was considered the most promising genetic strategy to improve Al tolerance in plants (Ryan et al., 1993; Ryan et al., 2001; Kochian et al., 2004, Kochian et al., 2015). However, it was very well documented that Al-sensitive plants might exudate large amounts of OA from root tips to the rhizosphere (Ishikawa et al.; 2000; Piñeros et al., 2005; Zheng et al., 2005). Similarly, genetic manipulations on DNA-checkpoints components did not promote the survival of plants under long-term Al exposure. Thus, although certain mutant plants were tolerant to long-term Al exposure, they were more Al sensitive than wild-type in short-term exposure to elevated Al doses, showing a slow-growth recovery after the stress period (Chen et al., 2019). These examples provide further evidence of the relevance to identify environmental traits regulating pleiotropic mechanisms associated with improvements in Al tolerance (Siqueira et al., 2022b). Likewise, in some cases Al-tolerant species have their origin center on soils with alkaline pH, emerging the question that acid soils distribution is not able to fully explain the differential Al tolerance across the plant kingdom.

A diel variation was noted for pH in the ocean during Arctic summers when pH increases over the day and declines overnight (Krause-Jensen et al., 2016). The longer photoperiods are responsible to sustain the high pH in Arctic kelp forests allowing the expansion of the Arctic vegetation in response to warming (Krause-Jensen et al., 2016). Furthermore, photoperiod was recently demonstrated as the dominant factor controlling vegetation phenology and growing season in the African continent (Adole et al., 2019). In this continent, a large pH range varying from alkaline to extremely acid soils can be observed. Furthermore, in terms of root development, the cell death around the root apical meristem (RAM) exhibits an intrinsic relationship with shoot exposure to light (Raya-González et al., 2018), revealing the essentiality of day-length to regulate root responses to genotoxic elements such as Al. Thus, proper investigation of the photoperiod relevance on Al tolerance seems to be more than necessary to overcome this historical obstacle. Moreover, Al imposes serious barriers for developing countries to reach more sustainable agriculture, and therefore this knowledge would enable reductions in applications of correctives and fertilizers to work around the acid soils' low fertility, turning agriculture more sustainable in these countries.

Chapter 4. Metabolic and DNA checkpoints for enhancement of aluminum tolerance

Soils from developing countries are characterized by low fertility and excessive levels of Al cations, which historically has been limited food production. Despite liming having been successfully employed to overcome this barrier, limestone is rather unavailable in certain countries and even crops under liming still can absorb Al reducing their productivity. At these conditions, root elongation is inhibited whereas the most accepted strategy to cope with Al stress in plants refers to OA exudation from roots to the rhizosphere where those compounds neutralize the metal. In addition, over the last years, a growing body of evidence has indicated that loss-of-function at DNA-checkpoint regulators confers the maintenance of cell divisions under Al toxicity, supporting Al tolerance. However, OA exudation was demonstrated to be not sufficient to ensure Al tolerance, since Al sensitive genotypes still exudate large levels of these compounds. Similarly, modifications at DNA checkpoints were reported to be insufficient to improve Al tolerance, as revealed by the fact that their regulators are essential to growth recovery after exposure to higher Al doses. With this review, we provide the knowledge synthesis for potential interactions between OA metabolism and DNA-checkpoint regulators, wherein these interactions were reported to occur in microorganisms and animals coping with other genotoxic agents. Collectively, we introduce new ways to develop Al tolerant crops with reduced yield penalties.

Chapter 5. A long and stressful day: photoperiod shapes aluminum tolerance in plants

In our daily life is common that stressful days would be longer than pleasant days. Intriguingly, Al tolerance seems to mimetize this traditional knowledge, whereas longer days favor Al sensitivity. In general, long-day (LD) species are generally more Al-sensitive than short-day (SD) species, and the genetic conversion of plants for SD growth habit boosts Al tolerance. The reduced tolerance in plants growing under LD is associated with DNA duplication without mitosis occurrence, characterizing the endoreduplication that blocks root elongation. Moreover, genes associated with endoreduplication are induced only at LD conditions, which culminate in cell division stoppage. Indeed, these genes showed a genetic diversity correlated to day-length, revealing that *Arabidopsis thaliana* ecotypes from regions with shorter days manifested genes under positive selection. In this chapter, we describe the photoperiodic regulation of Al tolerance in plants, which can contribute to solving historical problems related to acidic soils

Chapter 6. The hidden half comes into the spotlight: peeking inside the black box of root developmental phases

Plant biologists have been discussing shoot developmental phases at least for the past three decades, whereas the root developmental program remained poorly discussed. Shoot and root have extremely contrasting meristems and habit discrepant environments, and as such shoot is exposed to a predominant gaseous atmosphere whereas roots develop in soils with solid, liquid, and gaseous particles. In this pioneering review, we described exclusive root developmental phases, which are independent of the shoot developmental program. We posited the presence of at least four distinct pathways mediating the root developmental program, and the availability of specific nutrients can either ensure or arrest developmental phases. Recent advances in root biology are discussed, with a particular focus on the development of next-generation crops that exhibit tailored root growth. The understanding of root developmental phases represents a new frontier in plant science and overcomes the inherent knowledge limitations that are currently available. Therefore, we presented novel and exciting tools to establish agricultural landmarks that will enhance nutrient use efficiency and reduce the use of chemical fertilizers in the next generation of crops.

REFERENCES

Aaboe, A. (1974) Scientific Astronomy in Antiquity», **Philosophical Transactions of the Royal Society London 276**, 21-42.

Adole, T. *et al.* (2019) Photoperiod controls vegetation phenology across Africa. **Commun. Biol. 2**: 391.

Chen, P. *et al.* (2019) A multi-level response to DNA damage induced by Aluminium. **Plant J. 98**, 479–491.

de la Fuente, J.M. et al. (1997) Aluminum tolerance in transgenic plants by alteration of citrate synthesis. **Science 276**, 1566-1568.

Doebley, J. *et al.* (1997) The evolution of apical dominance in maize. **Nature 386**, 485–488.

Doebley, J. et al. (2006) The Molecular Genetics of Crop Domestication. **Cell 127**, 1309-1321.

Dong, Z.B. *et al.* (2017) Ideal crop plant architecture is mediated by tassels replace upper ears1, a BTB/POZ ankyrin repeat gene directly targeted by TEOSINTE BRANCHED1. **Proc. Natl. Acad. Sci. USA 114**, E8656-E8664.

Eekhout, T. et al. (2017) Modification of DNA Checkpoints to Confer Aluminum Tolerance. **Trends Plant Sci. 22**, 102-105.

Faure, S. et al. (2012) Mutation at the circadian clock gene EARLY MATURITY 8 adapts domesticated barley (Hordeum vulgare) to short growing seasons**. Proc. Natl. Acad. Sci. USA 109**, 8328–8333.

Kloppstech, K. (1985). Diurnal and circadian rhythmicity in the expression of light-induced nuclear messenger RNAs. **Planta 165**, 502–506.

Kochian, L.V. et al. (2004) How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. **Annu. Rev. Plant Biol. 55**, 459-493.

Kochian, L.V. et al. (2015) Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. **Ann. Rev. Plant Biol. 66**, 571-598.

Krause-Jensen, D. et al. (2016) Long photoperiods sustain high pH in arctic kelp forests. **Sci. Adv. 2**: e1501938.

Ishikawa, S. *et al* (2000) Comparison of the amount of citric and malic acids in Al media of seven plant species and two cultivars each in five plant species. **Soil Sci. Plant Nutr. 46**:751–758.

Lemmon, Z.H. et al. (2018) Rapid improvement of domestication traits in an orphan crop by genome editing. **Nat. Plants 4**, 766–770.

McClung, C.R. and Kay, S.A. (1994) Circadian rhythms in Arabidopsis thaliana. In Arabidopsis, E.M. Meyerowitz and C.R. Somerville, eds (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 615–637.

Millar, A.J. and Kay, S.A. (1991) Circadian control of cab gene transcription and mRNA accumulation in Arabidopsis. **Plant Cell 3**, 541–550.

Millar, A.J. (2016) The intracellular dynamics of circadian clocks reach for the light of ecology and evolution. **Annu. Rev. Plant Biol. 67**, 595 – 618.

Minow, M.A.A. *et al.* (2018) Distinct gene networks modulate floral induction of autonomous maize and photoperiod-dependent teosinte. **J. Exp. Bot. 69**, 2937–2952.

Müller, N.A. et al. (2016) Domestication selected for deceleration of the circadian clock in cultivated tomato. **Nat. Genet. 48**, 89-93.

Müller, N.A. et al. (2018) Mutations in EID1 and LNK2 caused light-conditional clock deceleration during tomato domestication. **Proc. Natl. Acad. Sci. USA** 15, 7135-7140.

Nagy, F. et al. (1988) A circadian clock regulates transcription of the wheat Cab-1 gene. **Genes Dev. 2**, 376–382.

Park, S.J. *et al.* (2012) Rate of meristem maturation determines inflorescence architecture in tomato. **Proc. Natl. Acad. Sci. USA 109**, 639-644.

Navarro, J.A.R. et al. (2017) A study of allelic diversity underlying flowering-time adaptation in maize landraces. **Nat. Genetics 49**, 476-483.

Nezames, C.D. et al. (2012) The Arabidopsis cell cycle checkpoint regulators TANMEI/ALT2 and ATR mediate the active process of aluminum-dependent root growth inhibition. **Plant Cell 24**, 608–621.

Pankin, A. et al. (2018) Targeted resequencing reveals genomic signatures of barley domestication. **New Phytol. 218**, 1247–1259.

Papagiannakis, A. et al. (2017) Autonomous metabolic oscillations robustly gate the early and late cell cycle. **Mol. Cell 65**, 285-295.

Piñeros, M.A. et al. (2005) Aluminum resistance in maize cannot be solely explained by root organic acid exudation. A comparative physiological study. **Plant Physiol. 137**, 231-241.

Raya-González, J. *et al.* (2018) MEDIATOR18 influences Arabidopsis root architecture, represses auxin signaling and is a critical factor for cell viability in root meristems. **Plant J.** 96: 895–909.

Ryan, P.R. et al. (1993) Aluminum toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. **J. Exp. Bot. 44**, 437–446.

Ryan, P.R. et al. (2001) Function and mechanism of organic anion exudation from plant roots. **Annu. Rev. Plant Physiol. Plant Mol. Biol. 52**, 527–60.

Sauvage, C. et al. (2017) Domestication rewired gene expression and nucleotide diversity patterns in tomato. **Plant J. 91**, 631–645.

Schauer, N. *et al.* (2006) Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. **Nat. Biotechnol. 24**, 447–454.

Siqueira, J.A. *et al.* (2018) Unraveling Interfaces between Energy Metabolism and Cell Cycle in Plants. **Trends Plant Sci. 23**, 731-747.

Siqueira, J.A., *et al.* (2022a) The hidden half comes into the spotlight: Peeking inside the black box of root developmental phases. **Plant Comm. 3**: 100246.

Siqueira, J.A. *et al.* (2022b) Metabolic and DNA checkpoints for enhancement of Aluminum tolerance. **J. Hazard. Mat. 430**: 128366.

Sjögren, C.A. *et al.* (2015) Aluminum-dependent terminal differentiation of the Arabidopsis root tip is mediated through an ATR-, ALT2-, and SOG1-regulated transcriptional response. **Plant Cell 27**, 2501-2515.

Soyk, S. *et al.* (2017). Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. **Nat. Genet. 49**, 162–168.

Varshney, R.K. et al. (2017) Whole-genome resequencing of 292 pigeonpea accessions identifies genomic regions associated with domestication and agronomic traits. **Nat. Genet. 49**, 1088-1090.

Yang, K. et al. (2015) Genome sequencing of adzuki bean (Vigna angularis) provides insight into high starch and low fat accumulation and domestication. **Proc. Natl. Acad. Sci. USA 112**, 13213–13218.

Xu, C. et al. (2015) A cascade of arabinosyltransferases controls shoot meristem size in tomato. **Nat. Genet. 47**, 784–792.

Zheng, S.J. *et al.* (2005) Immobilization of aluminum with phosphorus in roots is associated with high aluminum resistance in buckwheat. **Plant Physiol. 138**, 297–303.

Zheng, X.M. et al. (2019) Genome-wide analyses reveal the role of noncoding variation in complex traits during rice domestication. **Sci. Adv. 5**, eaax3619.

Chapter 1

Plant Domestication: Setting Biological Clocks[1]

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Plant Domestication: Setting Biological Clocks

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Abstract

By domesticating wild plants our ancestors generated broad changes in their developmental and circadian clocks. These changes rendered modern crops more productive and adaptable to contrasting environments from the center of origin of the wild species, with concomitant reductions in both genetic variability and abiotic stress tolerance. Likewise, a complete restructuration of the metabolic timekeeping of plants seems to have occurred over crop domestication. Here, we highlight that contrasting timing among organs in wild relatives of crops allows to them have a faster recognition of environmental adversities. We further propose that the identification of connections among biological clocks, which were achieved during the plant domestication process, may represent a fundamental source of genetic variation to improve the resilience and yield of crops.

Biological Clocks in Wild Plants

Plant domestication over the past centuries has allowed the growth of the human population mainly by increasing calorific availability. During the domestication process, broad changes were observed not only in genes, proteins, and metabolites but also in both vegetative and reproductive structures in plants. For instance, in maize genetic selection during domestication targeted regulatory genes associated with both the cell cycle and the carbon metabolism (Doebley et al. 2006), which presumably coordinated the flowering time and circadian rhythms. As a consequence, imbalances occurred in stem cell proliferation and energy metabolism which apparently modified the circadian and developmental programs during domestication.

The domesticated tomato (*Solanum lycopersicum*) is characterized by an advanced state of maturation in meristems in its early phases of development (Park et al., 2012). Key proteins in developmental regulation, such as WUSCHEL (WUS) and CLAVATA (CLV), are dependent on arabinose to maintain meristem size (Xu et al., 2015). Therefore, it demonstrates the importance of arabinose in altering fruit development during the domestication process in *S. lycopersicum* (Xu et al., 2015). These findings are suggestive of the convergence of energy metabolism with developmental pathways during tomato domestication. Besides these examples, specific adaptations of the circadian and developmental clocks might also have emerged in parallel throughout the domestication process of plant species. The cultivated tomato is insensitive to day-length such that the photoperiod does not obligatorily regulate its flowering. However, this photoperiodic regulation of flowering is strictly required following loss-of-function mutations in a paralog of the florigen gene (Soyk et al., 2017a). Humans have continually selected tomatoes with slower circadian rhythms, allowing plants to grow and reproduce rapidly during longer days that are common for summers at higher latitudes of the northern hemisphere (Müller et al., 2016). Meanwhile, clocks from the shoot apex synchronize circadian rhythms in distal organs exhibiting variability in clocks precision and circadian synchrony, contributing also to environmental adaptation (Takahashi et al., 2015; Chen et al., 2020). Despite the evident human selection of developmental and circadian traits, the precise mechanism by which they were simultaneously selected during domestication remains unclear.

Timing influences all organisms' biology, and traits related to development, physiology, and metabolism are under constant spatiotemporal regulation. Beyond the circadian clock, an organism can establish temporal patterns associated with other biological clocks. Likewise, the developmental clock of an organism describes its set of chronometers that defines the emergence of cells, tissues, and organs in consonance with time, defining the unique genetic, biochemical, and morphological features of the organism (Siqueira et al., 2022). For instance, temporary cell aging is induced to arrest cell division rates in specific periods, whereas the divisions can be subsequently restored. This fact characterizes a disruption of temporal dynamics by the developmental clock and makes the time (*t*) pass differently for organisms from the same species or among different species. Meantime, the metabolic program also follows autonomous oscillations varying independently from environmental conditions (Papagiannakis et al. 2017; Siqueira et al., 2018), similar to the observed for the circadian clock. These autonomous metabolic oscillations in some moments appear to operate in coordination with cell cycle progression, supporting cell division and differentiation (Papagiannakis et al. 2017). Thus, the timing for complete oscillations seems to be different in setting the most diverse biological clocks of living organisms, which certainly affects their responsiveness to the environment. Despite considerable yield improvements, cultivated plants are currently far more sensitive to adverse environmental conditions than their wild counterparts (Zhang et al., 2018). Given this fact, it is desirable to identify regulatory mechanisms associated with circadian and developmental clocks that might have been lost during domestication, which would contribute to improving plant performance under abiotic stresses.

Along with advances in genomics, metabolomics, and variomics, knowledge concerning developmental and circadian clocks is being constantly improved, but that concerning the relationship between these clocks remains limited. In this perspective, we provide evidence that during plant domestication energy metabolism was more tightly coupled to the developmental and circadian clocks. We further suggest that the selection pressure on these oscillators generated the loss of a highly valuable source of genetic variability. Furthermore, this loss of genetic variability in the biological clocks of cultivated plants presents a major obstacle limiting both yield improvements and the development of abiotic stresses tolerant crop plants.

Rewiring Developmental Phases during Plant Domestication History

Since the establishment of civilization, humans have been selecting plants according to traits that they themselves desire. This process leads to our current crops which display altered development and yield characteristics in comparison to their wild relatives. Historically, plant breeding favored synchronicity in flowering time, optimization of plant architecture, and improvement in the number and mass of fruits/seeds per plant. Several studies further indicate that selective forces acted on specific genes during domestication, suggesting that genetic selection most likely occurred in response to particular geographical regions (Meyer and Purugganan, 2013). Indeed, such selective pressure is more prominent in genes associated with developmental and yield traits, regulators of meristem activity, changes in shoot architecture, and increases in productivity (Meyer and Purugganan, 2013). Therefore, it seems reasonable to postulate that the genetic selection culminated in a global alteration of the developmental clock, which modified both developmental transitions and yield in crop plants (Figure 1).

The Domestication of the Developmental Clock

Developmental transitions and Meristem regulation

Plants use endogenous and exogenous cues to set their developmental clock, thereby enabling them to properly complete their life cycle. Three important developmental transitions occur throughout plant life: *i*) the progression of the embryonic state to the postembryonic phase; *ii*) the transition from juvenile to adult phase during vegetative development; and *iii*) the flowering transition that enables the reproductive state (Bäurle and Dean, 2006). The developmental clock is mainly regulated by day-length, light cues, nutrients, and temperature (Bäurle and Dean, 2006). Plant domestication was exhaustively described to have synchronized the development of individuals from populations for the same domesticated species, yet this process appears to have also synchronized the entire development across the same plant (Figure 1).

Apical meristems play fundamental roles in promoting developmental transitions, and thereby they likely represent sites of step progression during plant domestication. Maize plants exhibit alterations in *cis* elements of the *Teosinte Branched 1 (TB1)* gene compared to their wild relative, teosinte (Doebley, et al., 1997).

These alterations contributed to shaping the architecture and development of plants, being a key event in maize domestication (Doebley, et al., 1997). The *TB1* gene encodes a transcriptional regulator of the *Teosynte Branched/Cycloidea/Proliferating Cell Factor* (TCP) family, which is responsible for inducing and repressing cell-cycle genes, thus coordinating meristematic activity and branch elongation (Cubas et al., 1999; Li et al., 2005). A transposable element was found in the regulatory region of the *TB1* gene which may explain, at least partially, the improvement of maize apical dominance in comparison with teosinte, further contributing to the unbranched architecture of domesticated maize (Studer et al., 2011). A number of further genes were identified that displayed signals of selection in maize, associating morphological and developmental changes during the domestication of this species (Hufford et al., 2012). Amongst these changes, the regulation of shoot apical meristem (SAM) development seems to have been essential for the transition from wild species to modern cultivars.

Apical meristems represent the source of pluripotent cells, establishing the integration of cell proliferation with endogenous and environmental cues (Park et al., 2014a). Human selection during the domestication of wild tomatoes mainly altered regulatory genes associated with the proliferation and expansion of cells in reproductive organs (Lin et al., 2014). Thus, the SAM maturation clock was demonstrated to play an important regulatory role in determining flowering time in tomato (Park et al., 2012; Park et al., 2014a). Domesticated tomato shows a more advanced status of maturation in the SAM than the wild species, resulting in the rapid transition from inflorescence meristem to flower meristem (Park et al., 2012). The genetic manipulation of the florigen (FT) gene resulted in improvements in cultivated tomato yield, which are associated with both heterosis and the absence of reproductive/vegetative balance. Notably, this genetic manipulation is the result of a recessive mutation that possibly altered gene dosage during tomato domestication (Krieger et al., 2010, Park et al., 2014b). Based on these findings, additional studies concerning changes in the flowering pathway which occurred during plant domestication are likely crucial for enhancing our understanding of crop evolution, and to aid attempts to improve yield in differing environments.

Flowering Pathway Domestication

Tight flowering time regulation is essential for successful plant life and, is also associated with more uniform flowering distribution and enhanced crop production. Early regulation of the flowering transition is accomplished in the SAM, where the maturation of meristem cells promotes inflorescence formation followed by flowering. Therefore, meristem fate was of crucial significance for maize domestication from teosinte, and one of the players in this process, the Tassels Replace Upper Ears (TRU1) protein, acts by influencing meristem activity through influencing mitosis in their cells (Dong et al., 2017). Meristem size was also largely influenced during tomato domestication via the alteration of stem cell proliferation (Rodríguez-Leal et al., 2017). Indeed, the manipulation of target genes regulating domestication has been demonstrated to have an enormous potential to improve floral organ numbers in cultivated tomato (Rodríguez-Leal et al., 2017). Notably, similar alterations in cell morphology in reproductive meristems seem to have contributed to rice domestication (Zhu et al., 2013). Two MADS-box transcription factors were demonstrated to play redundant roles in meristem maturation (Soyk et al., 2017b). Whilst one was selected during tomato domestication the other was selected by plant breeders during the tomato improvement phase. Moreover, combined mutations in these regulators of meristem maturation culminated in higher flower and fruit production (Soyk et al., 2017b). In this manner, the proliferation and differentiation of meristematic cells have been suggested as a central factor driving the domestication of wild species by our ancestors.

Indigenous Americans have selected plants to generate maize genotypes adapted to particular regions. Notably, photoperiod responsiveness is absent in teosinte, and it was further demonstrated that photoperiod has the biggest impact on the flowering of landrace maize genotypes (Hung et al., 2012). In many domesticated species, day length contributes to meristem differentiation, causing the flowering transition. Thus, species-specific pathways orientate the floral transition accordingly with photoperiod and meristem activity (Pfeiffer et al., 2014). On the other hand, the flowering repressor *Self-Pruning 5G* (*SP5G*), due to a variation in a *cis*-regulatory region of the gene, is induced in tomato wild species during long days but not in cultivated varieties (Soyk et al. 2017a). In addition, the expression of the *SP5G* gene in the cultivated tomato was lower in the transition stage of the meristem maturation (Soyk et al. 2017a).

The introgression of desirable traits (e.g. stress tolerance, higher nutrient use efficiency, and pathogens resistance) from wild relatives for cultivated plants have failed due to introgression barriers. Likewise, two novel conceptual approaches to explore genetic variability lost with domestication have been recently proposed. The first approach is termed '*de novo* domestication' of wild species, consisting of genome editing in wild-species to alter domestication genes related to agronomic traits such as plant architecture and flowering, fruit shape, size, and number (Li et al. 2018, Zsögön et al. 2018). The second approach is based on genome editing of orphan crops, which exhibit a large index for undesirable traits (e.g. excessive branching and desynchronized flowering) as observed in wild relatives (Lemmon et al., 2018). Consistently, both approaches showed that *Self-Pruning* (*SP*) and *SP5G* editions improve the yield of the wild tomato species *Solanum pimpinellifolium* (Li et al. 2018, Zsögön et al. 2018) and the orphan crop *Physalis pruinosa* (Lemmon et al., 2018), revealing their enormous potential for developing novel productive crops. It has been suggested that plant domestication modified meristem regulation and photoperiod response in parallel.

The circadian clock is among the most remarkable traits involved in the photoperiodic response, being able to finely regulate environmental and developmental responses in every plant organ. A gene ortholog of the *Arabidopsis thaliana* circadian clock regulator, *Early Flowering 3* (*ELF3*), regulates the transition to reproductive meristem and flowering-time in barley (*Hordeum vulgare*) according to day-length (Faure et al. 2012). Thus, it has been suggested that this gene was crucial to barley domestication (Faure et al. 2012). Analogously, *ELF3* variation exhibit a prominent role in the reduced photoperiod sensitivity of pigeonpea (*Cajanus cajan*) (Varshney et al. 2017). It has also been suggested that this gene facilitated the domestication of this species, enabling its cultivation in the most diverse photoperiods (Varshney et al. 2017). In maize landraces, different genes in the SAM and leaf contribute to flowering-time regulation, highlighting the coordination between genes of the circadian clock and the photoperiod pathway (Navarro et al. 2017). The greater interdependence of these two pathways might have been gradually lost during the maize domestication and its recovery could bring significant metabolic rearrangements in modern maize cultivars (Figure 2). Similarly, it was found that tomato domestication affected genes involved in carbohydrate metabolism such as regulators of sucrose and starch metabolism (Sauvage et al., 2017). Sucrose levels correlate with starch accumulation, and this accumulation is more affected by the photoperiod than by light intensity itself in *A. thaliana* (Mengin et al., 2017). Moreover, starch turnover is suggested to be controlled by the circadian clock to maintain carbon homeostasis (Seki et al., 2018). Therefore, this connection between the environment and endogenous cues promotes the transition from vegetative growth to flowering. It is believed that carbohydrate status is integrated with the SAM and the photoperiod for an adequate flowering time control (Wahl et al., 2013). It seems reasonable to suggest that the integration of development with photoperiod and the circadian clock might have been a pivotal factor in plant domestication. However, these factors are regulatory agents known to mediate the coupling between metabolism and the environment. Therefore, our ancestors might have selected plants with developmental features that converged to adapt metabolism to novel crop environments gradually leading to synchronous flowering in crop plant populations.

Metabolic changes occurring on Plant Domestication

An enormous environmental diversity is observed around farmlands worldwide. Furthermore, the centers of origin of many cultivated species are very different environmentally from the regions where they are now cultivated (Fernie and Yan, 2019). Domestication selected plants adaptable to a climate different from that of their origin region. For example, it has been suggested that rice domestication modified several genes involved in environmental responses (Li et al., 2017). The early factors regulating environmental responses are photosynthesis and respiration, which in turn trigger changes in metabolism and plant yield. Leaf growth rate is better determined by physiological than morphological features in tomato, whereas domesticated tomato exhibited accentuated differences in these components when compared with wild tomato species (Conesa et al., 2017). Therefore, knowingly the photosynthesis and respiration, affect widely the adaptation of plants to contrasting environments, and alterations in the plasticity of these traits may have been contributed to domesticating the tomato and other species.

Natural genetic variation in *Capsicum chinense* indicates that fruit set is directly associated with photosynthesis-related parameters (Rosado-Souza et al., 2015), while RuBisCO is the major enzyme responsible for CO_2 fixation in chloroplasts, exercising a central role in the photosynthesis of land plants. Thus, RuBisCO enzymes of undomesticated plants from diverse climate regions often show higher carboxylation capacity and plants harboring them often display a higher yield than cultivated plants (Orr et al., 2016). Differences in RuBisCO sequences were found among species exhibiting several amino acid substitutions (Orr et al., 2016). By evaluating the strength of natural selection on gene expression in rice, it was found that selection act on photosynthesis-related genes (e.g. the RuBisCO small subunit and RuBisCO activase) (Groen et al., 2020). Meanwhile, the expression of genes encoding proteins associated with photosynthesis exhibits a close correlation with leaf area, chlorophyll levels, and *fitness* (Groen et al., 2020). Additionally, tomato domestication triggered remarkable variation in gene expression of photosynthesis-related transcripts, and these transcripts showed an inverse correlation with leaf development-related transcripts (Ranjan et al., 2016). The altered leaf morphology alongside changes in photosynthesis and respiration most likely acted in a combined manner to regulate metabolism and plant yield (Nunes-Nesi et al., 2016). At the metabolome level, wheat (*Triticum turgidum*) domestication triggered a metabolic rewiring with changes in the levels of unsaturated fatty acids and amino acids characterizing the primary and secondary domestication events of this species, respectively (Beleggia et al., 2016). Tomato domestication, by contrast, resulted in the reduction of anti-nutritional compounds in fruit whilst breeding selections for larger fruits were documented to play a greater impact on the metabolome than the domestication (Zhu et al., 2018). These findings appear to indicate that domestication affected key metabolites as opposed to hundreds of compounds, correlating with a gradual adaptation to most distinct environments (Figure 2).

In this sense, the adzuki bean (*Vigna angularis*) seed is characterized to show high starch levels, which in turn is the result of strong selection during domestication (Yang et al., 2015). Starch metabolism genes were also central agents in barley domestication, exhibiting intrinsic correlations with genes of photoperiod-responses and the circadian clock (Pankin et al., 2018). Moreover, in contrast to wild cotton, in cultivated cotton, genes encoding Target of Rapamycin (TOR) and its binding partner

Raptor display a demethylated status which was reported to its metabolic regulation, playing a role in the loss of photoperiod sensitivity during cotton domestication (Song et al., 2017). Likewise, long noncoding RNAs (lncRNAs) modified the expression of genes related to energy metabolism during rice domestication, increasing grain starch levels (Zheng et al., 2019). The finding that the SP5G gene is a central player in tomato domestication, as well as underlying several metabolic QTLs (Schauer et al., 2006), indicates a possible parallel selection in these traits during tomato domestication. In good agreement, one of the pivotal proteins in maize domestication, TRU1 affects shoot meristem maturation and was suggested to control the flux of diffusible substances (Dong et al., 2017). Both transport and accumulation of carbohydrates were also suggested to be important factors in lncRNAs mediated phenotypic evolution during rice domestication (Zheng et al., 2019). Accordingly, shoot meristem regulation is directly altered by sugar metabolism and it is fundamental for the meristem cell growth (Xu et al., 2015). Indeed, this mechanism was suggested to control stem cell proliferation during tomato domestication, enhancing fruit size (Xu et al., 2015). Thus, specific metabolic signatures are found in the plant domestication process, varying mainly accordingly with organ development and environment of origin. For instance, a protein that interacted with dozens of proteins related to the circadian clock and development in wild species, with domestication began to interact with few proteins (Figure 2). In this respect, it will be instrumental to find specific connections between developmental and circadian clocks across the domestication process, given that these clocks are the major regulators of the energy metabolism as well as the environmental responses.

Circadian and Developmental Clocks Display Similar Features with Energy Metabolism in Plant Domestication Context

The diversification in the distribution of cultivated plants worldwide largely altered the physiology of these plants. As such plants which are more adapted to diverse environments were progressively exhibiting elevated phenotypic plasticity for physiological and developmental traits. In nature, the perennial species, *Echinacea angustifolia*, which is widespread in North America displays fire-synchronized flowering time with postfire formed populations exhibiting higher vegetative yield and fecundity than plants grown from unburnt seeds (Wagenius et al., 2019). This example sheds light on how environmental traits might set the biological clocks of wild plants, although additional endogenous cues must be considered for their adjustment. A pivotal mediator of physiological and developmental responses in plants is the circadian clock which triggers responses according to environmental conditions, mainly responses associated with photoperiod and temperature adaptations. In several instances, the mechanisms that orchestrate the circadian timekeeping occur regardless of transcriptional control, being sufficient to sustain the circadian clock (O'Neill et al., 2011). Markers for these adaptations are the maximum efficiency of photosystem II in light (F_v'/F_m') and heat dissipation by non-photochemical quenching (NPQ), which are photoprotective components. Recently, the role for the circadian clock in regulating environmental adaptation in *A. thaliana*, based on the control of photosynthesis light reactions and photoprotective mechanisms, was revealed (Yarkhunova et al., 2018). Wild barley shown variability for circadian traits as assessed by F_v'/F_m' variations, and it was suggested that environmental conditions likely helped to select the circadian rhythms during domestication (Dakhiya et al., 2017). In this sense, the circadian clock controls photosynthesis at different levels, either mediating CO_2 fixation (Dodd et al., 2005), regulating the metabolism (Sanchez-Villarreal et al., 2013), and/or at the level of chloroplast gene expression (Noordally et al., 2013). Apparently, a correlated evolution for circadian traits and photosynthetic rates indeed exists. In fact, *Brassica rapa* domestication involved a parallel selection of both circadian and photosynthetic traits (Yarkhunova et al., 2016). By contrast, chloroplast activity and cell-metabolism are largely dependent on mitochondrial respiration, which mediates the maintenance of photosynthesis under adverse environmental conditions (Nunes-Nesi et al., 2011), indicating a potential role of respiration in the circadian network. In support of this, the circadian clock controls mitochondrial respiration in mice allowing a proper synchronization of metabolic pathways with the diurnal cycle (Peek et al., 2013). In a similar vein, metabolic components may have been fundamental components in environmental adaptation during plant domestication, which have been mediated mainly through of circadian clock selection. Together, these studies suggest that key physiological traits are quickly remodeled with environmental and circadian oscillations, such as photosynthesis and respiration, which suffered several mechanistic adaptations over the plant domestication history.

Metabolic plasticity contributes to optimizing cell energy use, and energy sensors such as TOR and the non-fermenting 1 (Snf1)-related kinase 1 (SnRK1) complex are fundamental in this context. TOR and SnRK1 control mRNA translation, linking endogenous and exogenous cues in a circadian clock-dependent manner, to daily metabolic programing (Feeney et al., 2016; Shin et al., 2017). To corroborate this, endogenous sugar signals regulate the gene expression of circadian clock components according to photoperiod (Dalchau et al., 2011; Haydon et al., 2013). The programming of metabolic oscillations is of pivotal significance in circadian clock regulation (Dalchau et al., 2011; Haydon et al., 2013). Particular variations in circadian clock behavior were found in wild and domesticated plants, evidencing that natural and human selections might have acted differently to set this clock (Greenham et al., 2017) (Figure 1). On the other hand, it has been demonstrated that natural variation in circadian components conveys a growth advantage in wild plants when compared with domesticated plants, inducing precise changes in gene expression during day/night cycles (de Montaigu et al., 2015). Starch metabolism is widely known to be regulated according to day/night cycles and to influence plant growth and yield.

Changes in starch metabolism trigger signals for circadian adaptation in wild plants, where starch metabolism-related genes seem to show genetic covariation with circadian clock genes (Salmela et al., 2018). Recently, it was demonstrated that domestication slowed down the circadian clock of the cultivated tomato, improving its performance specifically under long day conditions, which enabled tomato cultivation under long summer days (Müller et al., 2016). In this sense, the decelerated circadian clock in cultivated tomato is suggested to be light-dependent requiring photoreceptor signaling (Müller et al., 2018). The relevance of human selection on the circadian clock of soybean (*Glycine max*) was also shown to be fundamental in allowing the flowering of domesticated soybean under long days (Lu et al., 2020). This selection empowered LHY proteins that suppress *E1* gene expression, with the E1 protein being a repressor of *FT* mobility to the SAM (Lu et al., 2020). Thus, domesticated soybean has the development synchronized in comparison with wild relatives owing to the impaired activity of E1 protein (Lu et al., 2020). Apparently, soybean domestication promoted an accurate communication between leaf and SAM, suggesting how biological clocks setting may have been important during the evolution of this crop. Intriguingly, the circadian period of older leaves is shorter than younger leaves, revealing a co-regulation of leaf aging and circadian rhythms re-orientation (Kim et al., 2016). Nevertheless, it is interesting to notice that each leaf tissue displays a particular clock contributing differentially to the global leaf clock. For instance, mesophyll and vascular clocks exhibit asymmetrically regulation of each other to regulate *A. thaliana* development (Endo et al., 2014). The dominant clock has been suggested to be the shoot apex circadian clock that synchronizes the circadian rhythms of different tissues, exhibiting a gradation in circadian communication (Takahashi et al., 2015). Likewise, shoot and root circadian clocks show distinct responses to ambient temperature - which is, by the way, a resetting cue of clocks - revealing the autonomy and specificity of both organs to regulate circadian clocks in response to the environment (Li et al., 2020). Therefore, communication between biological clocks appears pivotal for proper plant physiology and development, although how exactly this network was selected during crop domestication remains largely unknown.

At the cellular level, multicellularity improves the delay in anticipating outer cycles in *A. thaliana* (Masuda et al., 2017). Duckweed (*Lemna gibba*) growing under constant conditions exhibit cells working with individual clocks that oscillate autonomously and respond independently to external stimuli, revealing the relevance of light-dark cycles to correct the heterogeneity of circadian clocks in intact plants (Muranaka and Oyama, 2016). The heterogeneity among circadian clocks revealed that mice growing under a high-fat diet loosened the temporal coherence of circadian metabolism among eight different tissues, resulting in abnormal cell proliferation and unbalanced growth of these tissues (Dyar et al. 2018). Furthermore, an autonomous branch of the liver's circadian clock is independent of all other clocks yet still dependent on the light-dark cycle (Koronowski et al., 2019). In wild tomato species, a dominant locus promotes tolerance to continuous light, where the major genetic factor is the gene light harvesting *chlorophyll a/b binding protein 13* (CAB-13) (Velez-Ramirez et al., 2014). Introgression lines for this locus in domesticated tomato hybrid lines overcome the adaptation to day/night cycles by not disrupting carbohydrate metabolism and photosynthesis under constant light (Velez-Ramirez et al., 2014). Likewise, Sugars Will Eventually Be Exported Transporter 4c (SWEET4c) mediates hexose transport and seed filling in domesticated maize and rice, where the *SWEET4c* gene shows signatures of selection that occurred during species domestication (Sosso et al., 2015). Besides these examples, the transcription factor TB1 arrests axillary branching in the cultivated specie through altered energy balance in axillary bud tissue, which impairs cell proliferation at this site (Dong et al., 2019). Organism size raises a relevant question regarding animal and plant domestications. To domesticate farm animals, humans performed selections favoring specimens with intermediate to high size and low metabolic rates, while metabolism and size are largely uncoupled in plant domestication processes (Milla et al., 2018). To explain a part of this uncoupling in plants, the highly branched pattern in wild plants might be due to weak inter-tissue correlations for circadian metabolism, which was altered during the domestication of these species (Figure 1). From the findings of Milla et al. (2018), it is possible to note wild counterparts of crop plants displaying a broad spectrum relationship between metabolism and plant size, which suggests domestication set both metabolism and developmental clocks in plants (Figure 1). Notably, the recent findings suggest that the deceleration in circadian clock of tomato altered metabolism and development at the same time (Figure 2).

Concerning development, the circadian clock has been proposed to play intrinsic relationships with the developmental clock according to environmental conditions in animals, yeasts, and plants. In photosynthetic unicellular eukaryotes, cell division machinery maintains oscillations synchronized with circadian rhythms. Thus, unicellular red alga *Cyanidioschyzon merolae* exhibits cell cycle progression dependent on the circadian rhythms, and this network protects cells from oxidative stress (Miyagishima et al., 2014). In plants, cell cycle-related proteins were suggested to regulate energy balance at the same time that they regulate cell division and differentiation (Siqueira et al., 2018), while the circadian clock was demonstrated to set the time of the cell cycle (Fung-Uceda et al., 2018). Similarly, the domesticated potato shows selection signatures in genes regulating cell cycle, circadian clock, and energy metabolism, highlighting selection signals in genes associated with starch and sucrose metabolisms as well as in the energy sensor SnRK1 (Kloosterman et al., 2013; Hardigan et al., 2017).

Local and systemic responses to stresses in wild and domesticated genotypes

The phenotype is classically described to cover the relationships between genotype and environment, wherein genotype is more predictable than environmental conditions that occur during plant lifecycle, which are widely uncertain. Informative cues emanating from the environment are integrated into the signaling pathways of plants, and the ability to process and transmit coherent information between cells and organs is fundamental to coping with abiotic and biotic stresses (Chaiwanon et al., 2016). Communication skills orientate the improvement of water and nutrient capture as well as tolerance to diseases, flooding, and salinity (Bailey-Serres et al. 2019). Thus, a higher genetic diversity to improve crop tolerance for biotic and abiotic stresses is available on landraces and wild relatives of crops (Bailey-Serres et al. 2019). Domestication generated the loss of genes related to pattern recognition of pathogens (Nelson et al., 2018), whilst alleles that provide flooding survival might be found only in wild *Oryza* species (Bailey-Serres et al. 2019; Stein et al., 2018). Therefore, more heterogeneous clocks working independently might have a fast recognition of the dangerous conditions like pathogens attacks and environmental adversities.

Wild species such as teosinte and *Setaria viridis* are more able to sense local changes in soil water potential than their domesticated relatives, maize and *Setaria italica,* respectively (Sebastian et al., 2016). On analyzing the findings from Sebastian et al. (2016), it appears that wild species suppress the growth of the crown root causing minimal changes in leaf number under water limitations. Such imperturbable growth may be mediated by possible independence between leaf and root clocks. However, this is not occurring in crop species that exhibit high coordination among organs to maximize yield. Consequently, the salt tolerance of *S. pimpinellifolium* might be explained by the activity of transporter SlHAK20, which transports Na^+ and K^+ regulating the homeostasis of these elements (Wang et al., 2020). The gene encoding *SlHAK20* was selected during tomato domestication, and *SlHAK20* gene editing of *S. pimpinellifolium* rendered mutants that were not able to retain Na^+ in their roots, translocating more Na^+ to shoots turning the species sensitive to salinity (Wang et al., 2020). This finding suggests a candidate that couples clocks of root and shoot under salt stress, which was negatively selected against and thus lost functionality during tomato domestication.

Circadian signals derived from the shoot might move into the roots delivering temperature information to the subterraneous organ, thereby re-orientating the pacing of root circadian rhythms (Chen et al., 2020). Furthermore, the desert annual plant *Nicotiana attenuata* exhibit drought tolerance due to TOC1 gene expression in the shoot, wherein the expression of this gene on roots seems not to be sufficient to maintain plant fitness under drought conditions (Valim et al., 2019). As such, *S.*

pennellii growing under elevated CO_2 exhibit higher abundance for transcripts associated with translation comparatively to ambient CO_2, wherein *S. lycopersicum* has the same patterns for both conditions (Gray et al., 2020). The unperturbed translation in domesticated tomato seems to indicate cells working more coordinately are more difficult to redirect in terms of the translational regulatory modules. By contrast, wild tomato owing to independent biological clocks easily changes these modules for novel operating modules. By evaluating local and systemic responses to mechanical wounding of leaves of *S. lycopersicum* and *S. pennellii*, it was found that locally the wild tomato exhibits a wide induction and repression of gene expression compared to domesticated specie (Liu et al., 2018). Meanwhile, *Submergence Up-Regulated Families* (*SURFs*) genes are evolutionarily retained and activated during submergence and hypoxia (Reynoso et al., 2019). To estimate the conservation in modulating global gene expression, it was revealed more syntenic pattern for *SURFs* of *S. lycopersicum* than *S. pennellii* (Reynoso et al., 2019). Thus, distinct transcription factors co-regulate a higher number of genes in domesticated specie (Reynoso et al., 2019), indicating much more specific responses for *S. pennellii*. Over the past decades, a tremendous potential for understanding wild species' responses to the environment has been suggested as essential to enable to help us to improve crop tolerance to biotic and abiotic stresses.

The genomics of Genebanks has recently been introduced as a powerful tool to discover and transfer alleles from wild relatives and landraces into cultivated plants (Mascher et al., 2019). The phenotypic mapping of complex traits in germplasm banks, such as the synchrony of biological clocks and stress tolerance, is not a difficult task that genome-wide prediction may help provide insight into (Yu et al., 2016; Mascher et al., 2019). Initially, recognizing genomic and phenotypic features of ancient species could help us to direct the efforts to map phenotypes of specimens available in germplasm banks nowadays. Following, the spread of cultivated specimens worldwide such approaches have proven instructive when applied to the adaptation of maize to temperate North America and potato to the long summer days in Europe (Swarts et al., 2017; Gutaker 2019). That said we currently have a relative dearth of such genomic information on diverse wild species and landraces. A novel approach that circumvents this fact, based on short sequences - *k*-mers- is able to find a wider range of genetic variants related to phenotypic and genetic variability for a plethora of traits regardless of a reference genome (Voichek and Weigel, 2020). Thus, the variant origin is precisely localized and *k*-mers might be used to measure relatedness between individuals thus paving the way for GWAS analyzes in organisms without high-quality reference genomes (Voichek and Weigel, 2020). Taken together, the association of ancient genomics and *k*-mers mapping seems to be a powerful tool to re-orientate stress tolerance on domesticated crops, helping us to predict phenotypes already available in Genebanks.

Current estimates regarding future climate uncertainties impose restrictions to design crops for the next decades. By mapping genomic features for diverse individuals, polygenic signatures mediating adaptation to extreme drought were revealed more common in *A. thaliana* accessions from Mediterranean and Scandinavian regions (Exposito-Alonso et al., 2018). By contrast, populations from Central Europe might be slower than populations of the Mediterranean to adapt to climate changes at the end of the twenty-first century (Exposito-Alonso et al., 2018). Native populations from the Mediterranean to Temperate of Europe regions will can experience more-negative selection that will reduce their fitness and local adaptation, turning plant populations prone to evolutionary risk (Exposito-Alonso et al., 2019). The novel and exciting genomic approaches indicated here represent promising strategies to explore biological clocks within the context of domestication and crop improvement, We, therefore, posit that these tools might help us to predict the performance of current cultivars and varieties on crops of the next century, suggesting the most productive and stables genotypes during the environmental adversities anticipated for the future.

Concluding Remarks

Taken together, the results described providing compelling evidence that a convergent network encompassing developmental and circadian clocks mediated plant domestication. Perhaps more important, it was also revealed that these clocks may have been connected in several cases through energy metabolism driving environmental adaptations. Understanding and identification of connections in this regulatory network achieved along the evolution in domestication may contribute to increasing genetic diversity associated with mechanisms regulating plant yield, encouraging novel plant breeding programs, and further improving crop production under sub-optimal environmental conditions.

References

1. Doebley, J. *et al.* (2006) The Molecular Genetics of Crop Domestication. **Cell 127**, 1309-1321.
2. Park, S.J. *et al.* (2012) Rate of meristem maturation determines inflorescence architecture in tomato. **Proc. Natl. Acad. Sci. USA 109**, 639-644.
3. Xu, C. *et al.* (2015) A cascade of arabinosyltransferases controls shoot meristem size in tomato. **Nat. Genet. 47**, 784–792.
4. Soyk, S. *et al.* (2017a). Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. **Nat. Genet. 49**, 162–168.
5. Müller, N.A. *et al.* (2016) Domestication selected for deceleration of the circadian clock in cultivated tomato. **Nat. Genet. 48**, 89-93.
6. Takahashi, N. *et al.* (2015) A hierarchical multi-oscillator network orchestrates the Arabidopsis circadian system. **Cell 163**, 148–159.
7. Chen, W.W. *et al.* (2020) A mobile ELF4 delivers circadian temperature information from shoots to roots. **Nat Plants 6**, 416–426.
8. Siqueira, J.A. *et al.* (2022) The hidden half comes into the spotlight: Peeking inside the black box of root developmental phases. **Plant Comm. 3**: 100246.
9. Papagiannakis, A. *et al.* (2017) Autonomous metabolic oscillations robustly gate the early and late cell cycle. **Mol. Cell 65**, 285-295.
10. Siqueira, J.A. *et al.* (2018) Unraveling Interfaces between Energy Metabolism and Cell Cycle in Plants. **Trends in Plant Sci. 23**, 731-747.
11. Zhang, H. *et al.* (2018) Developing naturally stress-resistant crops for a sustainable agriculture. **Nat. Plants 4**, 989-996.
12. Meyer, R.S. and Purugganan, M.D. (2013) Evolution of crop species genetics of domestication and diversification. **Nat. Rev. Genet. 14**, 840- 852.
13. Bäurle, I. and Dean, C. (2006) The timing of Developmental transitions in Plants. **Cell 125**, 655-664.
14. Doebley, J. *et al.* (1997). The evolution of apical dominance in maize. **Nature 386**, 485–488.
15. Cubas, P. *et al.* (1999). The TCP domain: a motif found in proteins regulating plant growth and development. **Plant J. 18**, 215–222.
16. Li, C. *et al.* (2005). Arabidopsis TCP20 links regulation of growth and cell division control pathways. **Proc. Natl. Acad. Sci. USA 102**, 12978–12983.

17. Studer, A. *et al.* (2011) Identification of a functional transposon insertion in the maize domestication gene tb1. **Nat. Genet. 43**, 1160–1163.
18. Hufford, M.B. *et al.* (2012) Comparative population genomics of maize domestication and improvement. **Nat. Genet. 44**, 808–811.
19. Park, S.J. *et al.* (2014a) Meristem maturation and inflorescence architecture–lessons from the Solanaceae. **Curr. Opin. Plant Biol. 17**, 70–77.
20. Lin, T. *et al.* (2014) Genomic analyses provide insights into the history of tomato breeding. **Nat. Genet. 46**, 1220–1226.
21. Park, S.J. *et al.* (2014b) Optimization of crop productivity in tomato using induced mutations in the florigen pathway. **Nat. Genet. 46**, 1337–1342.
22. Krieger, U. *et al.* (2010) The flowering gene SINGLE FLOWER TRUSS drives heterosis for yield in tomato. **Nat. Genet. 42**, 459–463.
23. Dong, Z.B. *et al.* (2017) Ideal crop plant architecture is mediated by tassels replace upper ears1, a BTB/POZ ankyrin repeat gene directly targeted by TEOSINTE BRANCHED1. **Proc. Natl. Acad. Sci. USA, 114**, E8656-E8664.
24. Rodríguez-Leal, D. *et al.* (2017) Engineering Quantitative Trait Variation for Crop Improvement by Genome Editing. **Cell 171,** 470–480.
25. Zhu, Z. *et al.* (2013) Genetic control of inflorescence architecture during rice domestication. **Nat. Comm. 4**:2200
26. Soyk, S. *et al.* (2017b) Bypassing Negative Epistasis on Yield in Tomato Imposed by a Domestication Gene. **Cell 169**, 1142–1155.
27. Hung, H.Y. *et al.* (2012) ZmCCT and the genetic basis of day-length adaptation underlying the postdomestication spread of maize. **Proc. Natl. Acad. Sci. USA 109**, E1913–E1921.
28. Pfeiffer, A. *et al.* (2017) Beyond flexibility: Controlling stem cells in an ever changing environment. **Curr. Opin. Plant Biol. 35**,117–123.
29. Li, T. *et al.* (2018) Domestication of wild tomato is accelerated by genome editing. **Nat. Biotechnol. 36**, 1160–1163
30. Zsögön, A. *et al.* (2018) De novo domestication of wild tomato using genome editing. **Nat. Biotechnol. 36**, 1211–1216.
31. Lemmon, Z.H. *et al.* (2018) Rapid improvement of domestication traits in an orphan crop by genome editing. **Nat. Plants** 4, 766–770

32. Faure, S. *et al.* (2012) Mutation at the circadian clock gene EARLY MATURITY 8 adapts domesticated barley (*Hordeum vulgare*) to short growing seasons. **Proc. Natl. Acad. Sci. USA 109**, 8328–8333.
33. Varshney, R.K. *et al.* (2017) Whole-genome resequencing of 292 pigeonpea accessions identifies genomic regions associated with domestication and agronomic traits. **Nat. Genet. 49**, 1088-1090.
34. Navarro, J.A.R. *et al.* (2017) A study of allelic diversity underlying flowering-time adaptation in maize landraces. **Nat. Genet. 49**, 476-483.
35. Sauvage, C. *et al.* (2017) Domestication rewired gene expression and nucleotide diversity patterns in tomato. **Plant J. 91,** 631–645.
36. Mengin, V. *et al.* (2017) Photosynthate partitioning to starch in Arabidopsis thaliana is insensitive to light intensity but sensitive to photoperiod due to a restriction on growth in the light in short photoperiods. **Plant, Cell and Environ. 40**, 2608–2627.
37. Seki, M. *et al.* (2018) Adjustment of the Arabidopsis circadian oscillator by sugar signalling dictates the regulation of starch metabolism. **Sci. Reports 7**: 8305.
38. Wahl, V. *et al.* (2013) Regulation of flowering by trehalose-6-phosphate signaling in *Arabidopsis thaliana*. **Science 339**, 704-707.
39. Fernie, A.R. and Yan, J. (2019) De novo domestication: an alternative route toward new crops for the future. **Mol. Plant 12**, 615–631.
40. Li, L.F. *et al.* (2017) Signatures of adaptation in the weedy rice genome. **Nat. Genet. 49**, 811-816.
41. Conesa, M.A. *et al.* (2017) Growth capacity in wild tomatoes and relatives correlates with original climate in arid and semi-arid species. **Environ. and Exp. Bot. 141**, 181-190.
42. Rosado-Souza, L. *et al.* (2015) Exploring natural variation of photosynthetic, primary metabolism and growth parameters in a large panel of *Capsicum chinense* accessions. **Planta 242**, 677-691.
43. Orr, D. *et al.* (2016) Surveying Rubisco diversity and temperature response to improve crop photosynthetic efficiency. **Plant Physiol. 172**, 707–717.
44. Groen, S.C. *et al.* (2020) The strength and pattern of natural selection on gene expression in rice. **Nature 578**, 572–576.

45. Ranjan, A. *et al.* (2016) eQTL Regulating Transcript Levels Associated with Diverse Biological Processes in Tomato. **Plant Physiol. 172**, 328-340.
46. Nunes-Nesi, A. *et al.* (2016) Natural genetic variation for morphological and molecular determinants of plant growth and yield. **J. Exp. Bot. 67**, 2989–3001.
47. Beleggia, R. *et al.* (2016) Evolutionary metabolomics reveals domestication-associated changes in tetraploid wheat kernels. **Mol. Biol. Evol. 33**, 1740–1753.
48. Zhu, G. *et al.* (2018) Rewiring of the Fruit Metabolome in Tomato Breeding. **Cell 172**, 249–261.
49. Yang, K. *et al.* (2015) Genome sequencing of adzuki bean (Vigna angularis) provides insight into high starch and low fat accumulation and domestication. **Proc. Natl. Acad. Sci. USA 112**, 13213–13218.
50. Pankin, A. *et al.* (2018) Targeted resequencing reveals genomic signatures of barley domestication. **New Phytol. 218**, 1247–1259.
51. Song, Q. *et al.* (2017) Epigenomic and functional analyses reveal roles of epialleles in the loss of photoperiod sensitivity during domestication of allotetraploid cottons. **Genome Biol. 18**, 99.
52. Zheng, X.M. *et al.* (2019) Genome-wide analyses reveal the role of noncoding variation in complex traits during rice domestication. **Sci. Adv. 5**, eaax3619.
53. Schauer, N. *et al.* (2006). Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. **Nat. Biotechnol. 24**, 447–454.
54. Wagenius, S. *et al.* (2019) Fire Synchronizes Flowering and Boosts Reproduction in a Widespread but Declining Prairie Species. **Proc. Natl. Acad. Sci. USA 117**, 3000-3005.
55. O'Neill, J.S. *et al.* (2011). Circadian rhythms persist without transcription in a eukaryote. **Nature 469**, 554–558.
56. Yarkhunova, Y. *et al.* (2018) Circadian rhythms are associated with variation in photosystem II function and photoprotective mechanisms. **Plant Cell Environ. 41**, 2518-2529.
57. Dakhiya, Y. *et al.* (2017) Correlations between circadian rhythms and growth in challenging environments. **Plant physiology 173**, 1724-1734
58. Dodd, A.N. *et al.* (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. **Science 309**, 630-633.

59. Noordally, Z.B. *et al.* (2013) Circadian control of chloroplast transcription by a nuclear encoded timing signal. **Science 339**, 1316-1319.
60. Yarkhunova, Y. *et al.* (2016) Selection during crop diversification involves correlated evolution of the circadian clock and ecophysiological traits in *Brassica rapa*. **New Phytol. 210**, 1469-8137.
61. Peek, C.B. *et al.* (2013) Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. **Science 342**, 1243417.
62. Nunes-Nesi, A. *et al.* (2011) Targeting mitochondrial metabolism and machinery as a means to enhance photosynthesis. **Plant Physiol. 155**, 101–107.
63. Feeney, K.A. *et al.* (2016) Daily magnesium fluxes regulate cellular timekeeping and energy balance. **Nature 532**, 375–379.
64. Shin, J. *et al.* (2017) The metabolic sensor AKIN10 modulates the Arabidopsis circadian clock in a light-dependent manner. **Plant Cell Environ. 40**, 997–1008.
65. Dalchau, N. *et al.* (2011) The circadian oscillator gene GIGANTEA mediates a long-term response of the *Arabidopsis thaliana* circadian clock to sucrose. **Proc. Natl. Acad. Sci. USA 108**, 5104-5109.
66. Haydon, M.J. *et al.* (2013) Photosynthetic entrainment of the Arabidopsis thaliana circadian clock. **Nature 502**, 689–692.
67. Greenham, K. *et al.* (2017) Geographic variation of plant circadian clock function in natural and agricultural settings. **J. Biol. Rhythms 32**, 26-34.
68. de Montaigu, A., *et al.* (2015) Natural diversity in daily rhythms of gene expression contributes to phenotypic variation. **Proc. Natl. Acad. Sci. USA 112**, 905-910.
69. Salmela, M.J. *et al.* (2018) Circadian Rhythms and Reproductive Phenology Covary in a Natural Plant Population. **J. Biol. Rhythms 33**, 245-254.
70. Müller, N.A. *et al.* (2018) Mutations in EID1 and LNK2 caused light-conditional clock deceleration during tomato domestication. **Proc. Natl. Acad. Sci. USA** 15, 7135-7140.
71. Lu, S. *et al.* (2020) Stepwise selection on homeologous PRR genes controlling flowering and maturity during soybean domestication. **Nat. Genet. 52**, 428–436.
72. Kim, H. *et al.* (2016) Age-associated circadian period changes in Arabidopsis leaves. **J. Exp. Bot. 67**, 2665–2673.
73. Endo, M. *et al.* (2014) Tissue-specific clocks in Arabidopsis show asymmetric coupling. **Nature 515**, 419–422.

74. Li, Y. *et al.* (2020) Molecular investigation of organ-autonomous expression of *Arabidopsis* circadian oscillators. **Plant Cell Environ**. **43**, 1501–1512.
75. Masuda, K. *et al.* (2017) Multicellularity enriches the entrainment of Arabidopsis circadian clock. **Sci. Adv. 3**:e1700808.
76. Muranaka, T. and Oyama, T. (2016) Heterogeneity of cellular circadian clocks in intact plants and its correction under light-dark cycles. **Sci. Adv. 2**:e1600500.
77. Dyar, K.A. *et al.* (2018) Atlas of circadian metabolism reveals system-wide coordination and communication between clocks. **Cell 174**, 1571–1585.
78. Koronowski, K.B. *et al.* (2019) Defining the Independence of the Liver Circadian Clock. **Cell 177**, 1448-1462.
79. Velez-Ramirez, AI. *et al.* (2014) A single locus confers tolerance to continuous light and allows substantial yield increase in tomato. **Nat. Commun. 5**:4549.
80. Sosso, D. *et al.* (2015) Seed filling in domesticated maize and rice depends on SWEET-mediated hexose transport. **Nat Genet 47**, 1489–149.
81. Dong, Z. *et al.* (2019) The regulatory landscape of a core maize domestication module controlling bud dormancy and growth repression. **Nat. Commun. 10**:3810.
82. Milla, R. *et al.* (2018) Phylogenetic patterns and phenotypic profiles of the species of plants and mammals farmed for food. **Nat. Ecol. Evol. 2**, 1808–1817.
83. Miyagishima, S. *et al.* (2014) Translation-independent circadian control of the cell cycle in a unicellular photosynthetic eukaryote. **Nat. Commun. 5**: 3807.
84. Siqueira, J.A. *et al.* (2018) Unraveling Interfaces between Energy Metabolism and Cell Cycle in Plants. **Trends Plant Sci. 23**, 731–747.
85. Fung-Uceda, J. *et al.* (2018) The circadian clock sets the time of DNA replication licensing to regulate growth in Arabidopsis. **Dev. Cell 45**,101–113.
86. Kloosterman, B. *et al.* (2013) Naturally occurring allele diversity allows potato cultivation in northern latitudes. **Nature 495**, 246–250.
87. Hardigan, M.A. *et al.* (2017) Genome diversity of tuber-bearing Solanum uncovers complex evolutionary history and targets of domestication in the cultivated potato. **Proc. Natl. Acad. Sci. USA 114**, E9999–E10008.
88. Chaiwanon, J. *et al.* (2016) Information integration and communication in plant growth regulation. **Cell 164**, 1257–1268.

89. Bailey-Serres, J. *et al.* (2019) Genetic Strategies for Improving Crop Yields. **Nature 575**, 109–188.
90. Nelson, R. *et al.* (2018) Navigating complexity to breed disease-resistant crops. **Nat. Rev. Genet. 19**, 21–33.
91. Stein, J.C. *et al.* (2018) Genomes of 13 domesticated and wild rice relatives highlight genetic conservation, turnover and innovation across the genus *Oryza*. **Nat. Genet. 50**, 285–296.
92. Sebastian, J. *et al.* (2016) Grasses suppress shoot-borne roots to conserve water during drought. **Proc. Natl. Acad. Sci. USA 113**, 8861–8866.
93. Wang, Z. *et al.* (2020) Loss of salt tolerance during tomato domestication conferred by variation in a Na^+/K^+ transporter. **EMBO J. 39**: e103255.
94. Valim, H.F. *et al.* (2019) The clock gene TOC1 in shoots, not roots, determines fitness of *Nicotiana attenuata* under drought. **Plant Physiol.181**, 305–318.
95. Gray, S.B. *et al.* (2020) Translational regulation contributes to the elevated CO_2 response in two *Solanum* Species. **Plant J. 102**, 383-397.
96. Liu, M-J. *et al.* (2018) Regulatory Divergence in Wound-Responsive Gene Expression between Domesticated and Wild Tomato. **Plant Cell 30**, 1445–1460.
97. Reynoso, M.A. *et al.* (2019) Evolutionary flexibility in flooding response circuitry in angiosperms. **Science 365**, 1291–1295.
98. Mascher, M. *et al.* (2019) Genebank genomics bridges the gap between the conservation of crop diversity and plant breeding. **Nat. Genet. 51**, 1076–1081.
99. Yu, X. *et al.* (2016) Genomic prediction contributing to a promising global strategy to turbocharge gene banks. **Nat. Plants 2**: 16150.
100. Swarts, K. *et al.* (2017) Genomic estimation of complex traits reveals ancient maize adaptation to temperate North America. **Science 357**, 512–515.
101. Gutaker, R.M. *et al.* (2019) The origins and adaptation of European potatoes reconstructed from historical genomes. **Nat. Ecol. Evol. 3**, 1093–1101.
102. Voichek, Y. and Weigel, D. (2020) Identifying genetic variants underlying phenotypic variation in plants without complete genomes. **Nat. Genet. 52**, 534–540.
103. Exposito-Alonso, M. *et al.* (2018) Genomic basis and evolutionary potential for extreme drought adaptation in *Arabidopsis thaliana*. **Nat. Ecol. Evol. 2**, 352–358.

104. Exposito-Alonso, M. *et al.* (2019) Natural selection on the *Arabidopsis thaliana* genome in present and future climates. **Nature 573**, 126–129.

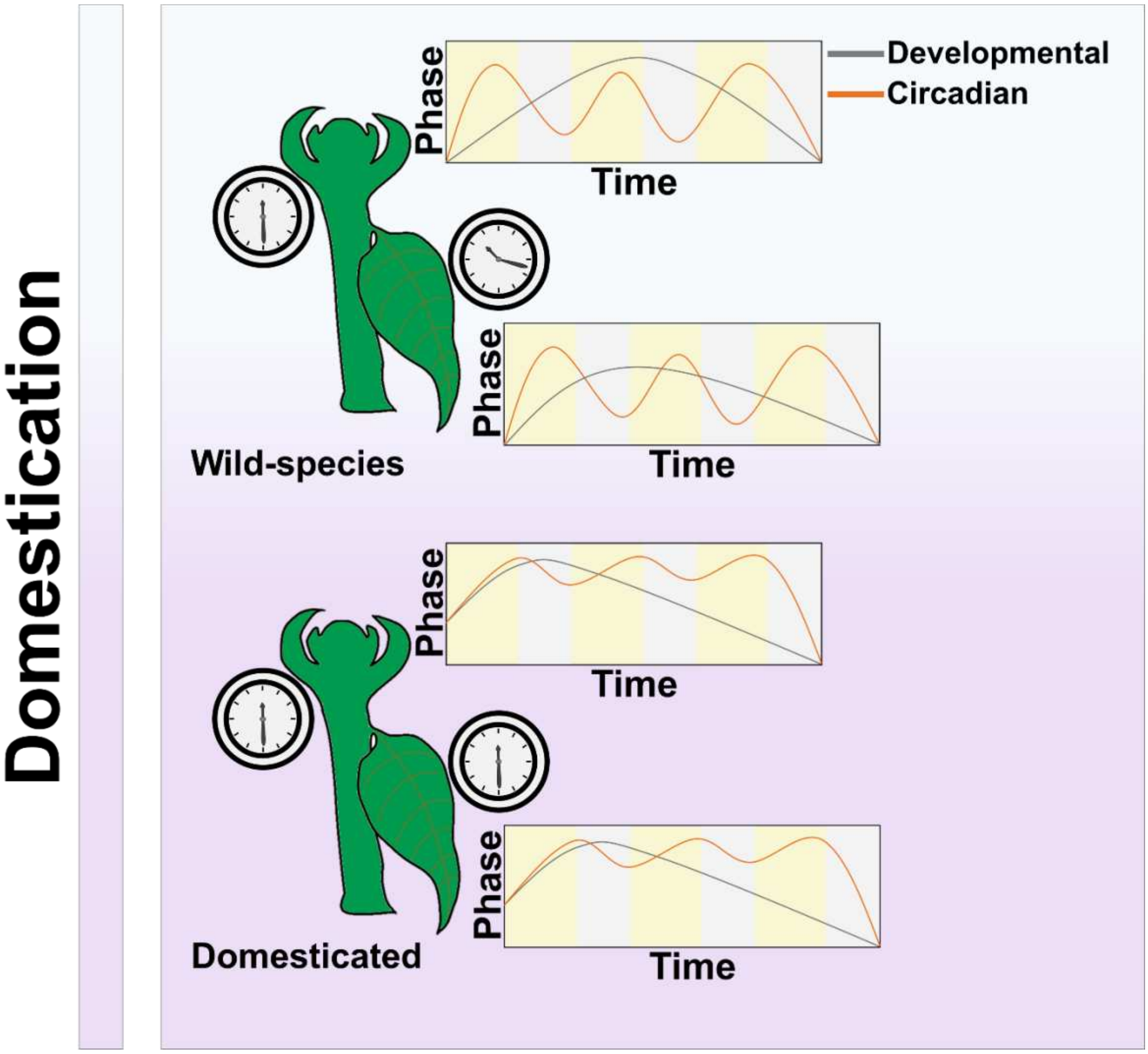

Figure 1. Domestication impacts on circadian and developmental clocks. The phase represents activity peaks of the clock components aligning with environmental cues, in this case, dawn and dusk. In another hand, time defines the plant life cycle, indicating the three major developmental transitions. Undomesticated plants might exhibit specific developmental clocks for each organ and a circadian clock with the more oscillatory behavior. Contrasting with this, domesticated species seem to display synchronized developmental clocks in whole plant, while the circadian clock exhibit minor oscillations comparatively with undomesticated plants. Collectively, these factors contribute to reduce the abiotic stress responses and to improve agronomic traits in domesticated plants (e.g. more and bigger fruits/seeds per plant as well as a larger productivity). Thus, the development in populations of domesticated plants was synchronized making crops more uniform.

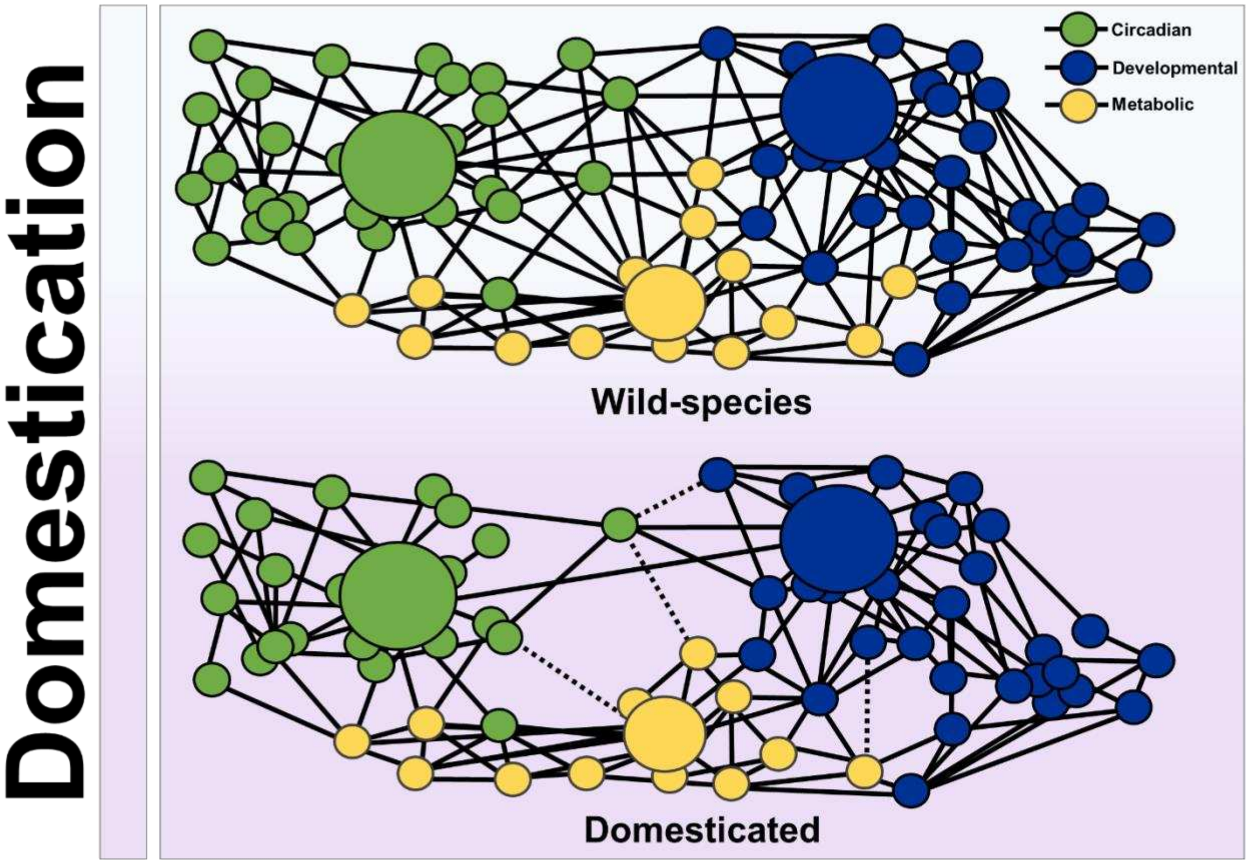

Figure 2. Hypothetical model for plant domestication evolution triggering losses on network covering biological clocks and metabolism. Wild-species exhibit complex networks between circadian and developmental clocks, and the metabolic components seems linking these clocks through intrinsic relationships. Therefore, the progression in domestication process triggered selective sweeps reducing interactions among circadian, development and metabolic components. The human selections wild relatives and landraces populations generated crop species with a larger distance among these components. Solid lines represent strong interactions while dotted lines refer to weak interactions.

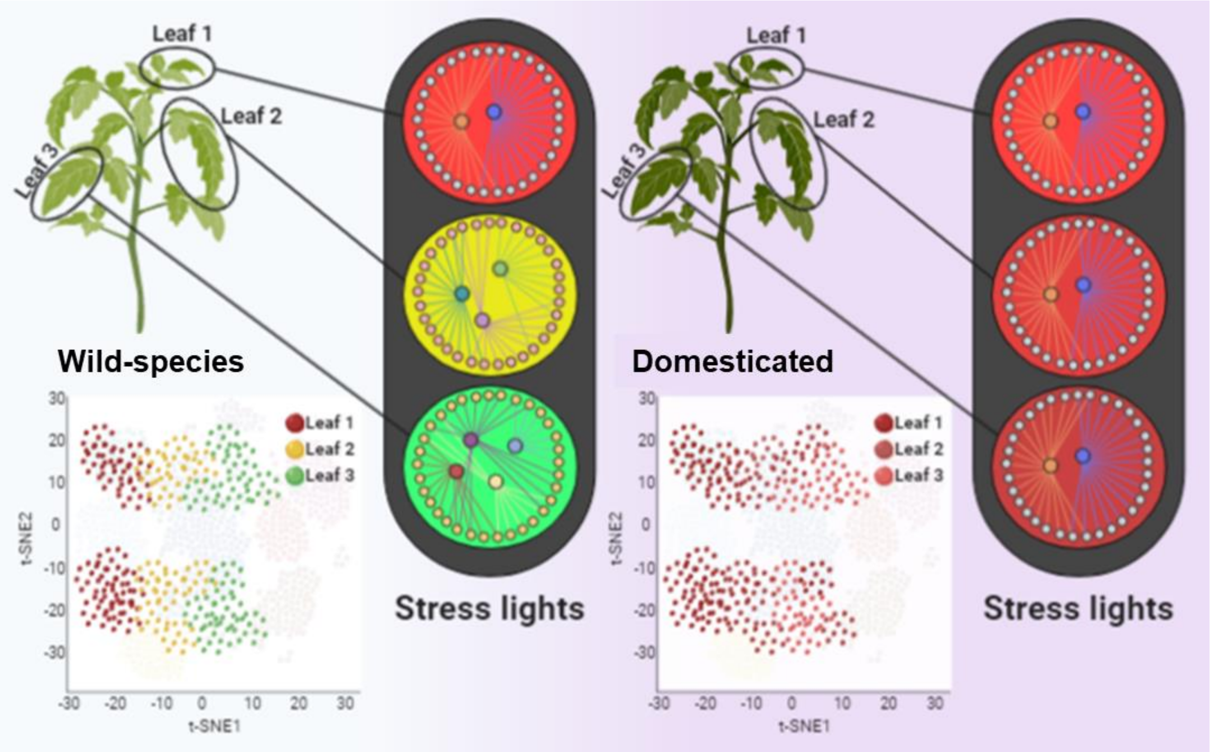

Figure 3. Stress lights to respond to biotic and abiotic adversities on wild and domesticated species. Wild-species turn on distinct stress lights according with leaf developmental status, exhibiting divergent transcriptional patterns (t-SNE) for leaves, which enables the deletion of compromised leaves under adverse conditions. In turn, domesticated species exhibit more synchronized clock wherein overall plant is found on stress, and compromised leaves fatally will affect stress tolerance response.

Chapter 2

The Synchronized Biological Clocks of Domesticated Tomato[1]

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[1] *Manuscript in preparation for submission*

The Synchronized Biological Clocks of Domesticated Tomato

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Abstract

Cells, tissues, and organs are characterized by complex systems allowing communication between each other. Plant domestication was demonstrated to have structured the circadian rhythms, and synchronized the flowering and metabolism, components that integrate the biological clocks. Here, we demonstrated that domesticated tomatoes (*Solanum lycopersicum*) have more synchronized rhythmicity across the whole plant. Consequently, the leaf development program is more coordinated in this species than in their wild relatives, wherein *S. lycopersicum* young leaves develop slowly in comparison to mature leaves. Young leaves from wild tomatoes display higher photosynthesis than mature, while large metabolite accumulations occur across plant segments. Diel metabolite levels are rather similar between young and mature leaves in the wild tomato *S. pennellii*, whereas the expression patterns for circadian clock genes are widely contrasting between both leaves. We further demonstrated that editions in genes related to domestication at wild tomato *S. pimpinellifolium* appear to synchronize the development of young and mature leaves similar to the observed for *S. lycopersicum*. Collectively, the strengthening of inter-organs relationships on domesticated tomato indicates synchronized biological clocks, which are most likely fundamental to explain, at least partially, their higher yield.

INTRODUCTION

Historically, human beings have been selecting plants to ensure their food security and support population growth, leading to drastic alterations in plant development. Most plant traits are affected by timing, demanding accurate mechanisms to record it. Accordingly, timekeeping mechanisms monitor geophysical time and record coincidences, which adjust the organism's development and frequently promote the anticipation of future environmental conditions. A single organism may have diverse timekeepers working either independently or in synchronicity, wherein can exist clocks associated with development, circadian rhythms, and metabolic oscillations (Zhu et al., 2020; Park, et al., 2012; Sanchez and Kay, 2016; Papagiannakis et al., 2017; Siqueira et al., 2018). Accordingly, the developmental clock defines the precise timing to induce cell fates related to the growth of organs and lately shifting their morphology (Zhu et al., 2020). The circadian clock, in turn, represents the timekeepers set that synchronizes the endogenous system of the organism with “solar time”, to regulate oscillating rhythms according to a period of 24 hours (Sanchez and Kay, 2016). Furthermore, autonomous metabolic oscillations may occur across diverse growth conditions independently from other oscillatory systems, characterizing the so-called metabolic clock (Papagiannakis et al., 2017; Siqueira et al., 2018). Remarkably, it remains rather unclear why exactly biological clocks in modern crops are characterized by flowering synchronization phenomenon, circadian clock deceleration, and broader metabolic adjustments than their wild relatives.

Domestication is assumed to have modified mainly the developmental clock of wild plants, altering shoot apical meristem (SAM) to both shape architecture and synchronize flowering time on plants (Doebley et al., 2006). SAM maturation clock in domesticated tomato (*Solanum lycopersicum*) shows a more advanced maturation status than in tomato wild species, culminating in a fast transition from inflorescence meristem to floral meristem (Park et al., 2012). Notably, photoperiod is a dominant factor orientating flowering induction and the ability to sense changes based on day length (Wahl et al., 2013), which allowed the spreading of most crop species worldwide. Varieties from cultivated tomato are insensitive to day length, while their wild relatives are not, and this difference is partially explained by variations in the cis-regulatory region of the gene *Self-Pruning 5G* (*SP5G*) (Soyk et al. 2017). Under long-day conditions, the induction of gene *SP5G* arrests the flowering of wild tomatoes, whereas the *SP5G* repression under those conditions enables the flowering of domesticated tomatoes (Soyk et al. 2017). Furthermore, photoperiod is a central factor governing the rhythmicity of circadian clocks, whereas tomato domestication selected plants with a slower circadian clock (Müller et al., 2016). The reproductive performance of *S. lycopersicum* is improved specifically over long-days owing to this selection, allowing their cultivation under long summer days at higher latitudes of the northern hemisphere (Müller et al., 2016). A remarkable component connecting developmental and circadian clocks is the metabolism, as revealed by metabolic signatures which are assumed to orientate the domestication of some species, including adzuki bean (*Vigna angularis*) and barley (*Hordeum vulgare*) (Yang et al., 2015; Pankin et al., 2018). Singularities at biological clocks are expected to occur across plant species, and as such temporal coincidences among developmental, circadian, and metabolic clocks must occur to enable the improved yield of domesticated tomato in comparison to its wild relatives.

Communication across tissues of complex biological systems occurs due to cell-to-cell relationships, contributing to multicellularity structuration in both animals and plants. Accordingly, mice (*Mus musculus*) submitted to a high-fat diet had losses in the temporal coincidences of circadian metabolism among eight distinct tissues, promoting aberrant cell proliferation and growth of these tissues (Dyar et al. 2018). Notably, mice liver exhibits an independent circadian clock oscillating autonomously from all other clocks, while this clock is dependent on the light/dark cycles to sustain the rhythmicity (Koronowski et al., 2019). In good agreement, each leaf tissue exhibit a particular clock that express differential contributions to the global leaf circadian clock, and the rhythmicity is asymmetrically coupled across tissues, ensuring leaf development in *Arabidopsis thaliana* (Endo et al., 2014). Recently, we suggested that domestication was an agent setting biological clocks on cultivated species, reducing the heterogeneity among distinct tissues (Siqueira et al., 2022). It seems reasonable to posit that this may have contributed to photoperiod adaptation and synchronized flowering on domesticated plants (Xiang et al., 2022; Siqueira et al., 2022). In an attempt to reveal potential synchrony among biological clocks on wild and domesticated tomatoes, we combined physiological, metabolic, and genetic assays to demonstrate differences among these species. Our results revealed that domesticated tomato has synchronized circadian rhythms across plant organs, a fact that is not observed for wild tomato *S. pimpinellifolium*. We additionally demonstrated that metabolism and gene expression are rather integrated between leaves of domesticated tomato, which appear to be a factor to help in explaining their fruit yield in comparison with their wild relatives.

RESULTS

The similar circadian rhythms at cotyledons of Solanum pimpinellifolium

Tomato domestication decelerated the circadian rhythms of cultivated tomato (Müller et al., 2016; Müller et al., 2018), and these rhythms may be variable across tissues of wild plants. The circadian clock of the human liver can oscillate even in absence of other clocks suggesting their independence, although liver circadian rhythms are still dependent on light/dark cycles (Koronowski et al., 2019). Likewise, each circadian clock seems to contribute to specific processes in the leaf, and differently from the centralized circadian clock of mammals, the plant circadian clock seems to not exhibit a clear centralization (Shimiziu et al., 2015). Nevertheless, shoot apex clocks apparently have the capacity to synchronize circadian rhythms across plant structures, affecting rather distant organs such as circadian oscillations of roots (Takahashi et al., 2015; Chen et al., 2020). Despite certain imprecision regarding circadian rhythms centralization of plants, it is consensus that circadian misalignment largely arrests the fitness of mammals and plants (Koronowski and Sassone-Corsi, 2021; Sorkin and Nusinow, 2021). Based on this aforementioned informations and uncertainty concerning clocks synchronization, we decided to assess circadian rhythms in domesticated and wild tomatoes.

The exposure of the two *A. thaliana* cotyledons to constant light revealed contrasting circadian rhythms for both over several days, indicating the independence of each cotyledon clocks (Thain et al., 2000). We, therefore, assessed circadian rhythms at cotyledon pairs of *S. lycopersicum* and *S. pimpinellifolium*, which revealed contrasting rhythms only for the wild tomato (Fig. 1A-B). Accordingly, the circadian period of cotyledon 2 was 0.8 h longer than cotyledon 1 of *S. pimpinellifolium*. Thereby, we turned our attention to identifying the developmental and physiological consequences of the absence of differences in circadian rhythms in domesticated tomato.

The developmental hierarchy of leaves at domesticated tomato

Most of the domestication and breeding of plants were performed based on reproductive yield, given that humans most likely selected plants according to desirable fruits and grains. Meantime, the role of leaves to ensure elevated crop productivities was rather neglected; even though leaf shape was recently demonstrated to be an excellent predictor for fruit production and quality in domesticated tomatoes (Rowland et al., 2020). In agreement, latitude and its relationship with day-length are closed associated with tomato domestication, allowing tomato production for longer days in the summer of the northern hemisphere (Müller et al., 2016; Soyk et al., 2017; Xiang et al., 2022), which may have affected differently tomato organs. We thus decided to investigate the major tomato traits affected by latitude using the data from the germplasm bank of Universidade Federal de Viçosa, Brazil (http://www.bgh.ufv.br/). Thus, we mapped the relationships among latitude with growth traits of distinct organs in *S. lycopersicum* genotypes. Our *in silico* assays revealed that leaf traits are correlated with latitude in approximately 100 tomato genotypes from diverse regions of the earth (Supplementary Fig. S1-2).

We next decided to turn our attention to leaf development and investigate whether the domesticated tomato could have a synchronized development of leaves. Although canopies of cultivated plants have homogenous leaves, they capture light heterogeneously (Long et al., 2015). Indeed, analyzes of leaf developmental trajectories in *S. lycopersicum* revealed a not evenly photosynthetic competence, which spreads heterogeneously across leaf zones (Martinez et al., 2021). Investigating the leaf development on wild tomatoes, it was revealed a complex behavior in leaf series, in which shade avoidance is ephemeral and leaf length and area are widely variable (Chitwood et al. 2012a). These traits tend to correlate with the shade avoidance index negatively at early development, becoming positive in later stages of development of leaves (Chitwood et al. 2012a). Thus, wild tomatoes exhibit unequal shade avoidance along of leaf developmental series, where each leaf might exhibit a distinct shade avoidance degree (Chitwood et al. 2012a; Chitwood et al. 2012b). Thus, domesticated and wild tomatoes are characterized by an ephemeral shade avoidance that is not continuous over leaves (Chitwood et al. 2015). Moreover, this indeterminacy is induced by light and reconfigures leaf morphology to promote shade avoidance (Chitwood et al. 2015). By analysing canopy and leaf patterns, we observed that the developmental plasticity seems to be higher in wild tomatoes in comparison with domesticated ones (Supplementary Fig. S3), indicating a leaf shape homogenization during the domestication of tomatoes. Thus, we postulated that a potential developmental overlap may exist covering the third and fourth leaves of wild (*S. pimpinellifolium*, *S. habrochaites*, *S. neorickii*, and *S. pennellii*) tomatoes which does not occur in the domesticated species. To reduce the impacts of variations in leaf shape and length, we performed analyzes mainly at older leaflets from third (L3) and fourth (L4) leaves, which allowed to us assess both developmental and physiological competence from 15 to 35 days after sowing (DAS). Intringuingly, the same developmental pattern was observed for overall wild tomatoes, wherein the L3 had higher leaf area than L4 only at the first observations, in general around 15 or 20 DAS (Figure 2A). In sharp contrast, domesticated tomato was always characterized by L3 with largest leaf area than L4 across all experimental observations (Figure 2A), suggesting a developmental coordination on *S. lycopersicum*. Specific leaf area (SLA) is an important physiological trait describing the plant ability to intercept photosynthetic irradiation. It is common that older leaves exhibit a lower SLA than young leaves, denoting a better photosynthetic ability and a more advanced development due to cell differentiation *status* (Baldazzi et al., 2013). SLA was virtually invariant between L3 and L4 over the early development on wild tomatoes, whereas it was clearly different between L3 and L4 during the 15 to 30 DAS in *S. lycopersicum* (Figure 2B). The physiological maturity indicates when a leaf or leaflet turn self-sufficient frequently describing source zones, while chlorophyll is an indicator for sink-to-source transition. We thus measured chlorophyll level over early development, founding a similar pattern observed for leaflet area and SLA, with differences practically absent between L3 and L4 even in early developmental stages (Figure 2C). Similarly, only domesticated species displayed differences in chlorophyll levels and chlorophyll *a*/*b* ratio (Figure 2C-D). Decreases in chlorophyll levels exert a direct role on reduction of the number of lateral branches, which seems to modulate shoot branching and also alter energy balance (Khangura et al., 2020). Likewise, we next determined the levels of sugars related to energy homeostasis and observed that highest sugar levels are present in L3 than L4 during early stages of the leaf development only in *S. lycopersicum* (Suplementary Fig. S4). This was supressed gradatively over development for glucose and fructose, wherein more elevated levels of these compounds were found later developmental stages for L4 in comparison to L3, a fact that was not observed for sucrose (Suplementary Fig. S4). Meanwhile, we did not observe a clear pattern for sugar concentration in wild tomatoes, and as such L3 and L4 frequantly had similar levels of glucose, fructose, and sucrose regardless of the developmental stage. Altogether, both development and metabolism are likely coordinated and synchronized in *S. lycopersicum* while wild tomatoes exhibited a clear independence between L3 and L4.

We further performed light and CO_2 curves using *S. lycopersicum* cv. M82, *S. pimpinellifolium*, and *S. pennellii* to compare gas exchanges responsiveness between L3 and L4. Net photosynthesis (A_N) is homogenous in leaflets of M82 and *S. pimpinellifolium*, whereas in *S. pennellii* L4 had higher A_N than L3 in most levels of light and CO_2 (Figure 3). In an attempt to further understand the differences observed, we splited seven segments from L3 to L4 of M82 and *S. pennellii* to map metabolic alterations across these segments. We observed that in M82 no metabolic variations across segments, while *S. pennellii* exhibited remarkable metabolite accumulations, highlighting metabolites related to sink including sucrose, citrate, and GABA (Figure 3). Collectively, those results indicated that wild tomatoes leaflets experience early emancipation, meaning that they are turning sources long before that observed for domesticated tomato.

Physiological traits seemingly determine more leaf growth than morphological features; in addition, domesticated tomatoes are more impacted by these traits than wild tomatoes (Conesa et al., 2017). Thus, we investigated leaflet physiology on M82, *S. pennellii*, and introgression lines (ILs) harbouring *S. pennellii* genomic fragments. These ILs allowed the identification of several quantitative trait loci (QTLs) (Eshed and Zamir, 1995), including the identification of regions intimately associated with tomato domestication, branching, flowering, photoperiodism, and circadian clocks. In this sense, IL5-4 have the genomic fragment harbouring the *SP5G* gene regulating architecture and flowering based on photoperiodism whereas IL9-2-6 and IL9-3 harbours the genes that promote circadian clock deceleration (Müller et al., 2016; Soyk et al., 2017). By investigating L3 and L4 development of *S. lycopersicum* (cv. M82), IL5-4, IL9-2-6, IL9-3, and *S. pennellii*, we observed a similar developmental window for these leaflets in both ILs and *S. pennellii* (Supplementary Table 1). Thus, around 35 DAS both leaflet area and mass were similar for L3 and L4 of the same plant at ILs and on wild species, which does not occur in *S. lycopersicum* that showed L3 with higher area and mass than L4 (Supplementary Table 1). Again, only in M82 plants, SLA from L3 was higher than L4 in good agreement with a coordinated development (Supplementary Table 1). By contrast, ILs and wild tomatoes showed similar SLA for both leaflets describing a similar ability to intercept radiation (Supplementary Table 1), which suggests the same developmental trajectory. In consonance with these developmental coincidences, we next decided to investigate whether ILs and *S. pennellii* would exhibit contrasting physiologies for each leafleat.

Heterogeneous physiology but similar metabolism between leaves of S. pennellii

Tomato domestication resulted in remarkable changes in photosynthesis-related genes, as revealed by an inverse correlation between transcripts related to leaf development and photosynthesis (Ranjan et al., 2016). Intriguingly, despite *S. pennellii* leaflets exhibiting the same SLA, a higher A_N for L4 in comparison with L3 was observed (Supplementary Figure S6). Meanwhile, our results further revealed that *S. pennellii* exhibited a differential V_{cmax} for the same leaf pair, with the highest values for L4 than L3 (Supplementary Figure S6). In agreement with these data, a similar pattern for both ILs and *S. pennellii* was observed for A_{N}, R_{d}, P_{R}, g_{m}, Vc_{max}, and biochemical limitations, supporting the notion of weak source-to-sink relationships in these plants (Supplementary Fig. S6-7). Taken together, our findings suggested that these genomic regions might regulate the metabolic synchronization between L3 and L4. To further elucidate this synchronization, we decided to perform metabolite analyzes on these leaflets in an attempt to identify the source-to-sink patterns.

Despite the higher A_N at L4, the diel metabolism glucose, fructose and sucrose revealed few differences between L3 and L4 for ILs and *S. pennellii* (**Figure 4** and Supplementary Figure S8). Interestingly, the coupling between organism size and metabolic rates displays few relationships with the selection of plants over domestication (Milla et al., 2018), which may be due to the weak inter-tissue metabolic relationships once wild species usually have shoots highly branched. Genetic factors regulate in parallel the branching and sucrose metabolism in wild maize (*Zea mays* ssp. *parviglumis*), whereas sucrose seems to activate branching in pea (*Pisum sativum*) (Dong et al., 2019; Mason et al., 2014). Genetic diversity for *Sucrose synthase* (*SuSy*) genes is virtually absent in landrace and cultivated tomatoes while wild species display a wide diversity (Dinh et al., 2018; Slugina et al., 2019). *SuSy1* expression level is rather invariable across flower, fruit, leaf, and root in wild tomatoes, whereas an expression gradient is apparently present in domesticated species, with the lower expression on source organ (leaf) than sinks (Slugina et al., 2019). To summarize, the incoherence between L3 and L4 to regulate sucrose/starch metabolism on ILs and *S. pennellii* appears to describe weak inter-tissue relationships, which were most likely selected during tomato domestication and/or breeding selections. Metabolite diversity in wild and domesticated *Phaseolus vulgaris* was observed revealing that tissue specificity is the major factor affecting the metabolite dataset, yet wild genotypes have more metabolite diversity (Souza et al., 2019). Meanwhile, considering a single tomato plant, metabolite specialization across different tissues seems to result from differential epigenetic regulation that in turn promotes the differential gene expression in mature and young organs (Zhong et al., 2013). Taken together, it seems reasonable to anticipate that gene expression might explain the contrasting metabolic regulation at L3 and L4 of M82 and *S. pennellii*.

Transcriptional regulation of leaves

The transcriptional variation in biological clocks-related genes was widely used to monitor circadian rhythms over the past years. Accordingly, we next assessed gene expression of M82 and *S. pennellii* growing under natural light/dark cycles to identify potential synchronicity between L3 and L4. *TIMING OF CAB EXPRESSION1* (*TOC1*) is a pivotal component in circadian clock regulation since it represses and is repressed by *LATE ELONGATED HYPOCOTYL* (*LHY*) (Nohales and Kay, 2016). Under natural conditions *TOC1* expression at shoots is sufficient to ensure plant survival under drought while expression in roots not ensure the same (Valim et al., 2019), supporting the notion of clock communication across plant organs. We observed contrasting expressions for *TOC1* during all diel period on L3 and L4 of M82 and *S. pennellii* (Figure 5). Circadian period decreases with leaf age progression, and the shorter period of older leaves is linked with *TOC1* expression in *A. thaliana* (Kim et al., 2016). Thus, the expression of *TOC1* on *S. pennellii* was always higher in L3 than L4, whereas it was higher in L4 than L3 under darkness in M82, with an almost similar pattern observed for *LHY* expression (Figure 5), suggesting an expression hierarchy based on the development for the wild species. *GIGANTEA* (*GI*) is a positive regulator of TOC1 and an integrator between circadian clock and development by contributing on flowering induction (Nohales and Kay, 2016). We found a homogenous *GI* expression between L3 and L4 on M82, whereas larger expressions at L3 than L4 were found *S. pennellii* (Figure 5). Together, the expression of *TOC1*, *LHY* and *GI* seems to indicate a coordinated regulation of circadian clock on leaflet of the domesticated species, while in wild relatives a more particular transcriptional regulation for clocks of each leaflet is observed.

Circadian clock deceleration in domesticated tomatoes occurred due selective pressures on genes EID1 and *NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED 2* (*LNK2*), both genes play function in light signalling and flowering (Müller et al., 2016; Müller et al., 2018; Rugnone et al., 2013). A sharp contrast was observed for *LNK2* expression on domesticated tomato, wherein L3 and L4 expression were distinct over diel period while it was virtually invariant in *S. pennellii* (Figure 5). In plants, *SP5G* program seasonal changes in consonance with day length, exhibiting a diel expression that generally culminate in flowering repression in wild tomatoes under long days (Soyk et al., 2017). *SP5G* expression was similar at both leaflets of M82, and widely contrasting at L3 and L4 of *S. pennellii* over overall diel period (Figure 5), supporting, at least in part, the notion of a particular developmental programming in each leaflet. *Light Harvesting Chlorophyll a/b Binding protein 13* (*CAB13*) obeys circadian dynamics, playing roles at photoinhibition mitigation and tolerance to constant light in wild tomatoes (Velez-Ramirez et al., 2014). No differences for *CAB13* between L3 and L4 from M82, whereas in *S. pennellii* L4 had usually higher *CAB13* expression when compared with L3 (**Figure 5**), supporting the notion of a differential photosynthesis regulation between L3 and L4 of wild species.

Domestication *de novo*, a promising strategy to re-structure synchronization of tomatoes

We next explored the domestication *de novo* concept, performing assays with *S. pimpinellifolium* multiplex lines harbouring editions on different genes related to tomato domestication. Complex genetic features are associated with the domestication syndrome linking fruit flavour and productivity of tomato. In addition, *S. pimpinellifolium* genome is rather complex than *S. lycopersicum*, and therefore gene editions on domesticated tomato may result in unpredictable phenotypes (Alonge et al., 2020; Rodríguez-Leal et al., 2017). Since wild species endure hostile environments, their biological clocks integrate a plethora of abiotic stresses responses (Markham and Greenham, 2021), *de novo* domestication describes the introduction of domestication genes and/or re-domestication into wild species, representing a promising strategy to develop crop ideotypes (Zsögön et al., 2018; Fernie and Yan, 2019; Yu et al., 2021). To exemplify the potential of this new concept, increased fruit size and number as well as higher nutritional value was observed in engineered *S. pimpinellifolium* lines (Zsögön et al., 2018). By growing some of these engineered lines side-by-side, we verified that the gene editions result also in rewiring of developmental coincidences between L3 and L4 (Figure 6). Briefly, edited lines showed the young leaflet with lowest length, with lower area and biomass than the mature leaflet, as well as variations in SLA resembling source-to-sink relationships similarly to previously observed for domesticated tomato. In summary, the *de novo* domestication presents itself with an enormous potential to re-structure biological clocks of new engineered crops in attempt to increase resilience againt stress in general.

Discussion

Our study sheds light in an unnoticed yet significant impact of tomato domestication, the synchronization of biological clocks that boost inter-tissue communication. It is important to mention, however, that our findings are in good agreement with observation in mammals that identified, for instance, the independence of liver circadian rhythms from central clock as well as that liver clock oscillates due light/dark cycles (Koronowski et al., 2019). In fact, aforementioned example revealed that the proper circadian clock communication elevated organism health, whereas highly caloric diets disrupts the coherence of circadian metabolism among tissues contributing for disordered growth in humans (Koronowski and Sassone-Corsi, 2021; Dyar et al., 2018). The situation in plants is rather complex since plants are largely environment dependent to orientate circadian rhythms, suggesting a different way in terms of inter-tissue synchronization, since leaf may maintain their growth independently from others organs/tissues. In this case, the exposure to constant light conditions promotes the desynchronization of circadian clocks whereas the increase multicellularity level precedes larger delays in anticipating outer cycles (Masuda et al., 2017; Muranaka and Oyama, 2016). Domestication of other species shaped differentially gas exchanges, *Brassica rapa* for example have the differentiation of their three morphotypes (leafy, turnip and oilseed) by gas exchange characteristics, wherein domestication impacted A_N and stomatal conductance only at oilseed varieties (Yarkhunova et al., 2016). Furthermore, oilseed have a shorter circadian period and higher Vc_{max} culminating in lower total biomass accumulation than leafy and turnip (Yarkhunova et al., 2016), remaining unclear the existence of organ (de)synchronization for oilseed. Otherwise, the domesticated specie *Nicotiana tabacum* (cv. Samsun) not exhibit differences for A_N and Vc_{max} between mature and developing leaves (López-Calcagno et al., 2020). Domesticated tomato in turn demonstrated to have a photosynthetic gradient according with leaf age, where mature leaves had much less A_N capacity and R_d than young leaves (Xu et al., 1997). Consequently, Rubisco activity (per g protein) is not different for mature and younger leaf of *S. lycopersicum* (Xu et al., 1997), indicating a physiological synchronization at leaves from this specie.

Autonomy to regulate genetic and metabolic traits is pivotal for organ development and to thus synchronize tissues across organism with environmental variations. The misalignment of circadian relationships among tissues causes desynchronized development prejudicing organism health (Dyar et al., 2018). Consequently, each *A. thaliana* leaf tissue has particular circadian rhythms and mesophyll and vascular clocks are the major drivers of global leaf clock (Endo et al., 2014). This asymmetry among clocks may be synchronized, and shoot apex clocks couples circadian rhythms of other organs, sending proteins for roots that increasing or decreasing circadian period according with temperature (Takahashi et al., 2015; Chen et al., 2020). The synchronization level among cells decreases with time progression and cell-to-cell communication probably enhance desynchronization, while individual plant cells under constant light manifest autonomous circadian oscillations (Masuda et al., 2017; Muranaka and Oyama, 2016). Circadian rhythms heterogeneity in intact plants may be corrected by light/dark cycles (Muranaka and Oyama, 2016), indicating that these cycles are extremely important to generate systemic responses of plants to environmental variations. Shifts in photoperiod responsiveness were obtained over tomato domestication process, whereas pivotal genes associate with this process have their expression intimately dependent from light/dark cycles (Xiang et al., 2022). Wild tomatoes have much more cells than domesticated tomato due to higher branching level, and they are classical examples that constant light not easily disrupt their phenotype, which rapidly occurs on domesticated tomato (Velez-Ramirez et al., 2014), suggesting the synchronization of clocks in wild species. In general, different organs of wild species display variations in circadian rhythms, highlighting differences observed in cotyledon-to-cotyledon, leaf-to-leaf and shoot-to-root, which extend to control of the organ development (Thian, 2000; Kim et al., 2016). This may ultimately contribute to biological advantage. It seems reasonable therefore that, when submitted to stressful conditions the wild species most likely suffer loss of leaves with lower commitment to the overall plant *fitness*. A very interesting example for synchronization may be observed for wild species *Echinacea angustifolia* that is perennial and have the flowering time synchronized by fire (Wagenius et al., 2020). In domesticated tomatoes, the synchronization seems to have been generated with human activity, ensuring the coordination between circadian rhythms and developmental for leaves. It would be interesting to determine whether, and to what extent, environmental factors over the evolution of domestication may have boosted inter-organs relationships in plants.

The results obtained here also point for developmental synchronization on domesticated tomatoes, which homogenize photosynthesis across leaves and establish stronger source-to-sink relationships. By contrast, *S. pennellii* seems to show a desynchronization of traits programing source-to-sink relationships, where each leaf would work rather independently. It is not surprisingly that the particular behavior for each leaf frequently results in a rather slow growth and reduced fruit mass yet a higher ability to tolerate environmental adversities is often observed. Notably, metabolism and organism size were largely uncoupled by plant domestication (Milla et al., 2018). For instance, the highly branched pattern of wild tomatoes might be due to weak inter-tissue correlations of the metabolism, which was synchronized over tomato domestication. From the findings of Milla et al. (2018) it is possible to notice that wild relatives of crop display a broad spectrum of relationships for metabolism *versus* plant size, suggesting that domestication indeed set developmental, circadian, and metabolic clocks in cultivated plants. Similarly, the elevated diurnal expression of *TOC1* leads ultimately to a higher starch turnover, improving biomass gain in *A. thaliana* polyploids when compared with their parental diploids (Ni et al., 2009). Consistently, *TOC1* expression and starch levels demonstrated similar patterns on domesticated tomato, whislt higher *TOC1* expression in L4 than L3 upon noon seems to be correlated with higher starch turnover and superior biomass gain of the young leaflet in wild relatives. Following, *GI* mediates changes on rhythmicity of shoot circadian clock, which may occur due to variations in sucrose (Dalchau et al., 2011). Thus, sucrose is likely able to reset circadian clock based on metabolic *status* acting as both signal and metabolite (Müller et al., 2014). *GI* expression was one of the more homogenous between L3 and L4 whereas sucrose levels were widely divergent for both leaflets over diel period in M82, indicating that sucrose apparently is not able to reset rhythms of L3 and L4. The opposite was observed for *S. pennellii* that shown a contrasting *GI* expression between L3 and L4, despite almost similar sucrose levels for both leaflets. It seems, therefore, that the contrasting development, physiology, and metabolism between older and younger leaves of *S. lycopersicum* likely program the source-sink relationships, which seems to have been reached during its domestication.

Over the past decades our understanding of circadian clocks, development and metabolism experienced significant advances, wherein the function of thousands genes, proteins and metabolites have been demonstrated (Greenham and McClung, 2015; Yu et al., 2015). Notwithstanding, the structuration of circadian, developmental, and metabolic clocks along plant species evolution is far to be fully understood. We posit that distinct plant tissues may exhibit variations on inter-relationships over plant development. Domesticated tomato is an interesting case that may contribute to start to solve how biological clocks are structured in plants, while *de novo* domestication provides an excellent opportunity to re-structure these clocks to engineer ideal crops. The results described here provide compelling evidence for a higher coherence among tissues from domesticated tomato, which seemingly does not occur on their wild relatives. Future studies must explore the (in)coherences among organs and tissues to generate ideal crops that are highly productive under changing environments, ensuring food production even in hostile environments.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of the tomato (*Solanum lycopersicum*, *Solanum pimpinellifolium*, *Solanum habrochaites*, *Solanum neorickii* and *Solanum pennellii*) and introgression lines (IL5-4, IL9-2-6 and IL9-3) in the genetic background M82 (*S. lycopersicum*) were obtained from Zamir et al. (1995). Seeds were surface-sterilized with 5% sodium hypochlorite for 10 min and then washed with running distilled water and subsequently sowed in a tray with commercial substrate (Tropstrato HT©). Seven days after germination (or following the appearance of the first true leaf), seedlings were transferred to 3.5-L pots containing the same commercial substrate supplemented with 5 g L−1 4:14:8 NPK. Plants were grown in a greenhouse located in Viçosa (20°45′S, 42°15′W, 650 m above sea level), south-eastern Brazil, with a minimum of 400-μmol photons m^{-2} s^{-1}. Plants were watered regularly and throughout the entire growth period were maintained under naturally fluctuating conditions of light intensity, temperature, and relative air humidity. All physiological, biochemical parameters analysed in the experiments were performed on the third and fourth leaves when source leaves were completely expanded, which occurred for 4-week-old plants. Additionally, the experiment was repeated at least two times (even in different growth facilities) with similar phenotypes observed each time.

Cotyledons movements and circadian rhythms determination

Seedlings were grown in controlled conditions of a growth chamber for four days under cool white fluorescent tubes (~100 μmol m^{-2} s^{-1}) under 12h light/ 12h dark and 20:18 °C temperature, which was transferred subsequently to constant light and temperature (25 °C). A polystyrene ball was attached to the tip of cotyledons of each seedling by using petroleum jelly according with Müller and Jiménez-Gómez (2016).

Growth analyses

Growth parameters were determined in 4-week-old plants by measuring leaf area, length, and mass as well as the specific leaf area (SLA). Leaf area was measured using a scanner (Hewlett Packard Scanjet G2410, Palo Alto, California, USA) and processing the resulting images on ImageJ. SLA was measured as described previously (Hunt et al., 2002). At the end of the experiment, plants were harvested by cutting the segments above ground level in the sense of the third leaf for fourth leaf, thus separating leaflets, petioles and stem.

Measurements of gas exchange and chlorophyll fluorescence

Gas exchange parameters were determined simultaneously with chlorophyll a (Chl a) fluorescence measurements as described in (Medeiros et al., 2016) using an open-flow infrared gas exchange analyser system (LI-6400XT; LI-COR Inc., Lincoln, NE) equipped with an integrated fluorescence chamber (LI-6400-40; LI-COR Inc.). Instantaneous gas exchanges were measured after 1-hr illumination during the light period under 1,000 µmol m^{-2} s^{-1} at the leaf level (light saturation) of photosynthetically active photon flux density (PPFD). The reference CO_2 concentration was set at 400-µmol CO_2 mol^{-1} air. All measurements were performed using the 2-cm2 leaf chamber at 25°C, as well as a 0.5 stomatal ratio (amphistomatic leaves), and leaf-to-air vapour pressure deficit was kept at 1.2 kPa, and the amount of blue light was set to 10% PPFD to optimize stomatal aperture. Briefly, the initial fluorescence emission (F0) was by illuminating dark-adapted leaves (1 h) with weak modulated measuring beams (0.03 µmol m^{-2} s^{-1}). A saturating white light pulse (8,000 µmol m^{-2} s^{-1}) was applied for 0.8 s to obtain the maximum fluorescence, from which the variable- to-maximum Chl fluorescence ratio was then calculated: Fv/Fm = [(Fm − F0)/ Fm)]. In light-adapted leaves, the steady-state fluorescence yield was measured with the application of a saturating white light pulse (8,000 µmol m^{-2} s^{-1}) to achieve the light-adapted maximum fluorescence (F m´). According to Genty et al. (1989), ΦPSII represents the number of electrons transferred per photon absorbed in the PSII, the electron transport rate (Jflu) was calculated. Dark respiration (R_d) was measured after 2 hour in the dark period (at night), using the same gas exchange system described above, and it was divided by two (Rd/2) to estimate the mitochondrial respiration rate in the light (RL). Determination of mesophyll conductance (g_m), maximum rate of carboxylation (V_{cmax}), maximum rate of carboxylation limited by electron transport (J_{max}), and photosynthetic limitations The CO_2 concentration in the carboxylation sites (C_c) was calculated according to (Harley et al., 1992). Briefly, this method uses the values of A_N, g_s, g_m, V_{cmax} and C_c, and permits the partitioning into the functional components of photosynthetic constraints related to stomatal (L_s), mesophyll (L_m), and biochemical (L_b) limitations.

Determination of metabolite levels

Tomato segments were harvested and immediately frozen in liquid nitrogen and stored at −80°C until further analysis. The extraction was performed by rapid grinding of tissue in liquid nitrogen and immediate addition of the appropriate extraction buffer. The levels of sucrose, fructose and glucose in the tissues were determined exactly as described previously (Fernie et al., 2001).

Statistical analyses

The experiments were randomized and designed with a minimum of three biological replicates of each treatment. Furthermore, experiments to describe phenotypes were repeated at least three times. Data were statistically tested for normality and subsequently examined using ANOVA ($P < 0.05$). Differences in the means ($P < 0.05$) displayed in figures and tables were examined by Student's *t-test*. All statistical analyses were performed using R statistical software (www.r-project.org).

References

Alonge, M. *et al.* (2020) Major impacts of widespread structural variation on gene expression and crop improvement in tomato. **Cell 182**, 145–161.

Baldazzi, V. *et al.* (2013) In-silico analysis of water and carbon relations under stress conditions. A multi-scale perspective centered on fruit. **Front. Plant Sci. 4**, 495.

Chen, W.W. *et al.* (2020) A mobile ELF4 delivers circadian temperature information from shoots to roots. **Nat Plants 6**, 416–426.

Chitwood, D.H. *et al.* (2012a) Native environment modulates leaf size and response to simulated foliar shade across wild tomato species**. PLoS One 7**: e29570

Chitwood, D.H. *et al.* (2012b) The developmental trajectory of leaflet morphology in wild tomato species. **Plant Physiol. 158**, 1230–1240.

Chitwood, D.H. *et al.* (2013) A quantitative genetic basis for leaf morphology in a set of precisely defined tomato introgression lines. **Plant Cell 25**, 2465–2481.

Chitwood, D.H. *et al.* (2015) Light-Induced Indeterminacy Alters Shade-Avoiding Tomato Leaf Morphology. **Plant Physiol. 169**, 2030–2047.

Conesa, M.A. *et al.* (2017) Growth capacity in wild tomatoes and relatives correlates with original climate in arid and semi-arid species. **Environ. Exp. Bot. 141**, 181-190.

Dalchau, N. *et al.* (2011) The circadian oscillator gene GIGANTEA mediates a long-term response of the *Arabidopsis thaliana* circadian clock to sucrose. **Proc. Natl. Acad. Sci. USA 108**, 5104-5109.

Dinh, Q. D. *et al.* (2018) Exploring natural genetic variation in tomato sucrose synthases on the basis of increased kinetic properties. **PLoS One 13**: e0206636.

Doebley, J. *et al.* (2006) The Molecular Genetics of Crop Domestication. **Cell 127**, 1309-1321.

Dong, Z. *et al.* (2019) The regulatory landscape of a core maize domestication module controlling bud dormancy and growth repression. **Nat. Commun. 10**:3810.

Dyar, K.A. *et al.* (2018) Atlas of circadian metabolism reveals system-wide coordination and communication between clocks. **Cell 174**, 1571–1585.

Endo, M. *et al.* (2014) Tissue-specific clocks in Arabidopsis show asymmetric coupling. **Nature 515**, 419-422.

Eshed, Y. and D. Zamir, D. (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. **Genetics 141**, 1147—1162.

Fernie, A.R. and Yan, J. (2019) De novo domestication: an alternative route toward new crops for the future. **Mol. Plant 12**, 615–631.

Greenham, K. and McClung, C.R. (2015) Integrating circadian dynamics with physiological processes in plants. **Nat. Rev. Genet. 16**, 598–610.

Harley, P.C. *et al.* (1992) Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. **Plant Physiol. 98**, 1429–1436.

Holtan, H.E.E. and Hake, S. (2003). Quantitative trait locus analysis of leaf dissection in tomato using *Lycopersicon pennellii* segmental introgression lines. **Genetics 165**, 1541–1550.

Khangura, R. *et al.* (2020) Variation in maize chlorophyll biosynthesis alters plant architecture. **Plant Physiol. 184**, 300–315.

Kim, H. *et al.* (2016) Age-associated circadian period changes in Arabidopsis leaves. **J. Exp. Bot. 67**, 2665–2673.

Koronowski, K.B. *et al.* (2019) Defining the Independence of the Liver Circadian Clock. **Cell 177**, 1448-1462.

Koronowski, K.B. and Sassone-Corsi, P. (2021) Communicating clocks shape circadian homeostasis. **Science 371**:eabd0951.

Long, S.P. *et al.* (2015). Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. **Cell 161**, 56–66.

López-Calcagno, P.E. *et al.* (2020) Stimulating photosynthetic processes increases productivity and water-use efficiency in the field. **Nat. Plants 6**, 1054–1063.

Markham, K. and Greenham, K. (2021) Abiotic stress through time. **New Phytol. 231**, 40–46

Martinez, C.C. *et al.* (2021) Spatial transcriptional signatures define margin morphogenesis along the proximal-distal and medio-lateral axes in tomato (*Solanum lycopersicum*) leaves. **Plant Cell 33**, 44-46.

Masuda, K. *et al.* (2017) Multicellularity enriches the entrainment of Arabidopsis circadian clock. **Sci. Adv. 3**:e1700808.

Mason, M.G. *et al.* (2014) Sugar demand, not auxin, is the initial regulator of apical dominance. **Proc. Natl Acad. Sci. USA 111**, 6092–6097.

Milla, R. *et al.* (2018) Phylogenetic patterns and phenotypic profiles of the species of plants and mammals farmed for food. **Nat. Ecol. Evol. 2**, 1808–1817.

Müller, N.A. *et al.* (2016) Domestication selected for deceleration of the circadian clock in cultivated tomato. **Nat. Genet. 48**, 89-93.

Müller, N.A. *et al.* (2018) Mutations in EID1 and LNK2 caused light-conditional clock deceleration during tomato domestication. **Proc. Natl. Acad. Sci. USA** 15, 7135-7140.

Muranaka, T. and Oyama, T. (2016) Heterogeneity of cellular circadian clocks in intact plants and its correction under light-dark cycles. **Sci. Adv. 2**:e1600500.

Ni, Z. *et al.* (2009) Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. **Nature 457**, 327–331.

Nohales, M.A. and Kay, S.A. (2016) Molecular mechanisms at the core of the plant circadian oscillator. **Nat. Struct. Mol. Biol. 23**, 1061–1069.

Pankin, A. et al. (2018) Targeted resequencing reveals genomic signatures of barley domestication. **New Phytol 218**, 1247–1259.

Papagiannakis, A. et al. (2017) Autonomous metabolic oscillations robustly gate the early and late cell cycle. **Mol. Cell 65**, 285-295.

Park, S.J. *et al.* (2012) Rate of meristem maturation determines inflorescence architecture in tomato. **Proc. Natl. Acad. Sci. USA 109**, 639-644.

Ranjan, A. *et al.* (2016) eQTL Regulating Transcript Levels Associated with Diverse Biological Processes in Tomato. **Plant Physiol. 172**, 328-340.

Rodríguez-Leal, D. *et al.* (2017) Engineering quantitative trait variation for crop improvement by genome editing. **Cell 171**, 470–480.

Rowland, S.D. *et al.* (2020) Leaf shape is a predictor of fruit quality and cultivar performance in tomato. **New Phytol. 226**, 851–865.

Rugnone, M.L. *et al.* (2013) LNK genes integrate light and clock signaling networks at the core of the *Arabidopsis* oscillator. **Proc. Natl. Acad. Sci. 110**, 12120–12125.

Sanchez, S.E., and Kay, S.A. (2016) The Plant Circadian Clock: From a Simple Timekeeper to a Complex Developmental Manager. **Cold Spring Harb. Perspect. Biol. 8**:a027748.

Shimizu, H. *et al.* (2015) Decentralized circadian clocks process thermal and photoperiodic cues in specific tissues. **Nat. Plants 1**:15163.

Siqueira, J.A. et al. (2018) Unraveling Interfaces between Energy Metabolism and Cell Cycle in Plants. **Trends Plant Sci. 23**, 731-747.

Siqueira, J.A. *et al.* (2022) Plant Domestication: Setting Biological Clocks. *Preparing for submission*.

Slugina, M.A. *et al.* (2019) Differences in the sucrose synthase gene SUS1 expression pattern between Solanum lycopersicum and wild tomato species. **Theor Exp. Plant Physiol. 31**, 455–462.

Sorkin, M.L. and Nusinow, D.A. (2021). Time will tell: Intercellular communication in the plant clock. **Trends Plant Sci. 26**, 706–719.

Souza, L.P. *et al.* (2019) Multi-tissue integration of transcriptomic and specialized metabolite profiling provides tools for assessing the common bean (*Phaseolus vulgaris*) metabolome. **Plant J. 97**, 1132–1153.

Soyk, S. *et al.* (2017). Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. **Nat. Genet. 49**, 162–168.

Takahashi, N. *et al.* (2015) A hierarchical multi-oscillator network orchestrates the Arabidopsis circadian system. **Cell 163**, 148–159.

Thain, S.C. *et al.* (2000) Functional independence of circadian clocks that regulate plant gene expression. **Curr. Biol. 10**, 951-956.

Valim, H.F. *et al.* (2019) The clock gene TOC1 in shoots, not roots, determines fitness of *Nicotiana attenuata* under Drought. **Plant Physiol.181**, 305–318.

Velez-Ramirez, AI. *et al.* (2014) A single locus confers tolerance to continuous light and allows substantial yield increase in tomato. **Nat. Commun. 5**:4549.

Wahl, V. *et al.* (2013) Regulation of flowering by trehalose-6-phosphate signaling in *Arabidopsis thaliana*. **Science 339**, 704-707.

Wagenius, S. *et al.* (2019) Fire Synchronizes Flowering and Boosts Reproduction in a Widespread but Declining Prairie Species. **Proc. Natl. Acad. Sci. USA 117**, 3000-3005.

Yang, K. et al. (2015) Genome sequencing of adzuki bean (*Vigna angularis*) provides insight into high starch and low fat accumulation and domestication. **Proc. Natl. Acad. Sci. USA 112**, 13213–13218.

Yarkhunova, Y. *et al.* (2016) Selection during crop diversification involves correlated evolution of the circadian clock and ecophysiological traits in *Brassica rapa*. **New Phytol. 210**, 1469-8137.

Yu, H. *et al.* (2021) A route to de novo domestication of wild allotetraploid rice. **Cell 184**, 1156–1170.

Yu, S. *et al.* (2015) Plant developmental transitions: the role of microRNAs and sugars. **Curr. Opi. Plant Biol. 27**, 1–7.

Xiang, Y. *et al.* (2022) Interaction between photoperiod and variation in circadian rhythms in tomato. bioRxiv preprint https://doi.org/10.1101/2022.01.14.476322

Xu, H-L. *et al.* (1997) Photosynthesis in leaves, fruits, stem and petioles of greenhouse-grown tomato plants. **Photosynthetica 33**, 113–123.

Zhong, S. *et al.* (2013) Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. **Nat. Biotechnol. 31**, 154–159.

Zhu, M. *et al.* (2020) Developmental clock and mechanism of de novo polarization of the mouse embryo. **Science 370**:eabd2703.

Zsögön, A. *et al.* (2018) De novo domestication of wild tomato using genome editing. **Nat. Biotechnol. 36**, 1211–1216.

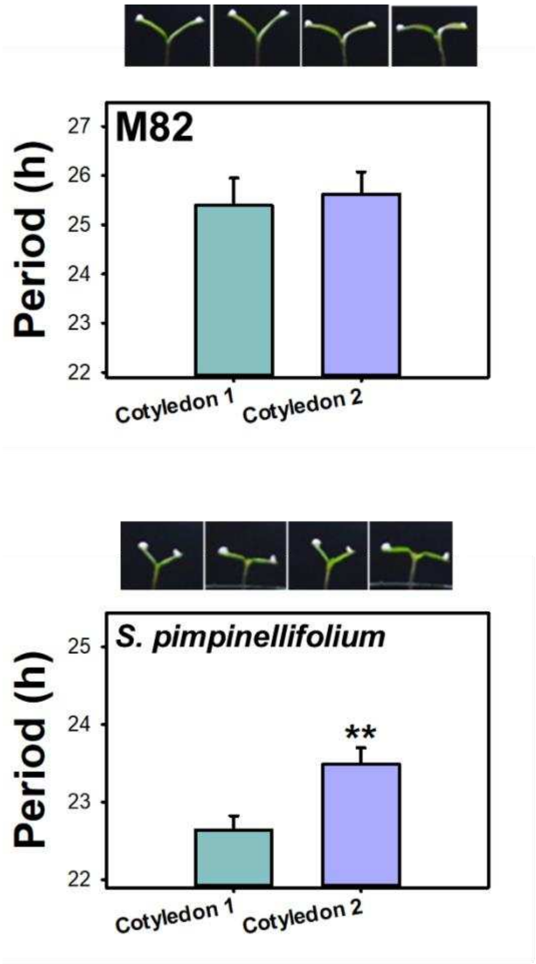


Figure 1. The circadian rhythms of cultivated tomato operate synchronously. Images showed cotyledons positions at 12, 36, 60 and 84 hours upon exposure to constant light conditions. Circadian period of the cotyledons from *Solanum lycopersicum* (cv. M82) and *Solanum pimpinellifolium*. Data presented are mean ± SE (n = 8), an asterisk (*) indicates different values that were determined by the two-sided Student's *t*-test to be different ($P < 0.05$) between cotyledon 1 and cotyledon 2.

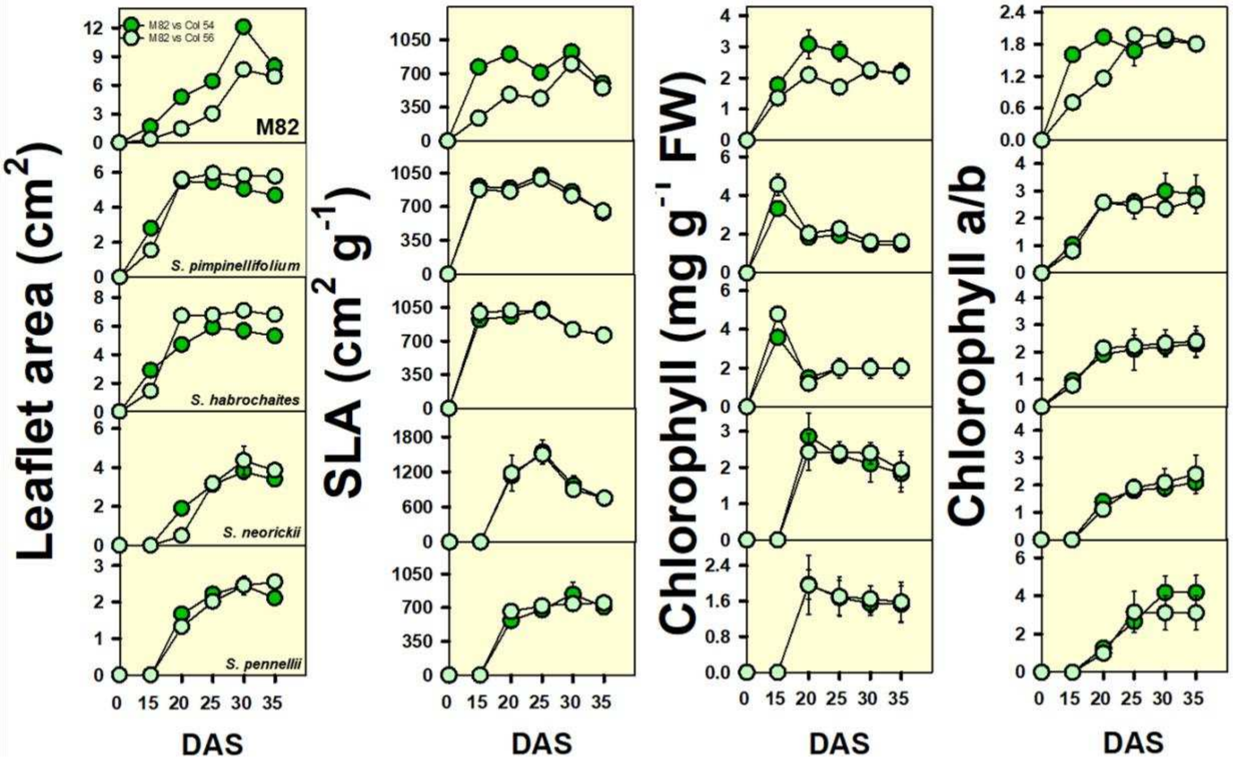


Figure 2. Developmental and physiological traits over development from domesticated and wild species of tomatoes. Leaflet traits were assessed in older leafleats of (dark-green) and fourth (pale-green) leaves from domesticated tomato (*Solanum lycopersicum*, Cv. M82), *S. pimpinellifolium*, *S. habrochaites*, *S. neorickii*, and *S. pennellii*. Data presented are mean ± SE (n = 7). Leaflet area (cm^2); SLA: specific leaf area (cm^2 g^{-1}); DAS: days after sowing.

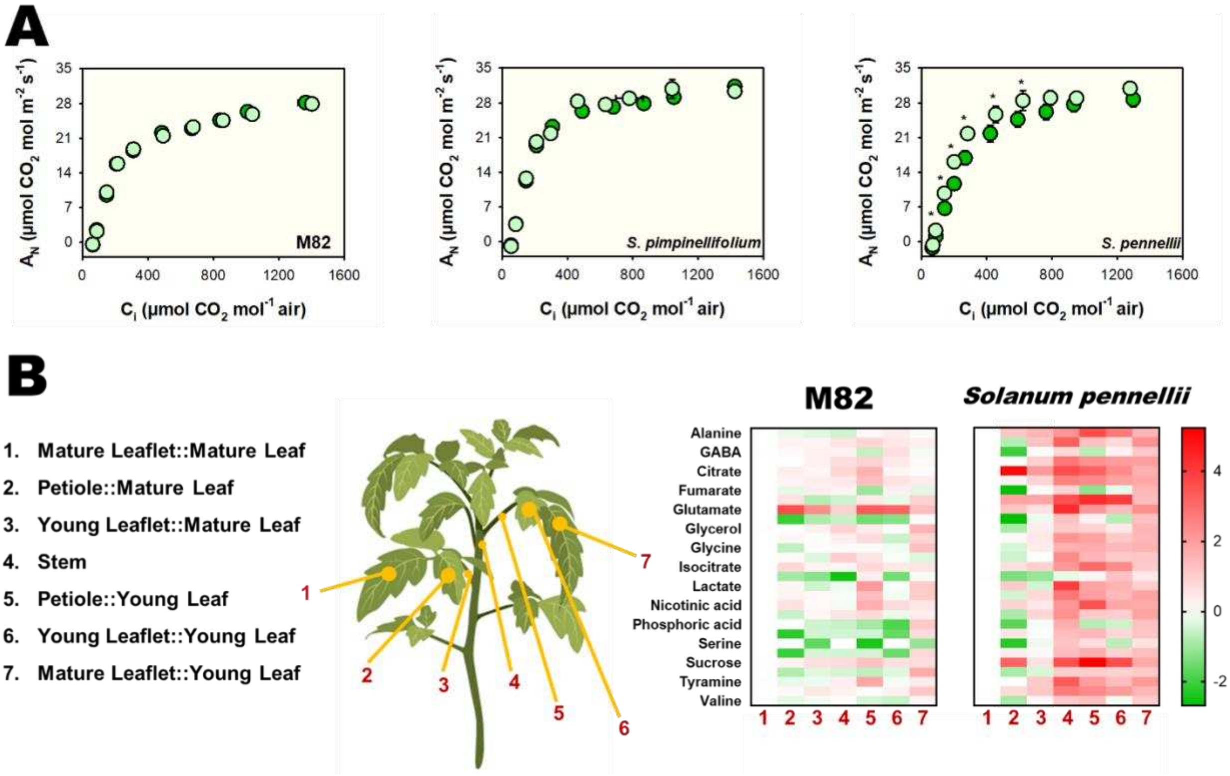


Figure 3. Net photosynthesis (A_N) curves in response to sub-stomatal (Ci) CO_2 concentration in domesticated and wild tomatoes. A, A_N/Ci curves of *Solanum lycopersicum* (Cv. M82), *S. pimpinellifolium*, and *S. pennellii* determined in leaflets of (dark-green) and fourth (pale-green) leaves. Data are means ± SE (n = 7), an asterisk (*) indicates different values that were determined by the two-sided Student's t-test to be different ($P < 0.05$) between third and fourth leaflets. **B,** Metabolite levels at middle day were assessed in seven segments. Samples were taken from the third and fourth leaf from the apex of 4-week-old plants. Data are means ± SE (n = 5).

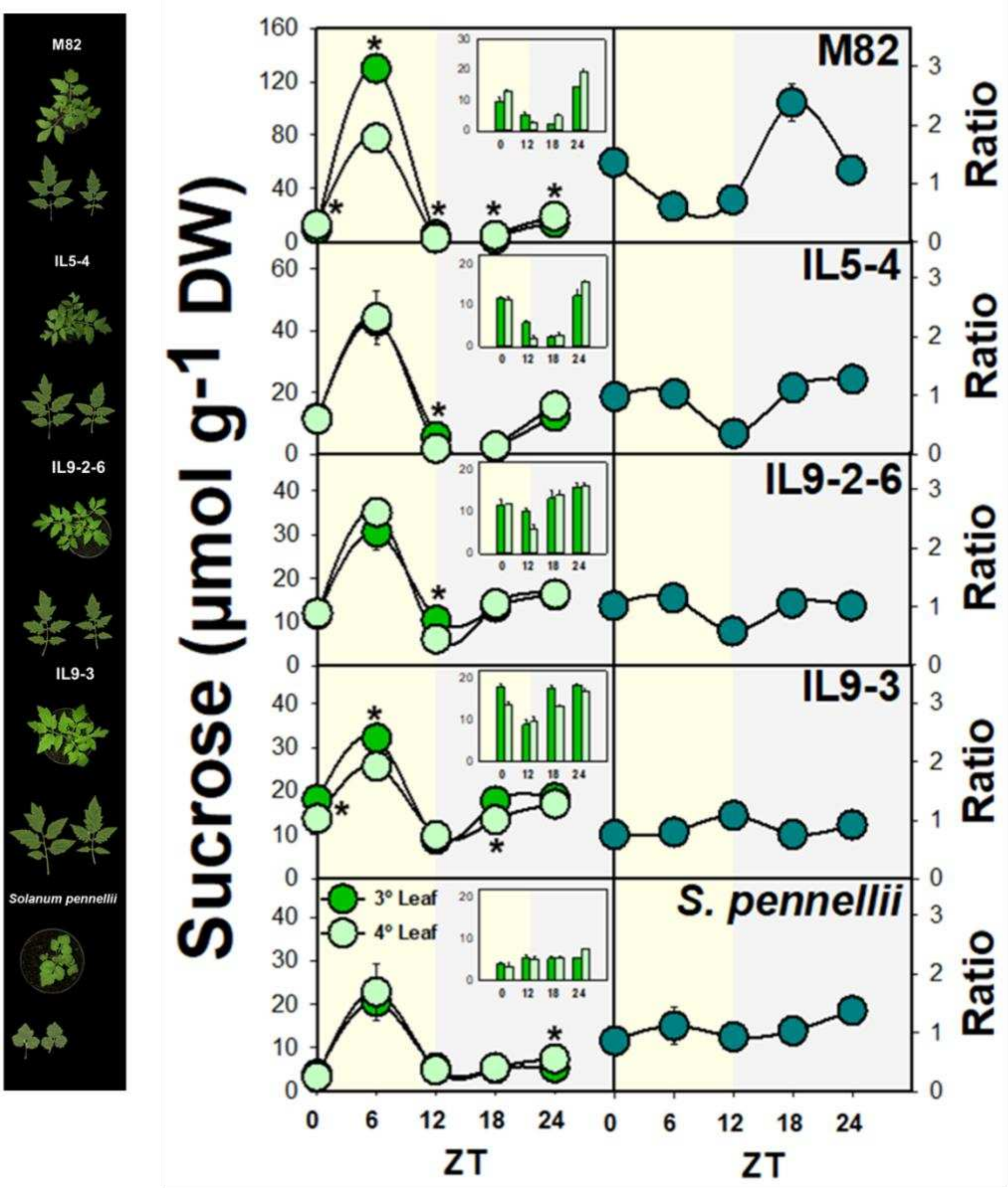


Figure 4. The diel regulation of sucrose metabolism on tomatoes. Older leafleats of (dark-green) and fourth (pale-green) leaves from domesticated tomato (Cv. M82), introgression lines (IL) and wild tomato *Solanum pennellii* were analyzed, as represented at left, while right are represented sucrose levels and the ratio between fourth and third leaflets. Sucrose levels were assessed in leaflets harvested every 6 hours over a diurnal cycle. Data are means ± SE (n = 5). Yellow bars indicate the light period while grey bars indicate the dark period. An asterisk (*) indicates different values that were determined by the two-sided Student's *t*-test to be different ($P < 0.05$) between third and fourth leaflets. DW: Dry weight.

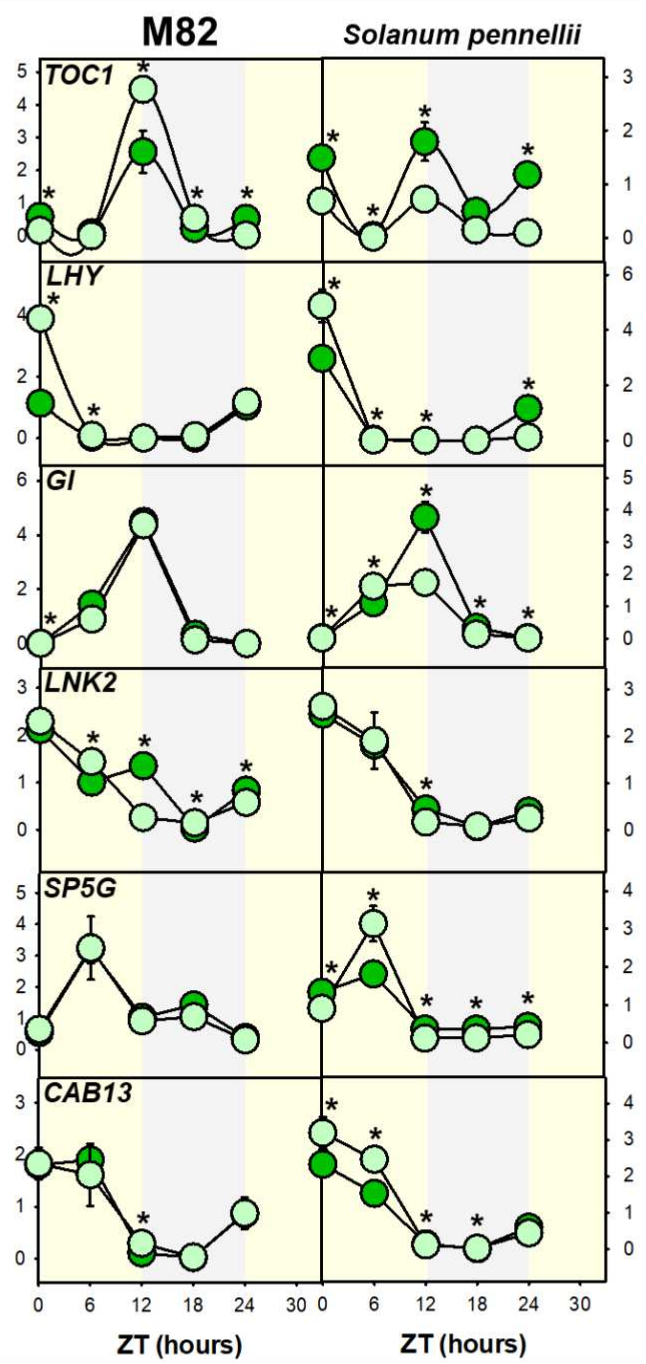


Figure 5. Expression profile of genes associated with tomato domestication in *Solanum lycopersicum* cv. M82 and *S. pennellii*. Diurnal oscillations of transcript levels were determined in leaflets of third (dark-green) and fourth (pale-green) leaves. An asterisk (*) indicates different values that were determined by the two-sided Student’s t-test to be different ($P < 0.05$) between third and fourth leaflets. Data represent the average expression of three biological replicates ± SE. TIMING OF CAB EXPRESSION 1 (TOC1); LATE ELONGATED HYPOCOTYL (LHY); GIGANTEA (GI); NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED 2 (LNK2); SELF PRUNING 5G (SP5G); CHLOROPHYLL AB BINDING PROTEIN 13 (CAB13).

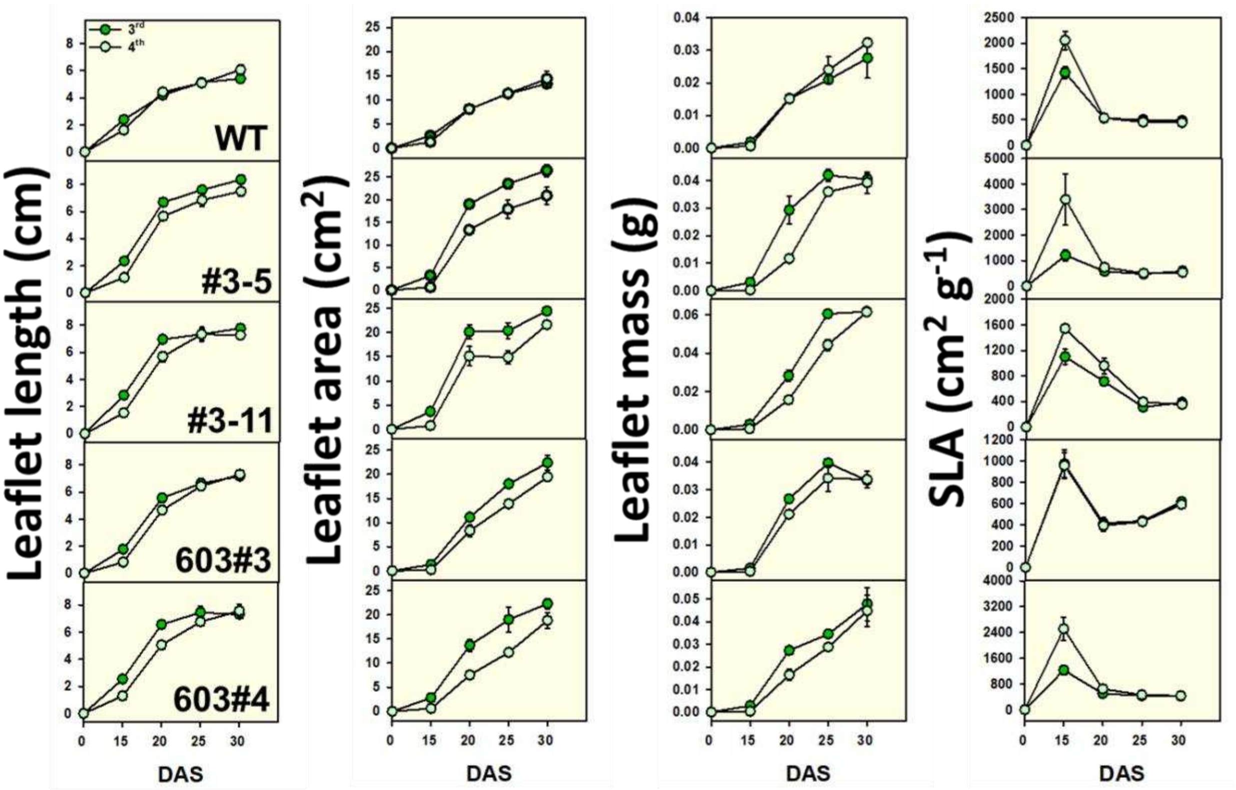
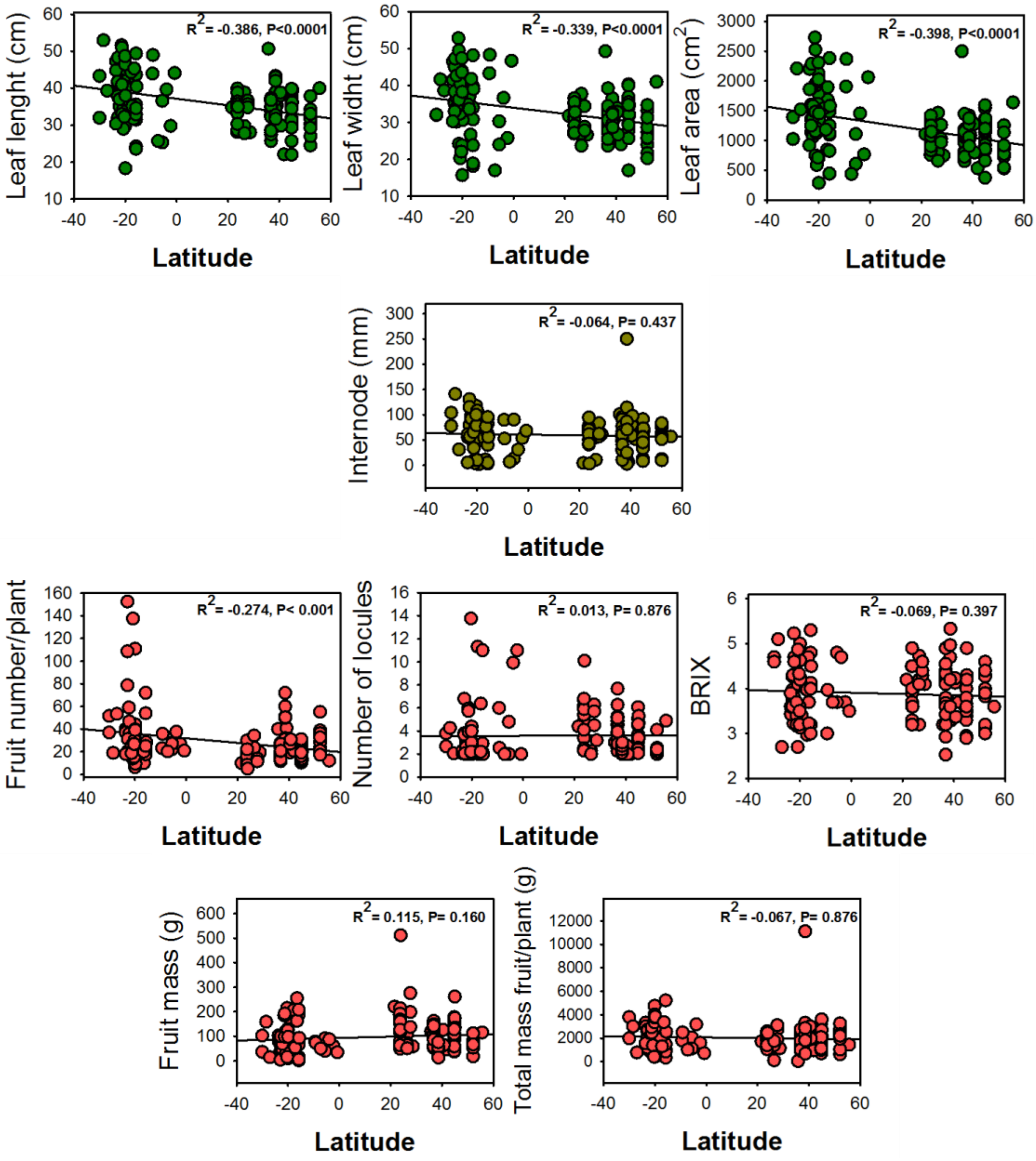
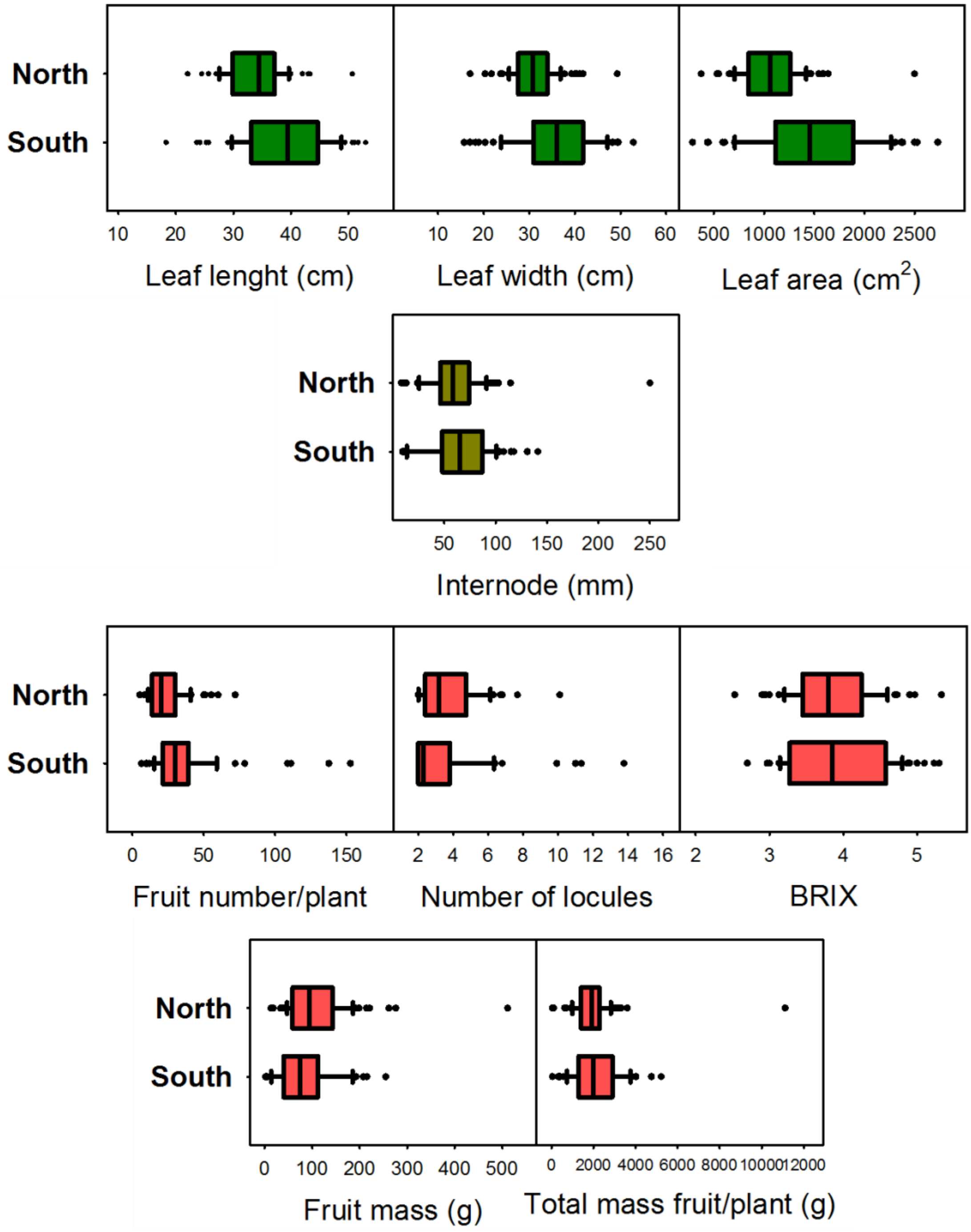


Figure 6. The establishment of leaf development hierarchy with domestication *de novo*. Leaflet traits were assessed in older leaflets of (dark-green) and fourth (pale-green) leaves from *Solanum pimpinellifolium* genotypes, wild-type (WT) and multiplex lines harbouring gene editions in key genes associated with domestication. Data presented are mean ± SE (n = 7). Leaflet length (cm); Leaflet area (cm^2); Leaflet mass (g); SLA: specific leaf area (cm^2 g^{-1}); DAS: days after sowing.

Supplementary Figures

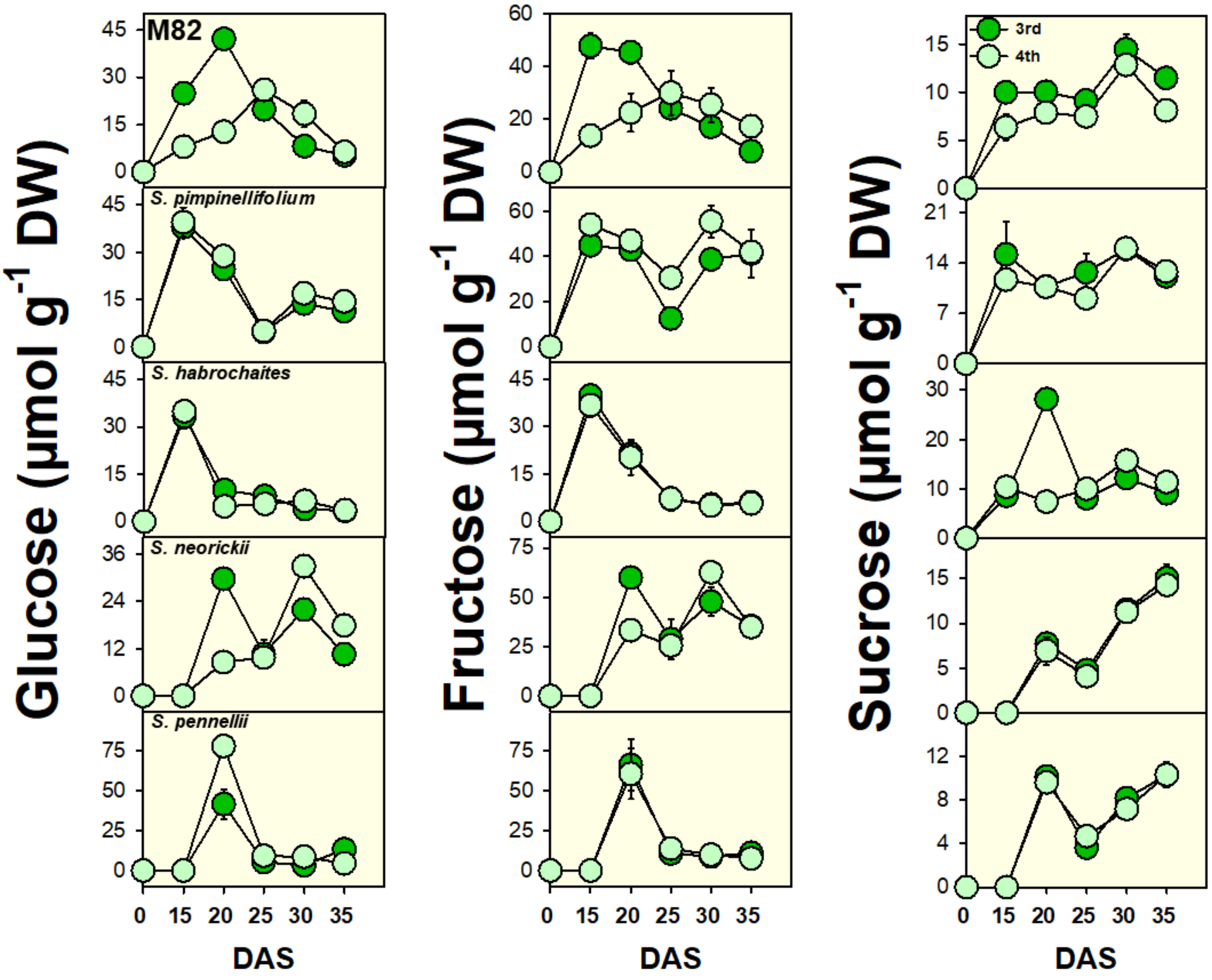


Supplementary figure S1. Pearson correlations for vegetative and reproductive traits of *Solanum lycopersicum* accessions. The data were obtained on germplasm bank of Universidade Federal de Viçosa, Brazil (http://www.bgh.ufv.br/). Values are presented as correlation index (R^2) and *p-value*.

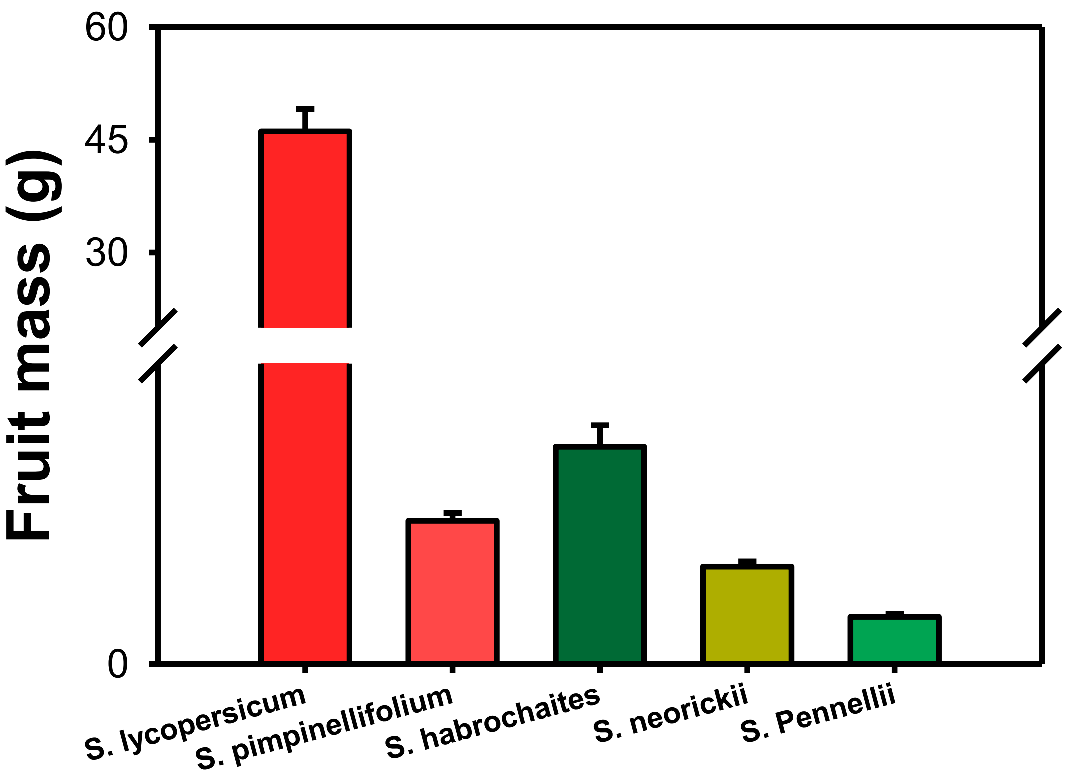


Supplementary figure S2. Comparisons for vegetative and reproductive traits of *Solanum lycopersicum* accessions originated from north and south hemisphere. The data were obtained on germplasm bank of Universidade Federal de Viçosa, Brazil (http://www.bgh.ufv.br/).

Suplemental Figure S3. Phenotypical coherence on tomato development. a, branching patterns (octave week) across *Solanum* species, where it are showed the wild tomatoes *Solanum pennellii*, *Solanum neorickii*, *Solanum habrochaites* and *Solanum pimpinellifolium* , and also the domesticated tomato *Solanum lycopersicum* (cv. M82). **b,** branching patterns and phenotype for 3rd and 4th leaves at fourth week, the image represent cv. M82 and *S. pennellii* as well as introgression lines (IL) on M82 containing genomic fragments of *S. pennellii*.



Suplemental Figure S4. The metabolism over leaf development on wild and domesticated tomatoes. Older leafleats of third (dark green) and fourth (pale-green) leaves from domesticated tomato (*Solanum lycopersicum*, Cv. M82), *S. pimpinellifolium*, *S. habrochaites*, *S. neorickii*, and *S. pennellii*. It is represented the levels of glucose, fructose, and sucrose. Sugar levels were assessed in leaflets harvested every five days over development. Data are means ± SE (n = 5). DW: Dry weight; DAS: Days after sowing.

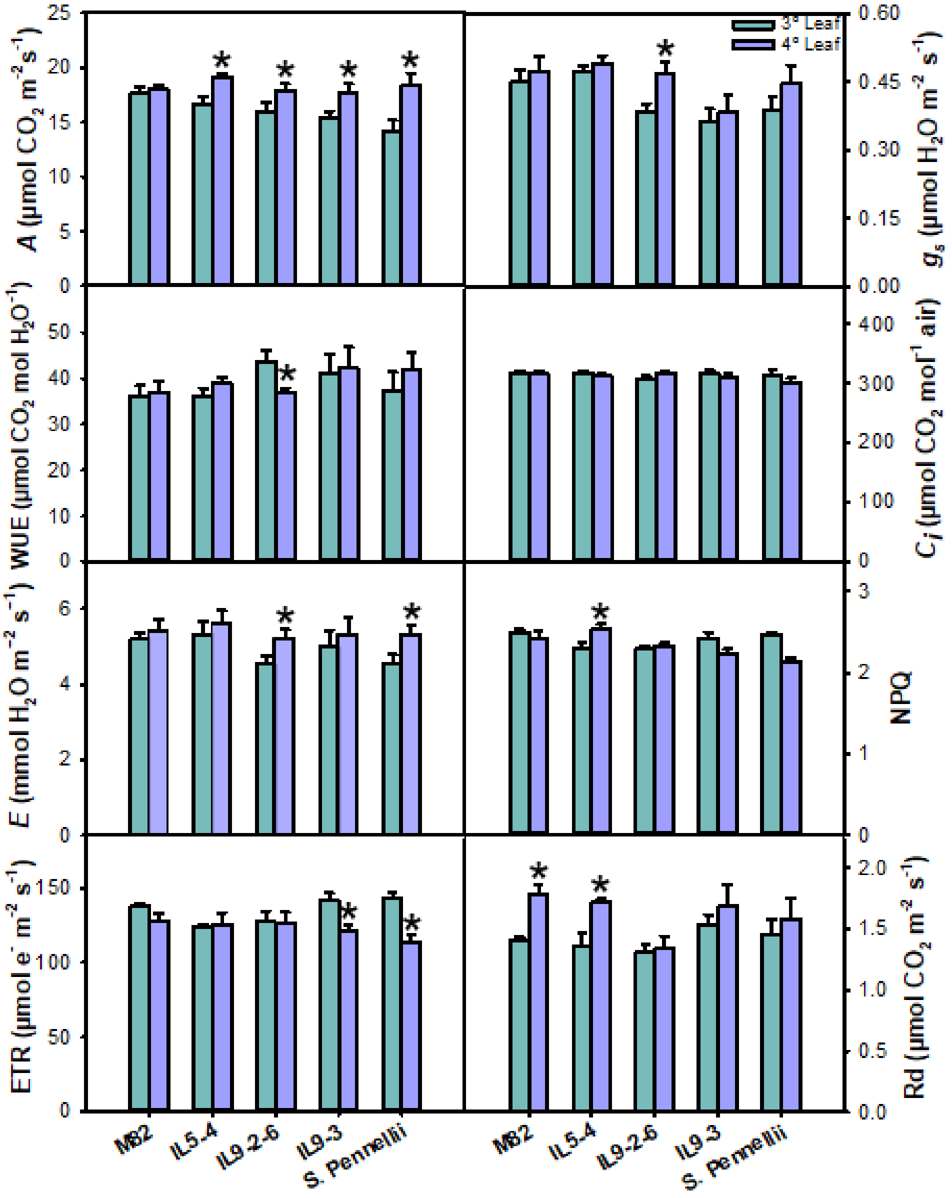


Supplementary figure S5. Fruit mass for tomato species (g). The fruits were harvested over plant development. Values are presented as means ± SE of individual fruits (n = 5 individual plants).

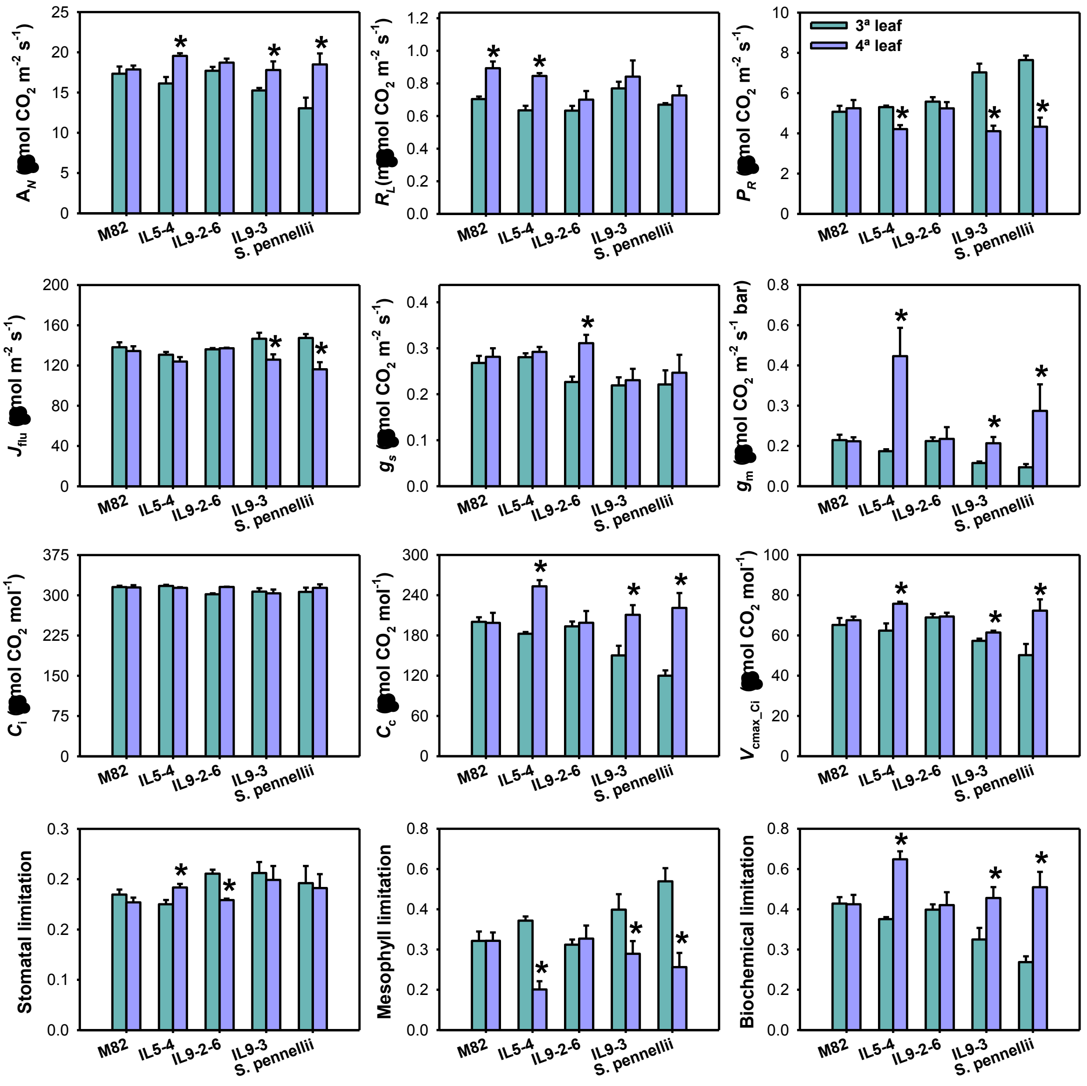
Trait*	M82		IL5-4		IL9-2-6		IL9-3		*S. pennellii*	
Leaf	3rd	4th	3rd	4th	3rd	4th	3rd	4th	3rd	4th
LA	23.9±2.4	**11.9±1.5**	21.5±2.2	15.4±2.1	18.7±1.9	15.6±1.5	25.9±2.9	15.6±1.5	5.4±0.5	5.1±0.7
LL	10.2±0.4	**7.7±0.4**	9.7±0.5	8.3±0.4	9.45±0.5	8.4±0.5	10.3±0.7	9.4±1.1	3.4±0.4	3.3±0.3
LM	34.1±3.9	**21.7±2.5**	35.4±3.5	26.0±3.3	29.11±2.9	22.6±2.8	34.0±4.9	27.7±4.7	6.2±1.0	6.5±0.9
SLA	671±17	**601±14**	639±9	627±14	680±11	**630±5**	647±49	714±68	770±38	714±36

Suplementary Table 1. Leaf growth traits on *Solanum lycopers*icum (cv. M82), *Solanum pennellii* and on introgression lines (IL). Data presented are mean ± SE (n = 7) obtained in at least two independent assays. Bold numbers describe differences $P < 0.05$ between 3rd and 4th leaves for each genotype, which it was calculated by two-sided Student's *t*-test.

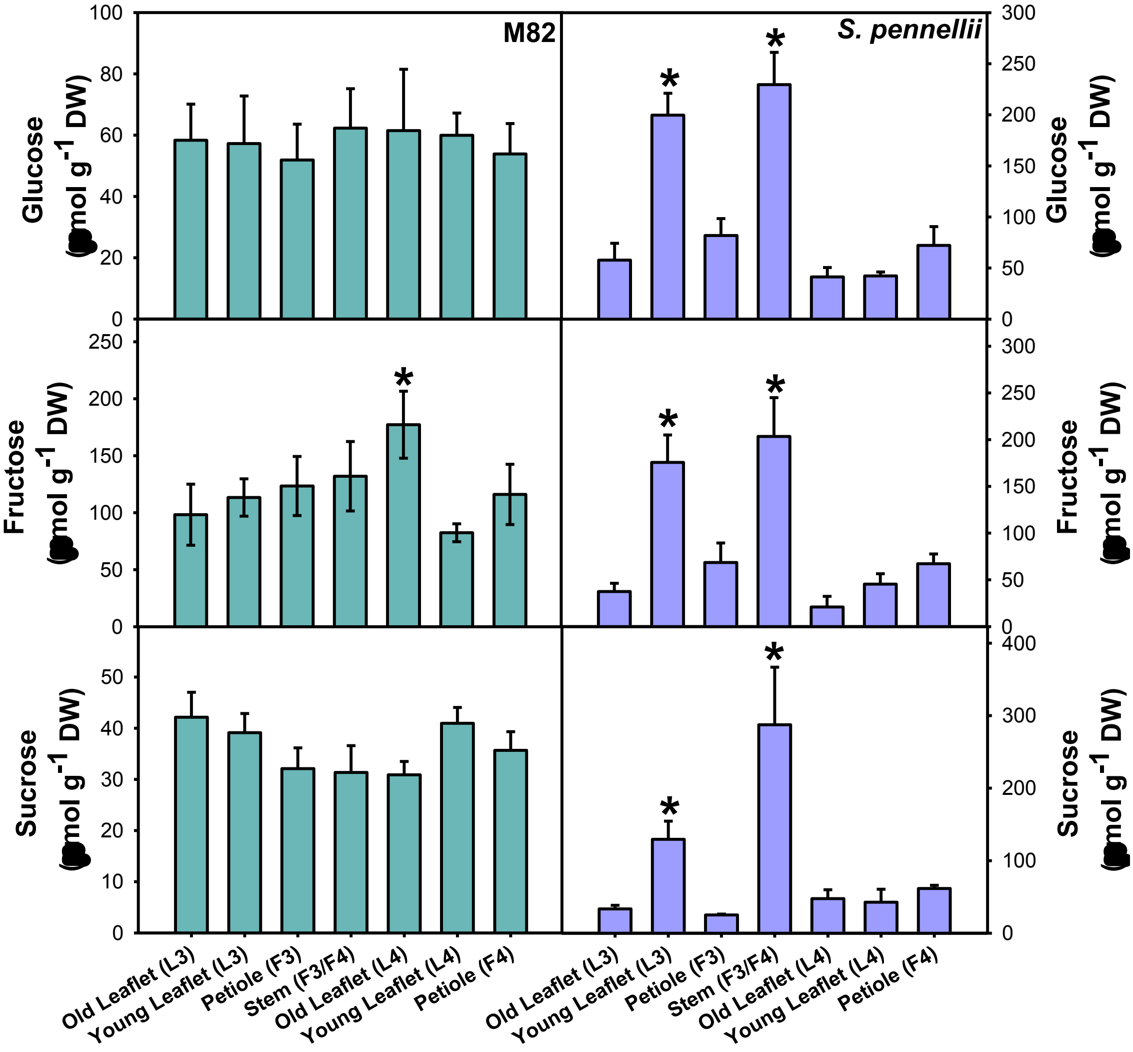
* LA: leaf area (cm^2); LL: leaf length (cm); LM: leaf mass; SLA: specific leaf area (cm^2 g^{-1}).



Supplementary Figure S6. Gas exchange parameters exhibit a differential regulation. Data were obtained in 4-week-old plants Solanum lycopersicum (cv. M82), Solanum pennellii and on introgression lines (IL) growing under optimal conditions. Values are presented as means ± SE (n = 5). Asterisks describe differences $P < 0.05$ between 3rd and 4th leaves for each genotype, which it was calculated by two-sided Student’s *t*-test. *A*: photosynthesis rate; g_S: stomatal conductance; WUE: intrinsic water-use efficiency; C_i: internal CO$_2$ concentration; E: transpiration rate; NPQ: non-photochemical quenching; ETR: electron transport rate; R_d: dark respiration.



Supplementary Figure S7. Influence of tomato domestication on photosynthetic efficiency of different plant leaves. Estimative for photosynthesis traits, where the data were obtained in 4-week-old plants *Solanum lycopersicum* (cv. M82), *Solanum pennellii* and on introgression lines (IL) growing under optimal conditions. Values are presented as means ± SE (n = 5). Asterisks describe differences $P < 0.05$ between 3rd and 4th leaves for each genotype, which it was calculated by two-sided Student's *t*-test. A_N: Net photosynthesis rate; R_L: light respiration; P_R: photorespiration rate; J_{flu}: electron transport rate estimated by chlorophyll fluorescence parameters; g_s: stomatal conductance CO_2; g_m: mesophyll conductance; C_i: internal CO_2 concentration; Cc: chloroplast CO_2 concentration; V_{cmax_Ci}: Rubisco maximum carboxylation capacity based on C_i.



Supplementary Figure S8. Leaf metabolite levels are altered on tomato segments. Key metabolite content in leaves of tomato plants: glucose, fructose, and sucrose at middle day. Samples were taken from the third and fourth leaf from the apex of 4-week-old plants. Data are means ± SE (n = 5), asterisks describe differences *P* < 0.05 between 3rd and 4th leaves for each genotype, which it was calculated by two-sided Student's *t*-test. DW: Dry weight.

Supplementary references

Fernie, A.R. *et al.* (2001). Fructose 2, 6-bisphosphate activates pyrophosphate: fructose-6-phosphate 1- phosphotransferase and increases triose phosphate to hexose phosphate cycling in heterotrophic cells. **Planta 212**, 250–263.

Genty, B. *et al.* (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. **Biochimica et Biophysica Acta (BBA) 990**, 87–92.

Hunt, R. *et al.* (2002) A modern tool for classical plant growth analysis. **Annals of Botany 90**, 485–488.

Medeiros, D. B. *et al.* (2016) Enhanced photosynthesis and growth in atquac1 knockout mutants are due to altered organic acid accumulation and an increase in both stomatal and mesophyll conductance. **Plant Physiology 170**, 86–101.

Chapter 3

Dusk watering accelerates tomato development without yield penalty[1]

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[1] *Preparing for the resubmission for Plant Physiology*

Dusk watering accelerates tomato development without yield penalty

Dear Editor,

Plants respond to water availability by operating at spatial and temporal scales, once water exchanges between soil-plant-atmosphere vary over minutes, hours or days. In fact, water evaporation from soil and plant varies temporarily within a day, and as such, leaf physiology is widely variable over day (Tardieu et al., 2018). Recently, the term 'chronoculture' was suggested as a way to conserve water in croplands by applying water in soil during specific day periods which could improve plant water-use efficiency (WUE) (Steed et al., 2021). In this context, such results offer the promise of a better understanding of potential connections between photoperiod and WUE on crops, which is a target of future engineering and optimization efforts.

In maize (*Zea mays*), hybrids irrigated at early periods of the day reduced grain productivity from 9-to-20% (Urrego-Pereira et al., 2016; Dong et al., 2016). Similarly, in tomato (*Solanum lycopersicum*) 5 to 26% higher yield was observed with evening irrigation when compared to morning irrigation (Ismail et al., 2008; Yacoubi et al., 2010). A common phenomenon encompassing WUE and day-length is drought escape, in which flowering time acceleration occurs under water deprivation, ensuring completion of the plant life cycle. In this sense, the expression of the *FLOWERING LOCUS T* (*FT*) gene is dependent on photoperiod, with expression peaks at the end of day culminating in flowering induction via a coincidence model (Song et al., 2015). *SINGLE FLOWER TRUSS* (*SFT*), an *FT* ortholog in tomato, is the major player involved in flowering, while also responsible of triggering the drought escape phenomenon, by modulating stomatal conductance (g_s) independently from abscisic acid (ABA) (Robledo et al., 2020). Accordingly, selection on *SELF-PRUNING 5G* (*SP5G*), a repressor of *SFT* in wild tomatoes under long-days conditions, occurred over tomato domestication reducing *S. lycopersicum* flowering sensitivity to day-length (Soyk et al., 2017). Intriguingly, the drought-resistant wild relative of tomato *S. pennellii* is able to maintain plant growth under constant light conditions due to an unperturbed photosynthetic system that is not observed in *S. lycopersicum* (Velez-Ramirez et al., 2014). It is important to note, however, that plant watering at different day periods might alter development and yield of domesticated tomato and this indicates an attractive avenue for research aiming to improve WUE and targets for engineering of stress tolerance.

To understand the significance of water supply at different periods of day on tomato yield, we exposed *S. pennellii* and tomato (cv. M82) plants to different watering regimes: at dawn (DAW), dawn/dusk (DDW) and dusk (DUW) (Supplemental data S1). We noted that the DUW treatment promoted a significant reduction on the number of leaves only in M82, whereas watering treatments did not altered the plant height in both genotypes (Figure 1a). This result is interesting, since leaves coordinate overall plant growth by being the sites of photosynthesis, and also by producing the SFT protein, which can relocate to the shoot apical meristem, where it triggers flowering (Corbesier et al., 2007; Jaeger and Wigge, 2007; Périlleux et al., 2019). Furthermore, *FT* deficient plants (*sft*) grow more leaves and a have higher intrinsic WUE (WUE$_i$), i.e. the ratio between net photosynthesis (A_n) and stomatal conductance (g_s) (Robledo et al., 2020), which prompted us to investigate diel watering responses in plants with alterations in *SFT* pathway. To further understand how watering at different day periods may impact tomato leaf physiology, we next compared tomato plants (cv. Micro-Tom [MT]), with its isogenic *sft* mutant, and a near-isogenic line harboring the *S. pennellii* allele of *SP5G* ($SP5G^{pen}$) that exhibits higher WUE (Siqueira et al., 2021).

Compelling evidence for a role for nocturnal physiology modulating WUE is provided by increases in leaf temperature and water evaporative demand. Indeed, this ultimately leads to a reduced A_n in the subsequent day that also increases the energy costs to build new leaves (for details*, see* Sadok and Jagadish, 2020). This prompted us to search for a potential interaction between diel physiology and the *FT*-related pathway by investigating gas exchange on the genotypes MT, *sft* and $SP5G^{pen}$. Higher A_n was observed for MT and $SP5G^{pen}$ plants under DUW treatment in comparison with DAW treated ones, whereas for MT plants lower g_s was found under the DAW (Figure 1B). Furthermore, higher WUE$_i$ was observed under DAW than DDW and DUW for MT, whereas for $SP5G^{pen}$ higher WUE$_i$ was observed under DUW (Figure 1B). Dark respiration was virtually invariant between watering treatments (Figure 1B), indicating no disruption of CO_2 release by watering treatments. (Figure 1B). However, dark respiration of $SP5G^{\mathrm{pen}}$ plants under DUW treated was higher (Figure 1B).

In *Eucalyptus camaldulensis*, g_s responsiveness follows a sunrise anticipation rule, wherein highest g_s values are usually observed before dawn and are related to higher plant biomass (Dios et al., 2016). Collectively, these results suggest that the end of the previous day may be fundamental to orchestrate this programming. Accordingly, alterations in nocturnal leaf physiology hamper the anticipation phenomena, reducing yield and grain quality in rice (*Oryza sativa*) (Desai et al., 2021), which apparently may be mitigated by DUW treatment. In consonance with our previous results obtained for M82 development, and MT and *sft* physiology, we next turned our attention to the impacts of treatments during vegetative and reproductive development of tomato. To this end, we incorporated an *SFT* over-expressing (SFT^{ox}) line in these experiments.

By analyzing height and number of leaves in the genotypes MT, SFT^{ox}, and $SP5G^{pen}$, we observed an overall trend revealing that DUW treatment resulted in smaller plants with less leaves than DAW and DDW (Figure 2A). Notably, no alteration in height or number of leaves over of experimental period was observed in *stf* mutant plants (Figure 2A). Changes in height or number of leaves were more drastic in SFT^{ox}, even though this genotype manifests an extremely early phase change from vegetative to reproductive stage, indicating that the developmental program may be disrupted under DUW treatment. Next, we assessed the cumulative number of flowers determined after the first flower appearance to verify whether the differential watering treatments modify reproductive developmental program. Our results indicated that under DUW treatment at MT, SFT^{ox} and $SP5G^{pen}$ displayed an early flowering with a higher number of flowers at early developmental stages, while this number decayed much more precociously under DUW than under DAW and DDW, culminating in a higher fruit number at late developmental stages under DUW (Figure 2A). It has been previously demonstrated that the acceleration of flowering time reduces WUE without penalizing the reproductive yield of *Arabidopsis thaliana* (Ferguson et al., 2019), revealing that WUE is likely associated with biomass allocation. Thus, at 100 days after germination (DAG), we assessed biomass of leaves, stem, roots, specific leaf area (SLA), relative water content (RWC) and WUE based on fruit production (WUE_{fruit}), which reflects each g of fruit dry mass produced per L of water supplied per plant during the experimental period. Under DUW treatment, reduced biomass of leaves, stem and roots was observed in both MT and SFT^{ox} while *sft* mutant was characterized by higher leaf biomass under DDW and DUW than DAW (Figure 2B). In consonance with our previous gas exchanges results, lower SLA was observed under DDW and DUW treatments on both MT and *SFT*^{ox}, indicating that these treatments support resource conservation of leaves and reduce water-losing surface exposed to the atmosphere. Regardless of watering treatments, RWC was not affected, suggesting that plants were not under water deficit (Figure 2B). Remarkably, plants from MT and *SP5G*^{pen} genotypes exhibited higher WUE_{fruit} whereas *sft* mutant plants exhibited lower WUE_{fruit} under DUW (Figure 2B). Our results are in good agreement with previous studies that demonstrated a lowest WUE_{fruit} for *sft* in comparison to MT (Vicente et al., 2015), indicating that despite a higher WUE_i (Robledo et al., 2020), the *sft* mutant displays lower productive yield which is aggravated by watering in the end of day.

In conclusion, our analyses of the impacts from different periods of watering during the day suggested that watering tomato near dusk can significantly improve crop yield. Similarly, satellite observations on croplands indicated that WUE_i is higher in the afternoon (2-5 pm) than in the morning (8-11 am) (Xiao et al., 2021). *SFT* expression is regulated over the course of the day, allowing the transition from vegetative to reproductive phase (Corbesier et al., 2007; Song et al., 2015), and watering plants in different periods of day offers a promising approach to ensure a tailored developmental program for crops. Whilst there are doubts concerning evening watering and an increase of pathogens infections, evening watering not necessarily will increase infections since water can be applied in the soil and stomata are programed to have lowest aperture at night. Altogether, the diel watering management may contribute to establish crops with higher WUE exploring natural resource in a better manner, which may be a new frontier in the next agricultural revolutions. The somewhat surprising alterations in fruit yield and WUE_{fruit} makes it tempting to speculate that crop yield is highly regulated at the whole-plant level in a manner that allows the plant to prioritize biomass production during development in response to differential watering.

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Author contributions

J.A.S., A.N-N., and W.L.A., designed experiments; J.A.S., T.W., L.R., M.F.S., J.W., A.O.M., performed experiments; J.A.S., A.Z., A.R.F., AN-N., and W.L.A. analysed data; and J.A.S., A.N-N., and W.L.A. wrote the paper.

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References

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, Coupland G (2007) FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. Science 316: 1030–1033.

Desai JS, Lawas LMF, Valente AM, Leman AR, Grinevich DO, Jagadish SVK, Doherty CJ (2021) Warm nights disrupt transcriptome rhythms in field-grown rice panicles. Proc Natl Acad Sci USA 118: e2025899118.

Dios VR, Loik ME, Smith R, Aspinwall MJ, Tissue DT (2016) Genetic variation in circadian regulation of nocturnal stomatal conductance enhances carbon assimilation and growth. Plant Cell Environ. 39: 3–11.

Dong X, Xu W, Zhang Y, Leskovar D (2016) Effect of irrigation timing on root zone soil temperature, root growth and grain yield and chemical composition in corn. Agronomy 6: 34–44.

Ferguson JN, Meyer RC, Edwards KD, Humphry M, Brendel O, Bechtold U (2019) Accelerated flowering time reduces lifetime water use without penalizing reproductive performance in Arabidopsis. Plant Cell Environ. 42: 1847–1867

Ismail M, Kiyoshi O, Khondaker A (2008) Influence of single and multiple water application timings on yield and water use efficiency in tomato (var. *First power*). Agric Water Manage 95: 116–122.

Jaeger KE, Wigge PA (2007) FT protein acts as a long-range signal in Arabidopsis. Curr Biol 17: 1050–1054.

Périlleux C, Bouché F, Randoux M, Orman-Ligeza B (2019) Turning meristems into fortresses. Trends Plant Sci. 24: 431–442.

Robledo JM, Medeiros D, Vicente MH, Azevedo AA, Thompson AJ, Peres LEP, Ribeiro DM, Araújo WL, Zsögön A (2020) Control of water-use efficiency by florigen. Plant Cell Environ. 43: 76–86.

Sadok W, Jagadish SK (2020) The hidden costs of nighttime warming on yields. Trends Plant Sci. 25: 644–651.

Siqueira JA, Araújo TW, Batista-Silva W, Vicente MH, Silva JCF, Coutinho J, Clarindo W, Zsögön A, Peres LEP, Veylder L, Fernie, AR, Nunes-Nesi A, Araújo WL (2021) Photoperiod shapes aluminium tolerance in plants. bioRxiv, https://doi.org/10.1101/2021.02.12.430934

Song YH, Shim JS, Kinmonth-Schultz HA, Imaizumi T (2015) Photoperiodic flowering: time measurement mechanisms in leaves. Ann Rev Plant Biol 66: 441–464.

Soyk S, Muller NA, Park SJ, Schmalenbach I, Jiang K, Hayama R, Zhang L, Van Eck J, Jimenez-Gomez JM, Lippman ZB (2017) Variation in the flowering gene *SELF PRUNING 5G* promotes day-neutrality and early yield in tomato. Nat Genet. 49: 162–168.

Steed G, Ramirez DC, Hannah MA, Webb AAR (2021) Chronoculture, harnessing the circadian clock to improve crop yield and sustainability. Science 372, eabc9141.

Tardieu F, Simonneau T, Muller B (2018) The physiological basis of drought tolerance in crop plants: a scenario-dependent probabilistic approach. Ann Rev Plant Biol 69: 733–759.

Urrego-Pereira YF, Martínez-Cob A, Cavero J (2013) Relevance of sprinkler irrigation time and water losses on maize yield. Agron J. 105: 827–853.

Velez-Ramirez AI, van Ieperen W, Vreugdenhil D, van Poppel PM, Heuvelink E, Millenaar FF (2014) A single locus confers tolerance to continuous light and allows substantial yield increase in tomato. Nat Commun. 5, 4549.

Vicente MH, Zsögön A, de Sá AFL, Ribeiro RV, Peres LEP (2015) Semi-determinate growth habit adjusts the vegetative-to-reproductive balance and increases productivity and water-use efficiency in tomato (*Solanum lycopersicum*). J. Plant Physiol. 177: 11–19.

Yacoubi S, Zayani K, Zapata N, Zairi A, Slatni A, Salvador R, Playán E (2010) Day and night time sprinkler irrigated tomato: irrigation performance and crop yield. Biosyst. 107: 25–35.

Xiao J, Fisher JB, Hashimoto H, Ichii K, Parazoo NC (2021) Emerging satellite observations for diurnal cycling of ecosystem processes. Nat. Plants 7: 877–887.

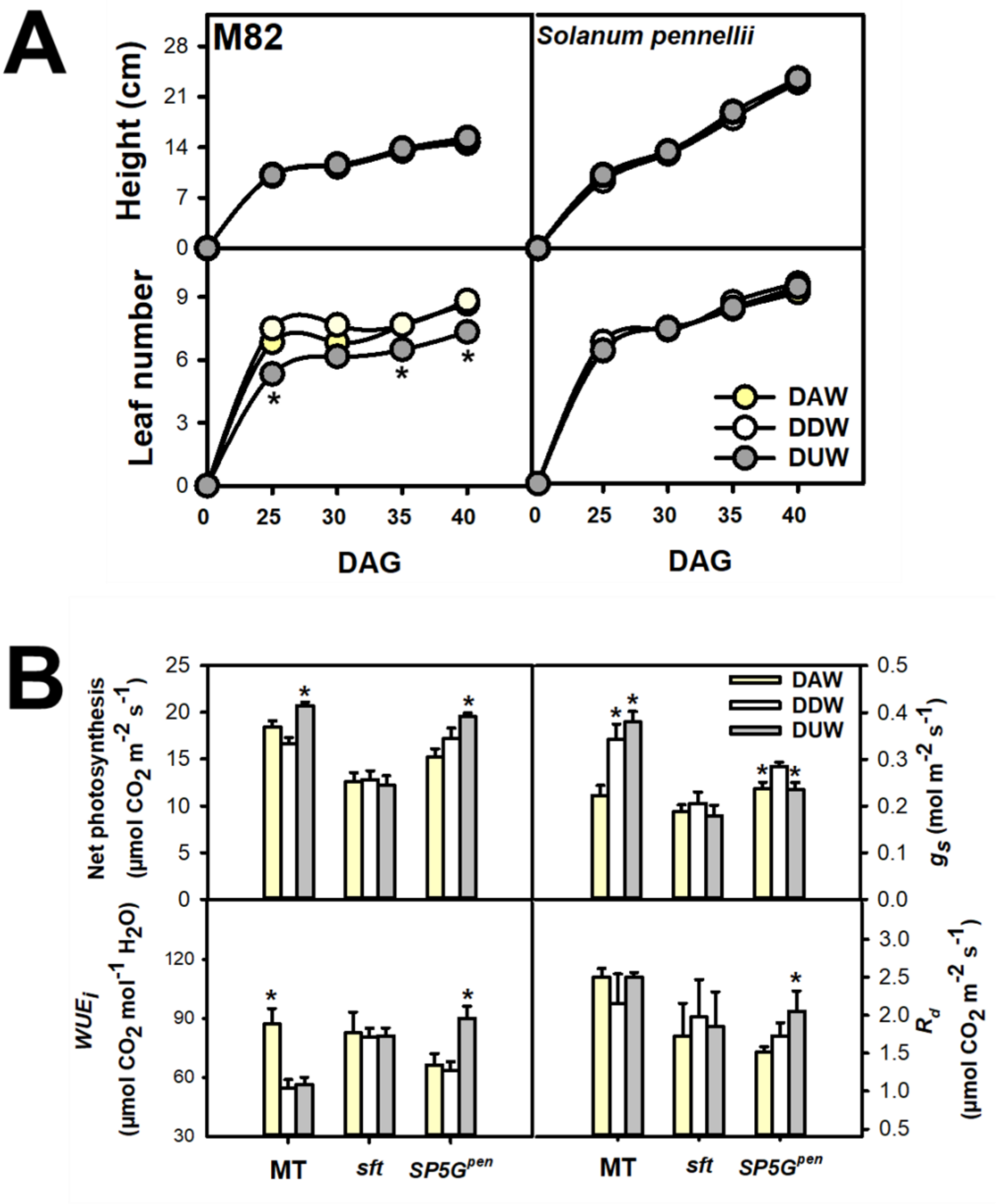

Figure 1: Dusk watering modify overall leaf production and physiology. Plants were exposed to daily watering at dawn (DAW), dawn/dusk (DDW) or dusk (DUW). A, *Solanum lycopersicum* (cv. M82) and *S. pennellii* were cultivated for 40 days after germination (DAG), and the height (cm) and leaf number were assessed. B, Net photosynthesis, stomatal conductance (g_s), intrinsic water use efficiency (WUE$_i$) and dark respiration (R_d) were determined in *S. lycopersicum* (cv. Micro-Tom [MT]), *SINGLE FLOWER TRUSS* (*sft*) and a near-isogenic line harboring the *S. pennellii* allele of *SELF-PRUNING 5G* (*SP5G^{pen}*) in the same genetic background of MT. Asterisks (*) indicate values that were determined by the two-sided Student's *t*-test to be different ($P < 0.05$) between watering treatments.

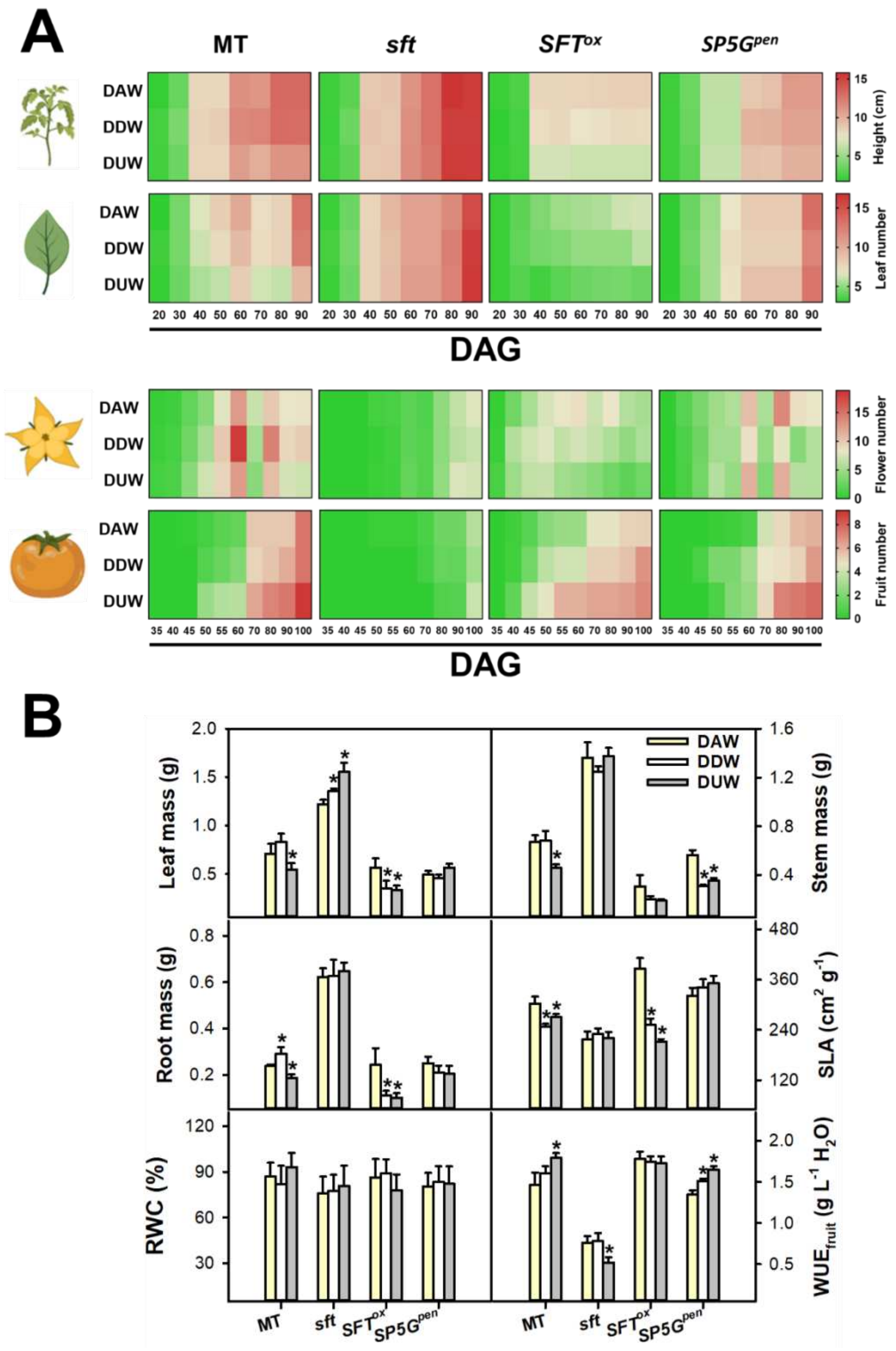

Figure 2: Dusk watering alters both vegetative and reproductive program improving yield and water use efficiency (WUE) in tomato. By using *Solanum lycopersicum* (cv. Micro-Tom [MT]), mutant *SINGLE FLOWER TRUSS* (*sft*), overexpression line of *SFT* (*SFT*^{ox}), and in a near-

isogenic line harboring the *S. pennellii* allele of *SELF-PRUNING 5G* (*$SP5G^{pen}$*) in the same genetic background of MT, we exposed the plants to daily watering at dawn (DAW), dawn/dusk (DDW) or dusk (DUW). A, plant height and numbers of leaves, flowers and fruits were assessed until 100 days after germination (DAG). B, the dry biomass (g) of leaves, roots and stem, as well as specific leaf area (SLA), relative water content (RWC) and WUE based on fruit dry mass (WUE_{fruit}) were measured at plants with 100 DAG. Asterisks (*) indicate values that were determined by the two-sided Student's *t*-test to be different ($P < 0.05$) between watering treatments.

Chapter 4

Metabolic and DNA checkpoints for enhancement of aluminum tolerance

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Review article

Metabolic and DNA checkpoints for the enhancement of Al tolerance

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UN: United Nations; QC: quiescent center; NIP: nodulin 26-like intrinsic protein; Nramp: natural resistance-associated macrophage protein; ALS1: ALUMINUM SENSITIVE 1; ALMT1: aluminum-activated malate transporter 1; MATE1: multidrug and toxic compound extrusion; GABA: gamma-aminobutyric acid; RAM: root apical meristem; ART1: aluminum resistance transcription factor 1; STOP1: sensitive to proton rhizotoxicity 1; CDK: cyclin-dependent kinase; CYC: cyclin; RBR1: retinoblastoma-related 1; DDR: DNA damage response; ATR: ataxia telangiectasia mutated and RAD3-related; SUV2: sensitive to UV2; ALT2: aluminum tolerant 2; SOG1: suppressor of gamma response; ATM: ataxia telangiectasia mutated; MDH: malate dehydrogenase; TCA: tricarboxylic acid; VDAC1: transporter voltage-dependent anion channel 1; HKII: hexokinase II; mETC: mitochondrial electron transport chain; SnRK1: SNF1-related protein kinase 1; ROS: reactive oxygen species; UCPs: uncoupling proteins

Abstract

Acidic soils are a major limiting factor for food production in many developing countries. High concentrations of soluble Al cations, particularly Al^{3+}, inhibit cell division and root elongation in plants. Al^{3+} damages several biomolecules, including DNA, impairing gene expression and cell cycle progression. Notably, the loss-of-function mutants of DNA checkpoints may mediate Al tolerance. Furthermore, mitochondrial organic acids play key roles in neutralizing Al^{3+} within the cell and around the rhizosphere. Here, we provide knowledge synthesis on interactions between checkpoints related to mitochondrial organic acid homeostasis and DNA integrity mediating Al tolerance in land plants. These interactions, coupled with remarkable advances in tools related to metabolism and cell cycle, may facilitate the development of next-generation productive crops under Al toxicity.

Keywords: Al response, cell cycle, DNA checkpoint, root exudates, organic acids

I. Al tolerance and need for suitable agriculture

During the last decades, considerable efforts have been made to ensure global food safety while achieving sustainable agriculture, which represent two of the major human necessities. In fact, these are the central goals of the United Nations (UN) to be achieved over the next 15 for extinguishing hunger worldwide (UN, 2015). A vast majority of soils in the tropical and subtropical regions present a recurrent problem of acidity (pH ≤ 5.5), which culminates in the solubilization of Al cations, particularly Al^{3+}. Under these conditions, Al^{3+} suppresses root elongation impairing nutrient and water uptake, ultimately leading to substantial yield losses (Kochian et al., 2004; Kochian et al., 2015). Moreover, intracellular Al^{3+} can bind several biomolecules, such as DNA, RNA, proteins, lipids, and carbohydrates, thereby significantly impeding gene expression, protein biosynthesis, and membrane integrity (Kochian et al., 2004; Kochian et al., 2015). Notably, in maize (*Zea mays*), Al promoted microfilament fragmentations and microtubule alterations, which reorganize and stabilize cytoskeleton, thus arresting root elongation (Blancaflor et al., 1998; Sivaguru et al., 1999; Kochian et al., 2004).

The most accepted mechanism for enhancing plant Al tolerance involves metal neutralization in both the intracellular space and around the rhizosphere (Nunes-Nesi et al., 2014; Kochian et al., 2015). To achieve this, organic acids (*e.g.*, citrate, malate, and oxalate) produced in mitochondrion-related reactions bind and neutralize Al cations (Nunes-Nesi et al., 2014; Kochian et al., 2015), allowing root growth. Thus, reprogramming of mitochondrial metabolism may be essential to maintain root elongation under Al stress (Nunes-Nesi et al., 2014). Nevertheless, the loss-of-function mutants of genes involved in DNA checkpoint regulation are characterized by cell cycle progression in the presence of Al (Eekhout et al., 2017). This ensures maintenance of the root **quiescent center** (**QC**), supporting root elongation under Al stress (Eekhout et al., 2017). Therefore, both organic acid-related metabolism and DNA checkpoint regulation are promising strategies to develop Al-tolerant crops. In this context, molecular interactions among these mechanisms can enhance plant tolerance to Al toxicity. Under genotoxic conditions, proteins involved in DNA repair disrupt mitochondrial metabolism in animal cells (Sahin et al., 2011; Kruiswijk et al., 2015;

Rufini et al., 2013). Thus, DNA repair and mitochondrial metabolism may be responsible for mediating cell cycle progression and survival under adverse conditions (Rufini et al., 2013; Qin et al., 2015). Although genes encoding organic anion transporters have already been established as the targets for enhancing Al^{3+} tolerance, other recently discovered mechanisms provide additional options for engineering mitochondrial metabolism and cell cycle progression to enhance Al^{3+} tolerance (**Table 1**).

In this review, by integrating molecular and metabolic changes occurring under Al stress, we highlight how DNA checkpoint regulation coupled with mitochondrial metabolism manipulation is linked to plant Al tolerance. Here, we discuss the currently accepted mechanisms of Al^{3+} tolerance, describing recent findings on Al perception and transport within plant cells. Additionally, we provide circumstantial evidence for the targeted genetic manipulation of DNA checkpoints in vascular plants and its association with changes in mitochondrial metabolism as a suitable metabolic approach to improve plant Al^{3+} tolerance. Furthermore, we posit that attempts to understand the metabolic response of Al^{3+} tolerance have largely been limited thus far, and comprehensive characterization of these responses and their unnoticed, although crucial, connections may provide an exciting scientific avenue to be pursued in future research. Finally, we consider evidence highlighting potential interactions among these mechanisms to showcase the relevant approaches for enhancing plant Al tolerance, contributing to develop a suitable agriculture for acidic soils.

II. Al perception and transport

Al is the third most abundant element of the Earth, and it is directly associated with acidic and low-fertility soils, presenting negative relationships with some essential elements for plants. Therefore, the “acid soil syndrome” constitutes a set of plant nutritional disturbances, including toxicity triggered by high Al, Mn, and Fe concentrations as well as limited P availability (Kochian et al., 2004). Under such conditions, mechanisms promoting the perception of Al toxicity are required for the activation of transcriptional, post-transcriptional, and metabolic responses in plant roots.

Among cellular sites for Al perception, the plasma membrane anchors the majority of the proteins related to this process and is evidently crucial. Cytosolic levels of Al increase due to the influx of Al^{3+} from the rhizosphere into the root cells through plasma membrane transporters (Kochian et al., 2015). Members of the **nodulin 26–like intrinsic protein** (**NIP**) and **natural resistance-associated macrophage protein** (**Nramp**) families are the central components of this mechanism, characterizing initial Al perception (Kochian et al., 2015). An Nramp-type transporter encoded by *OsNrat1* in rice (*Oryza sativa*) and an aquaporin encoded by *HmPALT1* in hydrangea (*Hydrangea macrophylla*) are involved in Al^{3+} influx from the rhizosphere into the root cells (Xia et al., 2010; Negishi et al., 2012). Meanwhile, calcineurin B-like calcium sensors (CBLs) likely play pivotal roles in Al perception (Ligaba-Osena et al., 2017). Following Al exposure, the loss-of-function mutant *cbl1* accumulated more Al and exhibited more severe defects in transcriptional modifications, thus suppressing root elongation (Ligaba-Osena et al., 2017). Furthermore, the aquaporin NIP1;2 favors the transport of Al complexed with malate from the root cell wall into the root symplasm, although this was not observed for Al^{3+} ions retained in the root cell wall (Wang et al., 2017). Subsequently, NIP1;2 mediates Al xylem loading and root-to-shoot translocation, which are crucial processes in Al internal detoxification (Wang et al., 2017). Accordingly, Al migrates across intercellular spaces and the metal is sequestrated from the cell wall into the vacuole through the activity of ALUMINUM SENSITIVE 1 (ALS1) and ALS3 transporters, culminating in the neutralization of Al toxicity (Huang et al., 2012; Larsen et al., 2005; Larsen et al., 2007; Dong et al., 2017).

Nonetheless, intercellular Al levels often exceed the internal cellular detoxification capacity, triggering differential Al accumulation in diverse cellular subcompartments. In this context, the enhancement of intercellular Al levels may alter the activity of several transcription factors, such as Al resistance transcription factor 1 (ART1), sensitive to proton rhizotoxicity 1 (STOP1), and WRKY46 (Yamaji et al., 2009; Iuchi et al., 2007). In *Arabidopsis thaliana*, STOP1 is a major regulator of genes related to Al tolerance, such as *ALS3* and organic acid transporters **aluminum-activated malate transporter 1** (***AtALMT1***) and **multidrug and toxic compound extrusion**

(***AtMATE1***) (Liu et al., 2009; Sawaki et al., 2009). *MATE* multiallelic promoter transactivation and ChIP analyses revealed fewer binding sites for transcription factors in sorghum (*Sorghum bicolor*) genotypes with Al-sensitive genetic backgrounds (Melo et al., 2019). Moreover, NIP1;2 and ALMT1 synergistically promote malate exudation from roots into the rhizosphere, which excludes Al and enables internal Al detoxification (Wang et al., 2017). Malate is essential for the regulation of these processes, and emerging evidence indicates its role in the root protection and ion translocation under nutrient deficit (**Box 1**). Under Al stress, non-protein amino acid gamma-aminobutyric acid (GABA) negatively affected ALMT1 activity (Ramesh et al., 2015). GABA-mediated regulation of ALMT1 is associated with the impairment of malate exudation and root elongation under Al toxicity (Ramesh et al., 2015). Meanwhile, ALMT1 plays crucial roles in the metabolic cellular communication within roots, mediating malate transport and reducing GABA intracellular levels to facilitate their transport to the rhizosphere (Ramesh et al., 2018). Furthermore, elements related to the acid soil syndrome, including Fe and P, exhibit transport interactions to promote proliferation and expansion in the stem cell niche. Under P-limiting conditions, the STOP1–ALMT1 axis induces malate exudation into the apoplast of cells around the meristematic zone, thus inhibiting cell division (Balzergue et al., 2017). Similarly, under Al toxicity, the primary lesions are apoplastic, which alter auxin distribution and suppress root cell elongation (Mora-Macías et al., 2017), reiterating malate relevance in protecting roots against Al primary targets.

In STOP1 and ALMT1 mutants, cell elongation under P-limiting conditions was rescued, although cell division was not (Balzergue et al., 2017). Overall, regardless of the mechanisms that confer the phenotypes described above, malate acts as a mediator of root growth and development (**Box 1**). Thus, roots that can cope with the acid soil syndrome present stringently coordinated activity of STOP1–ALMT1, which promotes malate exudation from the cell interior into the apoplast, arresting cell proliferation and inducing cell differentiation in the root apical meristem (RAM) (Mora-Macías et al., 2017). Thus, malate is a developmental checkpoint that reprograms root growth under P limitation (Mora-Macías et al., 2017), which is consistent with the notion that malate is a pivotal regulator of root *fitness*. In a broader context, emerging evidence suggests that in acidic soils, the regulation of *energy status* and *cell division* entails singular interactions modulating growth and detoxification responses

(Balzergue et al., 2017; Mora-Macías et al., 2017; Ramesh et al., 2018; Hamasaki et al., 2019; Upadhyay et al., 2019; Duan et al., 2020; Siqueira et al., 2021). Thus, elucidating the molecular mechanisms underlying interactions between metabolic and cell division checkpoints may aid the improvement of crop yield under Al stress.

III. Loss-of-function of DNA repair-related proteins confers root quiescence and Al tolerance

Genotoxicity is implicated in DNA damage, affecting genome integrity and impairing DNA replication, which arrest growth due to the blockage of cell cycle progression (Hu et al., 2016). Notably, this blockade often occurs as a result of **cyclin-dependent kinase** (**CDK**)/**cyclin** (**CYC**) inhibition at specific cell cycle phases, such as the G1–S, intra-S, and G2–M checkpoints (Hu et al., 2016). In plants, **retinoblastoma-related 1** (**RBR1**), which blocks G1–S transition, plays a crucial role in **DNA damage response** (**DDR**) under genotoxic conditions (Horvath et al., 2017; Biedermann et al., 2017). Similarly, to ensure proper DDR, the suppression of genes related to G2–M transition is also essential, which inhibits cell division in roots (Chen et al., 2017). However, studies covering DNA damage triggered by Al are almost exclusively limited to plants. Nonetheless, potential Al genotoxicity in other organisms, particularly humans, following chronic exposure through dietary ingestion of Al loaded foods has been suggested (Chen et al., 2019). Despite the genotoxicity of Al in microorganisms, animals, and plants, the molecular mechanisms underlying DNA damage triggered by this metal remain poorly understood.

Current evidence indicates that Al alters the conformational topology of DNA, rendering it vulnerable to endogenous mutagens (Hu et al., 2016). In barley (*Hordeum vulgare*) roots, Al-induced DNA damage reduced cell number in the S phase but increased cell number in the G2–M phase, ultimately delaying cell division (Jaskowiak et al., 2018). RBR1 plays an essential role in entry to the S phase, and its loss-of-function reduced the rate of DNA repair and increased Al sensitivity in *Arabidopsis* (Biedermann et al., 2017). A recent study revealed that RBR1 binds nearly 1,000 genes (Bouyer et al., 2018), several of which are involved in cell cycle progression, DNA repair and replication, and chromatin organization. Furthermore, two highly sensitive genotypes carrying mutations in the KNOTEN1 (*KNO1*) gene have been identified (Bouyer et al., 2018). As such, the *kno1-1* mutant exhibits defective DNA damage repair signal transduction, indicating that the KNO1 protein is essential for the localization of the DNA repair machinery in regions with abundant DNA lesions (Bouyer et al., 2018). Therefore, RBR1 plays vital roles in mediating DDR under Al toxicity. In contrast, the loss-of-function of components related to DNA integrity checkpoints is linked to improved plant tolerance of Al (**Box 2**). For instance, knockdown of the ***ataxia telangiectasia mutated and RAD3-related*** (***ATR***) gene enhanced cell division and root QC maintenance, sustaining root elongation even under Al stress (Rounds and Larsen, 2008). Additionally, **sensitive to UV2** (***SUV2***) a component of ATR-dependent checkpoint has been implicated in Al stress response, and the loss-of-function of SUV2 triggered Al response similar to that observed in *atr* mutants (Sjogren and Larsen, 2017). Furthermore, **aluminum tolerant 2** (**ALT2**) another DNA checkpoint regulator is involved in the perception of and response to Al-induced DNA damage (Nezames et al, 2012). Upon Al exposure, the loss-of-function mutants *atr*, *suv2*, and *alt2* exhibited a higher root elongation rate and a lower QC stem cell differentiation rate than the wild-type plants (Rounds and Larsen, 2008; Nezames et al, 2012; Sjogren and Larsen, 2017). In the Al-related DNA checkpoint pathway, the transcription factor **suppressor of gamma response** (**SOG1**) induced the expression of several DNA repair-related genes in an Al-dependent manner, suppressing root elongation under Al toxicity (Sjögren et al., 2015). Overall, ATR, SUV2, ALT2, and SOG1, through an Al-dependent pathway, regulate DNA repair and cell cycle arrest to suspend root growth under Al stress (Sjogren et al., 2015). Therefore, Al likely induces a specific pathway to mediate DNA repair and cell cycle arrest, in which the functional loss of components involved in this pathway promotes root elongation under Al toxicity (**Box 2**). Taken together, these reports indicate the crucial role of DNA repair checkpoints in plant tolerance of Al. Thus, further identification and targeted manipulation of specific DNA checkpoints may offer novel suitable strategies for the development of Al-tolerant crops (Eekhout et al., 2017).

Typically, the release of organic acids by roots is linked to **Al resistance**, whereas most previous studies on Al resistance have focused on root elongation at the early stages of development, and only a few studies have evaluated plant productivity. In this context, the extent to which organic acids release mediates root protection and maintenance of plant productivity remains controversial. Notwithstanding, large amounts of organic acids are secreted by the Al-sensitive genotypes of several species, including buckwheat (*Fagopyrum esculentum*), maize, rice, and sorghum (Ishikawa et al.; 2000; Piñeros et al., 2005; Zheng et al., 2005). Indeed, citrate release from the roots to the rhizosphere through the activity of *ZmMATE1* was not sufficient to explain the differences in Al tolerance across maize genotypes (Matonyei et al., 2020). Furthermore, *trans*-acting elements in the promoter region of *MATE* can modulate gene expression, contributing to the variable phenotypes of Al tolerance in the corresponding genetic backgrounds (Melo et al., 2013). Similarly, *MATE* expression depends on genomic repeat variants, which widely differ according to the genetic background (Melo et al., 2019). Since different genomes present abundant genetic diversity, organic acids metabolism and cell division may be shaped differentially across diverse genotypes. Given the genetic diversity in *MATE* and the differential importance of organic acids in specific genotypes, Al tolerance cannot be completely explained by organic acid exudation *per se*. Therefore, a combination of mechanisms may be essential to engineer Al tolerance in most crops, since these plants possess diverse root systems from morphological and cellular perspectives (Ezaki et al., 2013; Nunes-Nesi et al., 2014; Siqueira et al., 2022).

Root cellular identity is fundamental to activate stress response pathways (*e.g.*, responses to Fe deficiency and salinity exposure), and singular pathways are induced differently in distinct cell layers of the root (Dinneny et al., 2008). Thus, differential transcriptional patterns are established depending on the cell differentiation status across distinct root cell types (Brady et al., 2007), implying in a unique metabolic behavior of each cell group population. Therefore, mechanisms underlying Al tolerance appear to involve both organic acid metabolism and DNA repair in specific root cell groups, presenting a reliable yet complex opportunity for the improvement of Al tolerance. For instance, in the Al-hypersensitive mutant *als3-1* complemented with the loss-of-function mutant *alt2*, Al tolerance was rescued, leading to ALMT1 RNA and Al accumulation in roots (Nezames et al, 2012). Therefore, in addition to playing prominent role in Al exclusion from roots, ALMT1 probably is involved in the re-induction of cell division in RAM, as previously demonstrated (Balzergue et al., 2017). Consistently, the loss-of-function of ATR restored Al tolerance in *Arabidopsis* mutants of genes involved in Al perception and transport (*als3-1* and *star1*) (Zhang et al., 2018). In contrast, the *almt1atr* and *stop1atr* double mutants were Al-sensitive (Zhang et al., 2018), whereas *sog1* loss-of-function mutants could tolerate mitochondrial ATP synthesis blockage, which did not occurs in the wild-type plants (Hamasaki et al., 2019); this observation, to a certain extent, is similar to the finding for Al tolerance. Conversely, the exposure of *sog1* and *atm* (***ataxia telangiectasia mutated***) mutants to extremely high Al concentrations resulted in Al hypersensitivity, contrary to the report of the higher Al tolerance of these mutants in previous studies evaluating responses of more long-term (Chen et al., 2019). Therefore, a multi-level response to Al-induced DNA damage was proposed. Since the magnitude of Al stress in plants depends on Al concentration in soil *versus* exposure time to Al, under Al exposure in soils around the world, mechanisms underlying the tolerance of this metal may be activated before the induction of DDR. Apparently, both Al detoxification and DNA checkpoint regulation are indispensable for plant growth under Al stress. These findings indicate the presence of a possible convergent network between DNA checkpoint control and organic acid homeostasis within root cells, which likely contributes to the maximization of crop tolerance of Al (**Figure 1**). Further studies are warranted to experimentally elucidate how and to what extent these mechanisms interact to sustain Al tolerance and whether this interaction may result in any undesirable yield penalty.

IV. Convergent network between DNA repair control and organic acid metabolism mediating Al tolerance

Cell cycle and mitochondrial bioenergetics play intrinsic roles in enhancing DNA repair and survival of organisms under genotoxic conditions. Proteins relevant to cell cycle progression, such as CYCB1/CDK1, are localized in the mitochondrial matrix, promoting ATP synthesis in yeast (Wang et al., 2014). This improves cell bioenergetic efficiency for G2–M transition, enhancing DNA repair and survival of yeast cells under genotoxic conditions (Wang et al., 2014; Qin et al., 2015). In plants, *cdkb1* and *cycb1* mutants were Al-sensitive and exhibited delayed growth recovery under Al stress. Conversely, the loss-function mutants *atr*, *atm*, and *sog1* were relatively more Al-tolerant, with only *atm* and *sog1* exhibiting delayed growth recovery following Al exposure (Chen et al., 2019). Therefore, how genetic alterations in these intricate components generate distinct phenotypes in response to Al remains unclear, highlighting the need for unveiling the interactions between DNA repair proteins and other players that improve Al tolerance, which may expand our knowledge of cellular responses to Al stress.

In animals, the SOG1 analog **p53** mediates DNA repair and cell cycle checkpoint regulation (Kruiswijk et al., 2015). In the presence of DNA damage, the transcription factor p53 modifies mitochondrial activity and impairs the metabolite flux of certain mitochondrion-related pathways (Schwartzenberg-Bar-Yosephet al., 2004; Kondoh et al., 2005; Bensaad et al., 2006; Jiang et al., 2011; Rufini et al., 2013). Consistently, malate metabolism provides cues that can sustain p53 activation, mediating pivotal cell fates for the proper development of human and mouse cells (Lee et al., 2009 ; Jiang et al., 2013). Overall, there is ample evidence of the presence of a convergent network between p53 and the mitochondrial operational system in mammals, which promotes developmental progression under genotoxic conditions (**Figure 1**). Meanwhile, less progress has been realized in this context for the interactions between the genotoxic response system and mitochondrial pathways in plants. However, plant malate dehydrogenase (MDH) exhibits a more linear correlation with respiration, triggering non-cyclic flux of the tricarboxylic acid (TCA) cycle under stress (Araújo et al., 2012). Thus, a fundamental role of malate in the regulation of intracellular energy status and root cell length has been proposed; as such, plants with lower malate levels exhibited higher root elongation rates (van der Merwe et al., 2009). Al is one of the major genotoxic factors reducing cell elongation rates. In addition, STOP1 and ALMT1, which are pivotal proteins determining cell fates at RAM and inhibiting cell division under Al stress, are affected by malate transport from root cells to the rhizosphere (Balzergue et al., 2017; Kopittke et al., 2015; Mora-Macías et al., 2017). Organic acids transport regulates metabolic homeostasis and blocks specific cell cycle phases (Bauer et al., 2005). Specifically, transporter voltage-dependent anion channel 1 (VDAC1) transports malate and citrate under optimal conditions for cell proliferation (Shoshan-Barmatz et al., 2017). Moreover, hexokinase II (HKII) and CYCD1 compete for binding to a specific site on VDAC1 (**Figure 1**), and CYCD1 binding inhibited VDAC1 activity in yeast (Tchakarska et al., 2011). Similarly, increased p53 protein levels reduced VDAC1 protein levels in a dose-dependent manner, which was related to the higher mortality rate of human cells (Vaseva et al., 2012). In animal cells, mitochondrial metabolism and DNA repair mechanisms can act synergistically, favoring cell survival under adverse conditions (**Table 1**). Intriguingly, in *Neurospora crassa*, VDAC1 activity was inhibited by micromolar concentrations of Al (Dill et al., 1987). Since the VDAC1 protein is highly conserved among eukaryotes, it may be involved in mediating plant Al tolerance (**Figure 1**). With more comprehensive understanding of the link between mitochondrial metabolism and DNA repair, simultaneous genomic manipulation of genes involved in these mechanisms using TALEN or CRISPR/Cas9 system (Songstad et al., 2017; Pramanik et al., 2020) holds enormous potential to enhance crop tolerance of Al.

Functional connections between mitochondrial bioenergetics and DNA repair following the G2–M transition were recently posited in plants (Siqueira et al., 2018). In *Arabidopsis*, targeted inhibition of specific mitochondrial reactions resulted in the downregulation of cell cycle-related genes involved in G2–M transition (Jardim-Messeder et al., 2015). Additionally, deficient splicing of NAD4 intron 3 in mitochondrial electron transport chain (mETC) complex I led to cell cycle arrest at the G2 phase (Yang et al., 2014), indicating the role of mitochondrial complex I in cell cycle progression. Moreover, the RUG3 protein (RCC1–UVR8–GEF-Like 3) plays a crucial role in mitochondrial complex I functionality and mitosis in *Arabidopsis* (Kühn et al., 2011). As such, the loss-of-function of RUG3 enhanced DDR, blocking the cell cycle specifically at the G2–M phase in RAM (Su et al., 2017). Further, RUG3 physically interacts with ATM the central regulator of DDR in plants and this interaction between RUG3 and ATM regulated mitochondrial function and DDR in RAM to coordinate root elongation in *Arabidopsis* (Su et al., 2017). Likewise, antimycin A-induced inhibition of mETC complex III promotes the interaction between the SNF1-related protein kinase 1 (SnRK1) kinase complex and SOG1 (Hamasaki et al., 2019). This interaction occurring under low ATP levels triggers the expression of cell cycle-related genes CYCA3 and CYCD3;3 to arrest root cell proliferation (Hamasaki et al., 2019). Furthermore, canonical alterations in the chloroplast genome increase the levels of reactive oxygen species (ROS), which substantially affect the SOG1 pathway and, in turn, block cell cycle and mediate endoreduplication (Duan et al., 2020), a phenomenon based on which Al-sensitive plants can be distinguished from the Al-tolerant ones. Genome-wide transcriptomic analyses of leaves and seedlings exposed to gamma radiation revealed that the transcription factor SOG1 can bind specific MATE genes, in addition to the genes related to energy metabolism (Ogita et al., 2017, Bourbousse et al., 2018). Plant genomes harbor several MATE genes, and only a few of them are directly related to Al tolerance (Takanashi et al., 2014; Santos et al., 2017; Upadhyay et al., 2019). Although the potential interaction between SOG1 and MATE remains to be unequivocally demonstrated, these proteins may, in fact, interact in some plant species.

Surprisingly, quantitative phosphoproteomics of mature *Arabidopsis* rosettes aimed at identifying proteins phosphorylated by ATM/ATR kinases in response to ionizing radiation did not identify SOG1 protein as one of the targets (Roitinger et al., 2015). In particular, SOG1 is primarily expressed around meristematic regions, and in its loss-function mutants, the QC cells remain in an undifferentiated state (Yoshiyama et al., 2013, Sjögren et al., 2015), which explains the failure to identify SOG1 as one of the phosphorylation targets of ATM/ATR (Roitinger et al., 2015). In this light, the functions of SOG1 in specific cell groups must be studied. Furthermore, *ATM*, *ATR*, and *SOG1* exhibit a diel expression pattern, peaking near dusk, which demonstrates the relevance of photoperiod for inducing DNA checkpoint response (Siqueira et al., 2021). Thus, the absence of SOG1 protein may also be explained by sampling at time when the transcripts were not translated, suggesting DNA checkpoint regulators must be explored by taking photoperiodic regulation into account.

SOG1 mediates cell fates according to the degree of DNA damage, and MATE transporters play crucial roles in protecting cells around the root apex. These observations raise a question regarding the potential crosstalk among pathways involved in DNA checkpoint regulation and organic acids metabolism, which depend on the cell differentiation status. To this end, introducing genomic changes in specific root cell types may be useful to address this question, which may offer a means to improve Al tolerance of crops beyond the current limitations and further our understanding of Al toxicity. For instance, to cope with Al toxicity in specific root regions, genetic manipulation of the superior alleles of *MATE* and *ALMT* or TCA cycle genes in endodermal or epidermal cells (**Box 1**) may reduce Al uptake from soil or promote Al neutralization within root cells. Similarly, expression of the loss-of-function alleles of *SOG1*, *ATR*, and *ATM* in RAM cells would ensure the maintenance of root cell divisions under Al stress without impacting shoot growth (**Box 2**). A previous study using chromatin immunoprecipitation demonstrated the binding of RBR1 to mitochondrial metabolism-related genes, such as MDHs, nucleotide carriers, and uncoupling proteins (UCPs) (Bouyer et al., 2018). However, the precise significance of this association in response to Al remains unclear, due largely to the complications in dissecting the many possible impacts of this enigmatic metalloid.

Prolonged exposure of Al-tolerant specie *Citrus sinensis* to Al stress increased carbon flow to organic acids metabolism in both leaves and roots, whereas elevated malate and citrate levels in roots suggested an increased release of these compounds into the rhizosphere (Yang et al., 2020). In general, TCA cycle enzymes, such as MDH and citrate synthase, are differentially affected in roots and leaves under Al toxicity. Specifically, in *C. sinensis*, the leaves of Al-treated plants showed higher enzyme activities, while the roots showed lower activities (Yang et al., 2020). These differential responses culminated in differential biomass allocation to leaves, stems, and roots under Al stress (Yang et al., 2019), which was seemingly not associated with changes in net carbon assimilation but rather to the differential distribution of Al across these organs. Moreover, *C. sinensis* leaves exhibited higher levels of VDAC1 protein upon Al exposure (Li et al., 2016), which is intriguing because this protein is implicated in Al tolerance in non-plant organisms (Table 1). Furthermore, VDAC1 was regulated by proteins related to cell cycle and DNA repair control in animals and yeast (Tchakarska et al., 2011; Vaseva et al., 2012; Shoshan-Barmatz et al., 2017). Further exploration of molecular mechanisms underlying natural Al tolerance may further our understanding to improve crop Al tolerance. Collectively, these reports emphasize the potential of genetic engineering at the transcriptional level of both mitochondrial metabolism and DNA repair for improving crop Al tolerance. Genetic engineering aimed at cell type-specific modifications and comparative deep sequencing can expand the current knowledge of how diverse cell types coordinate Al tolerance at the functional level.

V. Concluding remarks and future perspectives

For over three decades, researchers have been investigating the mechanisms of Al tolerance in plants. The most characterized set of mechanisms involves the exudation of malate and citrate from the roots into the rhizosphere. Moreover, malate is known be an essential component for the perception and translocation of Al in meristematic cells, acting as a metabolic checkpoint for growth in acidic soils. Meanwhile, current evidence indicates that the functional loss of specific proteins related to DNA checkpoint control allows for the maintenance of cell division under Al toxicity. Therefore, metabolic and genetic checkpoints appear to share similar features of regulation under Al stress, allowing root elongation even under adverse conditions. Reports on heterotrophic yeast and animal cells indicate the presence of a synergistic network between malate/citrate metabolism-related enzymes and DNA repair proteins. While the advances highlighted in this regard clearly indicate that a new era of Al tolerance engineering has dawned, a number of substantial challenges remain to be addressed. Therefore, novel genetic, biochemical, and physiological approaches must be integrated to maximize our understanding of the impact of Al on root fitness, which can aid the improvement of crop Al tolerance without yield penalties.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abd El-Moneim D., Contreras, R., Silva-Navas, J., Gallego, F.J., Figueiras, A. M., Benito, C. 2015. On the consequences of aluminium stress in rye: repression of two mitochondrial malate dehydrogenase mRNAs. Plant Biol. (Stuttg) **17**:123–133.

Araújo, W.L., Nunes-Nesi, A., Nikoloski, Z., Sweetlove, L.J., Fernie, A.R. 2012. Metabolic control and regulation of the tricarboxylic acid cycle in photosynthetic and heterotrophic plant tissues. Plant Cell Environ. **35**:1–21.

Balzergue, C., Dartevelle, T., Godon, C., Laugier, E., Meisrimler, C., Teulon, J. M., et al. 2017. Low phosphate activates STOP1-ALMT1 to rapidly inhibit root cell elongation. Nat. Comm. **8**:15300.

Bauer, D.E., Hatzivassiliou, G., Zhao, F., Andreadis, C., Thompson, C.B. 2005. ATP citrate lyase is an important component of cell growth and transformation. Oncogene **24**:6314–6322.

Bensaad, K., Tsuruta, A., Selak, M.A., Vidal, M.N., Nakano, K., Bartrons, R., Gottlieb, E., Vousden, K.H. 2006. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell **126**:107-120.

Biedermann, S., Harashima, H., Chen, P., Heese, M., Bouyer, D., Sofroni, K., Schnittger, A. 2017. The retinoblastoma homolog RBR1 mediates localization of the repair protein RAD51 to DNA lesions in Arabidopsis. EMBO J. **36**:1279–1297.

Blancaflor, E.B., Jones, D.L., Gilroy, S. 1998. Alterations in the cytoskeleton accompany aluminum-induced growth inhibition and morphological changes in primary roots of maize. Plant Physiol. **118**:159-172.

Bouyer, D., Heese, M., Chen, P., Harashima, H., Roudier, F., Grüttner, C., et al. 2018. Genome-wide identification of RETINOBLASTOMA RELATED 1 binding sites in Arabidopsis reveals novel DNA damage regulators. PLoS Genet. **14**:e1007797

Bourbousse, C., Vegesna, N., Law, J.A. 2018. SOG1 activator and MYB3R repressors regulate a complex DNA damage network in Arabidopsis. Proc. Natl. Acad. Sci. USA **115**:E12453–E12462.

Brady, S.M., Orlando, D.A., Lee, J-Y., Wang, J.Y., Koch, J., Dinneny, J.R,. Mace, D., Ohler, U., Benfey, P.N. 2007. A high-resolution root spatiotemporal map reveals dominant expression patterns. Science **318**: 801– 806.

Chen, P., Sjogren, C.A., Larsen, P.B., Schnittger, A. 2019. A multi-level response to DNA damage induced by Aluminium. Plant J. **98**:479–491

Chen, P., Takatsuka, H., Takahashi, N., Kurata, R., Fukao, Y., Kobayashi, K., et al. 2017. Arabidopsis R1R2R3-Myb proteins are essential for inhibiting cell division in response to DNA damage. Nat. Commun. **8**:635

Dill, E.T., Holden, M.J., Colombini, M. 1987. Voltage gating in VDAC is markedly inhibited by micromolar quantities of aluminium. J. Membr. Biol. **99**: 187–196.

Dinneny, J.R., Long, T.A., Wang, J.Y., Jung, J.W., Mace, D., Pointer, S., Barron, C., Brady, S.M., Schiefelbein, J., Benfey, P.N. 2008. Cell identity mediates the response of Arabidopsis roots to abiotic stress. Science **320**: 942–945.

Dong, J., Piñeros, M.A., Li, X., Yang, H., Liu., Y, Murphy, A.S., Kochian, L.V., Liu, D. 2017. An Arabidopsis ABC transporter mediates phosphate deficiency-induced remodeling of root architecture by modulating iron homeostasis in roots. Mol. Plant **10**:244–259.

Duan, S., Hu, L., Dong, B., Jin, H.L., Wang, H.B. 2020. Signaling from Plastid Genome Stability Modulates Endoreplication and Cell Cycle during Plant Development. Cell Rep. **32**:108019.

Eekhout, T., Larsen, P., De Veylder, L. 2017. Modification of DNA checkpoints to confer aluminum tolerance. Trends Plant Sci. **22**:102–105

Ezaki, B., Jayaram, K., Higashi, A, Takahashi, K. 2013. A combination of five mechanisms confers a high tolerance for aluminum to a wild species of Poaceae, Andropogon virginicus L. Environ. Exp. Bot. **93**:35–44.

Kruiswijk, F., Labuschagne, C.F., Vousden, K.H. 2015. p53 in survival, death and metabolic health: A lifeguard with a licence to kill. Nat. Rev. Mol. Cell Biol. **16**:393–405

Finkemeier, I., König, A. C., Heard, W., Nunes-Nesi, A., Pham, P. A., Leister, D., et al. 2013. Transcriptomic analysis of the role of carboxylic acids in metabolite signaling in Arabidopsis leaves. Plant Physiol. **162**:239–253.

Garner, E., Raj, K. 2008. Protective mechanisms of p53-p21-pRb proteins against DNA damage-induced cell death. Cell Cycle **7**:277–282.

Guimaraes, C.T., Simoes, C.C., Pastina, M.M., Maron, L.G., Magalhães, J.V,Vasconcellos, R.C.C., Guimaraes, L.J.M., Ubiraci, G.P.L., Tinoco, C.F.S., Noda, R.W., et al. 2014. Genetic dissection of Al tolerance QTLs in the maize genome by high density SNP scan. Genomics **15**:153–167.

Gutierrez-Alanis, D., Ojeda-Rivera, J.O., Yong-Villalobos, L., Cardenas-Torres. L., Herrera-Estrella, L. 2018. Adaptation to phosphate scarcity: tips from Arabidopsis roots. Trends Plant Sci. **23**:721–730.

Hamasaki, H., Kurihara, Y., Kuromori, T., Kusano, H., Nagata, N., Yamamoto, Y.Y., Shimada, H., Matsui, M. 2019. SnRK1 kinase and the NAC transcription factor SOG1 are components of a novel signaling pathway mediating the low energy response triggered by ATP depletion. Front. Plant Sci. **10**: 503.

Hilton, B.A., Li, Z., Musich, P.R., Wang, H., Cartwright, B.M., Serrano, M., et al. 2015. ATR plays a direct antiapoptotic role at mitochondria, which is regulated by prolyl isomerase Pin1. Mol. Cell **60**:35–46.

Horvath, B.M., Kourova, H., Nagy, S., Nemeth, E., Magyar, Z., Papdi, C., Ahmad, Z., Sanchez-Perez, G.F., Perilli, S., Blilou, I., Pettkó-Szandtner, A., Darula, Z., Meszaros, T., Binarova, P., Bogre, L., Scheres, B. 2017. Arabidopsis RETINOBLASTOMA RELATED directly regulates DNA damage responses through functions beyond cell cycle control. EMBO J. **36**:1261–1278.

Hu, Z., Cools, T., De Veylder, L. 2016. Mechanisms used by plants to cope with DNA damage. Annu. Rev. Plant Biol. **67**:439–462.

Huang, C.F., Yamaji, N., Chen, Z., Ma, J.F. 2012. A tonoplast-localized half-size ABC transporter is required for internal detoxification of aluminum in rice. Plant J. **69**:857–867.

Ishikawa, S., Wagatsuma, T., Sasaki, R., Ofei-Manu, P. 2000. Comparison of the amount of citric and malic acids in Al media of seven plant species and two cultivars each in five plant species. Soil Sci. Plant Nutr. **46**:751–758.

Iuchi, S., Koyama, H., Iuchi, A., Kobayashi, Y., Kitabayashi, S., Kobayashi, Y., Ikka, T., Hirayama, T., Shinozaki, K., Kobayashi, M. 2007. Zinc finger protein STOP1 is critical for proton tolerance in Arabidopsis and co-regulates a key gene in aluminum tolerance. Proc. Natl. Acad. Sci. USA **104**:9900–9905.

Jardim-Messeder, D., Caverzan, A., Rauber, R., de Souza, F. E., Margis-Pinheiro, M., Galina, A. 2015. Succinate dehydrogenase (mitochondrial complex II) is a source of reactive oxygen species in plants and regulates development and stress responses. New Phytol. **208**:776–789.

Jaskowiak, J., Tkaczyk, O., Slota, M., Kwasniewska, J., Szarejko, I. 2018. Analysis of aluminum toxicity in *Hordeum vulgare* roots with an emphasis on DNA integrity and cell cycle. PLoS One **13**:e0193156

Jebelli, J.D., Hooper, C., Garden, G.A., Pocock, J.M., 2012. Emerging roles of p53 in glial cell function in health and disease. Glia **60**:515–525.

Jiang, P., Du, W., Mancuso, A., Wellen, K.E., Yang, X. 2013. Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence. Nature **493**:689–693.

Jiang, P., Du, W., Wang, X., Mancuso, A., Gao, X., Wu, M., Yang, X. 2011. p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase. Nat. Cell Biol. **13**:310–316.

Kochian, L.V., Pineros, M.A., Liu, J.P., Magalhaes, J.V. 2015. Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. Ann. Rev. Plant Biol. **66**:571–598.

Kochian, L.V., Hoekenga, A.O., Piñeros, M.A. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. Ann. Rev. Plant Biol. **55**:459–493.

Kondoh, H., Lleonart, M.E., Gil, J., Wang, J., Degan, P., Peters, G., Martinez, D., Carnero, A., Beach, D. 2005. Glycolytic enzymes can modulate cellular life span. Cancer Res. **65**:177–85

Kopittke, P.M., Moore, K.L., Lombi, E., Gianoncelli, A., Ferguson, B.J., Blamey, F.P., Menzies, N.W., Nicholson, T.M., McKenna, B.A., Wang, P et al. 2015. Identification of the primary lesion of toxic aluminum in plant roots. Plant Physiol. **167**:1402-1411

Kühn, K., Carrie, C., Giraud, E., Wang, Y., Meyer, E. H., Narsai, R., et al. 2011. The RCC1 family protein RUG3 is required for splicing of nad2 and complex I biogenesis in mitochondria of *Arabidopsis thaliana*. Plant J. **67**:1067–1080.

Larsen, P.B., Cancel, J., Rounds, M., Ochoa, V. 2007. *Arabidopsis ALS1* encodes a root tip and stele localized half type ABC transporter required for root growth in an aluminum toxic environment. Planta **225**:1447–1458.

Larsen, P.B., Geisler, M.J.B., Jones, C.A., Williams, K.M., Cancel, J.D. 2005. *ALS3* encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in *Arabidopsis.* Plant J. **41**:353–363.

Lee, S.M., Kim, J.H., Cho, E.J., Youn, H.D. 2009. A nucleocytoplasmic malate dehydrogenase regulates p53 transcriptional activity in response to metabolic stress. Cell Death Differ. **16**:738–748.

Lemire, J., Mailloux, R., Auger, C., Whalen, D., Appanna V.D. 2010. Pseudomonas fluorescens orchestrates a fine metabolic-balancing act to counter aluminium toxicity. Environ. Microbiol. **12**:1384-1390.

Li, H., Yang, L.T., Qi, Y.P., Gu, P., Lu, Y. B., Chen, L.S. 2016. Aluminum-toxicity-induced alterations of leaf proteome in two citrus species differing in aluminum-tolerance. **Int. J. Mol. Sci. 17**:1180

Ligaba-Osena, A., Fei, Z., Liu, J., Xu, Y., Shaff, J., Lee, S.C., Luan, S., Kudla, J., Kochian, L., and Piñeros, M. 2017. Loss-of-function mutation of the calcium sensor CBL1 increases aluminum sensitivity in Arabidopsis. New Phytol. **214**:830-841.

Liu J., Magalhaes J.V., Shaff J., Kochian L.V. 2009. Aluminum-activated citrate and malate transporters from the MATE and ALMT families function independently to confer Arabidopsis aluminum tolerance. Plant J. **57**:389–399.

Lü, J., Gao, X., Dong, Z., Yi, J., An, L. 2012. Improved phosphorus acquisition by tobacco through transgenic expression of mitochondrial malate dehydrogenase from *Penicillium oxalicum*. Plant Cell Rep. **31**:49–56.

Ma, J.F., Hiradate, S., Nomoto, K., Iwashita, T., Matsumoto, H. 1997. Internal detoxification mechanism of Al in hydrangea. Identification of Al form in the leaves. Plant Physiol. **113**:1033–1039.

Ma, J.F., Hiradate, S., Matsumoto, H. 1998. High aluminum resistance in buckwheat. II. Oxalic acid detoxifies aluminum internally. Plant Physiol. **117**:753–755.

Magalhaes, J.V., Liu, J., Guimarães, C.T., Lana, U.G.P., Alves, V.M.C., Wang, Y. H., et al. 2007. A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. Nat. Genet. **39**:1156–1161.

Maron, L.G., Guimarães, C.T., Kirst, M., Albert, P.S., Birchler, J.A., Bradbury, P., Buckler, E.S., Coluccio, A.E., Danilova, T.V., Kudrna, D., Magalhaes, J.V., Piñeros, M.A., Schatz, M.C., Wing, R., Kochian, L.V. 2013. Aluminum tolerance is associated with higher MATE1 gene copy-number in maize. Proc. Natl. Acad. Sci. USA **110**: 5241–5246.

Matonyei, T.K., Barros, B.A., Guimaraes, R.G.N., Ouma, E.O., Cheprot, R.K., Apolinário, L.C., Ligeyo, D.O., Costa, M.B.R., Were, B. A., Kisinyo, P.O., Onkware, A.O., Noda, R.W., Gudu, S.O., Magalhaes, J.V., Guimaraes, C.T. 2020. Aluminum tolerance mechanisms in Kenyan maize germplasm are independent from the citrate transporter ZmMATE1. Sci. Rep.**10**:7320.

Mathupala, S.P, Heese C., Pedersen, P.L. 1997. Glucose catabolism in cancer cells. The type II hexokinase promoter contains functionally active response elements for the tumor suppressor p53. J. Biol. Chem. **272**:22776–22780.

Melo, J.O., Lana, U.G., Piñeros, M.A., Alves V.M.C., Guimarães C.T., Liu J., Zheng Y., Zhong S., Fei Z., Maron L.G., Schaffert R.E., Kochian L.V., Magalhaes JV. 2013. Incomplete transfer of accessory loci influencing SbMATE expression underlies genetic background effects for aluminum tolerance in sorghum. Plant J. **73**:276–288

Melo, J.O., Martins, L.G., Barros, B.A., Pimenta, M.R., Lana, U.G., Duarte, C.E., Pastina, M.M., Guimaraes, C.T., Schaffert, R.E., Kochian, L.V., et al. 2019. Repeat variants for the SbMATE transporter protect sorghum roots from aluminum toxicity by transcriptional interplay in cis and trans. Proc. Natl. Acad. Sci. USA 2019, **116**:313–318.

Mora-Macias, J., Ojeda-Rivera, J. O., Gutierrez-Alanis, D., Yong-Villalobos, L., Oropeza-Aburto, A., Raya-Gonzalez, J., et al. 2017. Malate-dependent Fe accumulation is a critical checkpoint in the root developmental response to low phosphate. Proc. Natl. Acad. Sci. USA **114**:E3563–E3572.

Negishi, T., Oshima, K., Hattori, M., Kanai, M., Mano, S., Nishimura, M., Yoshida, K. 2012. Tonoplast- and plasma membrane-localized aquaporin-family transporters in blue hydrangea sepals of aluminum hyperaccumulating plant. PloS One **7**:e43189

Nezames, C.D., Sjogren, C.A., Barajas, J.F., Larsen, P.B. 2012. The Arabidopsis cell cycle checkpoint regulators TANMEI/ALT2 and ATR mediate the active process of aluminium-dependent root growth inhibition. Plant Cell **243**:608–62.

Nunes-Nesi, A., Brito, D. S., Inostroza-Blancheteau, C., Fernie, A. R., Araújo, W.L. 2014. The complex role of mitocondrial metabolism in plant aluminum resistance. Trends Plant Sci. **19**:399–407

Ogita, N., Okushima, Y., Tokizawa, M., Yamamoto, Y. Y., Tanaka, M., Seki, M., et al. 2018. Identifying the target genes of SUPPRESSOR OF GAMMA RESPONSE 1, a master transcription factor controlling DNA damage response in Arabidopsis. Plant J. **94**:439–453.

Piñeros, M.A., Shaff, J.E., Manslank, H.S., Alves, V.M.C., Kochian, L.V. 2005. Aluminum resistance in maize cannot be solely explained by root organic acid exudation. A comparative physiological study. Plant Physiol. **137**:231–241.

Pramanik, D., Shelake R.M., Kim M.J., Kim J-Y. 2020. CRISPR-mediated engineering across the central dogma in plant biology for basic research and crop improvement. Mol. Plant ***in press***.

Qin, L., Fan, M., Candas, D., Jiang, G., Papadopoulos, S., Tian, L., et al. 2015. CDK1 Enhances Mitochondrial Bioenergetics for Radiation-Induced DNA Repair. Cell Rep. **13**:2056–2063

Ramesh, S., Kamran, M., Sullivan, W., Chirkova, L., Okamoto, M., Degryse, F., McLaughlin, M., Gilliham, M., Tyerman, S.D. 2018. Split personality of aluminum activated malate transporter family proteins: facilitation of both GABA and malate transport Plant Cell **30**:1147–1164.

Ramesh, S.A., Tyerman, S.D., Xu, B., Bose, J., Kaur, S., Conn, V., Domingos, P., Ullah, S., Wege, S, Shabala, S. et al. 2015. GABA signalling modulates plant growth by directly regulating the activity of plant-specific anion transporters. Nature Communications 6:7879.

Roitinger, E., Hofer, M., Köcher, T., Pichler, P., Novatchkova, M., Yang, J., Schlögelhofer, P., Mechtler, K. 2015. Quantitative phosphoproteomics of the ataxia telangiectasia-mutated (ATM) and ataxia telangiectasiamutated and rad3-related (ATR) dependent DNA damage response in *Arabidopsis thaliana*. Mol. Cell Proteomics **14**:556–571.

Rounds, M.A., and Larsen, P.B. 2008. Aluminum-dependent root-growth inhibition in Arabidopsis results from AtATR-regulated cell-cycle arrest. Curr. Biol. **18**:1495-1500.

Rufini, P., Tucci, I., Celardo, and Melino, G. 2013. Senescence and aging: the critical roles of p53, Oncogene **32**: 5129–5143.

Sahin, E., Colla, S., Liesa, M., Moslehi, J., Müller, F.L., Guo, M., Cooper, M., Kotton, D., Fabian, A.J., Walkey, C., Maser, R.S., Tonon, G., Foerster, F., Xiong, R., Wang, Y.A., Shukla, S.A., Jaskelioff, M., Martin, E.S., Heffernan, T.P., Protopopov, A., Ivanova, E., Mahoney, J.E., Kost-Alimova, M., Perry, S.R., Bronson, R., Liao, R., Mulligan, R., Shirihai, O.S., Chin, L., DePinho, R.A. 2011.

Telomere dysfunction induces metabolic and mitochondrial compromise. Nature **470**:359–365.

Santos, A.L., Chaves-Silva, S., Yang, L., Maia, L.G.S., Chalfun-Junior, A., Sinharoy, S., Zhao, L., Benedito, V.A. 2017. Global analysis of the MATE gene family of metabolite transporters in tomato. BMC Plant Biol. **17**:185.

Sawaki, Y., Iuchi, S., Kobayashi, Y., Kobayashi, Y., Ikka, T., et al. 2009. STOP1 regulates multiple genes that protect Arabidopsis from proton and aluminum toxicities. Plant Physiol. **150**:281–294.

Schwartzenberg-Bar-Yoseph, F., Armoni, M., Karnieli, E. 2004. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. Cancer Res **64**:2627–2633

Shoshan-Barmatz, V., Maldonado, E.N., and Krelin, Y. 2017. VDAC1 at the crossroads of cell metabolism, apoptosis and cell stress. Cell Stress **1**:11–36.

Siqueira, J.A., Hardoim, P., Ferreira, P.C.G., Nunes-Nesi, A., Hemerly, A.S. 2018. Unraveling interfaces between energy metabolism and cell cycle in plants. Trends Plant Sci. **23**:731–747.

Siqueira, J.A., Otoni, W.C., Araújo, W.L. 2022. The hidden half comes into the spotlight: Peeking inside the black box of root developmental phases. **Plant Comm. 3**: 100246.

Siqueira, J.A., Wakin, T., Batista-Silva, W., Silva, J.C.F., Vicente, M.H., Silva, J.C., Clarindo, W.R. , Zsögön, A., Peres, L.E.P., De Veylder, L., Fernie, A.R., Nunes-Nesi, A., Araújo, W.L. 2021. Photoperiod shapes aluminium tolerance in plants. bioRxiv preprint https://doi.org/10.1101/2021.02.12.430934

Sivaguru, M., Baluska, F., Volkmann, D., Felle, H.H., Horst W.J. 1999. Impacts of aluminum on the cytoskeleton of the maize root apex. Short-term effects on the distal part of the transition zone. Plant Physiol. **119**:1073-1082.

Sjogren, C.A., Bolaris, S.C., Larson, P.B. 2015. Aluminum-dependent terminal differentiation of the Arabidopsis root tip is medicated through an ATR-, ALT2-, and SOG1-regulated transcriptional response. Plant Cell **27**: 2510–2515.

Sjogren, C.A., Larsen, P.B. 2017. SUV2, which encodes an ATR-related cell cycle checkpoint and putative plant ATRIP, is required for aluminium-dependent root growth inhibition in Arabidopsis. Plant Cell Environ. **40**:1849–1860.

Songstad, D.D., Petolino, J.F., Voytas, D.F., Reichert, N.A. 2017. Genome editing of plants. Crit. Rev. Plant Sci. **36**:1–23.

Su, C., Zhao, H., Zhao, Y., Ji, H., Wang, Y., Zhi, L, Li, X. 2017. RUG3 and ATM synergistically regulate the alternative splicing of mitochondrial nad2 and the DNA damage response in *Arabidopsis thaliana*. Sci. Rep. **7**:43897

Sweetlove, L.J., Beard, K.F.M., Nunes-Nesi, A., Fernie, A.R., Ratcliffe, R.G. 2010. Not just a circle: flux modes in the plant TCA cycle. Trends in Plant Sci. **15**:462– 470.

Takanashi, K., Shitan, N., Yazaki K. 2014. The Multidrug and Toxic Compound Extrusion (MATE) family in plants. Plant Biotechnol. **31**:417–430.

Tateda, C., Watanabe, K., Kusano, T., Takahashi, Y. 2011. Molecular and genetic characterization of the gene family encoding the voltage-dependent anion channel in Arabidopsis. J. Exp. Bot. **62**:4773– 4785.

Tchakarska, G., Roussel, M., Troussard, X., Sola, B. 2011. Cyclin D1 inhibits mitochondrial activity in B cells. Cancer Res. **71**:1690-1699.

Upadhyay, N., Kar, D., Datta, S. 2019. A multidrug and toxic compound extrusion (MATE) transporter modulates auxin levels in root to regulate root development and promotes aluminium tolerance. Plant Cell Environ. **43**: 745-759.

Upadhyay, N., Kar, D., Deepak Mahajan, B., Nanda, S., Rahiman, R., Panchakshari, N., Bhagavatula, L., Datta, S. 2019. The multitasking abilities of MATE transporters in plants. J. Exp. Bot. **70**: 4643–4656.

van der Merwe, M.J., Osorio, S., Moritz, T., Nunes-Nesi, A., Fernie, A.R. 2009. Decreased Mitochondrial Activities of Malate Dehydrogenase and Fumarase in Tomato Lead to Altered Root Growth and Architecture via Diverse Mechanisms. Plant Physiol.**149**:653-669.

Valadez, N., Colli, J., Brito, L., Muñoz, J., Zuñiga, J., Castaño, E., Hernadez, T. 2007. Differential Effect of Aluminum on DNA Synthesis and CDKA Activity in Two *Coffea arabica* Cell Lines. Journal of Plant Growth Regulation **26**:69-77.

Valentin-Vega, Y.A., MacLean, K.H., Tait-Mulder, J., Milasta, S., Steeves, M., Dorsey, F.C., et al. 2012. Mitochondrial dysfunction in ataxia telangiectasia. Blood **119**:1490–500.

Vaseva, A.V., Marchenko, N.D., Ji, K., Tsirka, S.E., Holzmann, S., Moll, U.M. 2012. p53 opens the mitochondrial permeability transition pore to trigger necrosis. Cell **149**:1536 – 1548.

Wang, C., Li, Z., Lu, Y., et al. 2006. Cyclin D1 repression of nuclear respiratory factor 1 integrates nuclear DNA synthesis and mitochondrial function. Proc Natl Acad Sci USA **103**:11567–11572.

Wang, Z., Fan, M., Candas, D., Zhang, T.Q., Qin, L., Eldridge, A., WachsmaHogiu , S., Ahmed, K.M., Chromy, B.A., Nantajit, D., Duru, N., He, F., Chen, M., Finkel, T., Weinstein, L.S., and Li, J.J. 2014. Cyclin B1/Cdk1 coordinates mitochondrial respiration for cell-cycle G2/M progression. Dev. Cell **29**:217–232.

Wang, Y., Li, R., Li, D., Jia, X., Zhou, D., Li, J., et al. 2017. NIP1;2 is a plasma membrane-localized transporter mediating aluminum uptake, translocation, and tolerance in Arabidopsis. Proc. Natl. Acad. Sci. USA **114**:5047–5052.

Wang, Z.A., Li, Q., Ge, X.Y., Yang, C.L., Luo, X.L., Zhang, A.H., Xiao, J.L., Tian, Y.C., Xia, G.X., Chen, X.Y., Li, F.G., Wu, J.H. 2015. The mitochondrial malate dehydrogenase 1 gene GhmMDH1 is involved in plant and root growth under phosphorus deficiency conditions in cotton. Sci. Rep. **5**: 10343.

Weimer, A.K., Biedermann, S., Harashima, H., Roodbarkelari, F., Takahashi, N., Foreman, J., et al. 2016. The plant-specific CDKB1-CYCB1 complex mediates homologous recombination repair in Arabidopsis. EMBO J. **35**:2068–2086.

Xia, J., Yamaji, N., Kasai, T., and Ma, J.F. 2010. Plasma membrane-localized transporter for aluminum in rice. Proc. Natl. Acad. Sci. USA **107**:18381–18385.

Yamaji, N., Huang, C. F., Nagao, S., Yano, M., Sato, Y., Nagamura, Y., et al. 2009. A zinc finger transcription factor ART1 regulates multiple genes implicated in aluminum tolerance in rice. Plant Cell **21**:3339–3349.

Yang, T., Cai, L., Qi, Y., Yang, L., Lai, N., Chen, L. 2019. Increasing nutrient solution pH alleviated aluminum-induced inhibition of growth and impairment of photosynthetic electron transport chain in Citrus sinensis seedlings. BioMed Res. Int. **2019**: 9058715.

Yang, T.Y., Qi, Y.P., Huang, H.Y., Wu, F.L., Huang, W.T., Deng, C.L., Yang, L.T., Chen, L.S. 2020. Interactive effects of pH and aluminum on the secretion of organic acid anions by roots and related metabolic factors in *Citrus sinensis* roots and leaves. Environ Pollut. **262**:114303.

Yang, L., Zhang, J., He, J., Qin, Y., Hua, D., Duan, Y., et al. 2014. ABA-mediated ROS in mitochondria regulate root meristem activity by controlling PLETHORA expression in Arabidopsis. PLoS Genet. **10**:e1004791.

Yoshiyama, K.O., Kobayashi, J., Ogita, N., Ueda, M., Kimura, S., Maki, H., and Umeda, M. 2013. ATM-mediated phosphorylation of SOG1 is essential for the DNA damage response in Arabidopsis. EMBO Rep. **14**:817–822.

Zhang, Y., Fernie, A.R. 2018. On the role of the tricarboxylic acid cycle in plant productivity. J. Integr. Plant Biol. **60**:1199–1216.

Zhang, Y., Guo, J., Chen, M., Li, L., Wang, L., and Huang, C.-F. 2018. The cell cycle checkpoint regulator ATR is required for internal aluminum toxicity-mediated root growth inhibition in Arabidopsis. Front. Plant Sci. **9**:118.

Zheng, S.J., Yang, J.L., He, Y.F., Yu, X.H., Zhang, L., You, J.F., Shen, R.F., Matsumoto, H. 2005. Immobilization of aluminum with phosphorus in roots is associated with high aluminum resistance in buckwheat. Plant Physiol. **138**:297–303.

Zhou, Y., Yang, Z., Xu, Y., Sun, H., Sun, Z., Lin, B., et al. 2018. Soybean nadp-malic enzyme functions in malate and citrate metabolism and contributes to their efflux under Al stress. Front. Plant Sci. **8**:2246.

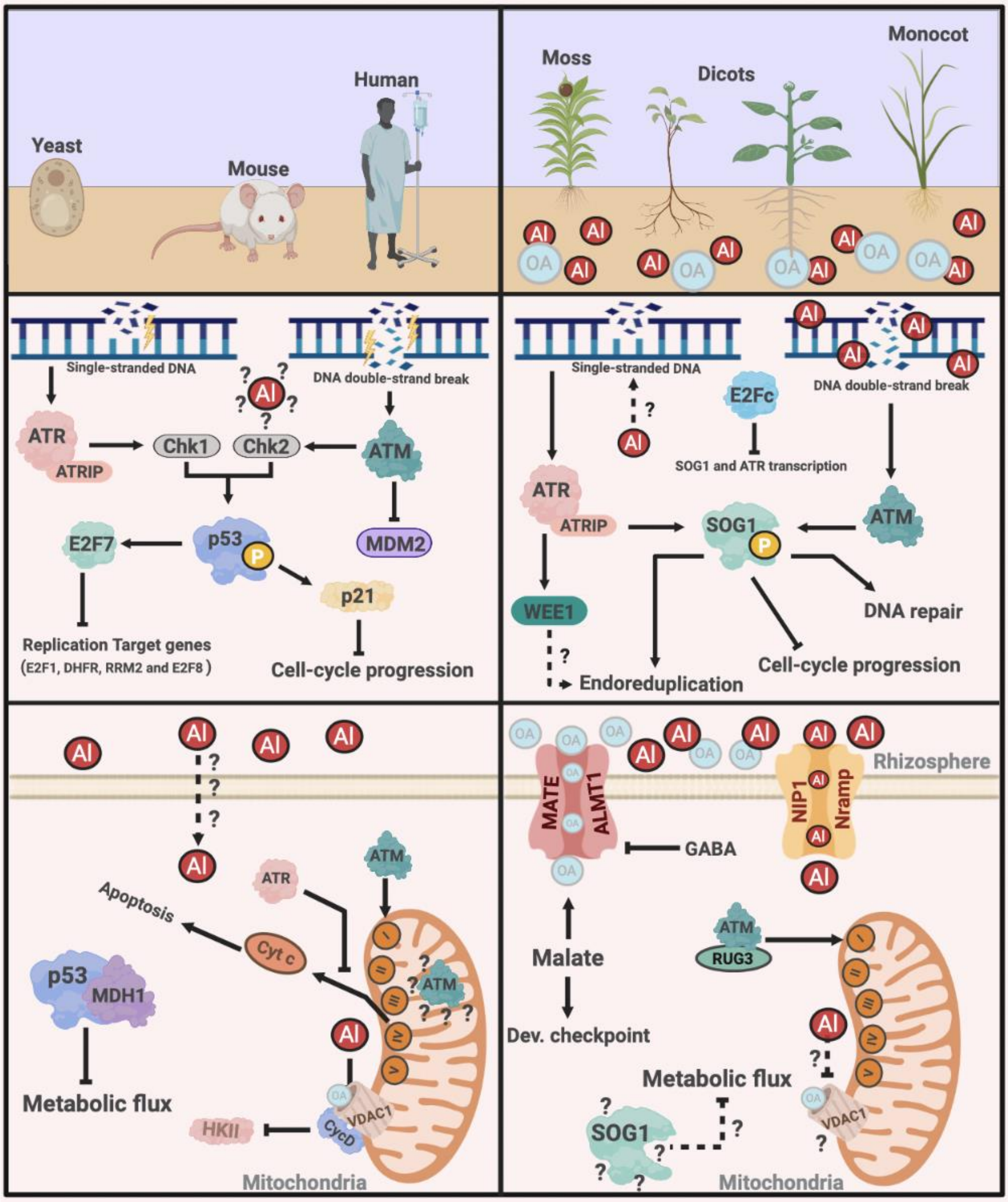
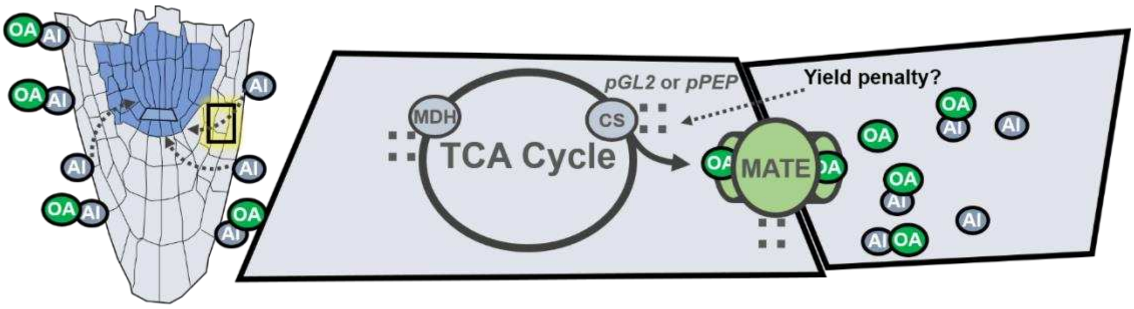


Figure 1. *Art state* of the mechanism of genotoxicity tolerance. Non-plant and plant systems display similar components for responding to genotoxic agents. While the non-plant systems (yeast, mice, and humans) exhibit broader components involved in DNA damage response (DDR), plant systems (mosses, dicots, and monocots) exhibit specific stress responses, specifically to Al toxicity, which damages roots. DNA single- and double-strand breaks are introduced in both systems, although Al is a well-established factor inducing DNA damage in plants (Chen et al., 2019). Specifically, Al is known to induce DNA double-strand breaks, but its relevance in inducing single-

strand breaks remains unclear. In non-plant models, following the induction of single-strand breaks, the ATR–ATRIP complex is induced, which activates ATR kinase to phosphorylate checkpoint kinase 1 (Chk1). Meanwhile, DNA double-strand breaks induce ATM protein, which activates Chk2 and inactivates MDM2—a negative regulator of the p53 transcription factor. Subsequently, Chk1 and Chk2 phosphorylate and stabilize p53, this enhancing p21 transcript levels and inhibiting cell cycle progression. Furthermore, p53 up-regulates the transcription factor E2F7, which promotes the repression of replication target genes (Carvajal et al., 2012). In contrast, in plant models, DNA single- and double-strand breaks activate ATR and ATM, respectively. Both proteins transcriptionally induce WEE1, which induces DNA replication in the absence of mitosis and endoreduplication. However, the relevance of WEE1 in triggering Al stress response is not well understood. The active form of this proteins promotes the activity of the transcription factor SOG1, which in turn induces genes related to DNA repair and endoreduplication but suppresses genes related to cell cycle progression. Consistently, under optimal conditions for cell proliferation, the transcription factor E2Fc suppresses *SOG1* and *ATR* genes, ensuring root growth. Under genotoxic conditions, however, the metabolic flux is rewired by specific DNA checkpoint regulators. In non-plant organisms, the ATM protein is essential for optimal mitochondrial electron transport chain (mETC) complex I function and ATP synthesis. Furthermore, the ATM protein is localized to the mitochondrial matrix, although its precise role is not completely understood. In cytosol, MDH1 binds p53 to block the metabolic flux of the major energy generation pathways associated with mitochondrial respiration. Specifically, HKII and CYCD1 compete for binding at specific sites of the mitochondrial organic acid transporter VDAC1. Under genotoxic stress, CYCD1 binds this transporter to block organic acid (malate and citrate) efflux from the mitochondria to cytosol, and Al rapidly suppresses VDAC1 activity. Subsequently, ATR blocks cytochrome c release from the mitochondria to cytosol, thus arresting apoptotic induction. In plant models, organic acids (OAs) exuded into the rhizosphere promote Al^{+3} neutralization in soil. These OAs are secreted by plasma membrane-localized transporters, such as multidrug and toxic compound extrusion (MATE) and aluminum-activated malate transporter 1 (ALMT1). The non-protein amino acid GABA may suppress ALMT1 activity under Al stress depending on energy availability. Under low soil pH, malate exudation is a root developmental checkpoint that promotes the maintenance of cell divisions. Conversely, internal Al detoxification mechanism triggers Al^{+3} influx mediated by nodulin 26-like intrinsic protein 1 (NIP1) and natural resistance-associated macrophage protein (Nramp) transporters and, posteriorly, Al is complexed with OAs in the cytosol. Contrary to its functional analog p53, the association of SOG1 with the rewiring of metabolic flux under genotoxic conditions remains unclear. Similarly, although Al-tolerant plants exhibited elevated VDAC1 protein levels under Al stress, the role of this transporter in mediating tolerance to this metal is not understood. Meanwhile, ATM interacts with RUG3 to promote mitochondrial complex I functionality and root meristem cell proliferation, demonstrating the possible dual role this protein in inducing DDR and mitochondrial metabolic response. Solid arrows indicate processes already demonstrated, and dotted arrows represent processes to be demonstrated.

Box 1. Mitochondrial metabolism manipulation and Al tolerance

Since the discovery of mechanisms mediating Al neutralization around the rhizosphere, growing evidence has reinforced this finding. Thus, root organic acid (*i.e.*, citrate, malate, and oxalate) exudation is associated with Al exclusion, and these exudates likely chelate Al in the soil (Kochian et al., 2015). Despite successful identification of superior alleles regulating Al tolerance based on early root elongation (Magalhaes et al., 2007; Maron et al., 2013; Guimaraes et al., 2014), the sensitive genotypes of different species release higher levels of organic acids (Ishikawa et al.; 2000; Piñeros et al., 2005; Zheng et al., 2005). In this context, genetic manipulation of enzymes associated with organic acid metabolism elevated the endogenous levels of malate and citrate in the roots and promoted the exudation of these organic acids from the roots to the rhizosphere (Lü et al., 2012; Wang et al., 2015; Yang et al., 2020). Indeed, in species with greater Al tolerance or Al accumulation, the endogenous levels of oxalic acid and citrate are extremely important for overall cell detoxification (Ma et al., 1997; Ma et al., 1998). Organic acids affect a wide range of physiological processes associated with productivity, including photosynthesis, respiration, amino acid biosynthesis, and root development (for more details, see Zhang and Fernie, 2018). In fact, diverse genotypes can present contrasting responses to genetic manipulations aimed at improving Al tolerance based on alterations of organic acid levels. For instance, species with C4 photosynthesis (*e.g.*, maize and sorghum) exhibited an elevated malate pump to support photosynthetic demands, even though malate showed differential importance, compared with other species exhibiting C3 photosynthesis. Therefore, genetic engineering of Al tolerance must take into account the relevance of organic acids to the target species, which would help improve Al tolerance and reduce yield penalties. Nonetheless, the extent of differential Al tolerance that can be explained by shifts in metabolism or exudation of organic acids remains unclear, since Al-sensitive genotypes also release large amounts of organic acids into the rhizosphere (Ishikawa et al., 2000; Piñeros et al., 2005; Zheng et al., 2005). The operation of non-cyclic fluxes of the TCA cycle according to environmental changes (Sweetlove et al., 2010) has been suggested to mediate Al tolerance in vascular plants (Nunes-Nesi et al., 2014) and microorganisms (Lemire et al., 2010). A previous metabolic flux control analysis indicated that mitochondrial MDH plays a major role in the regulation of the TCA cycle, and its non-cyclic fluxes likely operate under stress conditions (Araújo et al., 2012). For instance, tomato plants with genetic manipulations of TCA cycle enzymes showed reduced malate levels and altered energy status in the roots, which were associated with higher rates of cell and root elongation (van der Merwe et al., 2009). Higher Al^{3+} levels activate a specific STOP1–ALMT1 module that inhibits cell elongation, while malate efflux contributes to ion transport in the apoplastic space (Gutiérrez-Alanís et al., 2018). Reduced rates of cell elongation and proliferation in root tips trigger meristem exhaustion, characterized by the loss of proliferative capacity of the root meristem (Gutiérrez-Alanís et al., 2018). Consistently, nutrient limitation, similar to that observed under Al stress, arrests mitosis, leading to the premature differentiation of meristematic cells. This is related to the increased exudation of intracellular malate into the apoplastic space, which triggers cation accumulation in the meristem and arrested root elongation (Mora-Macías et al., 2017). Thus, endogenous malate homeostasis around QC appears to be essential for root growth under Al exposure. Perhaps to the loss of this homeostatic control did several studies fail to achieve Al tolerance by exclusively manipulating TCA cycle-related genes. This shortcoming can be overcome through the simultaneous manipulation of TCA cycle-related enzymes and organic acid transporters (*e.g.*, ALMT1 and MATE) under the control of promoters of genes expressed in root endoderm or epidermis. Of note, however, these approaches would allow for organic acid production and/or exudation in regions around the meristem, but not in the root section. Abbreviations: ALMT1: aluminum-activated malate transporter 1; CS: citrate synthase; MDH: malate dehydrogenase; MATE: multidrug and toxic compound extrusion; *pGL2*: GLABRA2 promoter; *pPEP*: root cortex elongation/maturation cell promoter.



Box 2. Association between DNA repair and cell cycle progression in the root quiescence center under Al stress.

Several factors, such as UV-B radiation and Al and B exposure, lead to DNA damage in plants. The roots of tropical and subtropical plants grow in Al-saturated soils, which may cause DNA damage, termed DNA single-stranded DNA (ssDNA) or double-strand DNA breaks (DSBs). To date, however, how Al is translocated to the meristematic cells, where it produces a major impact on DNA and cell division remains unclear. In the root apical meristem, the quiescent center (QC) ensures DNA integrity by conserving genomic identity and avoiding the transmission of damaged DNA to meristem-derived cells. Al exposure forces QC cell differentiation, inhibiting cell division in the meristem. Under such conditions, retinoblastoma-related 1 (RBR1) moves to regions rich in DNA damage to mediate the assembly of DNA-bound repair complexes (DRCs) in an ATM- and ATR-dependent manner. Al-induced DNA damage activates ATM and ATR kinases to correct the loci with ssDNA and DSBs, respectively. These kinases mediate the activation of the transcription factor SOG1, which induces DNA repair and cell cycle stoppage genes. Together, ATM, ATR, and SOG1 constitute an Al response pathway related to DNA damage triggered by this element. The loss-of-function of proteins involved in this Al response pathway prevents the accumulation of cyclin (CYC) B1 in the meristem and inhibits cell cycle progression and higher root elongation. Further, RBR1 prevents cell death and DNA damage priming, and meristematic cells may acquire the memory for Al-related damage.

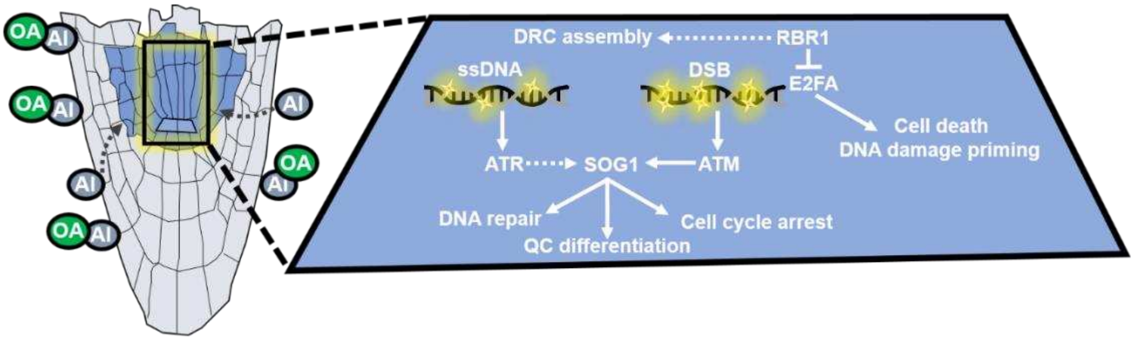


Table 1. Summary of studies covering the relationships between mitochondrial metabolism and DNA repair in non-plant and plant models

Protein	**Mechanisms**		**Ref**
	Non-Plant	Plant	
P53/SOG1*	Regulates genes and proteins related to DDR and mitochondrial metabolism. Genotoxic conditions promote p53 activation, which arrest cell cycle and TCA cycle.	SOG1 is activated upon ssDNA and DSB occurrence, regulating the transcription of hundreds genes that are associated to cell cycle block, DNA repair, endocycle and cell death. Al-induced DNA damage activates SOG1 impairing the root elongation and Al tolerance.	Kruiswijk et al., 2015; Sjogren et al., 2015
ATM*	ATM is activated under DSB, which ensures the inactivation of CDC25, arresting cell cycle at G1/S and G2/M checkpoints, as well as enabling p53 protein higher levels. Furthermore, ATM loss reduces mETC complex I levels, and a fraction of ATM protein is localized in mitochondria.	Similar to non-plant models, ATM upregulates SOG1 after DSB and induce WEE1, arresting cell cycle at intra-S checkpoint. Moreover, ATM might coordinate mETC complex I activity.	Valentin-Vega et al., 2012; Hu et al., 2016; Su et al., 2017
ATR*	ATR is essential for responses under conditions of blocked on DNA replication fork. ATR activity might occur independent of DNA checkpoint or kinase roles, where the protein localize on mitochondria deterring cytochrome c release and apoptosis.	Modulates the QC differentiation in Al-dependent DNA damage conditions. The ATR mutation restored Al tolerance in several Al-sensitive mutants, which did not occurred in the ALMT1 mutant that is deficient in malate transport from the root tip for rhizosphere.	Hilton et al., 2015; Zhang et al., 2018
ALT2*	WD-40 proteins are widely recognized in non-plant systems playing pivotal roles in DNA repair, while protein sharing domains and function with ALT2 still not found in organisms different than plants.	Factor responsible to stop root growth following Al-related DNA damages, coordinating the cyclinB1 in the root tip. Both, ALT2 and ATR force QC differentiation under Al stress.	Nezames et al., 2012
SUV2*	Despite ATR Interacting Protein (ATRIP) would be describe in non-plant systems recruiting ATR protein in sites with persistent single stranded DNA, protein with similar domains to SUV2 are not reported in these organisms.	An activator of ATR protein, that regulate the cell cycle checkpoint under Al stressful conditions, and might promote the QC differentiation and transition of mitotic phase from endocycle phase.	Sjogren and Larsen, 2017
pRb or RBR1*	It contributes for p53 protein stabilization in response to DNA damage, in which RBR1 activation prevents cell death.	It is recruited to sites with DNA damage through ATM- and ATR-dependent manner demonstrating an essential role in the assembly of DNA-bound repair complexes.	Garner and Raj, 2008; Jebelli et al., 2011; Bienderman et al., 2017; Howart et al., 2017
CDK	In DNA damage conditions, CDK1 is reallocated into mitochondrial matrix, where phosphorylates the mETC complex I core of proteins, which in turn improve energy generation and DNA repair rates ensuring cell divisions.	The plant-specific CDKB1 associates to CyclinB1, phosphorylating the DNA binding protein RAD51 (RADIATION SENSITIVE 1), which triggers the DNA repair and cell cycle arrestment. In Al-sensitive *Coffea arabica* cell lines, Al inhibited CDKA activity, while Al-tolerant cells improved DNA biosynthesis and CDKA activity.	Qin et al., 2015; Weimer et al., 2016; Valadez-González et al., 2007

Cyclin D	The Cyclin D1 abundance coordinates DNA biosynthesis and mitochondrial respiration through pRb inactivation.	Not reported playing similar function in plants.	Wang et al., 2006;
VDAC1*	Regulates the mitochondrial efflux of TCA cycle intermediaries. Adverse conditions (e.g. Al exposure) interrupts VDAC1 activity since Cyclin D and p53 might bind on VDAC1 specific sites. Therefore, the interruption of VDAC1 activity causes cell cycle arrestment and cell death.	Essential for growth regulation as well as in responses to biotic and abiotic stresses through regulation of hydrogen peroxide generation. Al tolerant plants might exhibit higher levels of this protein under Al stressful conditions.	Shoshan-Barmatz et al., 2017; Tchakarska et al., 2011; Vaseva et al., 2012; Dill et al., 1987; Tateda et al., 2011; Li et al., 2016
HKII	Expressed under p53 control, enhancing the glycolysis in cancer cells.	Not reported playing similar function in plants.	Mathupala et al., 1997
MDH*	Under adverse growth conditions, MDH1 activates p53, which block cell cycle progression reducing cell proliferation.	The reduction in mitochondrial MDH transcripts impair specifically root cell elongation arresting the growth. Indeed, MDH1 and MDH2 mRNA levels decrease in response to Al stress.	Lee et al., 2009; El-Moneim et al., 2015; van der Merwe et al., 2009
CS*	An impaired activity deregulates TCA cycle parallel to a blockage of G1 to S transition, reducing cell proliferation.	Citrate levels play the regulation of key transcripts involved in DNA synthesis. CS activity is related to citrate exudation of root tip from rhizosphere promoting the root elongation in several limiting environmental conditions.	Bauer et al 2005; Finkemeier et al 2013.

Chapter 5

A long and stressful day: photoperiod shapes aluminum tolerance in plants

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RESEARCH ARTICLE

A long and stressful day: photoperiod shapes aluminum tolerance in plants

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Brief heading: Aluminum is a major constraint for crop yield worldwide. We reveal that photoperiod acts as a barrier for Al tolerance in plants. This could ultimately contribute to improve crop growth and productivity, particularly in many developing countries where the majority of acid soils reside and Al toxicity limits crop production.

Summary

Aluminium (Al), a limiting factor for crop productivity in acidic soils (pH ≤ 5.5), imposes drastic constraints for food safety in developing countries. The major mechanisms that allow plants to cope with Al involve manipulations of organic acids metabolism and DNA-checkpoints. When assumed individually both approaches have been insufficient to overcome Al toxicity. On analysing the centre of origin of most cultivated plants, we hypothesised that day-length seems to be a pivotal agent modulating Al tolerance across distinct plant species. We observed that with increasing distance from the Equator, Al tolerance decreases, suggesting a relationship with the photoperiod. We verified that long-day (LD) species are generally more Al-sensitive than short-day (SD) species, whereas genetic conversion of tomato for SD growth habit boosts Al tolerance. Reduced Al tolerance correlates with DNA-checkpoint activation under LD. Furthermore, DNA-checkpoint-related genes are under positive selection in Arabidopsis accessions from regions with shorter days, suggesting that photoperiod act as a selective barrier for Al tolerance. A diel regulation and genetic diversity affect Al tolerance, suggesting that day-length orchestrates Al tolerance. Altogether, photoperiodic control of Al tolerance might contribute to solve the historical obstacle that imposes barriers for developing countries to reach a sustainable agriculture.

Keywords: cell-division; DNA-repair; day-length; genetic diversity; photoperiodism

Introduction

During the last decades, considerable research efforts have been placed to ensure global food safety and together achieve a sustainable agriculture, which are currently two of the major human necessities. In fact, these are central goals of the United Nations (UN) to be achieved over the next 15 years for extinguishing hunger worldwide (United Nations UN, 2015). Acidic soils (pH ≤ 5.5) promote the release of aluminum cations, imposing serious constraints on root development in farmland soils around the world. They not only impair nutrient and water uptake (Nunes-Nesi et al., 2014; Kochian et al., 2015) but also hinder food production which is required to meet the demands of the growing global population. Notably, soil acidity is a chronic problem in the temperate zones of eastern North America and throughout Europe (where acidic soils cover up to 80% of the total area). More than 50% of the potentially available arable land in the world is covered by acidic soils (Kochian et al., 2015), which results in significant losses in crop productivity.

Mechanisms that allow plants to cope with Al stress have previously been elucidated and include alterations in organic acid (OA) metabolism, cell wall modifications, Al sequestration vacuole (de la Fuente et al., 1997; Nunes-Nesi et al., 2014; Kochian et al., 2015), and modifications of DNA-repair checkpoints (Eekhout et al., 2017). Classically, the most accept mechanism to increase Al tolerance describes metal neutralization with OA (e.g. citrate, malate and oxalate) which bind to Al that is neutralized (Nunes-Nesi et al., 2014; Kochian et al., 2015). Notwithstanding, higher levels of OA not always means enhanced Al tolerance, once Al sensitive genotypes produce and secrete large amounts of Al (Piñeros et al., 2005; Guimarães et al., 2014). Additionally, the most recent mechanism discussed to improve Al tolerance is based on genetic manipulations of DNA checkpoints, which enables cell cycle progression in presence of Al (Eekhout et al., 2017). Collectively, DNA checkpoint related proteins are responsible to monitor DNA integrity deciding whether maintain or arrest cell divisions during Al stress. Briefly, during chronic Al toxicity loss of quiescent-center (QC) identity occurs culminating in root death (Eekhout et al., 2017). In the Al-related DNA checkpoint pathway, the transcription factor Suppressor of Gamma Response (SOG1) is phosphorylated by the kinases Ataxia Telangiectasia Mutated and RAD3-related (ATR) and Ataxia Telangiectasia Mutated (ATM) (Eekhout et al., 2017). This phosphorylation enables the induction of DNA replication without the occurrence of mitosis, characterizing endoreduplication, a process that distinguish Al sensitive from Al tolerant genotypes (Sjögren et al., 2015). Likewise, loss of function mutants for the genes *ATR* and *SOG1* are characterized by more cell divisions and root QC maintenance, which sustain root elongation under Al stress (Rounds and Larsen, 2008). Intriguingly, a multi-level response to Al-induced DNA damage was revealed, wherein loss of function mutants *atm and sog1* despite displaying a higher short-term Al tolerance also have a delay in root growth recovery following Al toxicity (Chen et al., 2019). Apparently, both mechanisms Al detoxification and DNA checkpoint are indispensable for plant growth under Al stressful conditions. However, when analyzed individually, neither approach has been demonstrated to be sufficient to overcome Al toxicity. For example, Al-sensitive plants exudate large amounts of OA from root cells to the rhizosphere (Piñeros et al., 2005; Guimarães et al., 2014), whereas genetic manipulation of the DNA checkpoint machinery does not mediate plant survival in the presence of high Al concentrations (Chen et al., 2019). These findings reinforce the need to identify factors capable of regulating the overall plant response to Al.

Day-length is a remarkable factor modulating growth and development of different organisms worldwide. For instance, water pH exhibits a diel fluctuation in Arctic summers with increases over day and declines during the night, and it has been shown that longer photoperiods sustain higher pH in the kelp forests supporting the expansion of Arctic marine vegetation in response to environment variations (Krause-Jensen et al., 2016). Furthermore, in the soils of the Africa continent, a large pH (from alkaline to extremely acidic) range are usually found (Figure S1), and photoperiod is a dominant factor that controls vegetation phenology and growing season (Adole et al., 2019). Indeed, in terms of root development, reduced cell death around root apical meristem (RAM) is observed in Arabidopsis seedlings growing under darkness, whereas shoot exposure to light imposes higher cell death levels in RAM cells (Raya-González et al., 2018). It seems reasonable to assume, therefore, that photoperiod is likely a candidate to modulate root development in responses to Al. Accordingly, an empirical correlation seems indicate that the centre of origin of cultivated plants exhibiting lower variations in day-length over year, generally summer days with 12 h or less, favour the natural Al tolerance across the plant kingdom (Fig. S1). Likewise, previous studies indicate that many Al-tolerant species are short-day (SD) plants (e.g., *Oryza sativa*, *Stylosanthes humilis*, and *Vigna unguiculata*), while long-day (LD) plants (e.g., *Hordeum vulgar*e, *Pisum sativum*, and *Lens culinaris*) are generally Al-sensitive (Table S1). On analysing the centre of origin of most cultivated plants, we hypothesized that day-length is a pivotal agent modulating Al tolerance across distinct plant species. Our findings revealed that diel regulation and genetic diversity affect Al tolerance, suggesting that day-length orchestrates Al tolerance.

Material and Methods

Plant material and growth conditions

Different plant species were used in this study. Briefly, we used: (i) Arabidopsis wild-type (WT) and mutant plants of DNA repair and cell cycle related genes, overall on the Columbia-0 ecotype (Col-0) background; (ii) tomato (*Solanum lycopersicum*) and loss of function mutants in the *SELF-PRUNING 5G* (*SP5G*) and *SINGLE FLOWER TRUSS* (*SFT*) as well as near-isogenic lines harbouring the *S. pennellii* allele of *SP5G* ($SP5G^{pen}$), on cultivar Micro-Tom (MT); and (iii) different leguminous species, namely *Stylosanthes humilis*, *Vigna unguiculata*, *Lupinus albus*, *Crotalaria juncea*, *Pisum sativum,* and *Lens culinaris*. Previously to realisation of experiments we grew plants in the same conditions to obtain seeds with similar vigour and to avoid photoperiodic impacts in the progenies. Whereas overall species were grew in neutral day (12-h light/12-h dark) conditions, Arabidopsis wild-type and mutants were grown at same conditions; tomato genotypes and leguminous species were grown in the same conditions, further details about genotype are provided in Table S2. Briefly, seeds were surface sterilized by agitation in 30% (v/v) commercial bleach (2.7% [w/v] sodium hypochlorite) for 15 min, followed by three rinses with sterile distilled water and kept in darkness to synchronise germination for 4 days. Seedlings of all plant species, except *Arabidopsis thaliana*, were cultivated in hydroponics solution by using Hoagland medium (Hoagland and Arnon, 1950) at pH 4.0, with modifications. Plants were grown in a half-strength solution (pH 4.0) with 100 µM $AlCl_3$ (+Al) and 0 µM (-Al) for 5 days, the solution was exchanged five times and pH adjusted daily to 4.0. Plants were cultivated in a temperature-controlled chamber (20 ± 1 °C for Arabidopsis, and 25 ± 1°C for other species) under 250 µmol photons m^{-2} s^{-1}, 60% relative humidity, and either under short-days (SD: 8-h light/16-h dark) or long-days (LD; 16-h light/8-h dark).

Excluding *A. thaliana*, root systems were photographed, and the total root elongation was measured using the ImageJ software (https://imagej.nih.gov/ij/). Thus, we obtained the ratio between SD and LD to assess differences associated with day-length, wherein we also calculated the ratio between control and Al-treated plants. In addition, root systems were harvested at 5 days after sowing (DAS) and further used for nutritional analyses. To investigate the long-term impacts of Al exposure, we transferred 10-day-old *in vitro* grown *A. thaliana* seedlings to pots containing washed sand. These plants were cultivated for more two weeks and received 7 mL of Murashige and Skoog (MS medium) (Murashige and Skoog, 1962) solution daily, with Al or +Al (50 µM $AlCl_3$) at pH 4.0.

For *in vitro* assays, seeds from *A. thaliana* were surface-sterilized and imbibed for 4 days at 4°C in the dark on 0.8% (w/v) agar plates containing half-strength MS medium (Sigma-Aldrich; pH 4.0), with different $AlCl_3$ concentrations (0, 50, and 100 µM). Next, seedlings were cultivated for 10 days in a growth chamber (POL-EKO APARATURA® Climatic Chamber KK 1200) under SD and LD at 22 ± 1°C, 60% relative humidity, and 150 µmol photons m^{-2} s^{-1}. In a second experiment, the steps described above were followed and in addition to the treatments mentioned, we used MS medium at pH 4.0 supplemented with zeocin (5 µM zeocin), methyl methanesulfonate (MMS) (50 ppm), or hydroxyurea (HU) (1 mM). The ratio of root elongation was determined *in vitro* in a similar manner to the hydroponics experiments.

Flow cytometry

Roots from *A. thaliana* seedlings were collected, and the root apical meristems (RAM) were excised. From ~30 RAM for each repetition, nuclei were isolated using grinding movements with a pestle in 0.2 mL OTTO-I lysis buffer (Otto, 1990) supplemented with 2.0 mM dithiothreitol (Sigma®), and then made up to 0.8 mL with the same buffer. The nuclei suspensions were then filtered through a 20 µm nylon mesh (Partec®) and centrifuged at 100*g* × g for 5 min. The obtained pellet was incubated for 10 min in 0.1 mL of OTTO-I lysis buffer and stained with 0.5 mL of OTTO-I:OTTO-II (1:2) solution, supplemented with 75 µM propidium iodide and 2.0 mM dithiothreitol (Sigma®). The nuclei suspensions were incubated for 30 min in the dark at room temperature. Each suspension was analysed using a BD Accuri C6 flow cytometer (Accuri cytometers, Belgium) equipped with a 488 nm laser source. FL2 (585/640) and FL3 (670 LP) filters were used to detect propidium iodide fluorescence. The BD Csampler software (Accuri Cytometers, Belgium) was used for histogram analyses to determine the DNA ploidy level of each G_0/G_1 peak. For this, we considered only the histograms with G_0/G_1 peaks exhibiting a coefficient of variation below 5% for the G_0/G_1 peak and at least 5,000 nuclei were counted for each nuclei suspension.

Nutrient analyses

To quantify the levels of Al, phosphorus, potassium, calcium, magnesium, the dry root tips (~0.5 cm) were subjected to a nitric-perchloric digestion (65% and 70%) (Miyazama et al. 1999). The samples were analysed using inductively coupled plasma optical emission spectroscopy (ICP-OES, Perkin-Elmer Optima 3000XL, Maryland, USA).

Expression analysis by qRT-PCR

Total RNA was extracted from root samples harvested (immediate snap-freezing in liquid nitrogen) at time points described in the specific figure captions. The RNA was isolated using the TRIzol reagent (Ambion, Life Technology), according to the manufacturer’s recommendations. It was then treated with DNase I (RQ1 RNase free DNase I; Promega). RNA integrity was analysed on 1% (w/v) agarose gels by measuring the RNA concentration with a Nanodrop spectrophotometer. Real-time PCR was performed with cDNA using a sequence detection system (Applied Biosystems Applera) using the Power SYBR Green PCR Master Mix according to Piques *et al.* (2009). The calculations for transcript abundance were performed with standard curves of each selected gene and normalised using the constitutively expressed gene ACTIN (AT2G37620). Data analyses were performed as described previously (Caldana et al., 2007). The primer sequences used are shown in Table S3. Melting curves were checked for unspecific amplification and primer dimerization.

Genetic structure analysis

We selected 287 *A. thaliana* accessions from the 1001 Genomes Consortium database (https://1001genomes.org/) (Alonso-Blanco et al., 2016) based on variable day-length from 8:11 to 23:53 of duration, calculated by http://www.solartopo.com/daylength.htm. Single Polymorphism Nucleotide (SNPs) were identified in genes related to cell cycle and DNA repair by using the POLYMORPH 1001 (https://tools.1001genomes.org/polymorph/). The genetic structure population was analysed using fast structure software (Raj et al., 2014). Unsupervised machine learning approaches were used to analyse the accession groups. The accession data were hierarchically clustered with the Orange Canvas software (http://orange.biolab.si/) using Pearson's correlation distance as the distance measure and the average linkage clustering option. The discriminant analysis of principal components (DAPC) was performed using the 'adegenet' package 1.4-1 (Jombart et al., 2010) in R studio V 2.3.2 (R Development Core Team, 2011) to confirm cluster numbers and to describe the global diversity for overlooking differences between groups.

Statistical Analyses

All experiments were designed in a completely randomised distribution with a minimum of three biological replicates of each treatment. Additionally, the experiments were repeated at least three times (even in different growth facilities) with similar phenotypes observed each time. Data were statistically tested for normality and subsequently examined using ANOVA ($P < 0.05$). Differences in the means ($P < 0.05$) displayed in figures and tables were examined by Student's *t-test*. All statistical analyses were performed using R statistical software (www.r-project.org).

Results

SD favours Al tolerance regardless of flowering pathway

Since we observed that photoperiod may play a role in Al tolerance, it was hypothesized that an endogenous system also modulates Al-responses. Different species of leguminous plants, some that flower under SD conditions (*Stylosanthes* *humilis*, *Vigna unguiculata*, and *Lupinus albus*) and others that flower under LD (*Crotalaria juncea*, *Pisum sativum*, and *Lens culinaris*), were thus selected and cultivated in the presence of Al. In general, we observed that root elongation in SD species, which are generally Al-tolerant, was insensitive to photoperiod variations, showing similar root elongation rates under both SD and LD conditions, regardless of the presence of Al (Fig. 1A). Meanwhile, LD plants, which are usually Al-sensitive, displayed higher root growth and Al sensitivity under LD than under SD conditions, revealing that these plants are indeed more sensitive to Al (Fig. 1B). It was further investigated whether fluctuations in day-length altered the mineral-nutrient concentration. Our results revealed that photoperiod influenced Al uptake as revealed by higher Al levels in plants cultivated under SD conditions compared to those cultivated under LD (Fig. S2-3). Intriguingly, even though SD led to a higher Al-tolerance, higher levels of Al were observed in plants growing on SD than under LD, while the levels of other nutrients were less affected by day-length (Fig. S2).

Tomato (*Solanum lycopersicum*) is a day-neutral species, whereas its wild relative *S. pennellii* is an SD species (Soyk et al., 2017). The photoperiod-neutrality of *S. lycopersicum* is caused by the loss of function in *SELF-PRUNING 5G* (*SP5G*) (Fig. 1C), which is a flowering repressor that acts on the florigen paralog *SINGLE FLOWER TRUSS* (*SFT*) (Soyk et al., 2017). We analysed near-isogenic lines harbouring the *S. pennellii* allele of *SP5G* ($SP5G^{pen}$) or a loss-of-function mutation in *SFT* (*sft*) in tomato cv. ‘Micro-Tom’ (MT). Based on shoot apical meristem (SAM) analyses, we observed that $SP5G^{pen}$ modified *S. lycopersicum* into an SD plant, whereas sft modified it into a photoperiod-insensitive variety, which not flowering neither under SD or LD conditions (Fig. S4). Additionally, $SP5G^{pen}$ plants exhibited a higher Al tolerance, as revealed by similar root growth under both SD and LD conditions and in the presence of Al, a response that was not observed in MT and *sft* plants (Fig. 1D, E, S5-10), distinguishing photoperiodic responses from flowering pathway. Thus, Al tolerance in tomato seemed to depend on its ability of roots to respond to photoperiod, and not of flowering dependent of day-length.

Long-term Al tolerance occurs under SD

To further explore the molecular connections between photoperiodic response and Al toxicity, we cultivated Arabidopsis thaliana (L.) Heynh. ecotype Columbia 0 wild-type (WT) under SD (8h light/16h dark), neutral days (ND - 12h light/12h dark) and LD (16h light/8h dark), in the presence and absence of Al. Al-induced root growth inhibition was photoperiod-dependent, as neither SD nor ND reduced root growth in the presence of Al. Whereas, since 5 days after germination (DAG) plants growing under LD experienced the sensitivity to Al-toxicity, wherein root elongation begun to be reduced (Fig. S11). It was further investigated whether day-length mitigated reduction in root elongation following either pH change or differential Al concentrations. SD did not ameliorate the reduction in root elongation evoked at lower pH (4.0) compared to the optimal pH (5.7) (Fig. 2A). SD-grown plants were also able to tolerate higher Al levels, showing lower reductions in root elongation than LD plants following increased Al levels (Fig. 2B). Consequently, a multi-level Al response was reported for *A. thaliana*. In addition, following long-term Al exposure, loss-of-function mutants for major DNA-checkpoint regulator genes were generally Al-tolerant, but showed a slower growth recovery after a short-term stress imposed by Al (Chen et al., 2019). In view of this, we further investigated if SD would mitigate plant growth losses resulting from long-term Al exposure. No difference in rosette growth or root elongation was observed in plants growing under SD regardless of Al exposure (Fig. 2C), indicating a likely permanent Al tolerance under SD.

Photoperiod specifically mitigates Al toxicity

Cell cycle arrest and DNA damage resulting from Al toxicity are two major cellular alterations affecting plant growth. Al stress culminates in cell cycle blockage and ultimately alters root DNA endoploidy that induces differentiation of root apical meristematic (RAM) cells, a process known as meristem exhaustion (Nezames et al., 2012; Sjögren et al., 2015). Spatiotemporal control of DNA endoploidy was demonstrated across root tissues, indicating an elevated endoploidy in roots coping with low pH (4.6) (Bhosale et al., 2018). Thus, we isolated RAMs to assess the DNA ploidy levels in response to Al stress, and monitored cell divisions in the root meristems of young seedlings using flow cytometry. Our results revealed the maintenance of potential proliferative capacity (2C – 32C cells) in roots exposed to Al-toxicity under SD, but not under LD conditions. It was also observed that SD suppressed the appearance of polyploid cells (4C, 8C, 16C, and 32C) on the RAM (Fig. 3A), indicating that Al tolerance under SD is likely associated with the downregulation of endoreduplication. Endoreduplication promotes an increase in the DNA ploidy level in several cell types due to changes in cell cycle control. It only occurs in metabolically active and highly specialised cells and allows DNA replication in the absence of mitosis, which increases the levels of DNA endoploidy and regulates root-cell fates (De Veylder et al., 2011). Our results revealed that under SD, there was a strong reduction in the endoreduplication index in the presence of Al, but it was not so under LD (Fig. 3B). We next turned our attention to identify whether SD promoted reductions in endoreduplication, specifically triggered by Al toxicity, or whether it was a general mechanism of roots under genotoxic conditions. Previous studies have demonstrated that endoreduplication in quiescent-center (QC) cells of the RAM disrupt cell-cycle progression, which might be attributed to the effects of Al toxicity or drugs such as hydroxyurea (HU), methyl methanesulfonate (MMS), and zeocin, leading to a reduced root elongation phenotype (Sjögren et al., 2015; Adachi et al., 2011; Takahashi et al., 2019). Day-length did not mitigate root elongation limitations triggered by HU, MMS, or zeocin (Fig. 3C), supporting our notion that photoperiod acted specifically in endoreduplication resulting from Al toxicity.

LD are required to arrest cell divisions under Al stress

Endoreduplication is an alternative pathway that avoids cell cycle arrest or cell death due to DNA damage (Endo et al., 2012). Thus, we investigated how SD promotes reductions in endoreduplication during Al toxicity. In response to Al, endoreduplication is mainly modulated by the transcription factor *SUPPRESSOR OF GAMMA RESPONSE1* (*SOG1*), which is activated by the kinases *ATAXIA TELANGIECTASIA MUTATED* (*ATM*) and *ATAXIA TELANGIECTASIA AND RAD3-*

RELATED (*ATR*), enhancing the expression of genes associated with cell cycle stoppage and DNA repair (Sjögren et al., 2015). *A. thaliana* loss-of-function mutants for *SOG1* and *ATR* displayed higher Al tolerance due to the inhibition of early endoreduplication onset (Sjögren et al., 2015). In silico analyses further revealed that most of the genes involved in DNA repair and endoreduplication regulation exhibited a significant correlation with latitude/day-length in 32 *A. thaliana* accessions (Fig. S12). In agreement with our in silico analyses (Fig. S13), qRT-PCR analysis of the major DNA-repair regulators (*SOG1*, *ATM*, and *ATR*) revealed a more coordinated gene expression under SD than under LD conditions (Fig. 4). Briefly, *SOG1*, *ATM*, and *ATM* expression increased in a light-dependent manner only during LD, reaching maximum expression peaks at points near dusk (Fig. 4). Based on the contribution of both *SOG1* and *ATR* checkpoint regulators on the Al-toxicity response, it was hypothesised that their diel regulation might have contributed to the observed photoperiodic Al-tolerance. We confirmed this by growing *A. thaliana* mutants defective in DNA checkpoint control, *sog1-1*, *atm-1*, and *atr-2*. As expected, *sog1-1*, *atm-1*, and *atr-2* showed an invariant Al tolerance following day-length variations, with similar root growth responses following SD and LD (Fig. S14). Collectively, SD seemed to support mitosis maintenance by repressing excessive DNA replication that would culminate in higher endoreduplication.

Circadian clock and genetic diversity impose constraints for Al-tolerance

Day-length is a more predictable, unperturbed, and non-oscillating factor than temperature, air humidity, and rainfall. Thus, A. thaliana accessions from high-latitude environments usually experience long photoperiods around summer solstice, exhibiting longer circadian periods (Salmela and Weinig, 2019). Casein kinase 2 (CK2) phosphorylates both circadian clock proteins related to the light cycle (Lu et al., 2011) and SOG1 protein on amino acid T423 (Wei et al., 2021). Correspondingly, the mutation of T423 into its phosphomutant A (T423A) mimics the Al-resistant phenotype of *A. thaliana sog1-101* mutant plants (Wei et al., 2021). Interestingly, *sog1-101* and *T423A*-27 knockout mutants displayed root elongation insensitive to day-length as well as higher Al-tolerance than WT plants, regardless of the photoperiod (Fig. S15). Therefore, we postulate that genetic diversity could occur in genes related to DNA and cell cycle in *A. thaliana*, which would be selected in response to day-length and light components of the circadian clock machinery. In keeping with this assumption, we analyzed two independent studies (Satbhai et al., 2017; Ristova et al., 2018) to verify whether latitudinal/day-length variation affected root elongation in *A. thaliana* accessions. Interestingly, we observed a positive and significant correlation between latitude/day-length and root length, revealing that accessions from higher latitudes displayed greater root length (Fig. S16). Regarding root development, only with light incidence on shoots, cell death was triggered around the RAM cells of *A. thaliana* seedlings (Raya-González et al., 2018). Furthermore, to identify the existence of genetic diversity for genes investigated here as members of the interface between Al-tolerance and day-length, we selected 287 *A. thaliana* accessions with the centre of origin regions widely varying in photoperiod. By grouping approaches, we found three distinct groups (G1, G2, and G3) based on day-length in the region origin, in which G1 exhibited more accessions with photoperiod around 12-14h while G3 had the majority of accessions from regions with day-length of around 20h (Fig. 5A-B). Assessing the relatedness of accessions using STRUCTURE, we were also able to discriminate individuals from three populations (Fig. 5C), revealing an essential absence of a mixture of accessions due to photoperiod-barriers. Therefore, the higher fitness of *A. thaliana* accessions from low-latitudes under drought conditions (Exposito-Alonso et al., 2019) and more coordinated oscillatory behavior in drosophilids (Bertolini et al., 2019) might be due to the lower photoperiod variability across the year. Likewise, the mapping of polymorphisms in genes involved in DNA repair, cell cycle, and endoreduplication revealed the existence of a characteristic genetic diversity for genes involved in Al tolerance in *A. thaliana*, which varied with day-length (Fig. S17). Furthermore, we found more non-synonymous and synonymous mutations in *ATM*, *ATR*, and *SOG1* genes in accessions derived from longer day regions (G3), the unique group in which these mutations were correlated with day-length (Fig. 5D-E and S18). In agreement with these findings, the global distribution of population genetic diversity across the animal and plant kingdoms reveal that only eudicots exhibit a significant correlation between population genetic diversity and latitude (De Kort et al., 2021). Notably, genetic diversity increases with distance to the equator within eudicots (De Kort et al., 2021). Strikingly, accessions from lower day regions (G1 and G2) were under positive selection ($dN/dS \geq 1$), meaning that the selective patterns are directly related to gene expression (Fig. 5F, S14, and S20). Therefore, photoperiod seems to have been imposing selection patterns in genes that are known to improve Al tolerance. Collectively, our results suggested that photoperiod is likely a factor that mediates plant Al tolerance.

Discussion

Day-length disrupts root growth enhancing Al sensitivity

Photoperiod has been extensively suggested as a pivotal factor modulating growth, development, and metabolic responses (Schaffer et al., 1998; Valverde et al., 2004; Lagercrantz, 2009; Gibon et al., 2009; Andrés and Coupland 2012; Sulpice et al., 2014; Nunes-Nesi et al., 2016). This fact aside, the significance of photoperiod in overall stress responses has been suggested to be day-length dependent. In fact, the burst of oxidative stress responses in *A. thaliana* is dependent on the light period as evidenced by the higher efficiency to cope with reactive oxygen species (ROS) of plants growing under SD than LD (Queval et al., 2007; Queval et al., 2012; Abuelsoud et al., 2020). Notwithstanding, this knowledge is seemingly not well explored for abiotic stress responses, since this apparent notion remained restricted for impacts of day-length on plant development. Here, by performing a multi-specie study, we explored the photoperiod relevance for Al tolerance. Our results suggested a crucial yet unreported significance of photoperiod and endogenous cues, which appear to play a critical role for photoperiod in shaping Al tolerance across the plant kingdom. Our data further indicated that SD higher Al-tolerance is mostly observed in SD-grown plants. Intriguingly, plants growing under SD could uptake higher Al levels than under LD, but with less physiological impact. Higher Al content in the roots of plants growing under SD indicates an internal immobilisation of Al in the apoplast. Further work is clearly required to assess the significance of biochemical changes in photosynthesis and respiration, which are the major processes that produce and consume photoassimilates. Reprogramming of the mitochondrial OA metabolism may mediate Al tolerance in both microorganisms and plants (de la Fuente et al., 1997; Nunes-Nesi et al., 2014; Kochian et al., 2015). Although Phosphorus (P) uptake was previously described to be dependent from red-light signalling on *A. thaliana* accessions (Sakuraba et al., 2018), here we have not found variations for P uptake between SD and LD conditions, which further suggest day-length as specific factor modulating Al tolerance.

Long-term Al tolerance occurs under SD

Environmental stresses impacts diverse aspects of plant development, arresting a wide range of traits associated with crop yield. Accordingly, Al toxicity have implications on metabolism and RAM meristem, as depicted by the Al sensitive genotypes that exhibit enhancement in responses classically described to improve Al tolerance yet they are extremely sensitive to Al toxicity (Piñeros et al., 2005; Guimarães et al., 2014; Chen et al., 2019). Our results revealed that SD boosts root growth under Al stress, wherein this was not observed for variations in pH in absence of Al, once no growth mitigation was found comparing root elongation at low (4.0) and optimal pH (5.7). In fact, increasing Al doses were less impacting for SD-grown *A. thaliana*, while these doses impacted more LD-grown plants during short- and long-term of Al toxicity. Additionally, root growth is substantially elevated following day-length extension in *A. thaliana*, wherein at late night root elongation reach the maximum rates (Yazdanbakhsh et al., 2011). Root growth may be inhibited during light periods whereas at dark periods, growth may be recovered (Ruts et al., 2012). In consonance, Al toxicity is manifested differentially over time, since a same genotype may be tolerant to short-term Al exposure and sensitive to Al long-term exposure (Chen et al., 2019), which apparently occur due to accumulation of light cycles that likely favour Al sensitiveness.

SD repress endoreduplication specifically triggered by Al toxicity

Cell divisions likely disarm replicative defences turning the genome more susceptible to replication errors and damages, a fact that is enhanced under Al toxicity that in turn activates DNA checkpoints to halt cell division progression (Hu et al., 2016). Usually, plant roots coping with Al toxicity experiences the formation of a greater number of DNA polyploidy cells, reducing RAM cells competence for division (Nezames et al., 2012; Sjögren et al., 2015). Our monitoring of DNA ploidy at root meristems of *A. thaliana* seedlings revealed that SD boosts proliferative ability by arresting the emergence of polyploidy cells leading ultimately to higher Al tolerance. Natural populations of *Arabidopsis arenosa* are composed by diploids and tetraploids individuals widespread from Germany to Sweden (Monnahan et al., 2019), whereas the elevation on latitude/day-length seems to favour the natural occurrence of tetraploids. Accordingly, shifts on cell volume impact directly final function of cells, in which RAM cells growing under LD conditions are characterized by reductions on proliferation rates triggered by low pH (4.0) (Pacifici et al., 2018). Although this pH range have a slight impact on RAM activity (Gujas et al., 2012), it generated a drastic rise on cell size at the meristematic zone indicating an altered cell differentiation programming (Pacifici et al., 2018). Notably, low pH induces higher endoreduplication index on roots (Bhosale et al., 2018), and it seems to match with the higher cell size responsible for tailoring root developmental program. Likewise, zeocin sensitivity directly implicates cell area enlargement with distancing from QC, which seems to induce endoreduplication further reducing root length (Adachi et al., 2011). Endoreduplication induced in response to genotoxic stress is assumed to be programmed (Adachi et al., 2011), wherein low pH seemingly disarm the natural programming. Thus, inducing QC cell proliferation the sensitiveness of QC cells to DNA damage is also elevated, resulting on impaired root growth after genotoxic stress (Cruz-Ramírez et al., 2013). We observed that plants with enhanced root elongation rates under LD are Al sensitive, indicating that root growth induction seems to turn those plants more sensitive to genotoxic events, emerging light relevance for stress responses modulation.

Diurnal gene expression and genetic diversity behind Al tolerance

Life of autotrophic organisms is most likely synchronized with light/dark cycles, supporting the notion of photoperiodism and development. A kingdom-wide evolutionary analysis of diurnal gene expression on photosynthetic organisms revealed that more than one-third of genome is diurnally regulated (Ferrari et al., 2019). Despite the conserved diurnal regulation, precise gene expression peaks are likely able to orientate organism biology, and as such cell division and photoperiodism emerge as divergent process across organisms (Ferrari et al., 2019). Momentary root growth rate display a robust diel oscillation, wherein the minimum growth is reached at 8 to 10 hours after dawn (Yazdanbakhsh et al., 2011). Interestingly, this is the exact period that we noted major peaks expression for genes *SOG1*, *ATM* and *ATR*. Thus, the extension of light period subsequently to these 8 to 10 hours upon dawn seems to be mandatory to arrest root elongation following endoreduplication induction under Al toxicity. Supporting this motion, it has been previously shown that SOG1 was not found among the targets of the kinases ATM and ATR (Roitinger et al., 2015). Accordingly, our results for the photoperiodic expression response of these genes may explain, at least partially, these previous controversial results. In fact, mutations at *SOG1*, *ATM* and *ATR* culminate on an Al tolerance that is insensitive to day-length in Arabidopsis. Photoperiod is an extremely predictable factor and yet it is seemingly capable of modulate a differential Al tolerance across species. Taken together with our results it seems reasonable to indicate a potential role for photoperiodism in modulating genetic diversity on genes related to Al responses.

DNA checkpoint regulators were demonstrated to be under periodicity and oscillating widely across diverse animals affecting fatally DNA repair efficiency and cell divisions (Stewart-Ornstein and Lahav, 2017; Stewart-Ornstein et al., 2017). Plants are usually exposed to most distinct environmental conditions coping with shorter and longer days, wherein diurnal regulation of genetic features is rather contrasting on *A. thaliana* from low and high latitudes (Salmela and Weinig, 2019). Investigating 191 Swedish *A. thaliana* accessions it was revealed that circadian period is dependent from both geographical localization and population sub-structure (Rees et al., 2020). Furthermore, a single non-synonymous polymorphism affects drastically circadian period and flowering, turning both longer and generating a selective pressure gradients across Sweden (Rees et al., 2020). Our genetic diversity mapping also suggested that (i) the lower competition among plant species at higher latitudes may enable the occurrence of species adapted to stressful conditions, and (ii) with increasing distance from the Equator, stressful environmental conditions may alter patterns of energy allocation from vegetative growth to reproduction, increasing the genetic diversity in populations from these regions (De Kort et al., 2021; LaManna et al., 2017). Day-length specifically influences cell cycle arrest and endoreduplication related to Al, modulating gene expression in a diel manner. Notwithstanding, it is interesting investigated what would be the most relevant association for Al tolerance evolution, the specific soil types or day length. Furthermore, since genetic variability within the sequence for these genes was altered according to photoperiod, it seems reasonable to question whether day-length regulates only Al responses, or it could also coordinate tolerance to other abiotic stresses. In addition, our results offer novel perspectives for understanding the underlying mechanisms of Al-tolerance in land plants. For example, under field conditions where plants are recurrently exposed to fluctuating conditions, Al-tolerance mechanisms involving different levels of complexity can play a pivotal role in helping plants to successfully cope with high levels of Al. Additional studies, which employ more sophisticated multi-disciplinary analyses, will likely be of fundamental importance in providing a holistic understanding of the underlying mechanisms that affect the evolution of Al-tolerance. Therefore, our findings suggest a wide yet unrecognised significance of day-length in orchestrating Al tolerance, paving the way to development of the next generation of productive crops under higher Al conditions.

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Competing Interest Statement

The authors declare that they have no competing interests.

Author Contributions

J.A.S., and W.L.A. designed the research; J.A.S. performed most of the research with the support of T.W. and W.B-S.; J.C., and W.C. performed cytometry flow analyses; J.A.S., and J.C.F.S. realized bioinformatics analyses; W.B-S., J.C., W.C., L.D.V., A.R.F., and A.N-N. contributed new reagents/analytic tools; J.A.S., L.D.V., A.N-N., and W.L.A. analyzed the data; and J.A.S., and W.L.A. wrote the article with input from all the others.

References

Abuelsoud W, Cortleven A, Schmülling T. 2020. Photoperiod stress induces an oxidative burst-like response and is associated with increased apoplastic peroxidase and decreased catalase activities. Journal of Plant Physiology 253: 153252.

Adachi S, Minamisawa K, Okushima Y, Inagaki S, Yoshiyama K, Kondou Y, Kaminuma E, Kawashima M, Toyoda T, Matsui M, Kurihara D, Matsunaga S, Umeda M. 2011. Programmed induction of endoreduplication by DNA double-strand breaks in *Arabidopsis*. Proceedings of the National Academy of Sciences, USA 108, 10004–10009.

Adole T, Dash J, Rodriguez-Galiano V, Atkinson PM. 2019. Photoperiod controls vegetation phenology across Africa. Communications Biology 2: 391.

Alonso-Blanco C, Andrade J, Becker C, Bemm F, Bergelson J, Borgwardt KMM, Cao J, Chae E, Dezwaan TMM, Ding W, *et al.* 2016. 1,135 genomes reveal the global pattern of polymorphism in Arabidopsis thaliana. Cell 166: 481–491.

Andrés F, Coupland G. 2012. The genetic basis of flowering responses to seasonal cues. Nature Reviews Genetics 13: 627–639.

Bertolini E, Schubert FK, Zanini D, Sehadová H, Helfrich-Förster C, Menegazzi P. 2019. Life at High Latitudes Does Not Require Circadian Behavioral Rhythmicity under Constant Darkness. Current Biology 29: 3928–3936.

Bhosale R, Boudolf V, Cuevas F, Lu R, Eekhout T, Hu Z, et al. (2018). A spatiotemporal DNA endoploidy map of the Arabidopsis root reveals roles for the endocycle in root development and stress adaptation. The Plant Cell 30: 2330–2351.

Caldana C, Scheible WR, Mueller-Roeber B, Ruzicic S. 2007. A quantitative RT-PCR platform for high-throughput expression profiling of 2500 rice transcription factors. Plant Methods 3: 7.

Chen P, Sjogren CA, Larsen PB, Schnittger A. 2019. A multi-level response to DNA damage induced by aluminium. The Plant Journal 98: 479–491.

Cruz-Ramírez A, Diaz-Trivino S, Wachsman G, Du Y, Arteaga-Vazquez M, Zhang H, Benjamins R, Blilou I, Neef AB, Chandler V, Scheres B. 2013. A SCARECROW-RETINOBLASTOMA protein network controls protective quiescence in the *Arabidopsis* root stem cell Organizer. PLOS Biology 1: e1001724.

De Kort H, Prunier JG, Ducatez S, Honnay O, Baguette M, Stevens VM, Blanchet S. 2021. Life history, climate and biogeography interactively affect worldwide genetic diversity of plant and animal populations. Nature Communications 12:516.

de la Fuente-Martínez JM, Ramirez-Rodriguez V, CabreraPonce JL, Herrera-Estrella L (1997) Aluminum tolerance in transgenic plants by alteration of citrate synthesis. Science 276:1566–1588

De Veylder L, Larkin JC, Schnittger A. 2011. Molecular control and function of endoreplication in development and physiology. Trends Plant Science 16: 624–634.

Eekhout T, Larsen P, De Veylder L. 2017. Modification of DNA Checkpoints to Confer Aluminum Tolerance. Trends Plant Science 22: 102–105.

Endo M, Nakayama S, Umeda-Hara C, Ohtsuki N, Saika H, Umeda M, Toki S. 2012. CDKB2 is involved in mitosis and DNA damage response in rice. The Plant Journal 69: 967–977.

Exposito-Alonso M, 500 Genomes Field Experiment Team, Burbano HA, Bossdorf O, Nielsen R, Weigel D. 2019. Natural selection on the Arabidopsis thaliana genome in present and future climates. Nature 573:126–129.

Ferrari C, Proost S, Janowski M, Becker J, Nikoloski Z, Bhattacharya D, Price D, Tohge T, Bar-Even A, Fernie A, *et al.* 2019. Kingdom-wide comparison reveals the evolution of diurnal gene expression in Archaeplastida. Nature Communications 10: 737.

Gibon Y, Pyl ET, Sulpice R, Lunn JE, Höhne M, Günther M, Stitt M. 2009. Adjustment of growth, starch turnover, protein content and central metabolism to a decrease of the carbon supply when Arabidopsis is grown in very short photoperiods. Plant, Cell & Environment 32: 859–874.

Guimarães CT, Simões CC, Pastina MM, Maron LG, Magalhães JV, Vasconcellos RC, *et al.* 2014. Genetic dissection of Al tolerance QTLs in the maize genome by high density SNP scan. BMC Genomics 15: 153.

Hoagland DR, Arnon DI. 1950. The water culture method for growing plants without soil. Circular **347**, University of California Agriculture Experimental Station, Berkeley.

Hu Z, Cools T, De Veylder L. 2016. Mechanisms used by plants to cope with DNA damage. Annual Review of Plant Biology 67:439–462.

Jombart T, Devillard S, Balloux F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11: 94.

Kochian LV, Piñeros MA, Liu JP, Magalhães JV. 2015. Plant Adaptation to Acid Soils: The Molecular Basis for Crop Aluminum Resistance. Annual Review Plant Biology 66: 571–598.

Krause-Jensen D, Marba N, Sanz-Martin M, Hendriks IE, Thyrring J, Carstensen J, Sejr MK, Duarte CM. 2016. Long photoperiods sustain high pH in arctic kelp forests. Science Advances 2: e1501938.

Lagercrantz U. 2009. At the end of the day: a common molecular mechanism for photoperiod responses in plants? Journal of Experimental Botany 60: 2501–2525.

LaManna JA, Mangan SA, Alonso A, Bourg NA, Brockelman WY, Bunyavejchewin S, Chang L-W, Chiang J-M, Chuyong GB, Clay K et al. 2017. Plant diversity increases with the strength of negative density dependence at the global scale. Science 356: 1389–1392.

Lu SX, Liu H, Knowles SM, Li J, Ma L, Tobin EM, Lin C. 2011. A role for protein kinase casein kinase2 α-subunits in the Arabidopsis circadian clock. Plant Physiology 157: 1537–1545.

Miyazama M, Pavan MA, Bloch MF, 1999. Análises químicas de tecido vegetal. In: Silva FC, et al, eds. *Manual de análises químicas de solos, plantas e fertilizantes*. Brasília: 171–224.

Monnahan P, Kolár F, Baduel P, Sailer C, Koch J, Horvath R, Laenen B, Schmickl R, Paajanen P, Srámková G et al. 2019. Pervasive population genomic consequences of genome duplication in *Arabidopsis arenosa*. Nature Ecology & Evolution 3: 457–468.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum **15**, 473–497.

Nezames CD, Sjogren CA, Barajas JF, Larsen PB. 2012. The *Arabidopsis* cell cycle checkpoint regulators TANMEI/ALT2 and ATR mediate the active process of aluminum-dependent root growth inhibition. The Plant Cell 24: 608–621.

Nunes-Nesi A, Nascimento VL, Silva FMO, Zsögön A, Araújo WL, Sulpice R. 2016. Natural genetic variation for morphological and molecular determinants of plant growth and yield. Journal of Experimental Botany 67: 2989–3001.

Nunes-Nesi A, Santos Brito DS, Inostroza-Blancheteau C, Fernie AR, Araújo WL. 2014. The complex role of mitochondrial metabolism in plant aluminum resistance. Trends Plant Science 19: 399-407.

OTTO FJ. 1990. DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. Methods in Cell Biol. 33, 105-110.

Pacifici E, Di Mambro R, Dello Ioio R, Constantino P, Sabatini S. 2018. Acidic cell elongation drives cell differentiation in the Arabidopsis root. EMBO Journal 37: e99134.

Piñeros MA, Shaff JE, Manslank HS, Alves VMC, Kochian LV. 2005. Aluminum resistance in maize cannot be solely explained by root organic acid exudation. A comparative physiological study. Plant Physiology 137: 231-241.

Piques M, Schulze WX, Höhne M, Usadel B, Gibon Y, Rohwer J, Stitt M. 2009. Ribosome and transcript copy numbers, polysome occupancy and enzyme dynamics in Arabidopsis. Molecular Systems Biology 5: 314.

Queval G, Issakidis-Bourguet E, Hoeberichts FA, Vandorpe M, Gakière B, Vanacker H, Miginiac-Maslow M, Van Breusegem F, Noctor G. 2007. Conditional oxidative stress responses in the Arabidopsis photorespiratory mutant *cat2* demonstrate that redox state is a key modulator of daylength-dependent gene expression and define photoperiod as a crucial factor in the regulation of H_2O_2-induced cell death. The Plant Journal 52: 640–657

Queval G, Neukermans J, Vanderauwera S, Van Breusegem F, Noctor G. 2012. Day length is a key regulator of transcriptomic responses to both CO_2 and H_2O_2 in *Arabidopsis*. Plant, Cell & Environment 35: 374–387.

R Development Core Team. 2011. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Raj A, Stephens M, Pritchard JK. 2014. fastSTRUCTURE: variational inference of population structure in large SNP data sets. Genetics 197: 573–589.

Raya-González J, Oropeza-Aburto A, Lopez-Bucio JS, Guevara-Garcia AA, de Veylder L, Lopez-Bucio J, Herrera-Estrella L. 2018. MEDIATOR18 influences Arabidopsis root architecture, represses auxin signaling and is a critical factor for cell viability in root meristems. The Plant Journal 96: 895–909.

Rees H, Joynson R, Brown JKM, Hall A. 2021. Naturally occurring circadian rhythm variation associated with clock gene loci in Swedish Arabidopsis accessions. Plant, Cell & Environment 44: 807–820.

Ristova D, Giovannetti M, Metesch K, Busch W. 2018. Natural genetic variation shapes root system responses to phytohormones in Arabidopsis. The Plant Journal 96, 468–481.

Roitinger E, Hofer M, Kocher T, Pichler P, Novatchkova M, Yang J, Schlogelhofer P, Mechtler K. 2015. Quantitative phosphoproteomics of the ataxia telangiectasia-mutated (ATM) and ataxia telangiectasia-mutated and rad3-related (ATR) dependent DNA damage response in *Arabidopsis thaliana*. Molecular & Cellular Proteomics 14: 556–571.

Rounds MA, Larsen PB. 2008. Aluminum-dependent root-growth inhibition in *Arabidopsis* results from AtATR-regulated cell-cycle arrest. Current Biology 18, 1495–1500.

Ruts T, Matsubara S, Wiese-Klinkenberg A, Walter A. 2012. Diel patterns of leaf and root growth: endogenous rhythmicity or environmental response? Journal of Experimental Botany 63: 3339–3351.

Sakuraba Y, Kanno, S, Mabuchi A, Monda K, Iba K, Yanagisawa S. 2018. A phytochrome-B-mediated regulatory mechanism of phosphorus acquisition. Nature Plants 4: 1089–1101.

Salmela MJ, Weinig C. 2019. The fitness benefits of genetic variation in circadian clock regulation. Current Opinion Plant Biology 49: 86–93.

Satbhai SB, Setzer C, Freynschlag F, Slovak R, Kerdaffrec E, Busch W. 2017. Natural allelic variation of *FRO2* modulates Arabidopsis root growth under iron deficiency. Nature Communication 8: 15603.

Schaffer R, Ramsay N, Samach A, Corden S, Putterill J, Carré IA, Coupland G. 1998. The late elongated hypocotyl mutation of Arabidopsis disrupts circadian rhythms and the photoperiodic control of flowering. Cell 93: 1219–1229.

Sjögren CA, Bolaris SC, Larsen PB. 2015. Aluminum-Dependent terminal differentiation of the arabidopsis root tip is mediated through an ATR-, ALT2-, and SOG1-Regulated transcriptional response. The Plant Cell 27: 2501–2515.

Soyk S, Muller NA, Park SJ, Schmalenbach I, Jiang K, Hayama R, Zhang L, Van Eck J, Jimenez-Gomez JM, Lippman ZB. 2017. Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. Nature Genetics 49: 162–168.

Stewart-Ornstein J, Lahav G. 2017. p53 dynamics in response to DNA damage vary across cell lines and are shaped by efficiency of DNA repair and activity of the kinase ATM. Science Signaling 10: eaah667.

Stewart-Ornstein J, Cheng HW, Lahav G. 2017. Conservation and divergence of p53 oscillation dynamics across species. Cell Systems 5: 410–417.

Sulpice R, Flis A, Ivakov A, Apelt F, Krohn B, Encke B, Abel C, Feil R, Lunn JE, Stitt M. 2014. Arabidopsis coordinates the diurnal regulation of carbon allocation and growth across a wide range of photoperiods. Molecular Plant 7: 137–155.

Takahashi N, Ogita N, Takahashi T, Taniguchi S, Tanaka M, Seki M, *et al.* 2019. A regulatory module controlling stress-induced cell cycle arrest in *Arabidopsis*. eLife 8: e43944.

UN. The millennium development goals report 2015. New York: United Nations, 2015.

Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G. 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science 303: 1003– 1006.

Wei P, Demulder M, David P, Eekhout T, Yoshiyama KO, Nguyen L, Vercauteren I, Eeckhout D, Galle M, De Jaeger G, *et al.* 2021. Arabidopsis casein kinase 2 triggers stem cell exhaustion under Al toxicity and phosphate deficiency through activation of the DNA damage response pathway. The Plant Cell 33, 1361–1380.

Yazdanbakhsh N, Sulpice R, Graf A, Stitt M, Fisahn J. 2011. Circadian control of root elongation and C partitioning in Arabidopsis thaliana. Plant, Cell & Environment 34: 877–894.

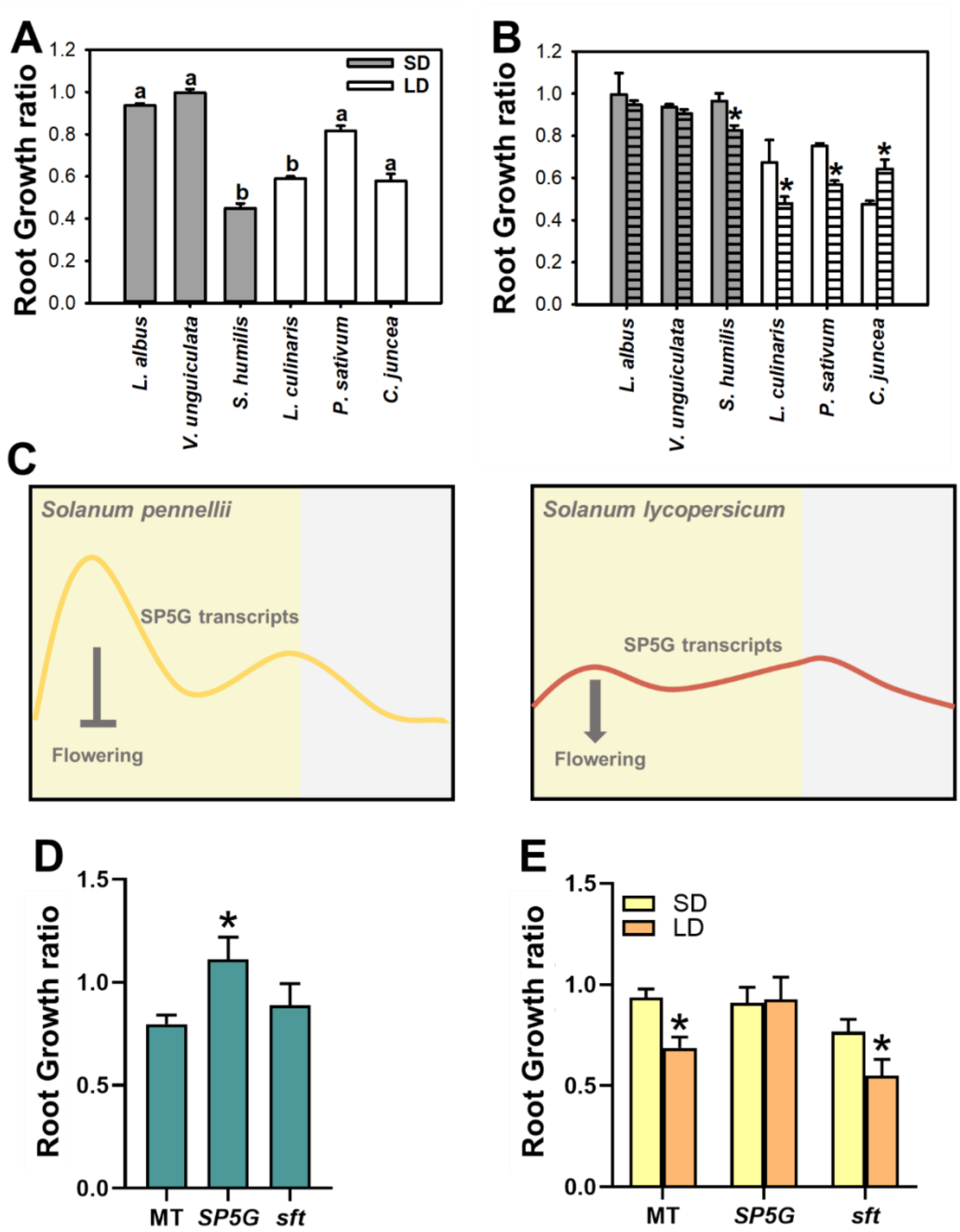

Figure 1. Photoperiod responses modulate root elongation and aluminium (Al) tolerance. (**A**) Root growth ratio was determined by the final root length measured in species from short-days (SD, grey) and long-days (LD, white) plants growing at SD and LD conditions under control conditions (absence of Al). Root elongation was determined in 5-day-old seedlings (*n* = 16 plants for each condition), whereas the growth ratio was calculated as followed: SD root elongation (cm) / LD root elongation (cm). Statistical groups were determined using a Tukey honest significant difference (HSD)

test (*P* <0.05) and are indicated with different letters. (**B**) Root growth ratio indicates the final root elongation measured in plants growing in the absence of Al^{3+} or in the presence of 100 μM Al^{3+} under either SD (clear bar) or LD (hatches bar) conditions. Growth ratio was calculated as follow: SD (-Al) root elongation (cm) / SD (+Al) root elongation (cm) (clear bars); and LD (-Al) root elongation (cm) / LD (+Al) root elongation (cm) (hatches bar). An asterisk (*) indicates different values that were determined by the two-sided Student's *t*-test to be different ($P < 0.05$) between SD and LD. SD species: *Lupinus albus*, *Vigna unguiculata* and *Stylosanthes humilis*; LD species: *Lens culinaris*, *Pisum sativum* and *Crotalaria juncea*. (**C**) In the wild tomato *Solanum pennellii* oscilations on the expression of *SELF-PRUNING 5G* transcripts are observed arresting flowering under LD conditions, which does not occur in the domesticated tomato *Solanum lycopersicum*. (**D**) Root growth ratio was assessed by the final root elongation determined in plants growing at SD or LD under optimal control conditions. Root elongation was determined in 5-day-old seedlings cultivated on hydroponics culture (n = 12 plants for each condition), and the growth ratio was calculated as followed: SD root elongation (cm) / LD root elongation (cm).. Asterisks (*) indicate different mean values that were determined by the two-sided Student's *t*-test to be different ($P < 0.05$) from the wild-type tomato Micro-Tom (MT). (**E**) Root growth ratio indicates the final root elongation measured in plants growing in the absence of Al^{3+} or in the presence of 100 μM Al^{3+} under either SD or LD. This ratio was calculated as follow: SD (-Al) root elongation (cm) / SD (+Al) root elongation (cm) (yellow bars); and LD (-Al) root elongation (cm) / LD (+Al) root elongation (cm) (orange bars). An asterisk (*) indicate values that were determined by the two-sided Student's *t*-test to be different ($P < 0.05$) between growth conditions (SD and LD). Abreviations: MT, *Solanum lycopersicum* cv. Micro-Tom; *SP5G*, *SELF-PRUNING 5G* allele from *Solanum pennellii* introgressed into MT; *SFT*, introgression of a loss-of-function mutation on *SINGLE FLOWER TRUSS*.

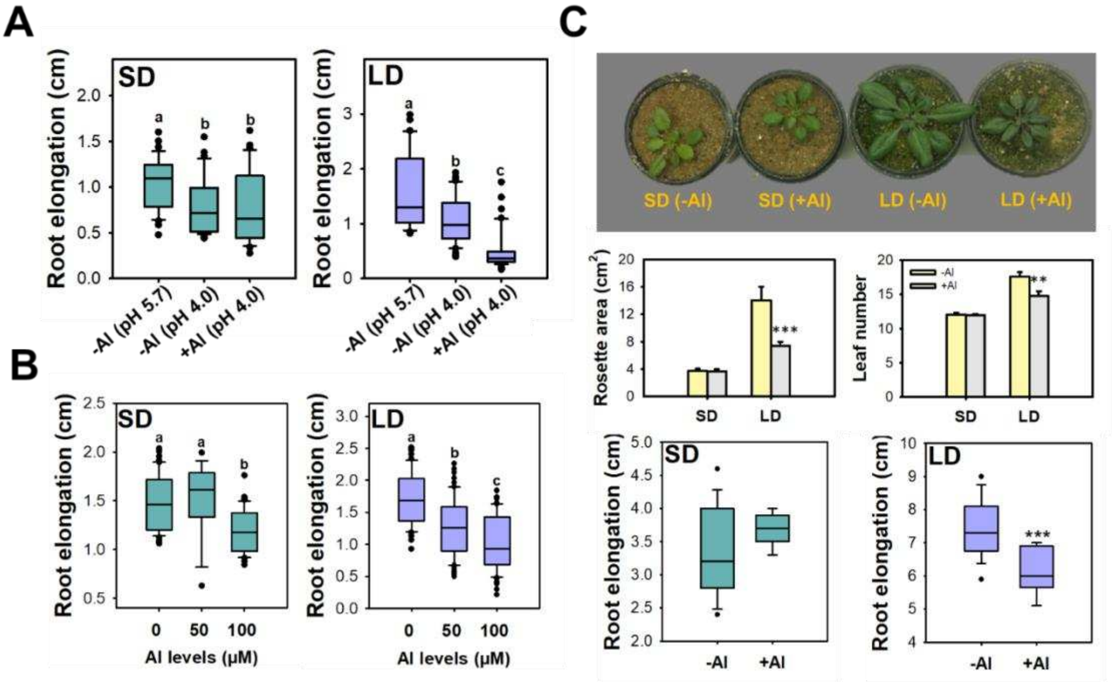

Figure 2. Higher severity of aluminium (Al)-toxicity under long-days (LD) in *Arabidopsis thaliana*. (**A**) Root elongation determined in seedlings growing at pH 5.7 (control) or 4.0 after 10 days, the seedlings were either cultivated with 0 (-Al) or 50 (+Al) µM $AlCl_3$ (n = 60 from three independent experiments). (**B**) Root elongation determined in seedlings growing at differential levels of $AlCl_3$ for 10 days (n = 60 from three independent experiments). Statistical groups were determined using a Tukey honest significant difference (HSD) test (P <0.05) and are indicated with letters. (**C**) Phenotype for Arabidopsis plants under Al treatment. Representative images of 3-week-old, short-day (SD) or long-day (LD) grown plants cultivated on sand in the absence [–Al (0 µM)] or the presence of Al [+Al (50 µM)]. Measurements from rosette area (cm^2), leaf number and root elongation for *A. thaliana* plants cultivated on sand (n = 30). Asterisks indicate values that were determined by Student's *t*-test to be different at P < 0.01 (**) or P < 0.001 (***) between Al levels.

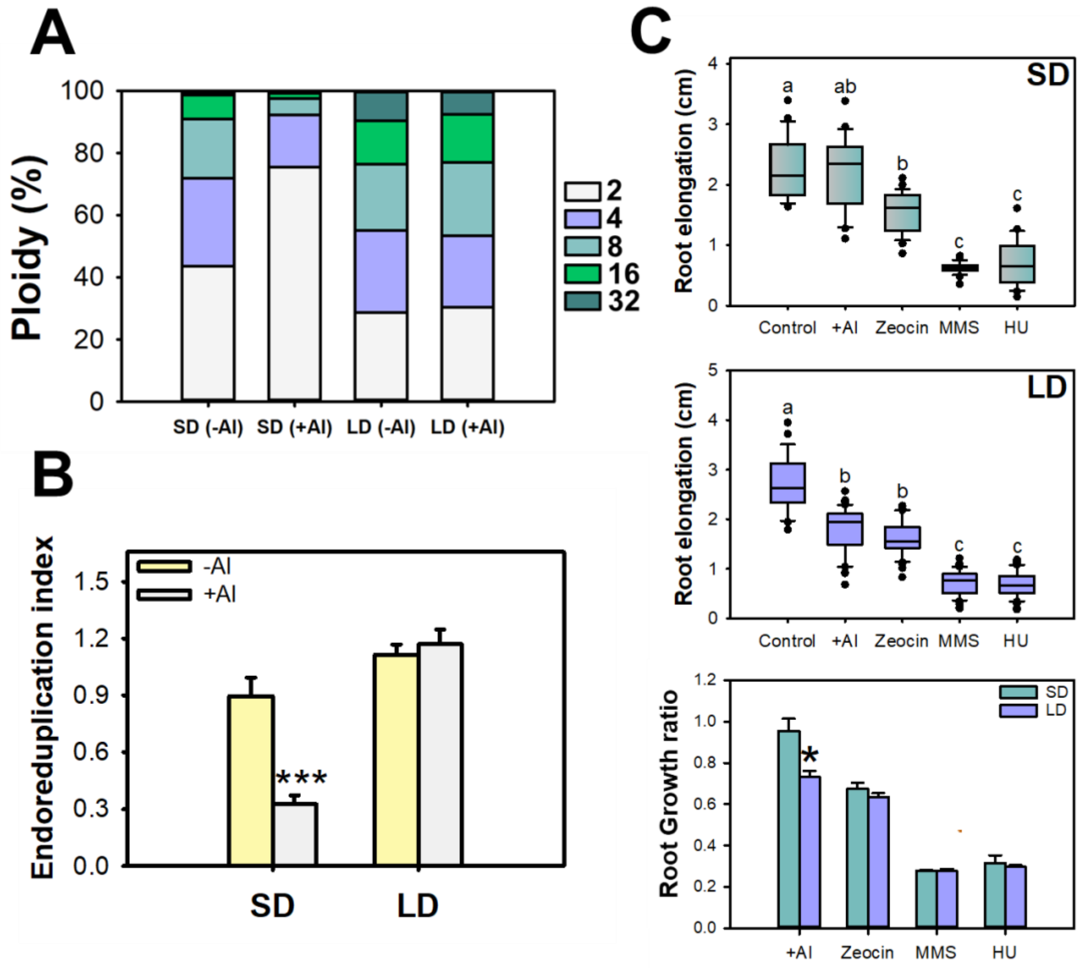

Figure 3. Differential DNA ploidy level and cell cycle regulation modulated by day-length on *Arabidopsis thaliana* seedlings growing under aluminum (Al) stress. DNA ploidy level (%) (**A**) and endoreduplication index (**B**) from root apical meristem (RAM) cells of 10-day-old, short-day (SD)- or long-day (LD) grown seedlings cultivated on –Al (0 μM) or +Al (50 μM). Data represent measurements for five replicates obtained with cells of approximately 30 RAM. (**C**) Seedlings (10-day-old) were submitted to +Al (50 μM), zeocin (5 μM zeocin), methyl methanesulfonate (MMS) (50 ppm) or hydroxyurea (HU) (1 mM), and root elongation (cm) was determined (n = 60 from three independent experiments). RGR (%) indicated root elongation in plants growing under cytotoxic and genotoxic treatments either under SD or LD. Root growth ratio was calculated as followed: SD (-Al) root elongation (cm) / SD (treatment) root elongation (cm) (green bars); and LD (-Al) root elongation (cm) / LD (treatment) root elongation (cm) (blue bars). Asterisks indicate values that were determined by Student's *t*-test to be different at $P < 0.05$ (*) or $P < 0.001$ (***) between Al levels. Statistical groups were determined using a Tukey honest significant difference (HSD) test (P value <0.05) and are indicated with different letters.

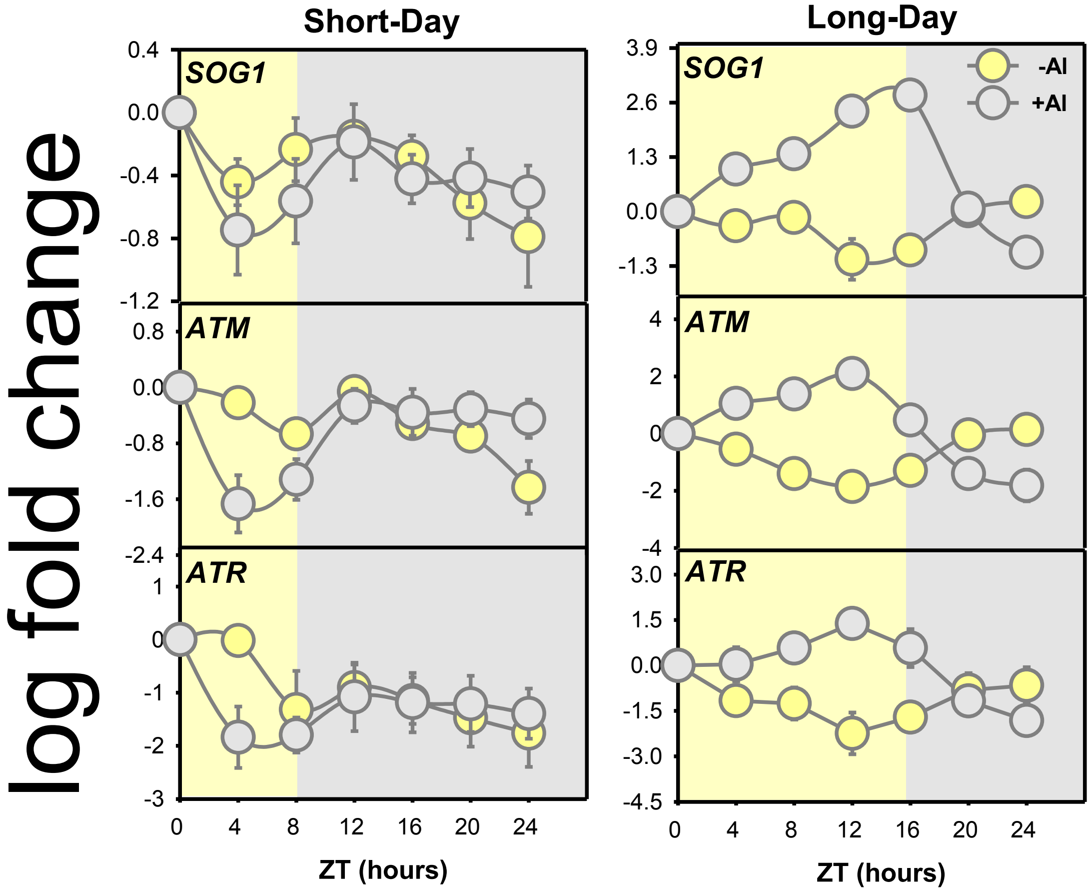

Figure 4. Expression profile of regulators of DNA checkpoints is variable in response to photoperiod and Al. Diurnal oscillations of transcript levels from *SOG1* (*SUPPRESSOR OF GAMMA RESPONSE1*), *ATM* (*ATAXIA TELANGIECTASIA MUTATED)* and *ATR* (*ATAXIA TELANGIECTASIA AND RAD3 RELATED*) were determined in roots of 3-week-old plants of *Arabidopsis thaliana* ecotype Columbia (Col-0) cultivated at –Al (0 µM) or +Al (50 µM) under short-days (SD) and long-days (LD). Light and dark rectangles denote day and night periods in SD (8 h light/16 h dark) and LD (16h light/8h dark), respectively. Data represent the average expression of three biological replicates ± SE.

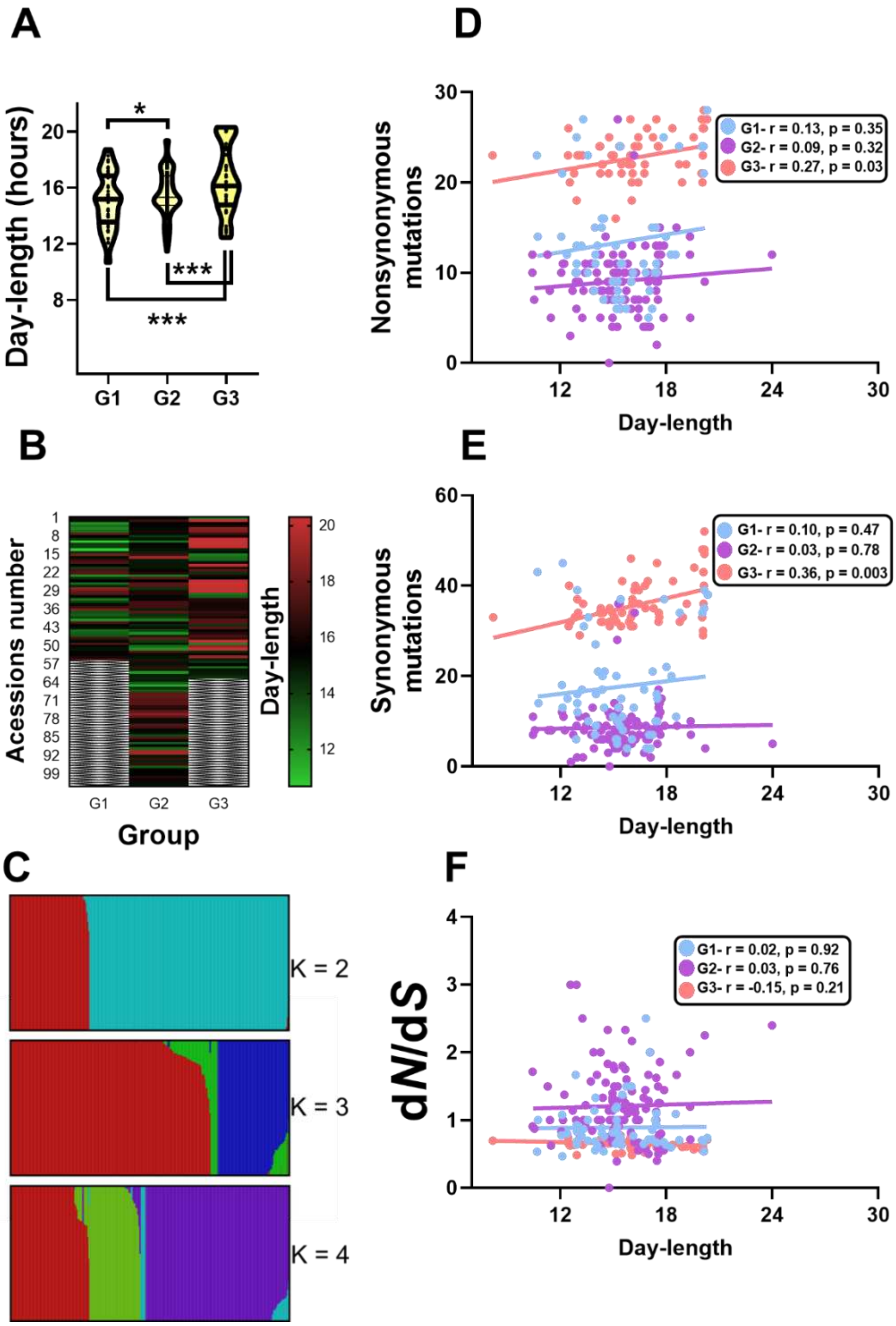

Figure 5. Photoperiods discriminate *Arabidopsis thaliana* accessions according with genetic variants of genes related to cell cycle and DNA checkpoints. 287 *A. thaliana* accessions from centre of origin varying in day-length from a duration of 8:11 to 23:53 were selected based on previous studies. (**A**) Violin-plots representing group analyses were clustered by the Orange Canvas software and a discriminant analysis of principal components, in which three major accession groups G1 (n = 59), G2 (n = 112) and G3 (n = 63) based on distinct day duration were formed. Remarkably, some accessions were not grouped into these three groups. Asterisks indicate values that were determined by Student's *t*-test to be different at $P < 0.05$ (*) or $P < 0.001$ (***) between groups. (**B**) Heat-map highlighting the differential composition of day-length on groups. (**C**) Population structure of *A. thaliana* accessions in

which each vertical bar represents an individual accession with single nucleotide polymorphism (SNP) on genes related to cell cycle and DNA checkpoints. K clusters indicate colours for fractional memberships. Further details concerning genes and accessions used in the analyses can be found in the supplementary material. (**D-F**) Accumulation of non-synonymous and synonymous mutations as well as the ratio of non-synonymous to synonymous fractions (*dN*/*dS*) and its correlations with day-length. By using the POLYMORPH 1001 (https://tools.1001genomes.org/polymorph/), polymorphism was detected on genes *ATAXIA TELANGIECTASIA MUTATED* (*ATM*), *ATAXIA TELANGIECTASIA AND RAD3 RELATED* (*ATR*), and *SUPPRESSOR OF GAMMA RESPONSE1* (*SOG1*) among groups G1, G2, and G3.

Supplementary Information for

Photoperiod shapes aluminium tolerance in plants

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This PDF file includes:

Supplementary text

Figures S1 to S18

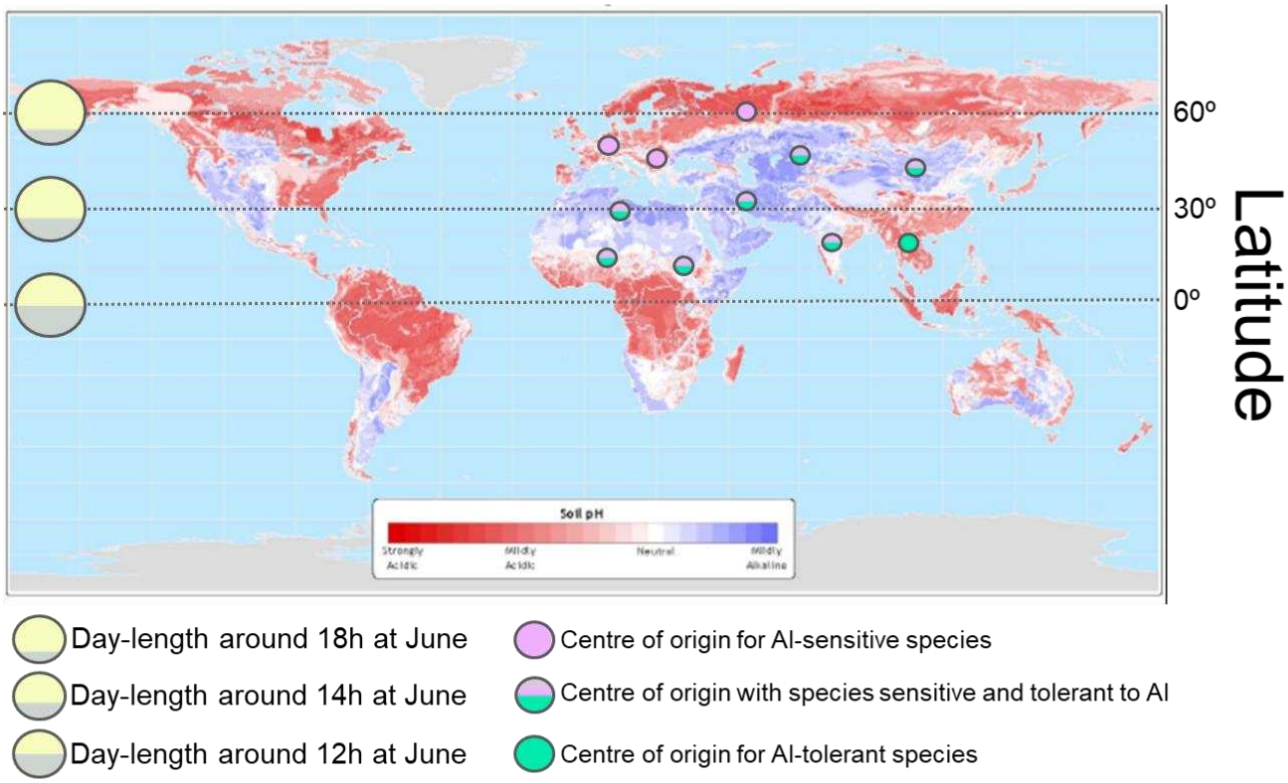
Tables S1 to S3

SI References

Other supplementary materials for this manuscript include the following:

Datasets S1 to S6

File S1

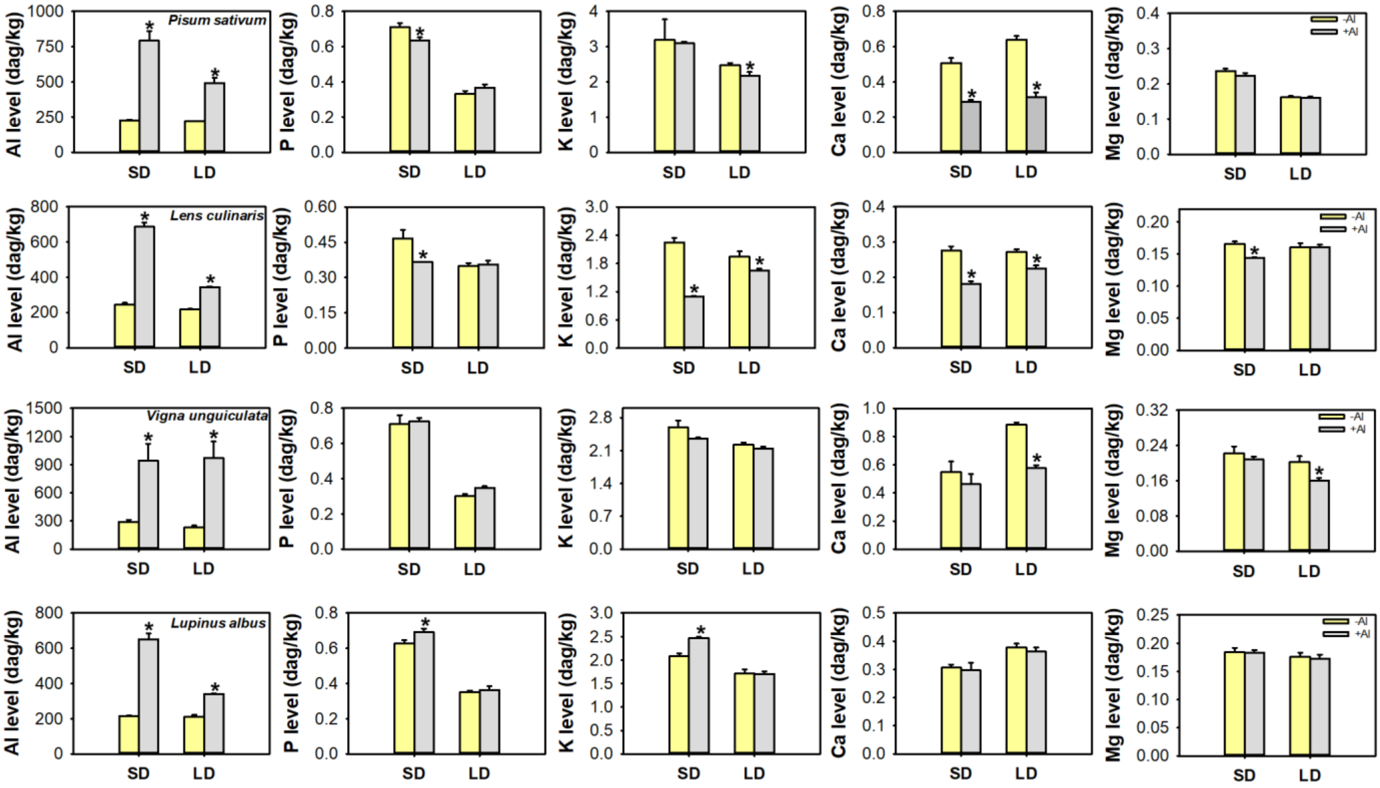


Supplementary Figure S1. Hypothetical scheme highlighting possible interfaces between photoperiod and aluminium (Al) tolerance. Apparently, the soil pH at the centre of origin of most plant species is not capable to adequately explain the differences in Al tolerance across the plant kingdom. By looking at the centre of origin of the major cultivated plants, Al tolerance is seemingly lost in species that originated further way from the Equator line. Therefore, photoperiod could explain, at least partially, the differential Al tolerance across the plant kingdom, since many origin centres harbouring Al tolerant plants are situated on regions with alkaline soils, which suggests the pivotal importance of photoperiod to select Al tolerance. Interestingly, centre of origin with higher latitudes, where day-length at summer solstice (June) reach 18 hours or more, are characterized predominantly by the presence of Al sensitive species. Taken together, we hypothesized that photoperiod can modulates plant responses to Al. The map is an adaptation from the Atlas of the Biosphere (Center for Sustainability and the Global Environment, University of Wisconsin, Madison) (https://nelson.wisc.edu/sage/data-and-models/atlas/maps.php).

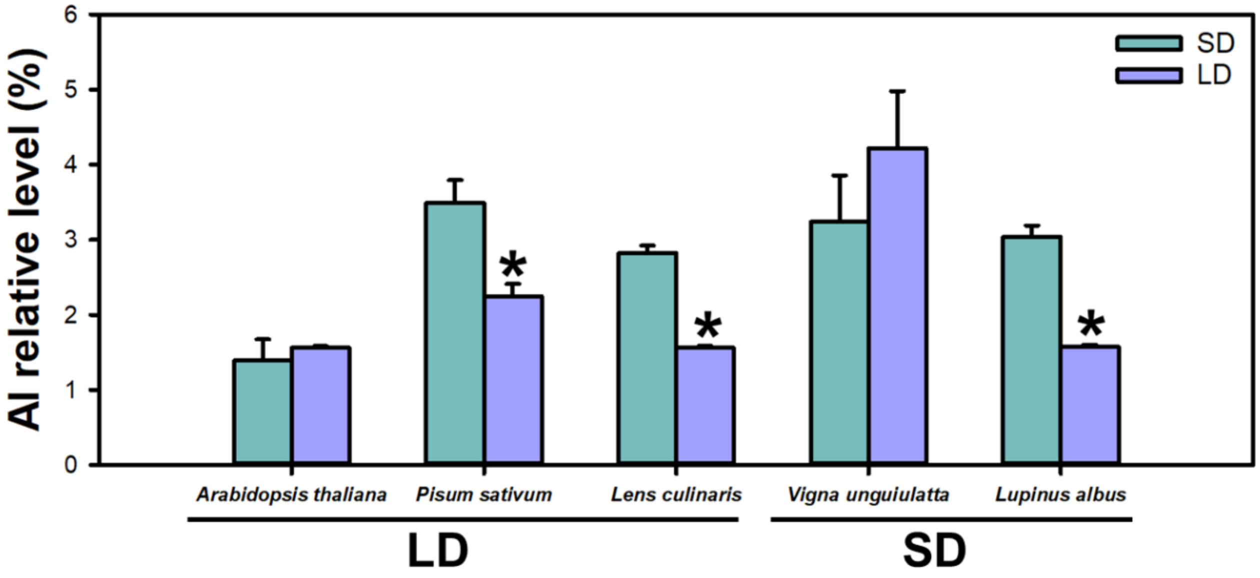
Supplementary Table S1. Relationship between aluminium (Al) tolerance levels and photoperiodism. We observed, across the plant kingdom, plants exhibiting the growing-season at origin centre with short-days (green), neutral-days (orange), and long-days (red). Long-day plants apparently exhibit a tendency to be more sensitive to Al, while short-day plants have propensity to display lower yield alterations under Al stress (higher tolerance). This table was constructed following Meda and Furlani (2005) and Garcia-Oliveira *et al.* (2016).

Al tolerance degree	Crop species*
Highly sensitive	**Alfalfa**, **barley**, **carrot**, **durum wheat**, **lettuce** and **pea**
Sensitive	**Oat** and **Wheat**
Moderately sensitive	**Cabbage**, **maize** and **sorghum**
Moderately tolerant	**Rice** and **rye**
Tolerant	**Soybean** and **pigeon pea**
Highly tolerant	**Tea**, **buckwheat**, **brachiaria**, **Vigna unguiculata**, and **Stylosanthes**

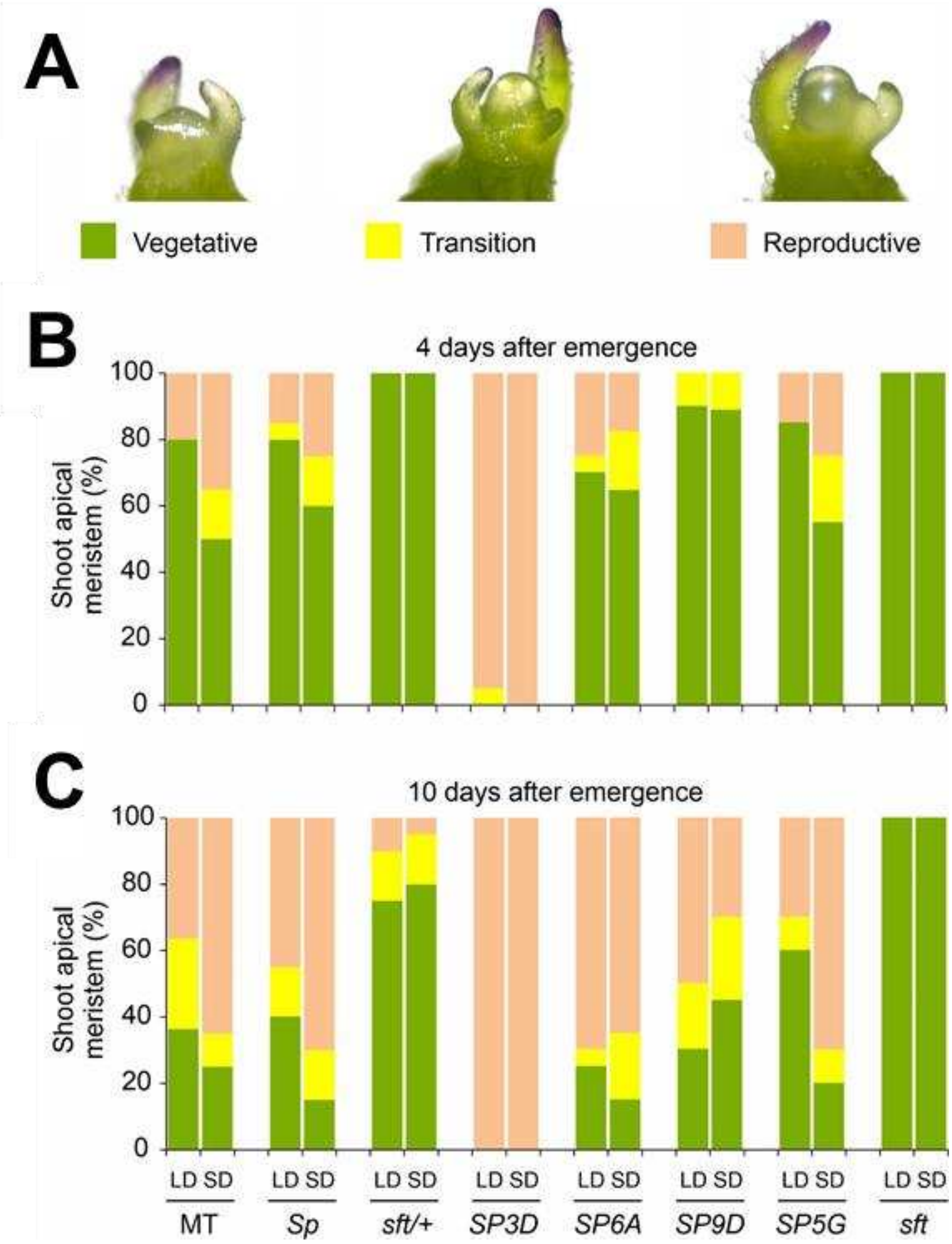
Crop species and their scientific names are as follow: Alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), *Brachiaria spp.*, buckwheat (*Fagopyrum esculentum*), cabbage (*Brassica oleracea* var. capitata), carrot (*Daucus carota* subsp. sativus), durum wheat (*Triticum durum*), lettuce, (*Lactuca sativa*) maize (*Zea mays*), oat (*Avena sativa*), pea (*Pisum sativum*), pigeon pea (*Cajanus cajan*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*), soybean (*Glycine max*), *Stylosanthes spp.*, Vigna unguiculata and wheat (*Triticum aestivum*).



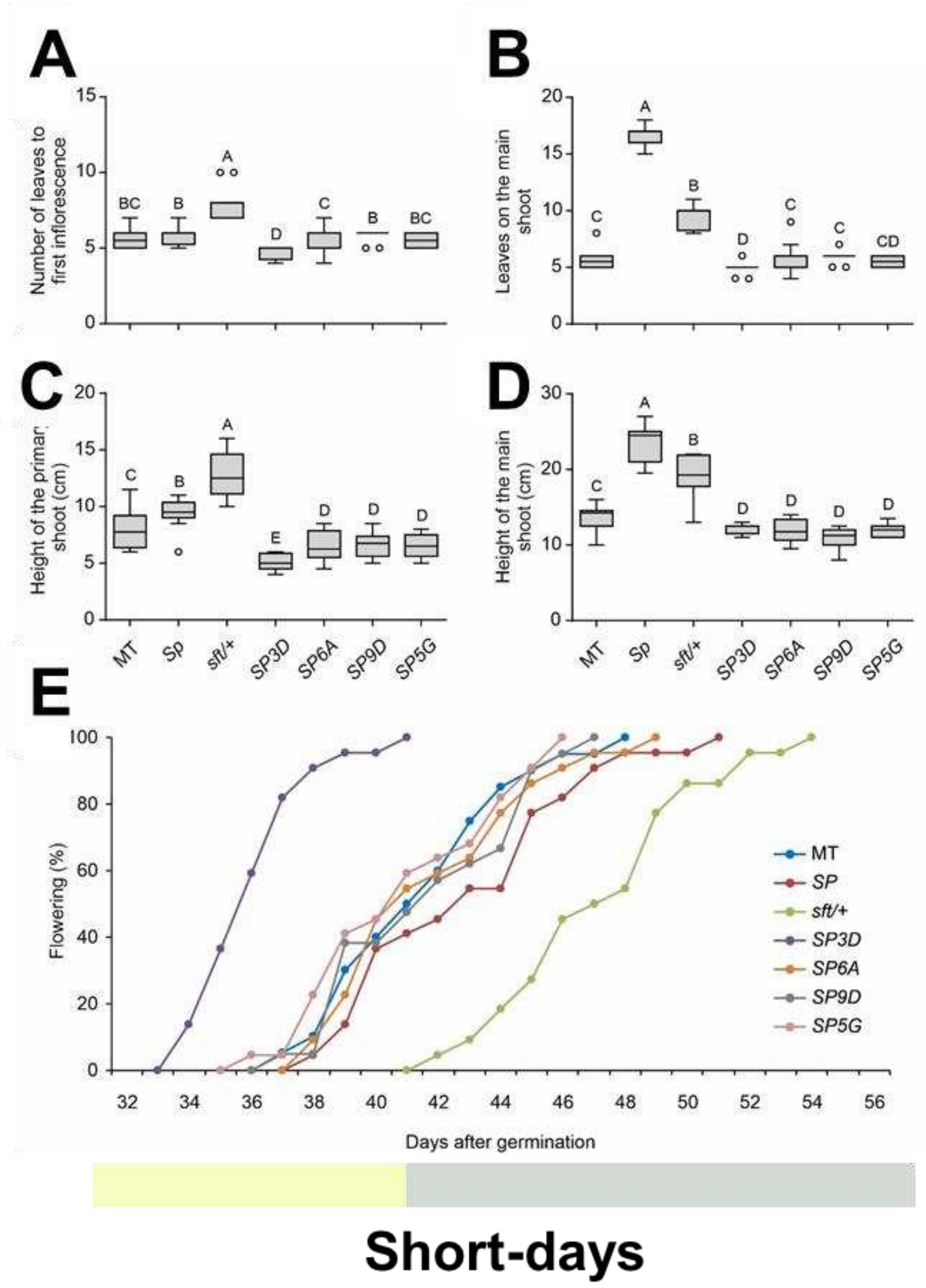
Supplementary Figure S2. Differential nutritional content in response to changes in photoperiod under Aluminium (Al) stress. The levels of Al, Phosphorus (P), Potassium (K), Calcium (Ca) and Magnesium (Mg) were determined on root samples (n = 4 samples for each condition) of plants growing at short-days (SD) or long-days (LD). Data represent nutrient levels determined in plants growing in the absence (-Al, yellow) or the presence 100 μM Al (+Al, grey) after five days on hydroponics culture. An asterisk (*) indicate values that were determined by the two-sided Student's *t*-test to be different ($P < 0.05$) between -Al and +Al.



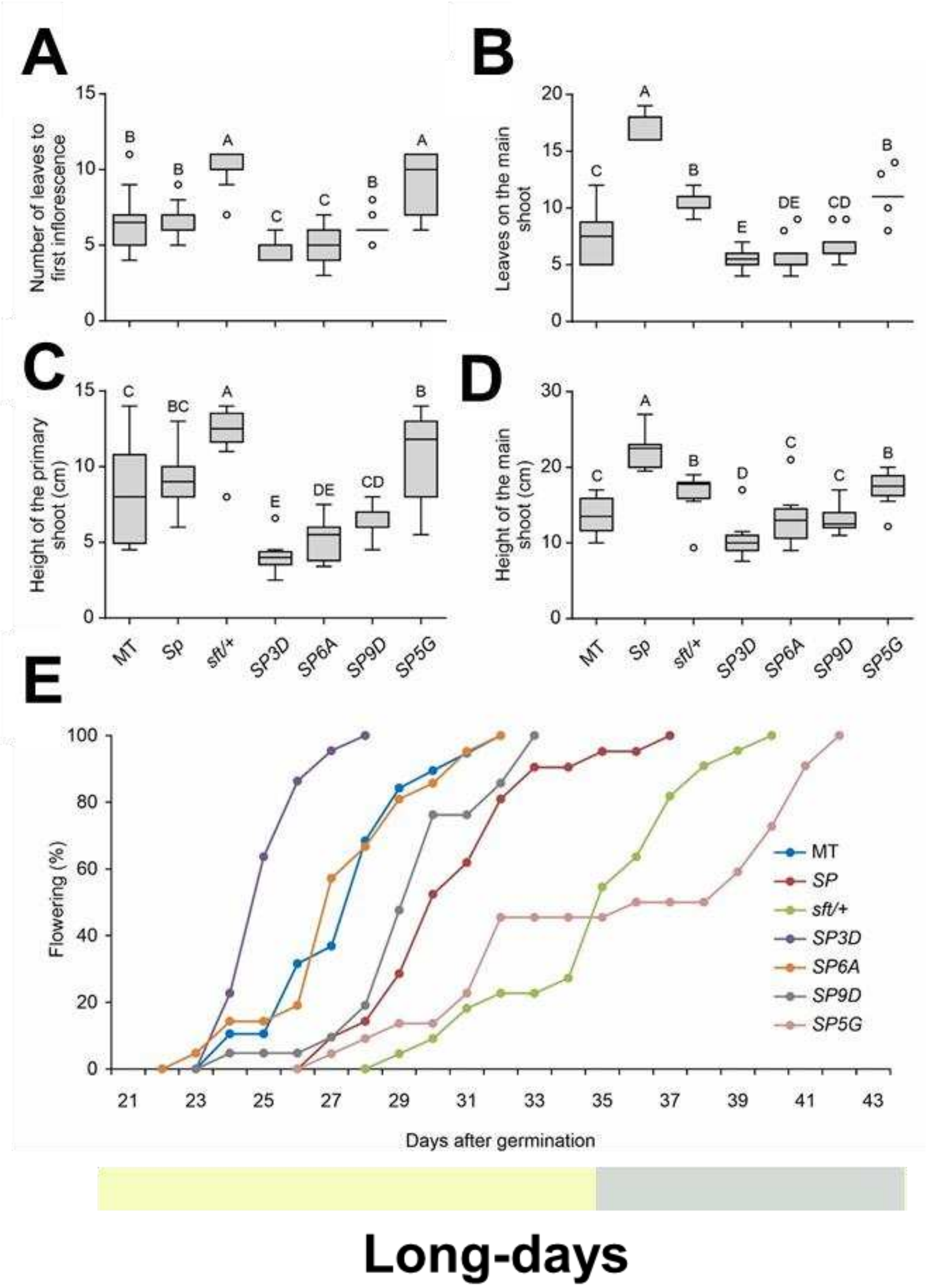
Supplementary Figure S3. Aluminium (Al) relative levels (%) is differentially affected by Short-days (SD) and long-days (LD). The levels of Al were determined on root samples (n = 4 samples for each condition) of plants growing at short-days (SD) or long-days (LD) and are additionally shown in Supplemental Figure S3. The relative levels were determined as follow: SD (-Al) Al levels / SD (+Al) Al levels (green bars); and LD (-Al) Al levels / LD (+Al) Al levels (blue bars). The plants were cultivated the absence of Al^{3+} (-Al) or in the presence of 100 µM Al^{3+} (+Al) under SD or LD by five days on hydroponics culture. An asterisk (*) indicate values that were determined by the two-sided Student's *t*-test to be different ($P < 0.05$) between SD and LD.



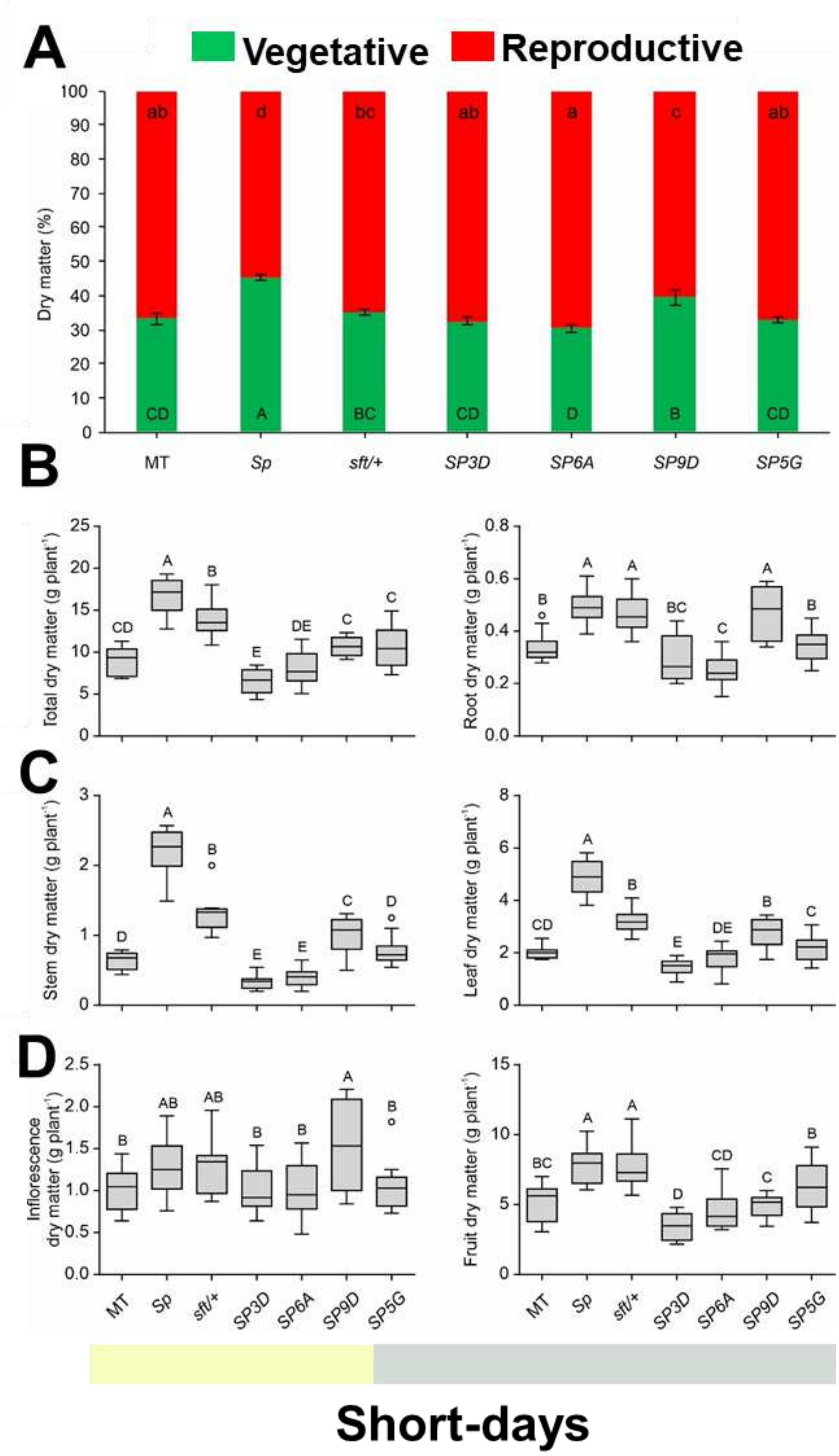
Supplementary Figure S4. Day-length regulates differently genes of the *SELF-PRUNING* family to orientate shoot apical meristem (SAM) transition. **A**, Representation of meristem maturation stages of SAM, vegetative (left), transition (center) and reproductive (right). **B-C**, SAM maturation at 4 (B) and 10 (C) days after germination on short (SD) and long (LD) days (n= 17-20 seedlings). Abbreviations: Micro-Tom (MT), *Self-pruning* (*Sp*), *Single flower truss* (*sft*/+ and *sft*), *Solanum pennellii* alleles *SELF-PRUNING 3D*, *6A*, *9D*, and *5G* (*SP3D*, *SP6A*, *SP9D*, and SP5G).



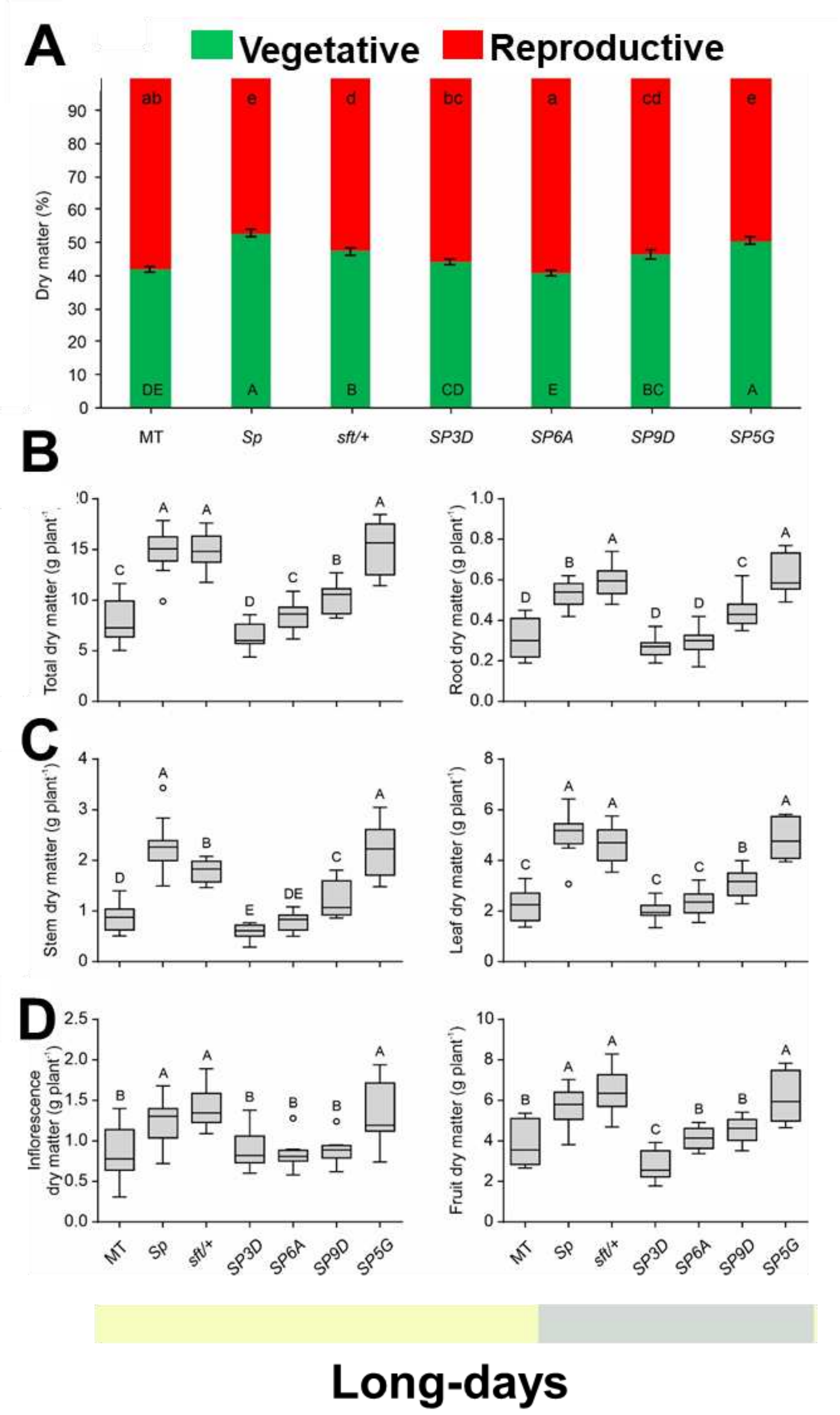
Supplementary Figure S5. Phenotype of tomato genotypes carrying distinct alleles from the *SELF-PRUNING* gene family under short-days. A, leaf number until the first inflorescence. **B**, leaf number on the main shoot. **C**, height of the primary shoot, or until the first inflorescence insertion. **D**, height of the main shoot. **E**, flowering time in days after germination. The values represent 20-22 plants per genotype, and statistical groups were determined using a Tukey honest significant difference (HSD) test (*P* <0.05) and are indicated with different letters.



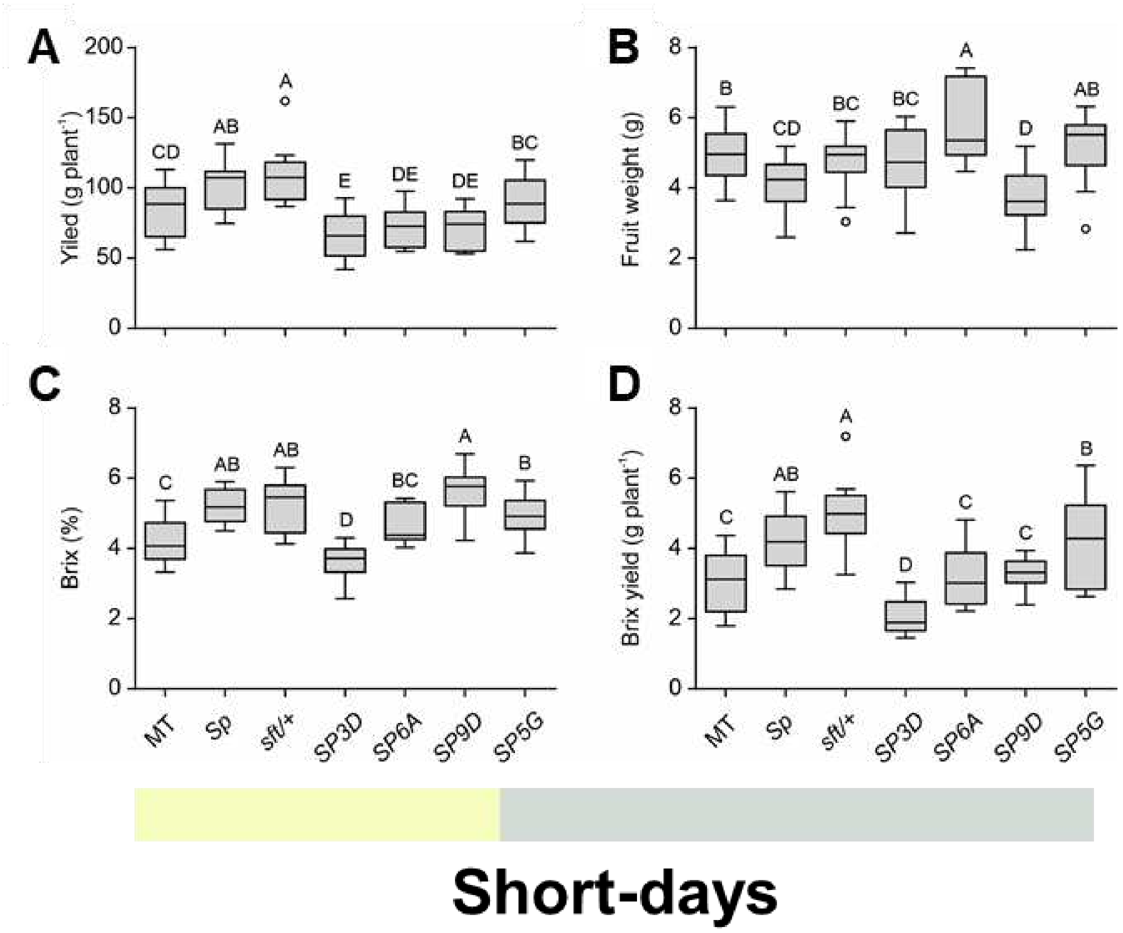
Supplementary Figure S6. Phenotype of tomato genotypes carrying distinct alleles from the SELF-PRUNING gene family under long-days. A, leaf number until the first inflorescence. **B**, leaf number on the main shoot. **C**, height of the primary shoot, or until the first inflorescence insertion. **D**, height of the main shoot. **E**, flowering time in days after germination. The values represent 20-22 plants per genotype, and statistical groups were determined using a Tukey honest significant difference (HSD) test (*P* <0.05) and are indicated with different letters.



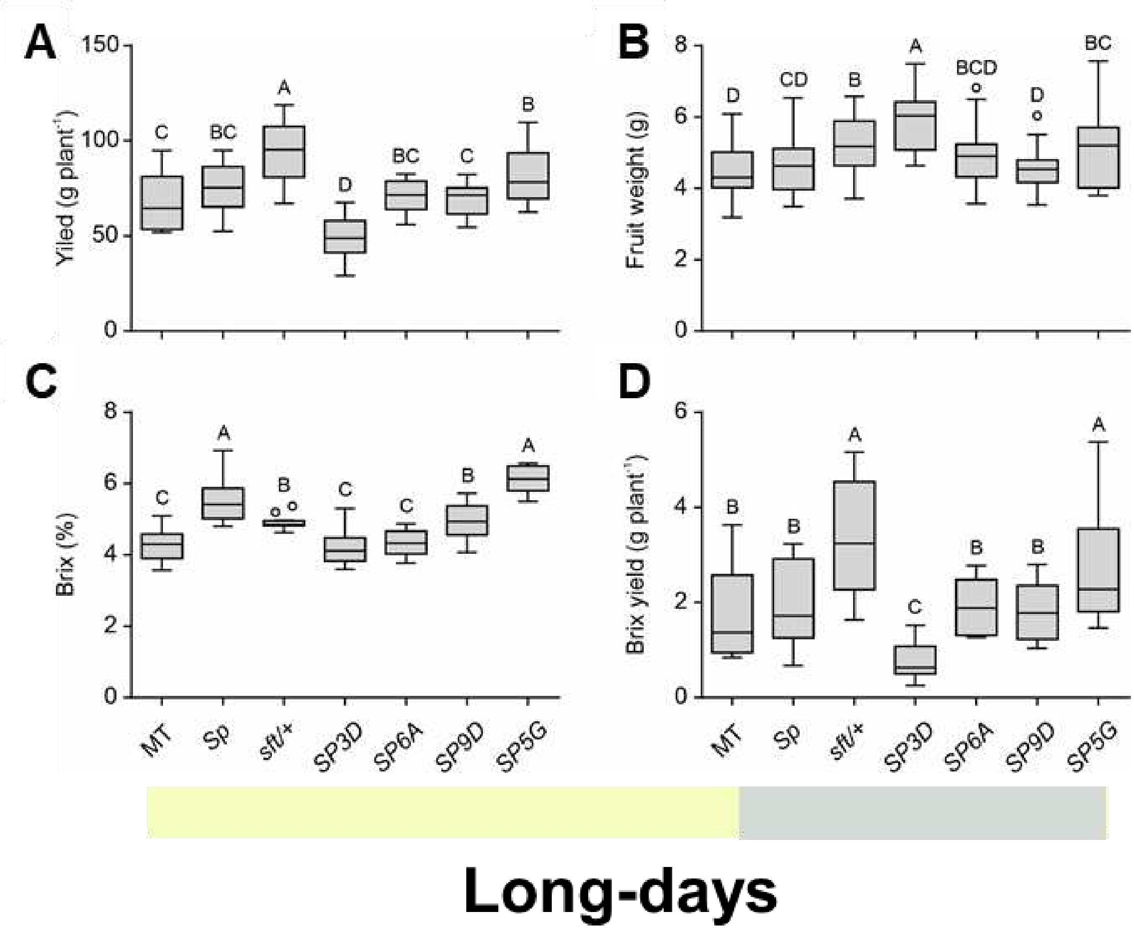
Supplementary Figure S7. Vegetative to reproductive balance on tomato genotypes carrying distinct alleles from the *SELF-PRUNING* gene family under short-days. A, dry matter (%) distribution between vegetative (roots, stem and leaves) and reproductive (inflorescences, flowers and fruits) organs. **B**, total and root dry matter of plants. **C**, shoot and leaf dry matter of plants. **D**, inflorescence and fruit dry matter of plants. The values were obtained from represent 8-12 plants per genotype, and statistical groups were determined using a Tukey honest significant difference (HSD) test (*P* <0.05) and are indicated with different letters.



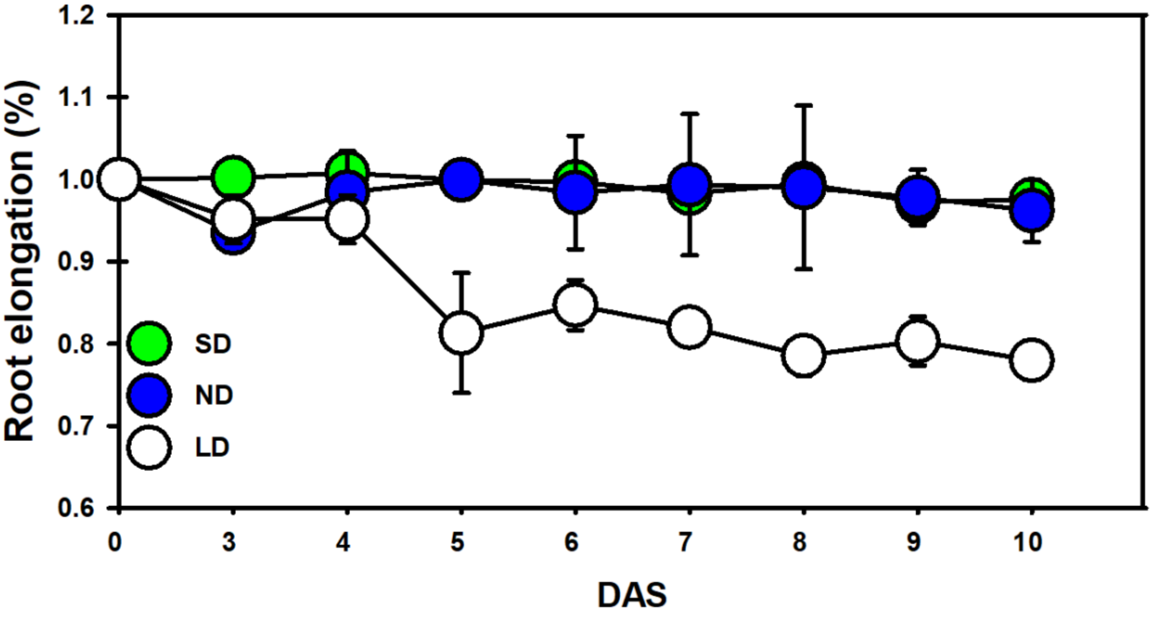
Supplementary Figure S8. Vegetative to reproductive balance on tomato genotypes carrying distinct alleles from the SELF-PRUNING gene family under long-days. **A**, dry matter (%) distribution between vegetative (roots, stem and leaves) and reproductive (inflorescences, flowers and fruits) organs. **B**, total and root dry matter of plants. **C**, shoot and leaf dry matter of plants. **D**, inflorescence and fruit dry matter of plants. The values were obtained from represents 8-12 plants per genotype, and statistical groups were determined using a Tukey honest significant difference (HSD) test (*P* <0.05) and are indicated with different letters.



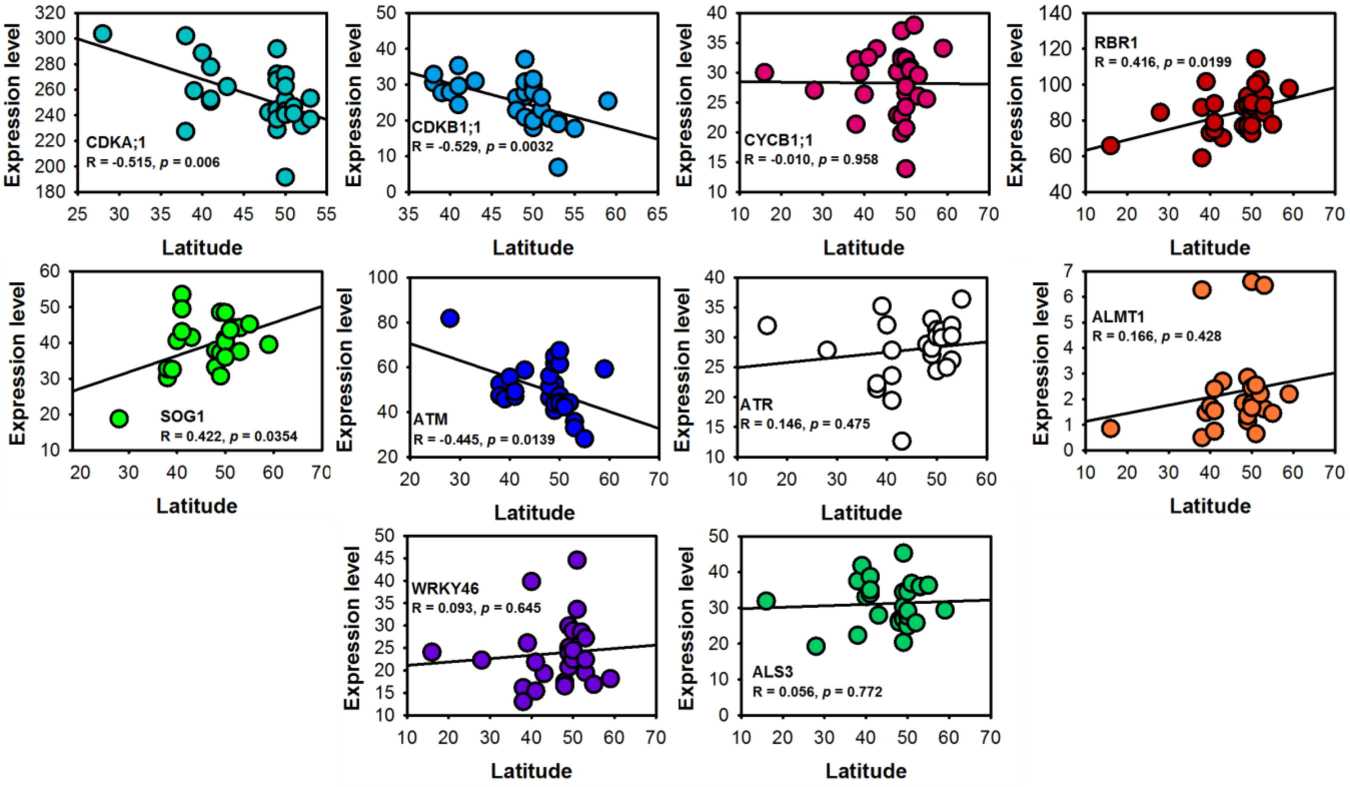
Supplementary Figure S9. Tomato productivity traits under short-days in tomato genotypes carrying distinct alleles from the *SELF-PRUNING* gene family. A, fruit production per plant (yield). **B**, mature fruit weight. **C**, percentage of total soluble solids for 30 fruits (Brix (%)). **D**, relationship between Brix (%) and mature fruits at 127 days after germination. The data were obtained from 8-12 plants per genotype, and statistical groups were determined using a Tukey honest significant difference (HSD) test (*P* <0.05) and are indicated with different letters.



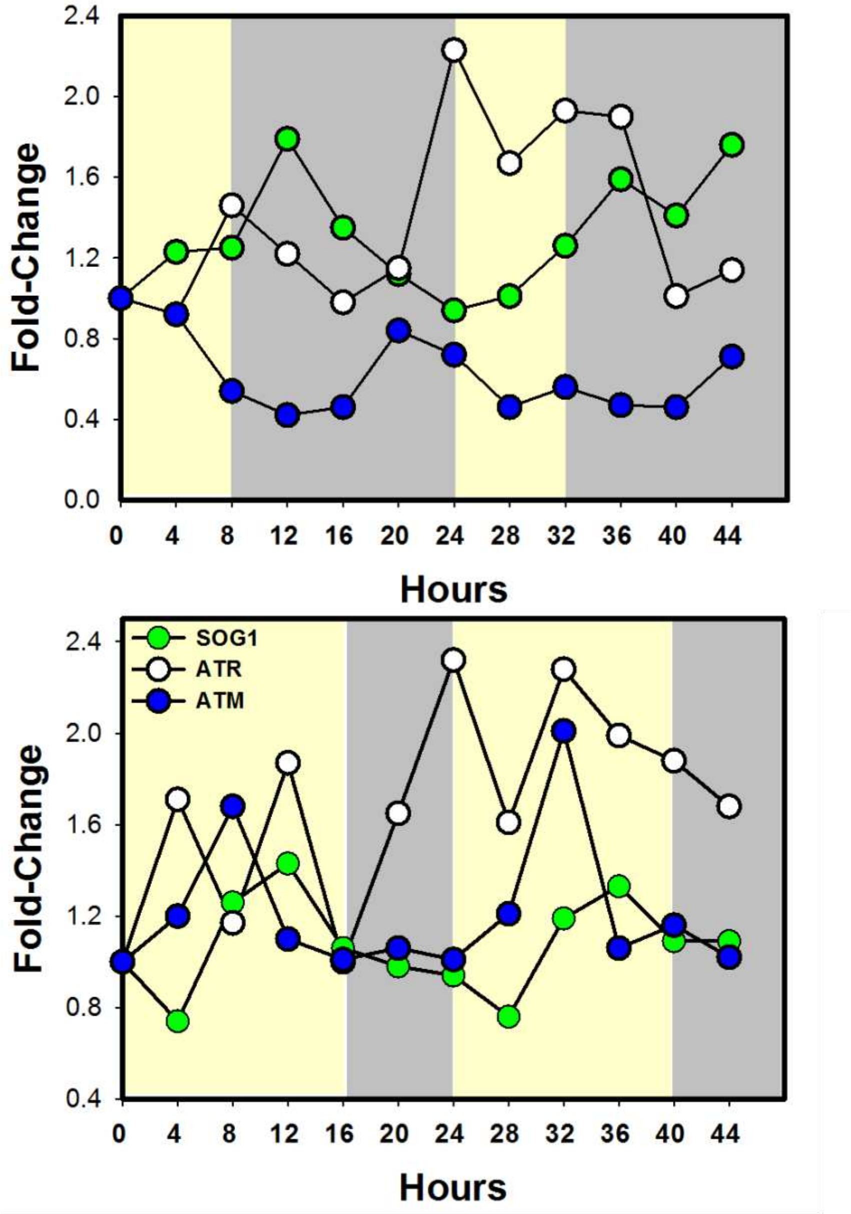
Supplementary Figure S10. Tomato productivity traits under long-days in tomato genotypes carrying distinct alleles from the SELF-PRUNING gene family. A, fruit production per plant (yield). **B**, mature fruit weight. **C**, percentage of total soluble solids for 30 fruits (Brix (%)). **D**, relationship between Brix (%) and mature fruits at 127 days after germination. The data were obtained from 8-12 plants per genotype, and statistical groups were determined using a Tukey honest significant difference (HSD) test (*P* <0.05) and are indicated with different letters.



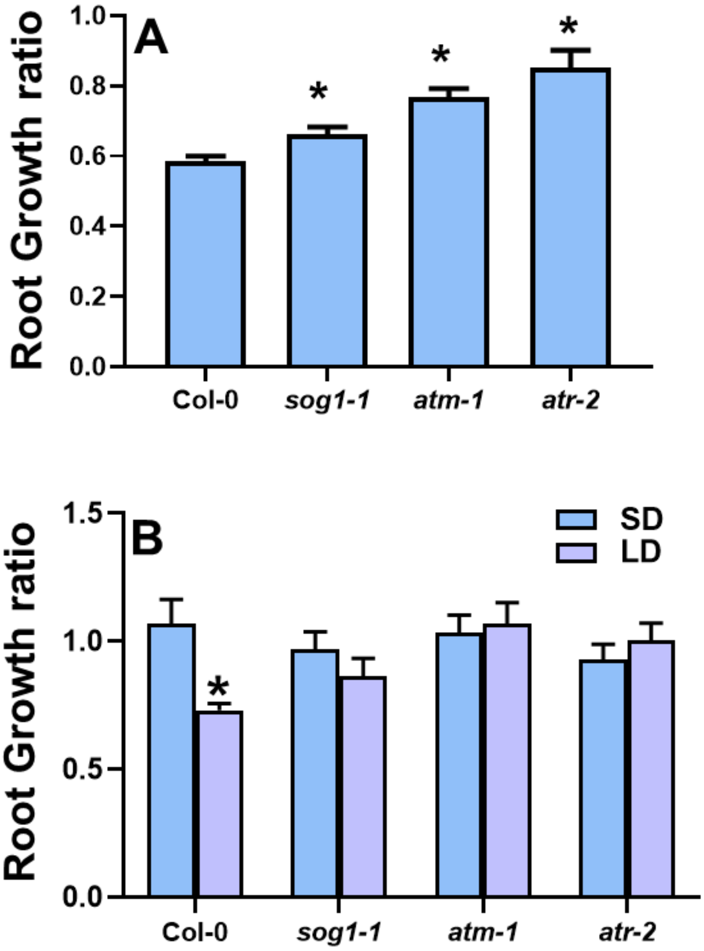
Supplementary Figure S11. Root elongation under aluminium (Al) stress is associated with day-length. Root elongation (%) in *Arabidopsis thaliana* ecotype Columbia (Col-0) was determined during 10days in seedlings growing at pH 4.0 in the absence of Al^{3+} (-Al) or in the presence of 50 µM Al^{3+} (+Al) under at either short-day (SD), neutral-day (ND), or long-day (LD) conditions (n = 60 from three independent experiments). Root elongation (%): -Al-root elongation (cm) / +Al-root elongation (cm).



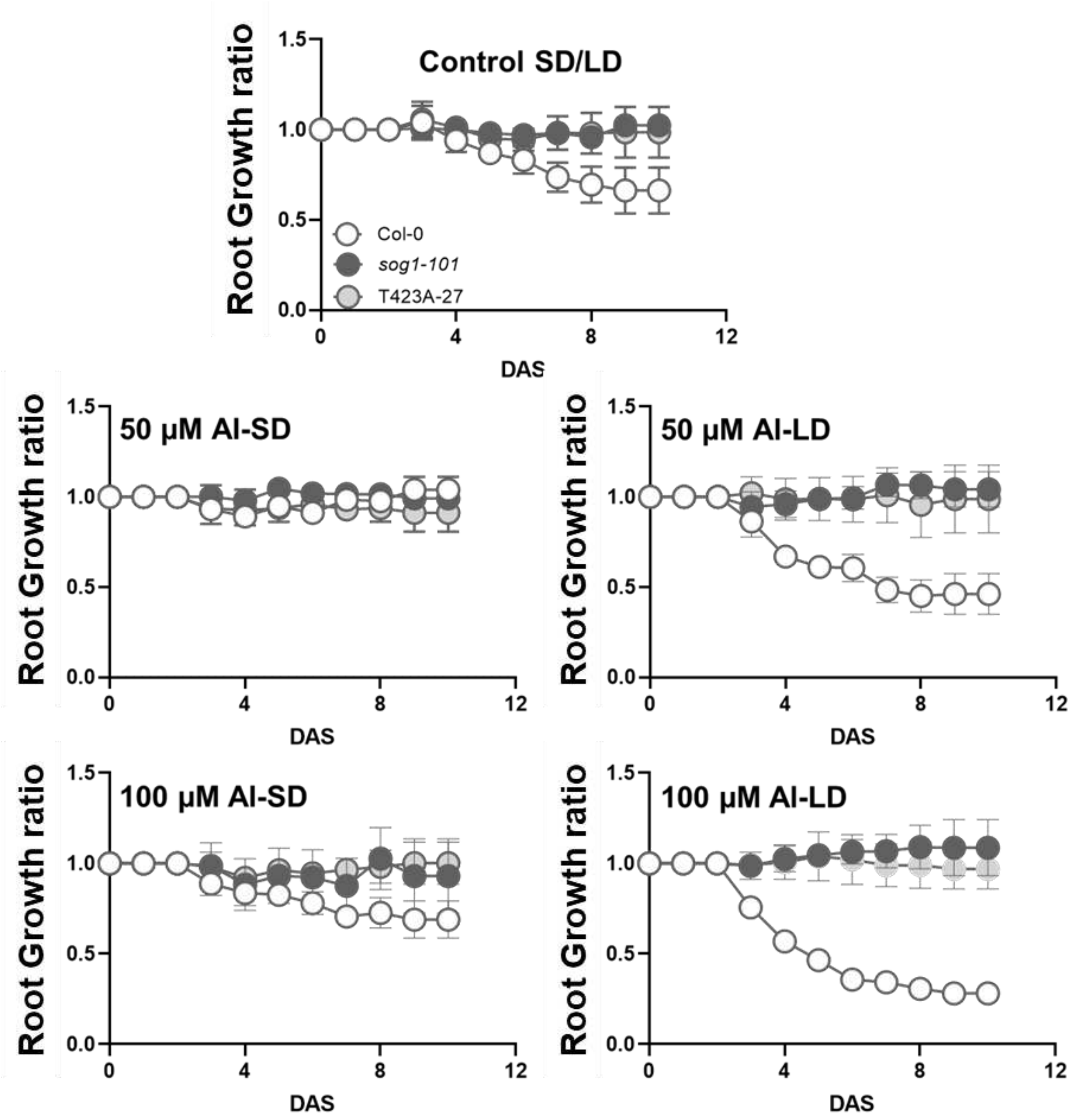
Supplementary Figure S12. Pearson Correlations between *in silico* gene expression and latitudinal variation. Data represent analyses using 30 *Arabidopsis thaliana* accessions with centre of origin from contrasting latitude (http://bar.utoronto.ca/). Abbreviations: *CDKA*;1 (Cyclin-*dependent* kinase *A;1*); *CDKB1;1* (Cyclin-*dependent* kinase *B1;1*); *CYCB1;1* (*Cyclin B1;1*); *Retinoblastoma-related 1* (*RBR1*); *SOG1* (*SUPPRESSOR OF GAMMA RESPONSE1*), *ATM* (*ATAXIA TELANGIECTASIA MUTATED*); *ATR* (*ATAXIA TELANGIECTASIA AND RAD3 RELATED*); *ALMT1* (*Aluminum-activated malate transporter 1*); *WRKY46* (*WRKY transcription factor 46*); *ALS3* (*ALUMINUM SENSITIVE 3*).



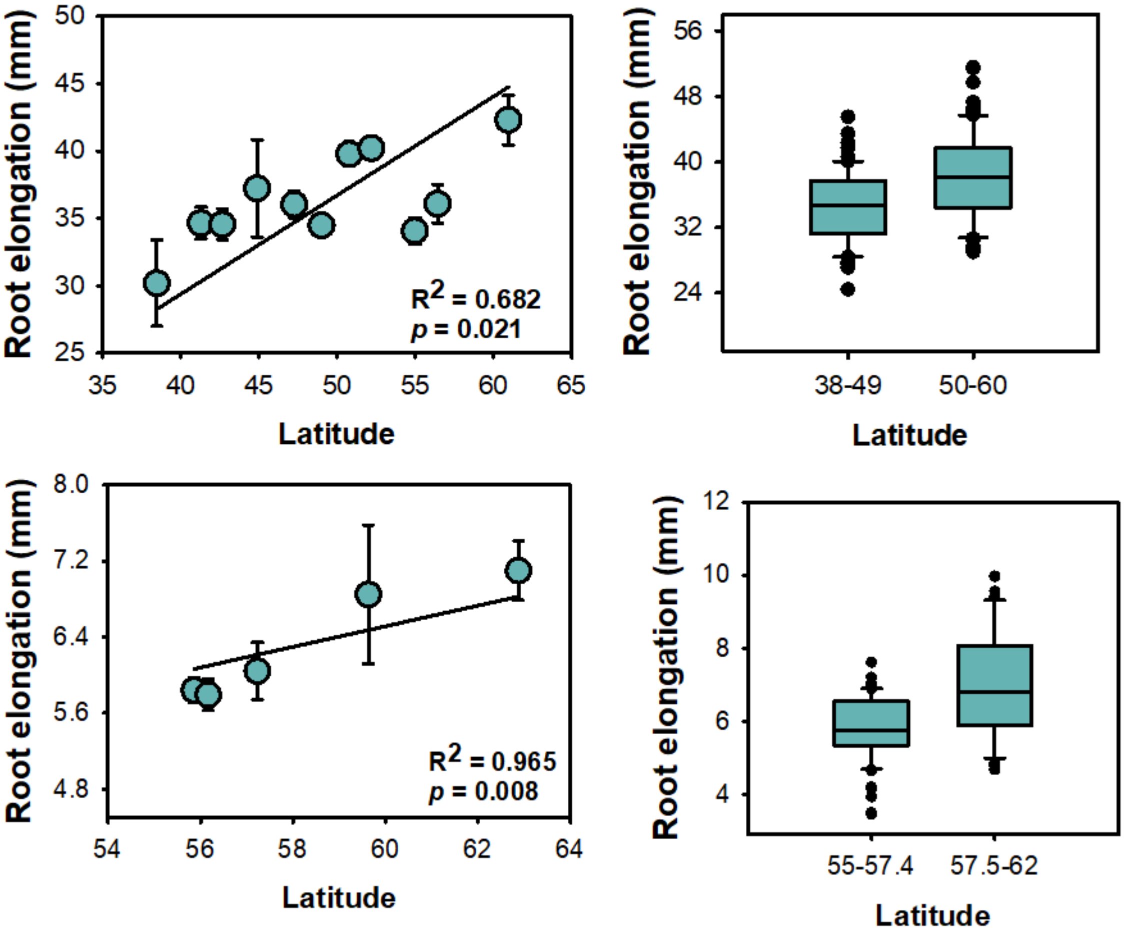
Supplementary Figure S13. ***In silico*** **gene expression for regulators of DNA checkpoints under different photoperiod length.** *In silico* transcripts profile for *SOG1* (*SUPPRESSOR OF GAMMA RESPONSE1*), *ATM* (*ATAXIA TELANGIECTASIA MUTATED*), and *ATR* (*ATAXIA TELANGIECTASIA AND RAD3 RELATED*) were obtained using the publicly data available at the (http://bar.utoronto.ca/). Light and dark rectangles denotes day and night periods under short-days (SD) and long-days (LD), respectively.



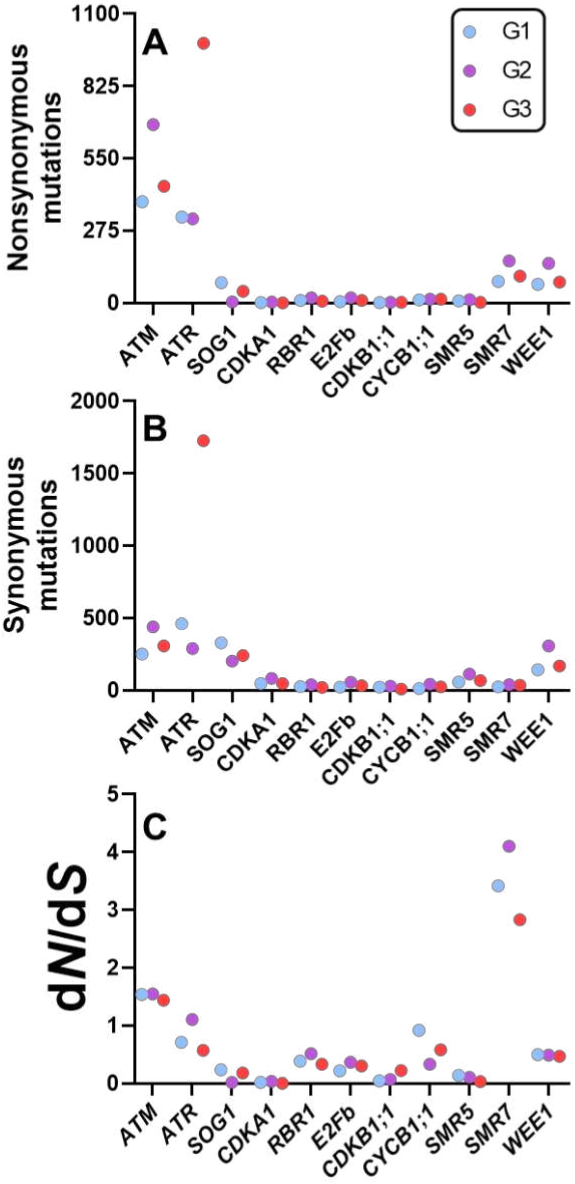
Supplementary Figure S14. DNA checkpoint and endoreduplication related genes are implicated on aluminium (Al) tolerance modulated by day-length. A, Root growth ratio was determined by the final root elongation measured in plants growing at either short-days (SD) or long-days (LD) under control conditions (absence of Al). Root elongation was determined in 10-day-old seedlings cultivated on MS medium, and growth ratio was calculated as followed: SD root elongation (cm) / LD root elongation (cm) (n = 40 plants for each condition). An asterisk (*) indicate values that were determined by the two-sided Student's *t*-test to be different ($P < 0.05$) to the *Arabidopsis thaliana* wild type ecotype Columbia-0 (Col-0). **B** Root growth ratio describes measurements of the root elongation in wild-type and *A. thaliana* mutants plants for DNA-repair and endoreduplication related genes growing in the absence of Al^{3+} or in the presence of 50 μM Al^{3+} under either SD or LD. RGR (%) was calculated as follow: SD (-Al) root elongation (cm) / SD (+Al) root elongation (cm) (blue bars); and LD (-Al) root elongation (cm) / LD (+Al) root elongation (cm) (orange bars) (n = 40 plants for each condition). An asterisk (*) indicate values that were determined by the two-sided Student's *t*-test to be different ($P < 0.05$) between SD and LD. Abbreviations: *sog1-1* (*Suppressor of Gamma Response1-1*), *atm1* (*Ataxia T Telangiectasia Mutated-1*), *atr-2* (*Ataxia Telangiectasia and Rad3 Related-2*).



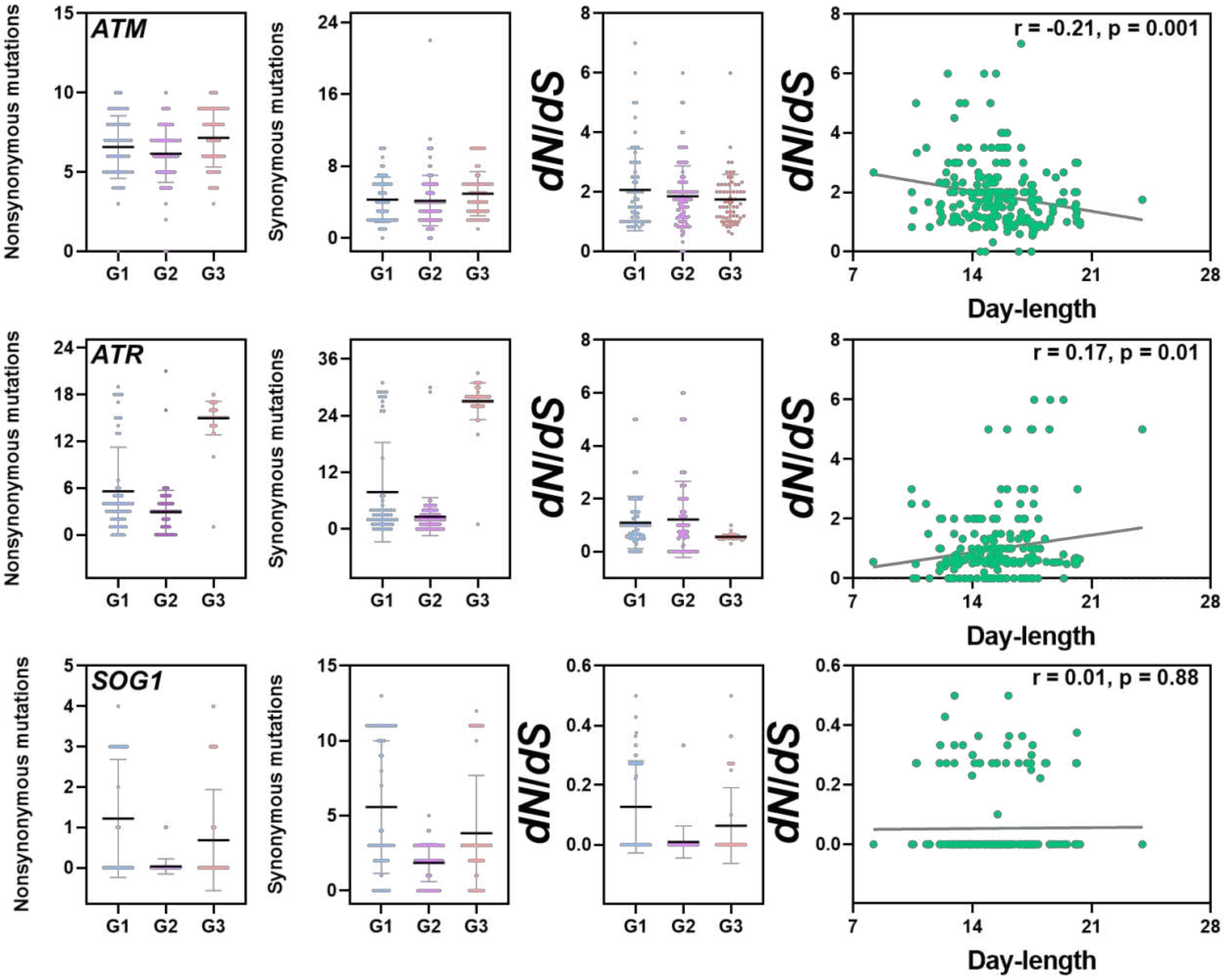
Supplementary Figure S15. Aluminium tolerance is dependent of the circadian clock related protein casein kinase 2 (CK2). Wild-type (Col-0), s*og1* knockout mutant *sog1-101* and phospho-mutant (*T423A-27*) on *sog1* gene, site phosphorylated by CK2. Relative growth rate (RGR -%) was determined in seedlings with 10-old-days growing at pH 4.0 in the absence of $AlCl_3$ or in the presence of 50 or 100 µM $AlCl_3$ under either short-days (SD) or long-days (LD) (n = 5, with 6 plants in each repeat) during 10 days after showing (DAS). Root growth ratio: -Al-root elongation (cm) / +Al-root elongation (cm) Abbreviations: *sog1-1* (*Suppressor of Gamma Response1-1*); SD, short-days; LD, long-days; DAS, days after sowing.



Supplementary Figure S16. Relationships between root elongation and latitudinal variation in *Arabidopsis thaliana* accessions. A-B, represent data from Ristova *et al.* (2018). **C-D**, represent data from Satbhai *et al.* (2017). **A**, **C** exhibit Pearson correlations between latitude and root elongation for groups obtained in the AraPheno database (https://arapheno.1001genomes.org/). **B**, **D** demonstrate the comparison between groups from two latitudinal ranges.



Supplementary Figure S17. Distribution of nonsynonymous, synonymous, and ratio between nonsynonymous and synonymous fractions. 287 *Arabidopsis thaliana* accessions were separated by grouping analyses, G1 (short-days), G2 (neutral), and G3 (long-days), and were further submitted to the POLYMORPH 1001 (https://tools.1001genomes.org/polymorph/) to check the existence of genetic variability on genes involved on DNA repair, cell cycle, and endoreduplication. Polymorphisms revealed the presence of (**A**) nonsynonymous, and (**B**) synonymous mutations on the aforementioned genes as well as the ratio of nonsynonymous to synonymous fractions (*dN*/*dS*) (**C**). The genes investigated were *ATM* (*Ataxia T Telangiectasia Mutated*), *ATR* (*Ataxia Telangiectasia and Rad3 Related-2*), *SOG1* (*Suppressor of Gamma Response1-1*), *CDKA1* (*Cyclin-dependent kinase A1*), RBR1 (*Retinoblastoma-related 1*), *E2Fb* (E2F transcription Factor B), *CDKB1;1* (*Cyclin-dependent kinase B1;1*), *CYCB1;1* (*Cyclin B1;1*), *SIAMESE-RELATED 5* (*SMR5*), *SIAMESE/SIAMESE-RELATED 7 (SMR7)* and *wee1-1* (Mitosis inhibitor protein kinase wee1).



Supplementary Figure S18. Comparison of nonsynonymous, synonymous and ratio of nonsynonymous to synonymous fractions between *Arabidopsis thaliana* groups. By using the POLYMORPH 1001 (https://tools.1001genomes.org/polymorph/), we compared polymorphisms occurring on the genes *ATAXIA TELANGIECTASIA MUTATED* (*ATM*), *ATAXIA TELANGIECTASIA AND RAD3 RELATED* (*ATR*), and *SUPPRESSOR OF GAMMA RESPONSE1* (*SOG1*) among groups G1, G2, and G3, in which 287 *A. thaliana* accessions were separated. Additionally, the ratio of nonsynonymous to synonymous fractions (*dN*/*dS*) was further correlated with day-length (hours).

Supplementary Table S2. List of plant material used in this study.

Specie	Genotype	Gene ID	Mutation	References
A. thaliana	Columbia-0 (Col-0)	-	Wild-Type	
A. thaliana	*atm-1*	AT3G48190	An insertion in the gene 3' end is found in this line, representing alternative allele. The T-DNA insertion is on exon 78 resulting in a 23-bp deletion at ATM sequence, and their background is Wassilewskija.	Garcia et al (2003)
A. thaliana	*atr-2*	AT5G40820 (SALK_032841)	The T-DNA is on ATR gene middle in fraction corresponding to exon 10, representing the Columbia background.	Culligan et al. 2004
A. thaliana	*sog1-1*	AT1G25580	A missense mutation occurs in the highly conserved amino acid region at NAC domain, wherein Gly (GGA)-155 is changed to Arg (AGA).	Yoshiyama et al. (2009)
A. thaliana	*sog1-101*	AT1G25580 (GABI_602B10)	Homozygous knockout mutant line.	Ogita et al. (2018)
A. thaliana	*sog1-1* (*T423A-27*)	AT1G25580	The threonine positioned at 423 amino acid was replaced to alanine (*T423A*). The resulting allele was used to complement knockout line *sog1-101*.	Wei et al. (2020)
A. thaliana	*e2fb-1*	AT5G22220 (SALK_103138)	A T-DNA insertion representing a knockout gene line, showing the inserted sequence in the exon 9 that abolish the gene expression.	Berckmans et al. (2011)
A. thaliana	*CDKB1*	AT3G54180	The codon GAT (Asp-161) was changed to AAT (Asn-161) by using *in vitro* mutagenesis, resulting the dominant negative *CDKB1;1.N161* allele. This allele was expressed under control of the constitutive 35S promoter of the Cauliflower mosaic virus (CaMV35S). The amino acid residue at position 161-Asp is required for correct ATP binding by CDK kinases, where the mutation compromises the kinase activity.	Boudolf et al. (2004)

A. thaliana	*smr5*	AT1G07500 (SALK_100918)	Line with a T-DNA insertion on the position 2304967bp of chromosome 1, representing a knockout line for the gene *SMR5.*	Yi et al. (2014
A. thaliana	*smr7*	AT3G27630 (SALK_128496)	Line with a T-DNA insertion on the position 10231203bp at chromosome 3, representing a knockout line for the gene SMR7.	Yi et al. (2014
A. thaliana	*wee1-1*	AT1G02970	The insertion of T-DNA is located on intron between exons 7 and 8, which result in a deletion of the last 197 amino acids, representing probably null alleles.	Schutter et al. (2007)
A. thaliana	*wee1-2*	AT1G02970	A T-DNA insertion on the exon 1, deleting most of the protein, also a probable null allele.	Schutter et al. (2007)
S. lycopersicum	MT	-	A cultivar containing the recessive allele self-prunning (*sp*) making the growth habit determinated and turning fruit ripening uniform. This transcription factor is at chromosome 6, and acts repressing the flowering.	-
S. lycopersicum	*Sp*	Solyc06g074350	Resulting from an introgression of allele *Self-pruning* (*Sp*) from cv. Moneymaker on MT cultivar. This result in a resumed vegetative growth after flower induction, which maintain the vegetative phase on plant.	Tomato esalq.usp seed-bank
S. lycopersicum	*sft*	Solyc03g063100	The MT cultivar was in introgressed with the loss-of-function mutation on single flower truss (*sft*) (LA2460) originating near-isogenic lines. The mutation promotes low flowering induction, and the transcription is at chromosome 3.	Tomato esalq.usp seed-bank
S. lycopersicum	SP5G	Solyc05g053850	The MT cultivar was in introgressed with the function allele of flowering repressor SELF-PRUNING 5G (SP5G) from *Solanum pennellii* (LA2460) originating near-isogenic lines. The allele repress the flowering under	Tomato esalq.usp seed-bank

			long-day conditions promotes low flowering induction, and the gene is located at chromosome 5.	
S. lycopersicum	M82		Wild-type	-
C. juncea	IAC-KR 1	-	Wild-Type	-
P. sativum	Amélia	-	Wild-Type	-
L. culinaris	Silvina	-	Wild-Type	-
S. humilis	BGH-UFV	-	Wild-Type	-
V. unguiculata	Pingo de Ouro	-	Wild-Type	-
L. albus	TRM 881	-	Wild-Type	-

Supplementary Table S3. Primer sequences used in qRT-PCR analyses performed in this study

Gene	Locus	Forward	Reverse
RBR1	AT3G12280	5’-GTGATCACCTCAGGCTATGAGC-3’	5’-CACTCTTTCTAACTGTGTTCGGTGT-3’
SOG1	AT1G25580	5’-CCATGAGGTTTCTCTTGCCGAGAC-3’	5’-TCAGGCCCAGAACTTGGTCTTTC-3’
ATR	AT5G40820	5’-GTGCCATTCAGATTGACCCAGAAC-3’	5’-TGCCCTCATATCCAGTGATGCC-3’
ATM	AT3G48190	5’- AACTTGATGGCTACGAGGGTGGT -3’	5’- CCAGGGAACATATGGGACAAACG -3’
CYCB1	AT4G37490	5’-ACCTCGCAGCTGTGGAATATGTG-3’	5’-ATCTCGTGGCCTCCATTCACTCTC -3’
CDKA1	AT3G48750	5’-ACTGGCCAGAGCATTCGGTATC -3’	5’-TCGGTACCAGAGAGTAACAACCTC-3’
CDKB1	AT3G54180	5’- ACTGGTGTTGACATGTGGTCTG -3’	5’- TTGGTGTTCCTAGCAACCTGAAG -3’

SI References

Berckmans, B. *et al.* (2011) Light-dependent regulation of DEL1 is determined by the antagonistic action of E2Fb and E2Fc. Plant Physiol. **157**, 1440-1451.

Boudolf, V. *et al.* (2004) B1-type Cyclin-dependent kinases are essential for the formation of stomatal complexes in *Arabidopsis thaliana*. Plant Cell **16**, 945-955.

Culligan, K. *et al.* (2004) ATR regulates a G2-phase cell-cycle checkpoint in Arabidopsis thaliana. Plant Cell **16**, 1091–1104.

Galbraith, D.W. *et al.* (1983) Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science **220**, 1049-1051.

Garcia, V *et al.* (2003) AtATM Is Essential for Meiosis and the Somatic Response to DNA Damage in Plants. Plant Cell **15**, 119-132.

Garcia-Oliveira, A.L. *et al.* (2016) Aluminium stress in crop plants. In Recent Advances in Plant Stress Physiology; Yadav, P., Kumar, S., Jain, V., Eds.; Daya Publishing House: New Delhi, India, 237–263.

Hoagland, D.R. and Arnon, D.I. (1950) The water culture method for growing plants without soil. Circular **347**, Univ. of Calif. Agric. Exp. Station, Berkeley.

Meda, A.R. and Furlani, P.R. (2005) Tolerance to aluminum toxicity by tropical leguminous plants used as cover crops. Braz. Arch. Biol. Technol. **48**, 309–17.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum **15**, 473–497.

Ogita, N. *et al.* (2018) Identifying the target genes of SUPPRESSOR OF GAMMA RESPONSE 1, a master transcription factor controlling DNA damage response in Arabidopsis. Plant J. **94**, 439-453.

Schutter, K. *et al.* (2007) Arabidopsis WEE1 kinase controls cell cycle arrest in response to activation of the DNA integrity checkpoint. Plant Cell **19**, 211-225.

Takahashi, N. et al. (2019) A regulatory module controlling stress-induced cell cycle arrest in *Arabidopsis*. Elife **8**:e43944.

Tomato esalq.usp seed-bank (http://www.esalq.usp.br/tomato/).

Yi, D. *et al.* (2014) The Arabidopsis SIAMESE-RELATED cyclin-dependent kinase inhibitors SMR5 and SMR7 regulate the DNA damage checkpoint in response to reactive oxygen species. Plant Cell **26**, 296-309.

Yoshiyama, K. *et al.* (2009) Suppressor of gamma response 1 (SOG1) encodes a putative transcription factor governing multiple responses to DNA damage. Proc. Natl. Acad. Sci. USA **106**, 12843–12848

Additional files

Data S1. Number of principal component analyses (PCA) retained: a−score optimisation − spline interpolation.

Data S2. Principal component analyses (PCA): Variance explained by PCA.

Data S3. Pop_Orange_Arabidopsis: Grouping analysis separating *Arabidopsis thaliana* accessions according with Orange Canvas software, for details see material and methods.

Data S4. Scatter plot: Representation of three group distinction.

Data S5. Barplot: % of reassignment to actual group.

Data S6. Compoplot: membership probability for three groups.

File S1. ***Arabidopsis thaliana*** **accessions details**

Chapter 6

The hidden half comes into the spotlight: peeking inside the black box of root developmental phases

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The hidden half comes into the spotlight: peeking inside the black box of root developmental phases

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Running title: Exploring root developmental phases

Abstract

Efficient use of natural resources (e.g., light, water, and nutrients) can be improved with a tailored developmental program that maximizes the lifetime and fitness of plants. In plant shoots, a developmental phase represents a time window in which the meristem triggers the development of unique morphological and physiological traits, highlighting the emergence of leaves, flowers, and fruits. Whereas developmental phases in plant shoots have been shown to enhance food production in crops, the phenomenon has remained poorly investigated in the roots. In light of recent advancements, we suggest that root development occurs in three main phases: root apical meristem appearance, foraging, and senescence. We provide compelling evidence suggesting that these phases are regulated by at least four developmental pathways: autonomous, non-autonomous, hormonal, and periodic. Root developmental pathways differentially coordinate organ plasticity, promoting morphological alterations, tissue regeneration, and regulation of cell death. Furthermore, we suggest how nutritional checkpoints may allow progression through developmental phases, thus completing the roots' life cycle. These insights highlight novel and exciting advances in root biology that could help maximize the productivity of crops through reduced use of chemical fertilizers and more sustainable agriculture.

Keywords: developmental transitions; plasticity; cell fates; nutritional checkpoints; root development; root clock

Roots: amazing organs with a unique developmental clock

The developmental clock describes a set of timekeepers defining the emergence of cells, tissues, and organs according to time. This clock defines the unique genetic, biochemical, and morphological features of the organism. Its complexity varies widely between or even within species and depends on embryonic patterns. Embryogenesis encompasses the formation of embryos and the completion of asymmetric cell divisions. Postembryonic development represents the process whereby an organism builds its body, forming new tissues and organs. Postembryonic development of entire organs is prominent in plants, but is much rarer in animals. Because they are sessile organisms, the uncoupling of embryos and organogenesis is crucial for plant survival under different environments. Accordingly, plants do not necessarily pre-establish the number and size of organs during embryogenesis; instead, both postembryonic development and developmental phases ensure that plants thrive under diverse environmental conditions.

In the last decades, factors controlling plant shoot development have been identified, and three developmental phase transitions have been recognized: seed germination-to-juvenile vegetative growth, juvenile vegetative-to-adult vegetative phase, and adult vegetative-to-reproductive phase (Poethig, 1990, 2003; Bäurle and Dean, 2006; Huijser and Schmid, 2011; Yu et al., 2015a). Seed germination denotes embryo-to-postembryonic transition. This is followed by juvenile vegetative growth, in which the plant remains unable to flower. Finally, during floral transition, the plant enters the reproductive phase, whose highlight is the occurrence of meiosis during gametogenesis.

The transcription factor SHORT VEGETATIVE PHASE (SVP), known to delay floral transition, has recently been suggested to negatively regulate cambial cell proliferation in roots and increase total xylem vessel number of Arabidopsis (*Arabidopsis thaliana*) (Zhang et al., 2019a). Furthermore, the circadian clock component EARLY FLOWERING 4 (ELF4) was demonstrated to move from shoots to roots, delivering circadian cues in a temperature-dependent manner in Arabidopsis (Chen et al., 2020). Thus, the proteins and temperature that control phase transition in shoots seem to regulate also root development and synchronize the two organs. The root apical meristem (RAM) undergoes self-renewal and continuous differentiation as it emerges from the meristematic zone. Programmed cell death (PCD) in the root cap promotes lateral root spacing (Möller et al., 2017; Motte et al., 2019) and shifts the cell arrangement in the plant roots, possibly contributing to the regulation of developmental phases.

The first layer of root cap cells was recently shown to be characterized by cell wall modifications resembling those seen in the plant cuticle of aerial plant organs. This cuticle is essential for root protection during seedling establishment and lateral root formation in Arabidopsis (Berhin et al., 2019). Suberin, a hydrophobic biopolymer with a long chain formed by the binding of fatty acids and glycerol, may be deposited on the cell walls of specific root parts, including the endodermis in response to environmental conditions, during a process called suberization. Although root suberization also occur in other root parts (Watanabe et al., 2013), nutritional changes can promote endodermal differentiation dictated by the suberization status of this cell layer, revealing functional and anatomical plasticity in adult roots of Arabidopsis (Barberon et al., 2016).

Unlike in animals, postembryonic development in plants relies on the asymmetric orientation of cell division planes, which determines specific cell fates. Thus, we posit that unique developmental features likely characterize different plant species. Root stem cells might be reconstituted from multiple cell types, whereby the rapid transition in cell identity precedes *de novo* stem cell formation and follows the same sequence seen during embryonic root formation in Arabidopsis (Efroni et al., 2016). This indicates that the regenerative potential of the root primordium is comparable to that of the shoot apical meristem (SAM) (Rosspopoff et al., 2017). A cell’s relative position in the root is closely associated with its identity, but may change during regeneration or restoration of tissue upon drastic damage (Efroni et al., 2018). The existing concept of cell differentiation has been the target of criticism within the plant biology community as a more fluid, flexible view of identity during cell transitions and differentiation is emerging (Sugimoto et al., 2011; Ikeushi et al., 2016; Efroni et al., 2018). The identity acquired by distinct root cell types is likely mediated by the stele-derived signaling peptide Casparian strip integrity factor 2 (CIF2), which stabilizes the transcription factor SHORT-ROOT (SHR) and promotes cell differentiation in the endodermis in Arabidopsis (Drapek et al., 2018). Studies on cell differentiation in young roots displaying a low level of branching have excluded the influence of root cell ontogeny on developmental time. Quiescent-center (QC) cells in the RAM are associated with spatial and developmental time *status*, whereby the distance from the QC is closely linked to cell differentiation potential in Arabidopsis (Wendrich et al. 2017). Accordingly, the QC proximal region produces two opposing gradients for transcriptional regulation, differentiation, and stemness (i.e., the ability of a cell to resume self-renewal), causing the expression of gradient-related genes to vary with root zone ontogeny (Wendrich et al. 2017). Collectively, the specific cell fates in the RAM likely dictate developmental decisions, hinting at distinct developmental phases for roots.

Numerous studies have described developmental phase transitions in shoots (Huijser and Schmid, 2011; Yu et al., 2015a), but not in roots. We posit the existence of at least three distinct developmental phases in the roots. First is the RAM appearance phase, which is characterized by higher rates of cell proliferation than differentiation. The root foraging phase that follows contains more differentiated than undifferentiated cells, together with higher nutrient uptake from the rhizosphere. Finally, the root senescence phase exhibits a constant declines and lately absence of cell proliferation/differentiation and nutrient uptake, but increasing cell death rates. Here, we further provide new insights on root biology, which might assist in the development of next-generation crops exhibiting tailored root growth, as well as improved uptake and use of nutrients to meet crop requirements. Accordingly, we posit that a late root foraging phase may improve nitrogen (N) uptake and utilization, whereas early branching likely improves efficient phosphorus (P) utilization.

Root developmental phases

Roots exhibit diverse forms and functions; they include fine, pioneer, coarse, absorptive, and transport roots (McCormack et al., 2015). A growing body of evidence suggests that their lifespan depends on the RAM stage, akin to regulation of the SAM in shoots (Poethig, 2003; Bäurle and Dean, 2006). Root developmental phases reflect cell fates associated with the RAM, regardless of the species or root class, and affect the entire root. This is likely similar to the situation described for shoot developmental phases, which display relatively little differentiation across flowering plant species (Pujar et al., 2006). Establishment of the QC promotes the maintenance and function of the whole root system, which may be observed in Arabidopsis and cereals, including rice (*Oryza sativa*), barley (*Hordeum vulgare*), and maize (*Zea mays*) (Strotmann and

Stahl, 2021). Although cereals likely harbor more complex root systems than Arabidopsis (Strotmann and Stahl, 2021), these results indicate that the QC in the RAM seems to modulate all root developmental phases.

RAM appearance phase

Following gamete fusion, embryos undergo asymmetric cell division. During early development, distinct hormonal response zones form in the Arabidopsis embryo, establishing stable cell division patterns and inducing the growth of root vasculature, such that a functional phloem exists prior to seed germination (De Rybel et al., 2014a,b). In response to adequate stimuli, seeds germinate, and the SAM and RAM give rise to the plant's aerial and terrestrial structures, respectively (**Figure 1**). Importantly, during the RAM appearance phase, the RAM utilizes seed reserves to sustain cell division.

The BIRD family of transcription factors organizes ground tissue during embryogenesis. BIRD and SCARECROW (SCR) transcription factors regulate postembryonic growth in roots, defining stem cell zones, cell divisions, and cell layers in Arabidopsis (Moreno-Risueno et al., 2015). Regardless of SHR and SCR, root epidermis identity is limited by SCHIZORIZA (SCZ), which suppresses subepidermal cell fates and root hair formation in Arabidopsis (Mylona et al., 2002). In contrast, ARABIDOPSIS CRINKLY 4 (ACR4) has direct implications in intercellular communication and is required for the formation of epidermis-related tissues in the outer layers (Tanaka et al., 2002; Watanabe et al., 2004). The *acr4* mutant displays additional divisions in the columella cell lineage, promoting root cap disorganization, formation of additional cell layers below the QC, and reduced emergence of lateral roots in Arabidopsis (De Smet et al., 2008). Hence, ACR4 is critical for completing the RAM appearance phase, confirming its role in maintaining QC integrity.

Primary root caps exhibit a cuticle only very early on, suggesting that the definition of root developmental and physiological traits occurs during this initial period. Later, this structure was found exclusively in the root cap of lateral roots at the start of the root foraging phase in Arabidopsis (Berhin et al., 2019). Taken together, these results indicate that the RAM appearance phase exhibits elevated cell division rates and strong QC identity. It seems reasonable to assume that a reduced nutrient uptake per root length seems linked to the absence of branches, which could increase the root surface and harbor nutrient transporters. Later on, roots experience an exponential increase in nutrient uptake, which most likely arises from an improved root branching *status* correlated with higher gene expression of nutrient transporters and complete differentiation of xylem and phloem, facilitating nutrient transport across the whole plant.

Root foraging phase

Transition to the root foraging phase follows progressive patterns of cell differentiation. During this phase, the root structure begins to be differentiated, displaying segments beyond the seminal root. Roots may emit protrusions along a longitudinal plane, which often coincides with the emission of lateral roots, while in other cases, adventitious roots may appear first (**Figure 1**). At this point, root cell layers might display increasing variability and greater complexity than shoots. Minimum requirements are likely necessary to induce root branching and promote cell differentiation in specific root zones. ROOT HAIR DEFECTIVE 2 (RHD2) mediates Ca^{2+} influx into the cytoplasm in response to reactive oxygen species and initiates root branching in Arabidopsis (Takeda et al., 2008). To sustain this event, SCR and SHR induce cell differentiation in roots, generating mature endodermis with a distinguished Casparian strip in Arabidopsis (Drapek et al., 2017). Intriguingly, feed-forward loops point to a transcription factor activation cascade, whereby SCR and SHR bound to regulatory regions induce the expression of a third transcription factor, MYB DOMAIN PROTEIN 36 (MYB36). This event results in the transition from proliferation to differentiation of endodermal cells, culminating in the formation of the Casparian strip in Arabidopsis (Liberman et al., 2015). In this process, SHORT HYPOCOTYL 2 (SHY2) plays a role in the repression of auxin signaling by reducing PIN-FORMED (PIN) transporter activity, which further stimulates endodermal cell differentiation in Arabidopsis (Dello Ioio et al., 2008; Di Mambro et al., 2017). Accordingly, in combination with stele-derived small peptides, SHR serves as a hub factor regulating the signaling cascade that reconstructs Casparian strips using non-endodermal cells and employing both apoplastic and symplastic communication to coordinate root cell fates in Arabidopsis (Li et al., 2018). CIF2 promotes SHR stabilization, which, together with MYB36, promotes the acquisition of endodermis identity. In addition, roots treated with CIF2 showed increased root hair density in Arabidopsis (Drapek et al., 2018), suggesting a role for this peptide in switching to the foraging phase.

Passage cells exist in most angiosperms, where they represent isolated unsuberized cells associated with meristematic patterning. Furthermore, older roots exhibit passage cells in the endodermis, which is characterized by thin walls and Casparian strips in Arabidopsis (Andersen et al., 2018). They play a prominent role in differential auxin and cytokinin responses inside the late endodermis circumference.

Cytokinin inhibition triggers almost complete loss of suberization, which correlates with endodermal cells becoming passage cells. Nevertheless, suberization likely persists around lateral root emergence sites, indicating insensitivity to cytokinins in Arabidopsis (Andersen et al., 2018). Reflecting a dynamic induction of the foraging phase, the transcription factor ROOT HAIR DEFECTIVE SIX-LIKE4 (RSL4) is synthesized in a 4-h pulse during root hair cell elongation and is then gradually degraded via the 26S proteasome in Arabidopsis (Datta et al., 2015). RSL4 synthesis is enhanced in response to low nutrient availability, resulting in a prolonged growth phase and the formation of long hair (Datta et al., 2015). The genetic and biochemical bases of root branching remain much less understood than shoot vegetative changes. Despite the existence of a root clock that positions the lateral root primordia in Arabidopsis (Marin et al., 2010; Wachsman et al., 2020; Xuan et al., 2020; Perianez-Rodriguez et al., 2021), the mechanism leading from branching to root secondary growth requires further investigation.

Root senescence phase

In the last phase, senescence may be progressively observed across the entire root. Cell proliferation and differentiation decline, while cell death rates increase, ultimately culminating in the plant’s death (**Figure 1**). Compared to leaves and petals, little is known about senescence in roots, even though morphological and cytological changes have been documented (Wojciechowska et al., 2018). It is important to mention that perennial species may live decades, centuries, or even millennia and, therefore, they may experience the yearly renewal and selective removal of root structures. As there seems to be little association in turnover timing between leaves and roots in biennial or perennial species (Heilmeier et al., 1986; Withington et al., 2006; Thomas, 2013), it is possible that root senescence is regulated independently from the shoot senescence program. This would imply the occurrence of unique developmental phases in roots in a species-specific manner.

The indeterminate meristem growth found in perennial species raises doubts whether senescence occurs at the whole-plant level, even though cellular and leaf senescence may occur simultaneously (Munné-Bosch, 2008). Trees are usually characterized by seasonal induction of root senescence and then shoot senescence, with the same pattern applied also to suppression of senescence (Munné-Bosch, 2007). In roots, the balance between growth and senescence is likely a pivotal trait defining tree mortality in temperate forests (Munné-Bosch, 2014). By evaluating the root turnover strategies of 19 monocot species, Courchesne et al. (2020) observed that species characterized by root overwintering blocked the development of new lateral roots during the late growing season. This strategy allowed them to preserve more nutrients and carbon than species, in which root senescence occurred in the autumn. Likewise, herbaceous perennial species with senescent leaves manifest different root senescence strategies. In *Sparganium emersum*, root senescence follows leaves senescence; whereas *Iris versicolor* does not display any root senescence symptoms despite showing leaf senescence over the winter period (Ryser et al., 2020). Moreover, roots from perennial plants are usually characterized by color shifts, such as in *Vitis labrusca* and *Populus trichocarpa*, and are likely associated with lower cell viability (Comas et al., 2000; Bagniewska-Zadworna et al., 2014). *P. trichocarpa* exhibits seasonal senescence of fine roots, akin to the vacuolar cell death of leaves and petals (Bagniewska-Zadworna et al., 2014; Wojciechowska et al., 2018). In the perennial species *Trifolium pratense*, a decline in longevity and respiration, as well as loss of membrane integrity in roots, correlates positively with loss of cell viability and negatively with a lower sugar content (Bingham and Rees, 2008; Webb et al., 2010; Bingham, 2012). In perennial species, nitrogen levels determine the overall content of live and dead fine roots (Kunkle et al., 2009). Notably, both the size of the meristem and its activity are highly relevant for senescence in shrubs and trees (Munné-Bosch, 2007). Therefore, root development seems to be involved in the senescence regulation of perennials, while organ size is relevant for acquiring senescence competence in annual plants.

Annual and biennial plant species must develop the competence to initiate senescence, with a terminal growth phase characterized by declining physiological efficiency. Ethylene becomes less effective in inducing senescence in older plants (Jing et al., 2005), indicating that once the plant acquires competence to senesce, acceleration of this process by exogenous factors is rather complex. It has been demonstrated that senescence progresses differently across root zones in annual species (Timilsina et al., 2019). QC cells are central modulators of senescence as their size becomes smaller and variations in cell divisions occur with root age (Timilsina et al., 2019), indicating a constant decline in QC identity (**Figure 1**). It has been recently demonstrated that root senescence may be explained by the gradual decrease in *WUSCHEL RELATED HOMEOBOX 5* (*WOX5*) expression over time, and consequent decline in QC identity in Arabidopsis (Wein et al., 2020). Because diverse structures and zones of maize roots display differential natural senescence, the latter is likely induced by aging and not in response to environmental conditions. The maize root cortex is characterized by abundant cell viability over the plant lifecycle, with root hair exhibiting a lifespan of 2–3 days and a constant renewal until the late grain-filling stage (Fusseder, 1987). This suggests that the entire root system enters the terminal senescence stage. Cell death in the cortex and stele of axial maize roots follows the onset of shoot senescence, exhibiting strict developmental synchrony (Wenzel and McCully, 1991). Liu et al. (2019a) reported root-specific senescence-related transcription factors in barley, some of which were not expressed in the leaves. This finding suggests that roots undergo an intrinsic, genetically-determined senescence program that is influenced by aging.

Cell death occurs during the entire root development, wherein root ontogeny regulation exhibits a noticeable dependence on PCD, which promotes active cell elimination (Bagniewska-Zadworna and Arasimowicz-Jelonek, 2016). Although ontogeny plays an important role in regulating root transcriptional gradients in Arabidopsis (Wendrich et al., 2017), its effect on PCD and senescence in distinct root zones has been less well explored. Senescent organs are defined by limited carbon availability, which is the case for roots during aging (Kosola et al., 2002). The shoot-derived carbon pool in roots has direct implications for root aging and turnover of *Sassafras albidum* (Adams and Eissenstat, 2014), and its remobilization is important during senescence. Intriguingly, PCD-dependent root cortical senescence in Triticeae species acts antagonistically to ethylene and lowers root respiration rates (Schneider et al., 2017a, b; 2018). This finding highlights the phenomena that may follow PCD during senescence. Whereas elevated cell death rates in the primary root tip trigger auxin release into proximal tissues, determining root cap turnover and root foraging in Arabidopsis (Xuan et al., 2016), it remains unclear whether lateral roots exhibit similar senescence patterns as leaves at the shoot.

Similar to shoot developmental phases, the final stage in the life cycle of roots also seems to be associated with loss of RAM quiescence. Specifically, the reduced ability of cells in the RAM QC to proliferate likely promotes the terminal growth of roots.

Overall, remarkable physiological changes seem to occur during root senescence, including limitations in carbon availability and respiration, remobilization of compounds, and increasing rates of cell death, in which ethylene is a pivotal triggering factor. Hence, a hormonal network seems to modulate root senescence as well as its early development and phase progression, but it is not clear how senescence might be triggered and whether it is determined or not.

Endogenous and exogenous cell cues rewiring root phases

Distinct developmental pathways fine-tune cell fate in response to endogenous and environmental cues. During development, this mostly involves cell cycle regulation to ensure appropriate cell proliferation and differentiation rates. A comparison of cell cycle duration (*T*) in 17 species revealed very narrow variations of *T* in the roots of the same species, irrespective of age or growth conditions (Zhukovskaya et al., 2018). Root systems of plants originating from the same genotype could exhibit strong developmental overlap with those of other rice genotypes (Fang et al., 2013). In contrast, root systems belonging to distinct genotypes tend to avoid each other while growing in the same soil region (Fang et al., 2013). Changes in cell size around the root meristem denote the onset of cell differentiation and identity acquisition, wherein root tips sense contrasting communication inputs to modulate root architecture of rice and Arabidopsis (Fang et al., 2013; Pacifici et al., 2018). Application of the synthetic clavata3/ESR-related 40 (CLE40) peptide causes meristem cell differentiation in Arabidopsis, rice, and barley; whereas columella stem cells become differentiated only in Arabidopsis (Kirschner et al., 2017). Thus, divergent pathways seem to support the maintenance of differentiation *status* in distal stem cell populations, indicating the existence of unrelated developmental pathways in the roots of different species.

Time and space can provide appropriate guidance for plant organ growth and architecture, forming intrinsic relationships with the environment to determine cell fates around meristematic zones (Scheres and van der Putten, 2017). Key mediators of root development, such as RETINOBLASTOMA-RELATED 1 (RBR1), WOX5, NAC PROTEINS FEZ, SOMBRERO, AUXIN RESPONSE FACTOR 10 (ARF10), and ARF16, provide independent inputs to regulate root development in Arabidopsis (Bennett et al., 2014). Autonomous, gibberellin, photoperiod, and temperature (vernalization) pathways act independently during developmental phase transitions in shoots, particularly during flowering. Interestingly, some of the cues regulating shoot development behave similarly during phase progression in the roots.

Cell-autonomous pathway

RBR1 plays a crucial role in the maintenance of stem cell integrity and performs unique functions in different cell types around the meristem of Arabidopsis (Wachsman et al., 2011). Specifically, RBR1 activity limits columella cell division and favors their differentiation, revealing cell-autonomous behavior in distinct cell types (Wachsman et al., 2011). SCR acts in a cell-autonomous manner, regulating fates around QC cells, where it interacts with RBR1 to modulate *de novo* cell formation in Arabidopsis (Sabatini et al., 2003; Cruz-Ramírez et al., 2013). Similarly, both MYB36 mRNA and protein display autonomous patterns in a small group of cells surrounding the lateral root primordia, and participate in the transition towards the root foraging phase in Arabidopsis (Fernández-Marcos et al., 2017). DNA methylation of specific root cell types, and particularly those in the columella root cap, could mediate root development through heterochromatin loss in Arabidopsis (Kawakatsu et al., 2016). As the expression gradient of PLETHORA (PLT) transcription factors declines in line with HISTONE ACETYLTRANSFERASE GCN5 activity in the columella stem cell layers, differentiation of the corresponding cells may be attenuated by differential histone acetylation in Arabidopsis (Kornet and Scheres, 2009). Local gene expression and protein activity indicate the existence of an autonomous pathway that promotes the induction of an early foraging phase (**Figure 2**). In contrast, mobile elements (e.g., mRNA, proteins, and hormones) might participate in the non-autonomous regulation of developmental pathways.

Non-cell-autonomous pathway

Certain elements can move across different root cell types in Arabidopsis, rice, and *Brachypodium distachyon* (Wu et al., 2014), suggesting a developmental pathway based on non-cell-autonomous regulation (**Figure 2**). For example, SHR is limited to the endodermis owing to inhibition by SCR, a conserved mechanism that favors a single endodermis in roots in Arabidopsis (Cui et al., 2014). In contrast, JACKDAW (JKD) is localized around QC cells, where it regulates asymmetric cell divisions and SHR movement, as well as the gene expression of epidermal cell fate regulators

GLABRA 2, *CAPRICE*, and *WEREWOLF* in Arabidopsis (Hassan et al., 2010). Non-cell-autonomous patterns revealed by JKD suggest that this protein affects signaling throughout the cortex, particularly in root hair cells (Hassan et al., 2010). Both miR165a and miR166b exhibit similar mobility in the roots as in the SAM, promoting the degradation of mRNAs encoding transcription factors that regulate endodermis and stele cell fates in Arabidopsis (Carlsbecker et al., 2010). When SHR moves into the endodermis and activates SCR, it enables miR165/6 to move and complete the non-cell-autonomous network (Carlsbecker et al., 2010). This suppresses *PHABULOSA* (*PHB*) gene expression in the peripheral stele, promoting xylem-related cell fate, and controlling the differentiation of various root cell types (Carlsbecker et al., 2010; Miyashima et al., 2011). Hormonal inputs occur in a non-cell-autonomous manner, as observed by cytokinin-induced expression of the mobile *PHLOEM EARLY DOF* (*PEAR*) transcription factor, which activates periclinal cell division and ensures root procambial tissue formation in Arabidopsis (Miyashima et al., 2019). The vascular patterns elicited by PHB, SHR, and miR165/6 are disrupted by cytokinin signaling in Arabidopsis (Muraro et al., 2014). The abscisic acid (ABA) receptor PYL8 assumes non-cell-autonomous features, displaying inter- and intracellular patterns in the epidermis and stele cells of the root meristem in Arabidopsis (Belda-Palazon et al., 2018). The non-cell-autonomous pathway is likely to regulate support features required during the foraging phase, as indicated by the formation and expansion of vascular tissue (**Figure 2**). Hormone gradients along distinct root zones ensure a specific developmental program.

Hormonal pathway

In plants, signal transduction inputs follow distinct hormonal leads through tissues, enabling the sequential development of various structures. Regulation of stem cell fate in roots (**Figure 2**) relies on a tight balance between independent hormonal inputs that promote root development in Arabidopsis (Bennett et al., 2014). A dynamic hormonal network in multiple cell types around the RAM regulates cell fate and tissue patterning. Specifically, auxin may concurrently induce and inhibit *WOX5* expression in distinct cell types of Arabidopsis (García-Gómez et al., 2017). In the embryonic stage, auxin controls cytokinin activation at specific sites by promoting cell divisions that allow vascular tissue growth and by organizing layer patterns in Arabidopsis (De Rybel et al., 2014b). The initial steps in root cell differentiation require changes in cell size of Arabidopsis; hence, cytokine indirectly modulates cell wall remodeling to drive cell differentiation (Pacifici et al., 2018). Cytokinin oxidase 2 (CKX2) controls cytokinin degradation and regulates the root system's radial distribution patterns based on anti-gravitropic signaling in Arabidopsis (Waidmann et al., 2019). The root transition zone separates dividing and differentiating cells, and the hormone cytokinin regulates auxin polar transport and degradation (Di Mambro et al., 2017). The ensuing basal auxin profile establishes the transition zone, defining division, and differentiation layers in Arabidopsis (Di Mambro et al., 2017). A local auxin maximum acts with CLASS III HOMEODOMAIN-LEUCINE ZIPPER (HD-ZIP III) transcription factors to regulate xylem identity acquisition, and form a stem cell organizer with its cellular quiescence in Arabidopsis (Smetana et al., 2019). Thus, auxin seems to be particularly important for the root foraging phase. Additionally, its dynamic behavior modulates Arabidopsis root elongation in the different root zones following cytokinin signaling repression (Andersen et al., 2018). ABA repression culminates in the absence of suberin in passage cell precursors (Andersen et al., 2018), demonstrating how hormonal balance enforces differential meristem patterning regulation. Following tissue formation and patterning establishment, root cells have to cope with a large number of damaging agents that impair growth and development, which might be overcome by regeneration pathways. In the Arabidopsis roots, cell replacement is guided by dynamic auxin and cytokinin domains that follow embryonic (Efroni et al., 2016). In contrast, the formation of a new ground tissue layer specifying the Arabidopsis middle cortex is precisely regulated by gibberellin and SCR via an additive effect and, independently, by SHR (Paquette and Benfey, 2005). Intriguingly, restorative divisions after damage and middle cortex formation do not seem to respond to gibberellin, suggesting that a divergent mechanism mediates these processes in Arabidopsis (Marhava et al., 2019). ETHYLENE RESPONSE FACTOR 109 (ERF109) acts at the interface between jasmonate signaling and auxin biosynthesis and transport, mediating Arabidopsis lateral root formation (Cai et al., 2014; Liu et al., 2019b) and subsequent foraging. Additionally, ERF109, ERF115, and jasmonate regulate cell division proteins to orient stem cell niche fates of Arabidopsis (Zhou et al., 2019).

In summary, multiple elaborate hormonal networks mediate distinct transition steps, indicating the multifaceted role of hormones in root development, coordinating not only both early and later stages of the foraging phase, but also entry in the senescence phase (**Figure 2**).

Periodic pathway

Oscillations in the expression and activity of genes, proteins, and metabolites promote tissue patterning in roots, suggesting that periodic events regulate the development of root structures. Root cap cells release auxin periodically over time, controlling a switch between developmental phases in these cells (Laskowski and Tusscher, 2017; Möller et al., 2017). The different root zones exhibit contrasting developmental times, hinting at periodic genetic and physiological regulation (**Figure 2**). Cell shape is determined through local positive feedback in root hair cells, with Ca^{2+} and RHD2 interacting at the growing point of the root section in Arabidopsis (Takeda et al., 2008). Therefore, growth location and stability at specific sites anticipate cell fate (Takeda et al., 2008). In contrast, the aggregation of ARF transcription factors causes cytoplasmic localization and inactivation, limiting auxin responsiveness in distinct root zones in Arabidopsis (Powers et al., 2019). Thus, the lateral root cap displays a multi-layered structure that does not obey the correspondence between cell age and distance from the QC. This contrasts with other root tissues and violates the spatio-temporal endoploidy model of the lateral root cap in Arabidopsis (Bhosale et al., 2018a). Cyclic transcriptional pulses establish periodic developmental regulation, which contributes to root pre-branch formation in Arabidopsis (Moreno-Risueno et al., 2010). Similarly, cyclic PCD enhances hormone signaling, which results in periodic lateral root induction and optimization of water and nutrient uptake in Arabidopsis (Xuan et al., 2016).

Multiple tissue-forming steps are regulated by the same set of transcription factors and their positional cues (Moreno-Risueno et al., 2010). Therefore, competition for the gradual transcriptional regulation of cell differentiation in Arabidopsis (Wendrich et al., 2017) seems to depend on cell ontogeny. Periodic pre-branch formation exhibits a lateral root clock that follows a specific spatial pattern in Arabidopsis (Van Norman et al., 2014). The vesicle trafficking regulator GNOM and repressor ARF-GTP-ACTIVATING PROTEIN DOMAIN (AGD3) are central agents controlling the functionality of the lateral root clock in Arabidopsis (Wachsman et al., 2020). These proteins regulate the differential distribution of esterified and de-esterified pectin across cells at pericycle-endodermis junctions, orchestrating oscillations in the lateral root clock that enable its emergence in Arabidopsis (Wachsman et al., 2020). Auxin exhibits a similar oscillatory circuit that regulates the root clock periodicity. Thus, gene expression oscillations across root pre-branch sites driving periodic cell division can induce lateral root spacing in Arabidopsis (Perianez-Rodriguez et al., 2021), highlighting a potential role for the root clock in the regulation of foraging phase progression. Regulation of the lateral root clock remains unclear; some reports have suggested periodic, age-dependent coordination among participating pathways. Deciphering the actual link between such pathways and their underlying mechanisms will expand our understanding of how the biological clock regulates lateral root formation. Overall, the periodic pathway seems capable of guiding root developmental phases; however, further efforts are required to fully decipher its regulatory nature during early and late development.

Involvement of developmental phases in root plasticity

Plants experience extremely contrasting environmental conditions. While the shoots exchange most of the gases, the roots exchange most of the remaining compounds due to a more aqueous external environment. The fossil roots of the lycopsid *Asteroxylon mackiei* revealed the absence of root caps, which were replaced by a continuous epidermis covering the entire meristem surface (Hetherington and Dolan, 2018). Hence, root cap acquisition is defined as a crucial moment in evolution that facilitated plant terrestrialization. Another feature that explains plant growth under oscillatory or extreme environments is root endoreplication across diverse cell types, which alters nuclear and cell wall features. Coordinated cell expansion highlights the contrasting characteristics of lateral root caps compared to most other root tissues of Arabidopsis (Bhosale et al., 2018a). Shifts in cell structure are coupled with cell division and expansion, enabling developmental progression.

The primary root cap cuticle promotes meristem protection during germination and facilitates the emergence and invasive growth of lateral roots during foraging in Arabidopsis (Berhin et al., 2019). The suberization layer is deposited in response to a range of nutrients by directing functional and anatomical plasticity in the adult endodermis of Arabidopsis (Barberon et al., 2016). While the number of cells in this layer tends to remain constant, suberization changes may occur in accordance with hormonal (ethylene and ABA) patterns. Thus, diffusion barriers in the endodermis respond to Casparian strip plasticity and differential suberization patterns (**Figure 3A**). This promotes delays in apoplastic barrier establishment and the arrest of cell development *in CASPARIAN STRIP MEMBRANE DOMAIN PROTEIN* (*CASP*) mutants, and particularly in *EXOCYST COMPLEX* (*EXO*) Arabidopsis mutants (Kalmbach et al., 2017). Recently, a specific gene encoding an exocytosis factor corresponding to the *EXO70* subunit was identified in Arabidopsis. This gene modulates root system architecture and depth, and affects root tip auxin transport, orienting root gravitropism to cope with variable rainfall patterns (Ogura et al., 2019). The origin and evolution of rapid root gravitropism were attributed to changes in amyloplast patterns within the root apex (**Figure 3B**). These patterns promote variations in the auxin transporter PIN2, whose alteration of auxin flow dynamics links gravity perception in the root cap to cell expansion in the root elongation zone in Arabidopsis (Zhang et al., 2019b). These molecular players promote and regulate root plasticity to improve the uptake of water and nutrients through precise communication between the root and rhizosphere.

Root tips can recognize soil obstacles by two means: (*i*) root-root recognition following root exudate patterns, and (*ii*) root-object recognition through physical contact. Crucially, both alter root architecture (Fang et al., 2013) and guide the response to soil texture, as exemplified by rice genotypes responding with differential plasticity to soil particle variations (Rogers et al., 2016). Root foraging is modulated by water availability in distinct root zones to prevent the emergence of lateral roots at sites with low water availability (**Figure 3C**). This hydropatterning strategy depends on water potential gradients across the root, resulting in differential lateral root growth (Robbins and Dinneny, 2018). Hydropatterning alters root architecture in response to water contact, eliciting SUMOylation of the auxin response factor ARF7 on air-exposed root sides of Arabidopsis (Orosa-Puente et al., 2018). This post-translational modification promotes the interaction between ARF7 and the auxin-responsive protein IAA3, which represses *LATERAL ORGAN BOUNDARIES DOMAIN 16* (*LBD16*) and prevents root branching initiation (Orosa-Puente et al., 2018). At the same time, lateral root formation is repressed and cell identity acquisition is blocked by ABA in soil air pockets under water scarcity in Arabidopsis, barley, and maize (Orman-Ligeza et al., 2018). Thus, the root foraging phase seems to react simultaneously to environmental cues such as hydropatterning and endogenous pathways via an on/off or rewiring response (**Figure**

3C). In this sense, root plasticity has a remarkable potential to trigger the regeneration of cells and tissues.

A complex conceptual framework defines regeneration and structural changes. To regenerate roots after complete root stem cell excision, complementary hormonal domains are established in root zones, forming sites that recapitulate embryogenesis steps and culminate in *de novo* generation of stem cells (Efroni et al., 2016). This ability allows the root primordium to regenerate the shoot meristem, with shoot removal triggering the root greening response that might otherwise be repressed by auxin sides in Arabidopsis (Kobayashi et al., 2017). According to this new concept, root cell identity transitions rely on positional and/or hormonal cues to identify differentiation patterns (Sugimoto et al., 2011; Efroni et al., 2016).

Lateral roots are sites of ongoing cell differentiation. WOX11 regulates root plasticity and mediates rooting in leaf and stem explants, as well as in hypocotyls. This, in turn, promotes proper rooting upon wounding and drought in Arabidopsis (Sheng et al., 2017). Accordingly, PLT transcription factors promote cell division to regulate *de novo* meristem formation and coordinate tissue patterning during lateral root outgrowth of Arabidopsis (Du and Scheres, 2017) (**Figure 3D**). Epidermal stem cell daughters rotate the orientation of the division plane to mimic the stem cell plane in Arabidopsis (Campilho et al., 2006; Marhava et al., 2019), suggesting that competent pre-existing cell populations are required to trigger cell regeneration steps upon root-tip excision (**Figure 3D**). The heterodimeric transcription factor complex ERF115-PHYTOCHROME A SIGNAL TRANSDUCTION 1 (PAT1) is pivotal in regenerating stem cells after cell death in Arabidopsis (Heyman et al., 2016). Hormone shifts cooperate in this process based on the type of damage (**Figure 3D**). Jasmonate coordinates wound signaling to enable *de novo* root regeneration from detached leaves via ERF109 activation and auxin biosynthesis induction in Arabidopsis (Zhang et al., 2019c). Specifically, jasmonate induces ERF109, which in turn stimulates CYCLIN D6;1 (CYCD6;1) expression and functions upstream of ERF115 to enable root regeneration of Arabidopsis (Zhou et al., 2019) (**Figure 3D**). Subsequently, JASMONATE ZIM-DOMAIN (JAZ) proteins inhibit ERF109, replacing constitutive regeneration with *de novo* regeneration (Zhang et al., 2019c). Intriguingly, these players are connected to SCR and RBR to adjust stem cell niche replacement and maintain QC quiescence of Arabidopsis (Zhou et al., 2019). During stem cell reactivation, a PLT expression gradient is formed in the roots, indicating regenerative competence of cells, together with accelerated cell cycle progression in Arabidopsis (Marhava et al., 2019) (**Figure 3D**). Cell division reprogramming for replacement differs across cell types. Generally, activation of SHR, SCR, and CYCD6;1 in the endodermis or near-endodermal cortex ensures correct cell fates in Arabidopsis (Marhava et al., 2019) (**Figure 3D**). As a countermeasure, cells might be selectively eliminated to maximize energy and nutrient usage, favoring developmental phase transitions.

Early cell descendants from root stem cells are preferentially eliminated under chilling stress. In contrast, columella stem cell daughters are highly exposed to DNA damage and even if the directional auxin flux from these cells is disrupted, the quiescent state of root stem cells is preserved in Arabidopsis (Hong et al., 2017). The sacrifice of these cells to sustain root survival explains how and to what extent alternative mechanisms regulate root development and direct responses to environmental cues. Thus, the precise timing of cell death represents a critical step that enables cell renewal and the entry of cells into the root elongation zone to ensure root growth and homeostasis in Arabidopsis (Fendrych et al., 2014). In short, plasticity allows the roots to cope with soil dynamics through hormone-mediated changes in root structure, architecture, and regeneration.

Nutritional checkpoints governing root developmental transitions

The most relevant environmental inputs affecting root development include water and nutrient uptake from the soil. Water is lost to the atmosphere through the stomata via several interconnected pathways; whereas nutrient uptake, transport, and use are regulated by numerous complex pathways. Agricultural revolutions over the last century have remarkably improved crop productivity, and plant breeding has focused on controlling shoot development to select plants with the best architecture (Bailey-Serres et al., 2019). Although traditionally neglected, the breeding of plants with differential root development and architecture can reduce nutrient imbalances (Cormier et al., 2016). The scarcity of some fertilizers, their elevated production costs, and environmental impact have limited sustainable agriculture. Crop yield is largely dependent on sufficient N and P. Because N fertilizers volatilize easily and release N_2 into the atmosphere, large amounts of them are required to sustain plant growth; whereas P is immobile and highly adsorbed in the soil. Although substantial advances have been made in understanding the mechanisms of uptake, transport, and efficient use of both N and P, only improved knowledge of how root developmental phase transitions respond to nutrients will help generate crops capable of more efficient nutrient use.

N modulates early root developmental phases

Nucleotides, amino acids, and proteins required for plant growth and development contain N, which is estimated to consume almost 2% of global energy, while N runoff leads to contamination of lakes and rivers (Sutton et al., 2013). In the soil, N is available mostly as ammonium (NH_4^+) and nitrate (NO_3^-), which stimulate contrasting pathways associated with root development (Motte et al., 2019; Meier et al., 2020). NH_4^+ blocks primary root growth, decreasing both meristem length and the number of proliferating cells, as well as reducing Arabidopsis root elongation zone (Liu et al., 2013). Successful seed germination depends on NO_3^- via NIN-like protein 8 (NLP8) and ABA catabolism in Arabidopsis (Yan et al., 2016). Similarly, the transcription factor teosinte branched 1/cycloidea/proliferating cell factor 1-20 (TCP20) implicated in Arabidopsis embryo development responds to NO_3^- levels with the effect of preferentially impairing lateral root growth (Guan et al., 2014) and the root foraging phase. Arabidopsis TCP20 interacts with both PLTs and SCR to precisely regulate *WOX5* transcripts (Shimotohno et al., 2018), indicating that NO_3^- regulates root transition from the embryonic to the RAM appearance phase (**Figure 4**). CLE peptides and CLAVATA1 (CLV1) receptors are fundamental for triggering root branching in response to N levels in the rhizosphere of Arabidopsis (Araya et al., 2014). CLE1, 3, and 7 are expressed preferentially in pericycle cells; whereas CLV1 is present in phloem companion cells (**Figure 4**). Under NO_3^- deficit, these cells increase the expression of CLE peptides and *CLV1* transcripts to halt root branching (Araya et al., 2014). Furthermore, auxin derived from shoots accumulates in root regions previously supplemented with NH_4^+, inducing the emergence of lateral roots in Arabidopsis to mediate root foraging along the “soil” surface (Meier et al., 2020).

A combinatorial network of interactions between N forms and hormones (auxin, cytokinin, and ABA) has been described in Arabidopsis roots, revealing the exact transcriptional regulation that drives ABA and NO_3^- responses (Ristova et al., 2016). Another study highlighted the role of cytokinin signaling in in Arabidopsis response to

N demand (Ruffel et al., 2011). Briefly, cytokinins induce *GLUTAREDOXINS* (*GRX*) expression to repress primary root elongation under elevated soil NO_3^- levels while, at the same time, promoting root branching in Arabidopsis (Patterson et al., 2016). The localized exposure of specific root sections to high NO_3^- levels triggers genes involved in both cell cycle progression and auxin efflux from the phloem to pericycle cells, further promoting root branching in maize (Yu et al., 2015b). Root-specific cell types experience particular transcriptional rearrangements based on differential NO_3^- content along the root length (Yu et al., 2015b). Thus, differential root branching patterns in roots allow plants to explore heterogeneous NO_3^- environments. Reduced root branching under low N conditions may result in greater N capture in maize (Zhan and Lynch, 2015), suggesting that a tailored root developmental program might improve N capture and utilization with reduced energy expenditure.

Soils are highly dynamic environments that impose diverse interactions between nutrients and physical, chemical, and biological components. A non-uniform root distribution pattern in the soil enables precise N sensing. Moreover, small C-terminally encoded peptides (CEPs) mediate long-distance communication, linking roots and shoots of Arabidopsis under N starvation conditions (Tabata et al., 2014). CEP1 and CEP5 are expressed mostly in the basal region of lateral roots, where elevated levels of these genes repress primary root growth (Tabata et al., 2014). Overall, multiple strategies to rewire the developmental program allow plants to cope with N starvation, along with other nutritional disturbances. Several avenues must be explored to better understand root development in response to N fluctuations. Considerable engineering feats will determine the precise transcriptional and post-translational regulation of multiple genes, which may shape root system architecture differentially across soil gradients.

On the connections between P and late developmental phase transitions of roots

Intensive food production over the past centuries has significantly altered the structure and chemical nature of soils, and modified the retention capacity towards certain nutrients. In particular, P sources have declined drastically, endangering food security. Most arable soils in the world have an acidic pH, under which P is in stable and difficult to assimilate forms: dihydrogen phosphate ($H_2PO_4^-$) and hydrogen phosphate (HPO_4^{2-}) (Kochian et al., 2004; López-Arredondo et al., 2014). Application of fertilizers might not be sufficient to meet the demand for P in crops. Hence, enormous efforts are being made to improve the efficiency of P uptake and its use by plants. Understanding how developmental root phases adjust in response to P is likely fundamental to ensuring food security.

Passage cells are commonly maintained in an unsuberized state, but this might change in response to P during meristem patterning, promoting expression of the P efflux protein PHO1 (Andersen et al., 2018). Consequently, vascular cell proliferation in roots is coordinated by the gene target of *MONOPTEROS 5 AND LONESOME HIGHWAY* (*TMO5/LHW*), which promotes the biosynthesis of mobile cytokinins in Arabidopsis (Andersen et al., 2018; Wendrich et al., 2020). TMO5/LHW protein re-orients both the length and the fate of epidermal cells to increase branching under low P conditions, indicating the important role of this protein in responding to P limitation during foraging. Under low phosphate conditions, synthesis of the transcription factor RSL4 surpasses its degradation, causing the formation of long root hair and demonstrating how protein stability determines hair size of Arabidopsis (Datta et al., 2015). In this context, auxin transport from the root apex to the differentiation zone in response to low P promotes root hair development, which is associated with induction of transcription factors *ARF19*, *RSL2*, and *RSL4* in Arabidopsis (Bhosale et al., 2018b). Under low external P, the rice auxin influx transporter *Os*AUX1 alters the root angle and promotes preferential foraging in the top soil layers, although this is not sufficient to improve P uptake (Giri et al., 2018). AUX1 is the main transporter for auxin in root hair, facilitating membrane depolarization at extracellular acidic pH and sensing external P in Arabidopsis (Dindas et al., 2018). Moreover, P limitation in the soil reduces secondary root growth while enhancing P uptake, a phenomenon that, in the common bean (*Phaseolus vulgaris*), varies according to the genetic background (Strock et al., 2018). P is likely a key factor in the transition from RAM appearance to the root foraging phase (**Figure 4**).

Taking into account the complexity of soil and the impact of interactions among nutrients on antagonistic or synergistic outcomes regarding nutrient use, better knowledge of how nutrient dynamics modulate phase transitions in roots is needed. Rice tightly coordinates N and P utilization, through interaction between the NO_3^- sensor NRT1.1B and the phosphate signaling repressor SPX4 (Hu et al., 2019). Arabidopsis NIGT1 is a transcriptional repressor of the NO_3^- transporter NRT2.1, and is itself stimulated by PHR1, the master regulator of P starvation responses, which limits nitrate uptake (Maeda et al., 2018). In maize, P and N drive root development to produce optimal lateral root branching density (Postma et al., 2014), allowing both soil foraging and root developmental plasticity. P and N modulate root elongation and branching; however, the effects of nutritional checkpoints on subsequent root developmental stages remain poorly understood. Barley root cortical senescence is ameliorated under P and N deficiency, whereby reduced respiration and nutrient content allow greater root growth (Schneider et al., 2017a,b). Conserved pathways related to local and long-distance N signaling control P starvation responses in *O. sativa* and *Triticum aestivum* (Medici et al., 2019). Expanding our understanding of how nutrients modulate root developmental programs may foster sustainable agriculture by decreasing the use of correctives and fertilizers while maximizing crop yield.

Concluding remarks

Over at least three decades, scientists have identified several factors that regulate developmental phase transitions in plant shoots. In comparison, much less attention has been given to root developmental phase transitions. However, this may be changing, as highlighted in the present review. We have described current evidence pointing to the existence of a unique developmental program for roots, which contrasts with that in shoots due to the absence of a reproductive phase. Root-specific development is based on tightly regulated generation and elimination of root structures. In addition, the ability of roots to alter their structure in response to the environment is associated with their regeneration capacity and gradual age-dependent loss owing to decreased QC identity.

Although the excellent publications surveyed here provide valuable blueprints on the current state-of-the-art, fully understanding developmental root phases will require additional theoretical and experimental capacities. Further progress will be related to plant hormones, as well as nutritional elements, and their function in the regulation of root growth, development, and overall plasticity.

Thus, a new era of root developmental biology is expected. Importantly, new knowledge will help maximize the use of nutrients and water by crops, thus reducing the need for correctives and fertilizers. Notably, uptake and utilization of nutrients linked to cell division (e.g., N and Ca) may be improved by prolonging early developmental phases. Instead, faster induction of the foraging phase would favor uptake and utilization of nutrients associated predominantly with cell differentiation (e.g., P and Fe). In summary, the mechanisms governing root developmental phases are only now emerging from the dark. Timely investments and research will likely bring returns in the form of an improved ability to carry out rational engineering of this “black box”.

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References

Adams, T.S., and Eissenstat, D.M. 2014.The continuous incorporation of carbon into existing *Sassafras albidum* fine roots and its implications for estimating root turnover. PLoS One **9**, e95321.

Andersen, T.G., Naseer, S., Ursache, R., Wybouw, B., Smet, W., De Rybel, B., Vermeer, J.E.M., and Geldner, N. 2018. Diffusible repression of cytokinin signalling produces endodermal symmetry and passage cells. Nature **555**, 529–533.

Araya, T., Miyamoto, M., Wibowo, J., Suzuki, A., Kojima, S., Tsuchiya, Y.N., Sawa, S., Fukuda, H., von Wirén, N., and Takahashi, H. 2014. CLE-CLAVATA1 peptide-receptor signaling module regulates the expansion of plant root systems in a nitrogen-dependent manner. Proceedings of the National Academy of Sciences, USA **111**, 2029–2034.

Bagniewska-Zadworna, A., Stelmasik, A., and Minicka, J. 2014. From birth to death–*Populus trichocarpa* fibrous roots functional anatomy. Biologia Plantarum **58**, 551–560.

Bagniewska-Zadworna, A., and Arasimowicz-Jelonek, M. 2016. The mystery of underground death: Cell death in roots during ontogeny and in response to environmental factors. Plant Biology **18**, 171–184.

Bailey-Serres, J., Parker, J.E., Ainsworth, E.A., Oldroyd, G.E.D., and Schroeder, JI. 2019. Genetic strategies for improving crop yields. Nature **575,** 109–118.

Barberon, M., Vermeer, J.E.M., De Bellis, D., Wang, P., Naseer, S., Andersen, T.G., Humbel, B.M., Nawrath, C., Takano, J., Salt, D.E., and Geldner, N. 2016. Adaptation of root function by nutrient-induced plasticity of endodermal differentiation. Cell **164**, 447-459.

Bäurle, I., and Dean, C. 2006.The timing of developmental transitions in plants. Cell **125**, 655-664.

Belda-Palazon, B., Gonzalez-Garcia, M.P., Lozano-Juste, J., Coego, A., Antoni, R., Julian, J., Peirats-Llobet, M., Rodriguez, L., Berbel, A., Dietrich, D., Fernandez, M.A., Madueño, F., Bennett, M.J., and Rodriguez, P.L. 2018. PYL8 mediates ABA perception in the root through non-cell-autonomous and ligand-stabilization–based mechanisms. Proceedings of the National Academy of Sciences, USA**115**, E11857-E11863.

Bennett, T., van den Toorn, A., Willemsen, V., and Scheres, B. 2014. Precise control of plant stem cell activity through parallel regulatory inputs. Development **141**, 4055-4064.

Berhin, A., de Bellis, D., Franke, R.B., Buono, R.A., Nowack, M.K., and Nawrath, C. 2019. The root cap cuticle: a cell wall structure for seedling establishment and lateral root formation. Cell **176**, 1367-1378.

Bhosale, R., Boudolf, V., Cuevas, F., Lu, R., Eekhout, T., Hu, Z.B., et al. 2018a. A spatiotemporal DNA endoploidy map of the Arabidopsis root reveals roles for the endocycle in root development and stress adaptation. Plant Cell **30**, 2330–2351.

Bhosale, R., Giri, J., Pandey, B.K., et al. 2018b. A mechanistic framework for auxin dependent Arabidopsis root hair elongation to low external phosphate. Nature Communications **9**, 1–9.

Bingham, I.J. 2012. Factors affecting the longevity of clover roots following shoot excision and its implications for managing N cycling in arable cropping systems. Soil Biology and Biochemistry **50**, 199-207.

Bingham, I.J., and Rees, R.M. 2008. Senescence and N release from clover roots following permanent excision of the shoot. Plant and Soil **303**, 229-240.

Cai, X.T., Xu, P., Zhao, P.X., Liu, R., Yu, L.H., Xiang, C.B. 2014. Arabidopsis ERF109 mediates cross-talk between jasmonic acid and auxin biosynthesis during lateral root formation. Nature Communications **5**, 5833.

Campilho, A., Garcia, B., Toorn, H.V., Wijk, H.V., Campilho, A., and Scheres B. 2006. Time-lapse analysis of stem-cell divisions in the *Arabidopsis thaliana* root meristem. Plant Journal **48**, 619–627.

Carlsbecker, A., Lee, J.Y., Roberts, C.J., Dettmer, J., Lehesranta, S., Zhou, J., Lindgren, O., Moreno-Risueno, M.A., Vaten, A., Thitamadee, S., Campilho, A., Sebastian, J., Bowman, J.L., Helariutta, Y., and Benfey, P.N. 2010. Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. Nature **465**, 316-321.

Chen, W.W., Takahashi, N., Hirata, Y., Ronald, J., Porco, S., Davis, S.J., Nusinow, D.A., Kay, S.A., and Mas, P. 2020. A mobile ELF4 delivers circadian temperature information from shoots to roots. Nature Plants **6**, 416–426.

Comas, L.H., Eissenstat, D.M., and Lakso, A.N. 2000. Assessing root death and root system dynamics in a study of grape canopy pruning. New Phytologist **147**, 171–178.

Cormier, F., Foulkes, J., Hirel, B., Gouache, D., Moënne-Loccoz, Y., and Le Gouis, J. 2016. Breeding for increased nitrogen-use efficiency: a review for wheat (*T. aestivum* L.). Plant Breeding **135**, 255–278.

Courchesne, D.N., Wilson, A.Z., and Ryser, P. 2020. Regional distribution patterns of wetland monocots with different root turnover strategies are associated with local variation in soil temperature. New Phytologist **226**, 86-97.

Cruz-Ramírez, A., Díaz-Triviño, S., Wachsman, G., Du, Y., Arteága-Vázquez, M., Zhang, H., Benjamins, R., Blilou, I., Neef, A.B., Chandler, V., and Scheres B. 2013. A SCARECROW-RETINOBLASTOMA protein network controls protective quiescence in the Arabidopsis root stem cell organizer. PLoS Biology **11**, e1001724.

Cui, H.C., Levesque, M.P., Vernoux, T., Jung, J.W., Paquette, A.J., Gallagher, K.L., Wang, J.Y., Blilou, I., Scheres, B., and Benfey P.N. 2007. An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. Science **316**, 421–425.

Datta, S., Prescott, H., and Dolan, L. 2015. Intensity of a pulse of RSL4 transcription factor synthesis determines Arabidopsis root hair cell size. Nature Plants **1**, 15138.

De Rybel, B., Adibi, M., Breda, A.S., Wendrich, J.R., Smit, M.E., Novák, O., Yamaguchi, N., Yoshida, S., Van Isterdael, G., Palovaara, J., Nijsse, B., Boekschoten, M.V., Hooiveld, G., Beeckman, T., Wagner, D., Ljung, K., Fleck, C., and Weijers, D. 2014a. Integration of growth and patterning during vascular tissue formation in Arabidopsis. Science **345**, 1255215.

De Rybel, B., Breda, A.S., and Weijers, D. 2014b. Prenatal plumbing–vascular tissue formation in the plant embryo. Physiologia Plantarum **151,** 126-133.

De Smet, I., Vassileva, V., De Rybel, B., Levesque, M.P., Grunewald, W., Van Damme, D., Van Noorden, G., Naudts, M., Van Isterdael, G., De Clercq, R., Wang, J.Y., Meuli, N., Vanneste, S., Friml, J., Hilson, P., Jürgens, G., Ingram, G.C., Inzé, D., Benfey, P.N., and Beeckman, T. 2008. Receptor-like kinase ACR4 restricts formative cell divisions in the Arabidopsis root. Science **322**, 594-597.

Dello Ioio, R., Nakamura, K., Moubayidin, L., Perilli, S., Taniguchi, M., Morita, M.T., Aoyama, T., Costantino, P., and Sabatini, S. 2008. A genetic framework for the control of cell division and differentiation in the root meristem. Science **322**, 1380-1384.

Di Mambro, R., De Ruvo, M., Pacifici, E., Salvi, E., Sozzani, R., Benfey, P.N., Busch, W., Novak, O., Ljung, K., Di Paola, L., Marée, A.F.M., Costantino, P., Grieneisen, V.A., and Sabatini, S. 2017. Auxin minimum triggers the developmental switch from cell division to cell differentiation in the Arabidopsis root. Proceedings of the National Academy of Sciences, USA **114**, E7641-E7649.

Dindas, J., Scherzer, S., Roelfsema, M.R.G., von Meyer, K., Müller, H.M., Al-Rasheid, K.A.S., Palme, K., Dietrich, P., Becker, D., Bennett, M.J., and Hedrich, R. 2018. AUX1-mediated root hair auxin influx governs SCF$^{TIR1/AFB}$-type Ca^{2+}signaling. Nature Communications **9**, 1174.

Drapek, C., Sparks, E.E., and Benfey, P.N. 2017. Uncovering gene regulatory networks controlling plant cell differentiation. Trends in Genetics **33**, 529–539.

Drapek, C., Sparks, E.E., Marhavy, P., Taylor, I., Andersen, T.G., Hennacy, J.H., Geldner, N., and Benfey, P.N. 2018. Minimum requirements for changing and maintaining endodermis cell identity in the Arabidopsis root. Nature Plants **4**, 586-595.

Du, Y., and Scheres, B. (2017) PLETHORA transcription factors orchestrate *de novo* organ patterning during Arabidopsis lateral root outgrowth. Proceedings of the National Academy of Sciences, USA **114**, 11709–11714.

Efroni, I., Mello, A., Nawy, T., Ip, P.L., Rahni, R., DelRose, N., Powers, A., Satija, R., and Birnbaum K.D. 2016. Root regeneration triggers an embryo-like sequence guided by hormonal interactions. Cell **165**, 1721-1733.

Efroni, I. 2018. A Conceptual framework for cell identity transitions in plants. Plant Cell Physiology **59**, 691-701.

Fang, S., Clark, R.T., Zheng, Y., Iyer-Pascuzzi, A.S., Weitz, J.S., Kochian, L.V., Edelsbrunner, H., Liao, H., and Benfey, P.N. 2013. Genotypic recognition and spatial responses by rice roots. Proceedings of the National Academy of Sciences, USA **110**, 2670-2675.

Fendrych, M., Van Hautegem, T., Van Durme, M., Olvera-Carrillo, Y., Huysmans, M., Karimi, M., Lippens, S., Guérin, C.J., Krebs, M., Schumacher, K., and Nowack, M.K. 2014. Programmed cell death controlled by ANAC033/SOMBRERO determines root cap organ size in Arabidopsis. Current Biology **24**, 931–940.

Fernández-Marcos, M., Desvoyes, B., Manzano, C., Liberman, L.M., Benfey, P.N., Del Pozo, J.C., and Gutierrez, C. 2017. Control of Arabidopsis lateral root primordium boundaries by MYB36. New Phytologist **213**, 105–112.

Fusseder, A. 1987.The longevity and activity of the primary root of maize. Plant Soil **101**, 257–265.

García-Gómez, M.L., Azpeitia, E., and Alvarez-Buylla, E.R. 2017. A dynamic genetic-hormonal regulatory network model explains multiple cellular behaviors of the root apical meristem of Arabidopsis thaliana. PLOS Computational Biology **13**, e1005488.

Giri, J., Bhosale, R., Huang, G., et al. 2018. Rice auxin influx carrier OsAUX1 facilitates root hair elongation in response to low external phosphate. Nature Communications **9**, 1408.

Guan, P.Z., Wang, R.C., Nacry, P., Breton, G., Kay, S.A., Pruneda-Paz, J.L., Davani, A., and Crawford, N.M. 2014. Nitrate foraging by Arabidopsis roots is mediated by the transcription factor TCP20 through the systemic signaling pathway. Proceedings of the National Academy of Sciences, USA **111**, 15267–15272.

Hassan, H., Scheres, B., and Blilou, I. 2010. JACKDAW controls epidermal patterning in the Arabidopsis root meristem through a non-cell-autonomous mechanism. Development **137**, 1523–1529.

Heilmeier, H., Schultze, E.D., and Whale, D.M. 1986. Carbon and nitrogen partitioning in the biennial monocarp *Arctium tomentosum* Mill. Oecologia **70**, 466-474.

Hetherington, A.J., and Dolan, L. 2018. Stepwise and independent origins of roots among land plants. Nature **52**, 381.

Heyman, J., Cools, T., Canher, B., Shavialenka, S., Traas, J., Vercauteren, I., Van den Daele, H., Persiau, G., De Jaeger, G., Sugimoto, K., et al. 2016. The heterodimeric transcription factor complex ERF115-PAT1 grants regeneration competence. Nature Plants **2**, 16165.

Hong, J.H., Savina, M., Du J., Devendran, A., Kannivadi Ramakanth, K., Tian, X., Sim, W.S., Mironova, V.V., and Xu J. 2017. A sacrifice-for-survival mechanism protects root stem cell niche from chilling stress. Cell **170**, 102–113.e14.

Hu, B., Jiang, Z., Wang, W., Qiu, Y., Zhang, Z., Liu, Y., Li, A., Gao, X., Liu, L., Qian, Y. et al. 2019. Nitrate-NRT1.1B-SPX4 cascade integrates nitrogen and phosphorus signalling networks in plants. Nature Plants **5**, 401–413.

Huijser, P., and Schmid, M. 2011. The control of developmental phase transitions in plants. Development **138**, 4117–4129.

Ikeuchi, M., Ogawa, Y., Iwase, A., and Sugimoto, K. 2016. Plant regeneration: cellular origins and molecular mechanisms. Development **143**, 1442–1451.

Jing, H.C., Schippers, J.H., Hille, J., and Dijkwel, P.P. 2005. Ethylene-induced leaf senescence depends on age-related changes and OLD genes in Arabidopsis. Journal of Experimental Botany **56**, 2915-2923.

Kalmbach, L., Hématy, K., De Bellis, D., Barberon, M., Fujita, S., Ursache, R., Daraspe, J., and Geldner, N. 2017. Transient cell-specific EXO70A1 activity in the CASP domain and Casparian strip localization. Nature Plants **3**, 17058.

Kawakatsu, T., Stuart, T., Valdes, M., Breakfield, N., Schmitz, R.J., Nery, J.R., Urich, M.A., Han, X., Lister, R., Benfey, P.N., et al. 2016. Unique cell-type-specific patterns of DNA methylation in the root meristem. Nature Plants **2**, 16058.

Kirschner, G.K., Stahl, Y., Von Korff, M., and Simon, R. 2017. Unique and conserved features of the barley root meristem. Frontiers in Plant Science **8**, 1240.

Kobayashi, K., Ohnishi, A., Sasaki, D., Fujii, S., Iwase, A., Sugimoto, K., Masuda, T., and Wada, H. 2017. Shoot removal induces chloroplast development in roots via cytokinin signaling. Plant Physiology **173**, 2340–2355.

Kochian, L.V., Hoekenga, O.A., and Piñeros, M.A. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Annual Review of Plant Biology **55**, 459-493.

Kornet, N., and Scheres, B. 2009. Members of the GCN5 histone acetyltransferase complex regulate PLETHORA-mediated root stem cell niche maintenance and transit amplifying cell proliferation in Arabidopsis. Plant Cell **21**, 1070–1079.

Kosola, K.R., Dickmann, D.I., and Parry, D. 2002. Carbohydrates in individual poplar fine roots: effects of root age and defoliation. Tree Physiology **22**, 741–746.

Kunkle, J.M., Walters, M.B., and Kobe, R.K. 2009. Senescence-related changes in nitrogen in fine roots: mass loss affects estimation. Tree Physiology **29**, 715-723.

Laskowski, M., and Ten Tusscher, K.H. 2017. Periodic lateral root priming: What makes it tick? Plant Cell **29**, 432–444.

Li, P., Yu, Q., Gu, X., Xu, C., Qi, S., Wang, H., Zhong, F., Baskin, T.I., Rahman, A., and Wu, S. 2018. Construction of a functional casparian strip in non-endodermal lineages is orchestrated by two parallel signaling systems in Arabidopsis thaliana. Current Biology **28**, 2777–2786.

Liberman, L.M., Sparks, E.E., Moreno-Risueno, M.A., Petricka, J.J., and Benfey, P.N. 2015. MYB36 regulates the transition from proliferation to differentiation in the Arabidopsis root. Proceedings of the National Academy of Sciences, USA **112**, 12099–12104.

Liu, R., Cai, X-T., Zhao, P-X., Xu, P., and Xiang, C-B. 2019b. Arabidopsis ERF109 regulates auxin transport-related genes in root development. *bioRxiv* preprint doi: 10.1101/725572.

Liu, Y., Lai, N., Gao, K., Chen, F., Yuan, L., and Mi, G. 2013. Ammonium inhibits primary root growth by reducing the length of meristem and elongation zone and decreasing elemental expansion rate in the root apex in *Arabidopsis thaliana*. PLoS One **8**, e61031.

Liu, Z., Marella, C.B., Hartmann, A., Hajirezaei, M.R., and von Wirén, N. 2019a. An age-dependent sequence of physiological processes defines developmental root senescence. Plant Physiology **181**, 993-1007.

López-Arredondo, D.L., Leyva-Gonzalez, M.A., Gonzalez-Morales, S.A., Lopez-Bucio, J., and Herrera-Estrella, L. 2014. Phosphate nutrition: improving low-phosphate tolerance in crops. Annual Review of Plant Biology **65**, 95-123.

Maeda, Y., Konishi, M., Kiba, T., Sakuraba, Y., Sawaki, N., Kurai, T., Ueda, Y., Sakakibara, H., and Yanagisawa, S. 2018. A NIGT1-centred transcriptional cascade regulates nitrate signalling and incorporates phosphorus starvation signals in Arabidopsis. Nature Communications **9**, 1376.

Marhava, P., Hoermayer, L., Yoshida, S., Marhavy, P., Benkova, E., and Friml, J. 2019. Re-activation of stem cell pathways for pattern restoration in plant wound healing. Cell **177**, 957–969.

Marin, E., Jouannet, V., Herz, A., Lokerse, A.S., Weijers, D., Vaucheret, H., Nussaume, L., Crespi, M.D., and Maizel, A. 2010. miR390, Arabidopsis TAS3 tasiRNAs, and their AUXIN RESPONSE FACTOR targets define an autoregulatory network quantitatively regulating lateral root growth. Plant Cell **22**, 1104–1117.

Medici, A., Szponarski, W., Dangeville, P., Safi, A., Dissanayake, I.M., Saenchai, C., Emanuel, A., Rubio, V., Lacombe, B., Ruffel, S. et al. 2019. Identification of molecular integrators shows that nitrogen actively controls the phosphate starvation response in plants. Plant Cell **31**, 1171–1184

Meier, M., Liu, Y., Lay-Pruitt, K.S., Takahashi, H., and von Wirén, N. 2020. Auxin-mediated root branching is determined by the form of available nitrogen. Nature Plants **6**, 1136-1145.

Miyashima, S., Koi, S., Hashimoto, T., and Nakajima, K. 2011. Non-cell-autonomous microRNA165 acts in a dose-dependent manner to regulate multiple differentiation status in the Arabidopsis root. Development **138**, 2303–2313

Miyashima, S., Roszak, P., Sevilem, I., Toyokura, K., Blob, B., Heo, J.O., Mellor, N., Help-Rinta-Rahko, H., Otero, S., Smet, W., et al. 2019. Mobile PEAR transcription factors integrate positional cues to prime cambial growth. Nature **565**, 490–494

Möller, B.K., Xuan, W., and Beeckman, T. 2017. Dynamic control of lateral root positioning. Current Opinion in Plant Biology **35**, 1–7.

Moreno-Risueno, M.A., Sozzani, R., Yardimci, G.G., et al. 2015. Transcriptional control of tissue formation throughout root development. Science **350**, 426–430.

Moreno-Risueno, M.A., Van Norman, J.M., Moreno, A., Zhang, J., Ahnert, S.E., and Benfey, P.N. 2010. Oscillating gene expression determines competence for periodic Arabidopsis root branching. Science **329**, 1306–1311.

Motte, H., Vanneste, S., and Beeckman, T. 2019. Molecular and environmental regulation of root development. Annual Review of Plant Biology **70**, 465–488.

Munné-Bosch, S. 2007. Aging in perennials. Critical Reviews in Plant Sciences **26**, 123-138.

Munné-Bosch, S. 2008. Do perennials really senesce? Trends in Plant Science **13**, 216-220.

Munné-Bosch, S. 2014. Perennial roots to immortality. Plant Physiology **166**, 720–725.

Muraro, D., Mellor, N., Pound, M.P., Help, H., Lucas, M., Chopard, J., Byrne, H.M., Godin, C., Hodgman, T.C., King, J.R., et al. 2014. Integration of hormonal signaling networks and mobile microRNAs is required for vascular patterning in Arabidopsis roots. Proceedings of the National Academy of Sciences, USA **111**, 857–862.

Mylona, P., Linstead, P., Martienssen, R., and Dolan, L. 2002. SCHIZORIZA controls an asymmetric cell division and restricts epidermal identity in the Arabidopsis root. Development **129**, 4327–4334.

Perianez-Rodriguez, J., Rodriguez, M., Marconi, M., Bustillo-Avendaño, E., Wachsman, G., Sanchez-Corrionero, A., et al. 2021. An auxin-regulable oscillatory circuit drives the root clock in Arabidopsis. Science Advances **7**:eabd4722. 10.1126/sciadv.abd4722.

Pujar, A., Jaiswal, P., Kellogg, E.A., Ilic, K., Vincent, L., Avraham, S., Stevens, P., Zapata, F., Reiser, L., Rhee, S.Y., Sach, M.M., Schaeffer, M., Stein, L., Ware, D., and McCouch, S. 2006. Whole-plant growth stage ontology for angiosperms and its application in plant biology. Plant Physiology **142**, 414-428.

Ogura, T., Goeschl, C., Filiault, D., Mirea, M., Slovak, R., Wolhrab, B., Satbhai, S.B., and Busch, W. 2019. Root system depth in Arabidopsis is shaped by EXOCYST70A3 via the dynamic modulation of auxin transport. Cell **178**, 400–412.

Orman-Ligeza, B., Morris, E.C., Parizot, B., Lavigne, T., Babé, A., Ligeza, A., Klein, S., Sturrock, C., Xuan, W., Novák, O., et al. 2018. The xerobranching response represses lateral root formation when roots are not in contact with water. Current Biology **28**, 3165–3173.

Orosa-Puente, B., Leftley, N., von Wangenheim, D., Banda, J., Srivastava, A.K., Hill, K., Truskina, J., Bhosale, R., Morris, E., Srivastava, M., et al. 2018. Root branching toward water involves posttranslational modification of transcription factor ARF7. Science **362**, 1407–1410.

Pacifici, E., Di Mambro, R., Dello Ioio, R., Constantino, P., and Sabatini, S. 2018. Acidic cell elongation drives cell differentiation in the Arabidopsis root. EMBO Journal **37**, e99134.

Paquette, A.J., and Benfey, P.N. 2005. Maturation of the ground tissue of the root is regulated by gibberellin and SCARECROW and requires SHORT-ROOT. Plant Physiology **138**, 636–640.

Patterson, K., Walters, L.A., Cooper, A.M., Olvera, J.G., Rosas, M.A., Rasmusson, A.G., and Escobar, M.A. 2016. Nitrate-regulated glutaredoxins control Arabidopsis primary root growth. Plant Physiology **170**, 989–999.

Poethig, R.S. 1990. Phase change and the regulation of shoot morphogenesis in plants. Science **250**, 923-930.

Poethig, R.S. 2003. Phase change and the regulation of developmental timing in plants. Science **301**, 334–336.

Postma, J.A., Dathe, A., and Lynch, J.P. 2014. The optimal lateral root branching density for maize depends on nitrogen and phosphorus availability. Plant Physiology **166**, 590–602.

Powers, S.K., Holehouse, A.S., Korasick, D.A., Schreiber, K.M., Clark, N., et al. 2019. Nucleo-cytoplasmic partitioning of ARF proteins controls auxin responses in *Arabidopsis thaliana*. Molecular Cell **76**, 177–190.

Ristova, D., Carré, C., Pervent, M., et al. 2016. Combinatorial interaction network of transcriptomic and phenotypic responses to nitrogen and hormones in the *Arabidopsis thaliana* root. Science Signaling **9**, rs13.

Robbins, N.E., and Dinneny, J.R. 2018. Growth is required for perception of water availability to pattern root branches in plants. Proceedings of the National Academy of Sciences, USA**115**, E822–E831.

Rogers, E.D., Monaenkova, D., Mijar, M., Nori, A., Goldman, D.I., and Benfey, P.N. 2016. X-ray computed tomography reveals the response of root system architecture to soil texture. Plant Physiology **171**, 2028–2040.

Rosspopoff, O., Chelysheva, L., Saffar, J., Lecorgne, L., Gey, D., Caillieux, E., Colot, V., Roudier, F., Hilson, P., Berthome, R., et al. 2017. Direct conversion of root primordium into shoot meristem relies on timing of stem cell niche development. Development **144**, 1187–1200.

Ruffel, S., Krouk, G., Ristova, D., Shasha, D., Birnbaum, K.D., and Coruzzi, G.M. 2011. Nitrogen economics of root foraging: transitive closure of the nitrate-cytokinin relay and distinct systemic signaling for N supply vs. demand. Proceedings of the National Academy of Sciences, USA **108**, 18524–18529.

Ryser, P., Puig, S., Mueller, M., and Munné-Bosch, S. 2020. Abscisic acid responses match the different patterns of autumn senescence in roots and leaves of *Iris versicolor* and *Sparganium emersum*. Environmental Experimental Botany **176**, 104097.

Sabatini, S., Heidstra, R., Wildwater, M., and Scheres, B. 2003. SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. Genes & Development **17**, 354–358.

Sheng, L., Hu, X., Du, Y., Zhang, G., Huang, H., Scheres, B., and Xu, L. 2017. Non-canonical WOX11-mediated root branching contributes to plasticity in Arabidopsis root system architecture. Development **144**, 3126–3133.

Scheres, B., and van der Putten, W.H. 2017. The plant perception connects environment to development. Nature **543**, 337–345.

Shimotohno, A., Heidstra, R., Blilou, I., and Scheres, B. 2018. Root stem cell niche organizer specification by molecular convergence of PLETHORA and SCARECROW transcription factor modules. Genes & Development **32**, 1085–1100.

Schneider, H.M., Postma, J.A., Wojciechowski, T., Kuppe, C., and Lynch, J.P. 2017a. Root cortical senescence improves growth under suboptimal availability of N, P, and K. Plant Physiology **174**, 2333–2347.

Schneider, H.M., Wojciechowski, T., Postma, J.A., Brown, K.M., Lücke, A., Zeisler, V., Schreiber, L., Lynch, J.P. 2017b. Root cortical senescence decreases root respiration, nutrient content and radial water and nutrient transport in barley. Plant, Cell and Environment **40**, 1392–1408.

Schneider, H.M., Wojciechowski, T., Postma, J.A., Brown, K.M., and Lynch, J.P. 2018. Ethylene modulates root cortical senescence in barley. Annals of Botany **122**, 95–105.

Smetana, O., Makila, R., Lyu, M., et al. 2019. High levels of auxin signaling define the stem-cell organizer of the vascular cambium. Nature **565**, 485–489.

Strock, C.F., de la Riva L.M., and Lynch, J.P. 2018. Reduction in root secondary growth as a strategy for phosphorus acquisition. Plant Physiology **176**, 691–703.

Strotmann, V.I., and Stahl, Y. 2021. At the root of quiescence - Function and regulation of the quiescent center in roots. Journal of Experimental Botany **72**, 1-11.

Sugimoto, K., Gordon, S.P., and Meyerowitz, E.M. 2011. Regeneration in plants and animals: Dedifferentiation, transdifferentiation, or just differentiation? Trends in Cell Biology **21**, 212–218.

Sutton, M.A., Bleeker, A., Howard, C.M., Bekunda, M., et al. 2013. Our nutrient world: The challenge to produce more food and energy with less pollution. *Global Overview of Nutrient Management*. Centre for Ecology and Hydrology, Edinburgh on behalf of the Global Partnership on Nutrient Management and the International Nitrogen Initiative.

Tabata, R., Sumida, K., Yoshii, T., Ohyama, K., Shinohara, H., and Matsubayashi, Y. 2014. Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. Science **346**, 343–346.

Takeda, S., Gapper, C., Kaya, H., Bell, E., Kuchitsu, K., and Dolan, L. 2008. Local positive feedback regulation determines cell shape in root hair cells. Science **319**, 1241–1244.

Tanaka, H., Watanabe, M., Watanabe, D., Tanaka, T., Machida, C., and Machida, Y. 2002. *ACR4*, a putative receptor kinase gene of *Arabidopsis thaliana*, that is expressed in the outer cell layers of embryos and plants, is involved in proper embryogenesis. Plant Cell Physiology **43**, 419–428.

Thomas, H. 2013. Senescence, ageing and death of the whole plant. New Phytologist **197**, 696-711.

Timilsina, R., Kim., J.H., Nam, H.G., and Woo, H.R. 2019. Temporal changes in cell division rate and genotoxic stress tolerance in quiescent center cells of Arabidopsis primary root apical meristem. Scientific Reports **9**, 3599.

Van Norman, J.M., Zhang, J., Cazzonelli, C.I., Pogson, B.J., Harrison, P.J., Bugg, T.D.H., Chan, K.X., Thompson, A.J., and Benfey, P.N. 2014. Periodic root branching in Arabidopsis requires synthesis of an uncharacterized carotenoid derivative. Proceedings of the National Academy of Sciences, USA **111**, E1300–E1309.

Wachsman, G., Heidstra, R., and Scheres, B. 2011. Distinct cell-autonomous functions of RETINOBLASTOMA-RELATED in Arabidopsis stem cells revealed by the brother of brainbow clonal analysis system. Plant Cell **23**, 2581–2591.

Wachsman, G., Zhang, J., Moreno-Risueno, M.A., Anderson, C.T., Benfey, P.N. 2020. Cell wall remodeling and vesicle trafficking mediate the root clock in Arabidopsis. Science **370**, 819–823.

Waidmann, S., Ruiz Rosquete, M., Schöller, M., et al. 2019. Cytokinin functions as an asymmetric and anti-gravitropic signal in lateral roots. Nature Communications **10**, 3540.

Watanabe, K., Nishiuchi, S., Kulichikhin, K., and Nakazono, M. 2013. Does suberin accumulation in plant roots contribute to waterlogging tolerance? Frontiers in Plant Science **4**: 178.

Watanabe, M., Tanaka, H., Watanabe, D., Machida, C., and Machida, Y. 2004. The ACR4 receptor-like kinase is required for surface formation of epidermis-related tissues in *Arabidopsis thaliana*. Plant Journal **39**, 298–308.

Webb, K.J., Jensen, E.F., Heywood, S., Morris, S.M., Linton, P.E., Hooker, J.E. 2010. Gene expression and nitrogen loss in senescing root systems of red clover (*Trifolium pratense*). The Journal of Agricultural Science **148**, 579-591.

Wein, A., Le Gac, A.L., and Laux, T. 2020. Stem cell ageing of the root apical meristem of *Arabidopsis thaliana*. Mechanisms of ageing and development **190**, 111313.

Wendrich, J.R., Möller, B.K., Li, S., Saiga, S., Sozzani, R., Benfey, P.N., De Rybel, B., and Weijers, D. 2017. Framework for gradual progression of cell ontogeny in the Arabidopsis root meristem. Proceedings of the National Academy of Sciences, USA **114**, 8922–8929.

Wendrich, J.R., Yang, B., Vandamme, N., Verstaen, K., Smet, W., Van de Velde, C., Minne, M., Wybouw, B., Mor, E., Arents, H.E., Nolf, J., Van Duyse, J., Van Isterdael, G., Maere, S., Saeys, Y., and De Rybel, B. 2020. Vascular transcription factors guide plant epidermal responses to limiting phosphate conditions. Science **370**: eaay4970.

Wenzel, C.L., and McCully, M.E. 1991. Early senescence of cortical cells in the roots of cereals. How good is the evidence? American Journal of Botany **78**, 1528–1541.

Withington, J.M., Reich, P.B., Oleksyn, J., and Eissenstat, D.M. 2006. Comparisons of structure and life span in roots and leaves among temperate trees. Ecological Monographs **76**, 381-397.

Wojciechowska, N., Sobieszczuk-Nowicka, E., and Bagniewska-Zadworna, A. 2018. Plant organ senescence - regulation by manifold pathways. Plant Biology (Stuttg) **20**, 167–181.

Wu, S., Lee, C.M., Hayashi, T., Price, S., Divol, F., Henry, S., Pauluzzi, G., Perin, C., and Gallagher, K.L. 2014. A plausible mechanism, based upon Short-Root movement, for regulating the number of cortex cell layers in roots. Proceedings of the National Academy of Sciences, USA, **111**, 16184–16189.

Xuan, W., Band, L.R., Kumpf, R.P., et al. 2016. Cyclic programmed cell death stimulates hormone signaling and root development in Arabidopsis. Science **351**, 384–387.

Xuan, W., De Gernier, H., and Beeckman, T. 2020. The dynamic nature and regulation of the root clock. Development **147**, dev181446. doi.org/10.1242/dev.181446.

Yan, D., Easwaran, V., Chau, V., et al. 2016. NIN-like protein 8 is a master regulator of nitrate-promoted seed germination in Arabidopsis. Nature Communications **7**, 13179.

Yeates, J.S., and Parker, C.A. 1986. Rate of natural senescence of seminal root cortical cells of wheat, barley and oats, with reference to invasion by *Gaeumannomyces graminis*. Transactions of the British Mycological Society **86**, 683–685.

Yu, P., Eggert, K., Von Wirén, N., Li, C., and Hochholdinger, F. 2015b. Cell-type specific gene expression analyses by RNA-Seq reveal local high nitrate triggered lateral root initiation in shoot-borne roots of maize by modulating auxin-related cell cycle-regulation. Plant Physiology **169**, 690–704.

Yu, S., Lian, H., and Wang, W. 2015a. Plant developmental transitions: the role of microRNAs and sugars. Current Opinion in Plant Biology **27**, 1–7.

Zhan, A., and Lynch, J.P. 2015. Reduced frequency of lateral root branching improves N capture from low-N soils in maize. Journal of Experimental Botany **66**, 2055–2065.

Zhang, G., Zhao, F., Chen, L., et al. 2019c. Jasmonate-mediated wound signalling promotes plant regeneration. Nature Plants **5**, 491–497.

Zhang, J., Eswaran, G., Alonso-Serra, J., Kucukoglu, M., Xiang, J., Yang, W., et al. 2019a. Transcriptional regulatory framework for vascular cambium development in Arabidopsis roots. Nature Plants **5**, 1033–1042.

Zhang, Y., Xiao, G., Wang, X., Zhang, X., and Friml, J. 2019b. Evolution of fast root gravitropism in seed plants. Nature Communications **10**, 3480.

Zhou, W., Lozano-Torres, J.L., Blilou, I., Zhang, X., Zhai, Q., Smant, G., Li, C., and Scheres, B. 2019. A jasmonate signaling network activates root stem cells and promotes regeneration. Cell **177**, 942–956.

Zhukovskaya, N.V., Bystrova, E.I., Dubrovsky, J.G., and Ivanov, V.B. 2018. Global analysis of an exponential model of cell proliferation for estimation of cell cycle duration in the root apical meristem of angiosperms. Annals of Botany **122**, 811–822.

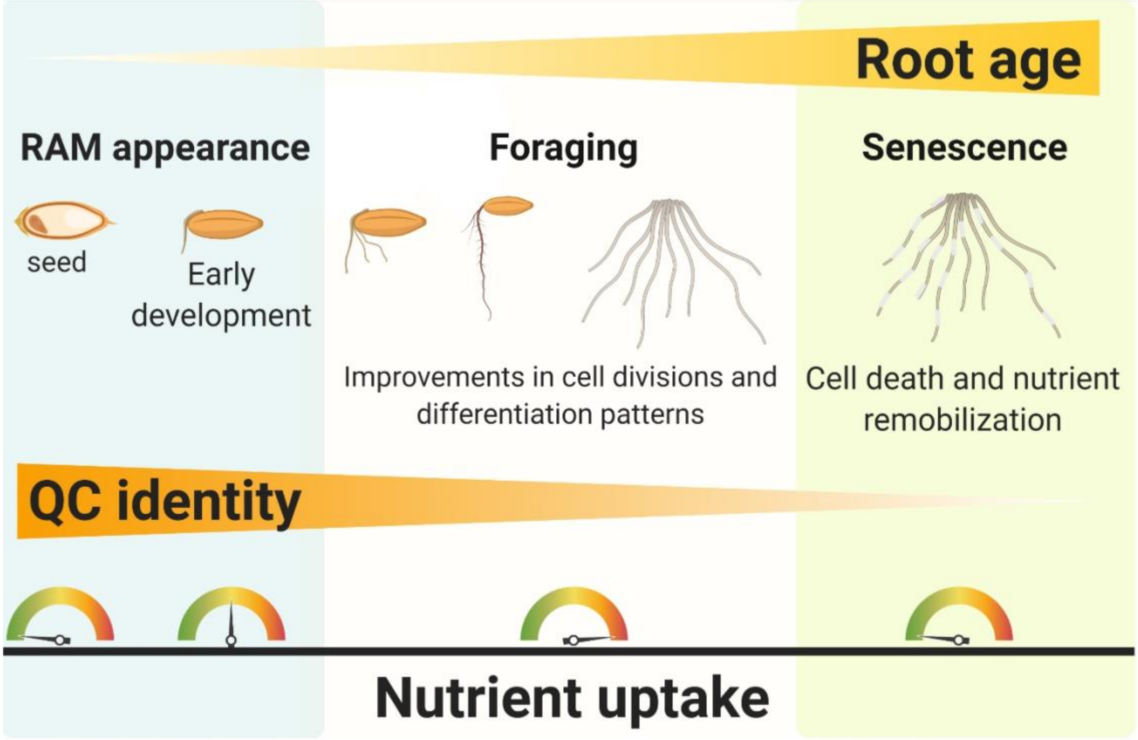

Figure 1. Root developmental phase progression. Following meiosis and the successful fertilization, in which seeds are originated and protect embryo. At this stage, stimuli promote seed germination and patterns of cell divisions are established, where root apical meristem (RAM) emerges to orientate cell divisions and growth during the RAM appearance phase. Thus, quiescent-center (QC) cells having the highest identity and the lowest rates of cell differentiation might be observed in this phase. Under adequate timing and environmental conditions, root foraging phase begins, with constant increases in cell differentiation and improvements in nutrient uptake, wherein at foraging phase nutrient uptake reaches maximum levels (red colour at speedometer) while minimum levels (green colour at speedometer) occurs during later developmental stages. Varying with tissue age, specie and root class, and following the maximum foraging time, the entire senescence phase is kicked off, in which cell proliferation and differentiation are most likely arrested while constant patterns of cell death might be observed. Thus, nutrient uptake reaches the minimal levels, which in older roots triggers losses of segments and nutrient remobilization, culminating in root death.

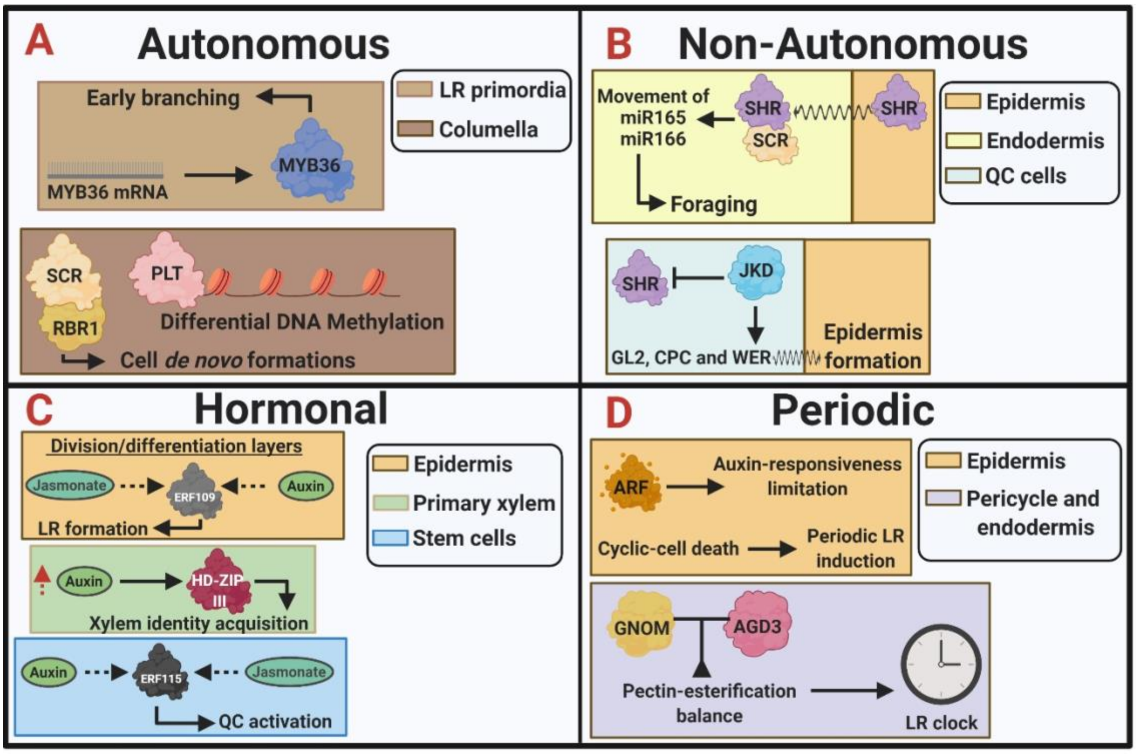

Figure 2. Pathways promoting root developmental phase transitions in *Arabidopsis thaliana*. A, The autonomous pathway seems to promote the root foraging phase, in which the transcription factor MYB36 mRNA is found only in specific cell-types, whereas the MYB36 protein induces genes to promote foraging. Similarly, PLETHORA (PLT) expression is variable across root zones, in which it has the highest levels around root stem cells, where differential DNA methylation patterns might promote the maximum activity of PLT. In these cells, the RETINOBLASTOMA-RELATED 1 (RBR1) protein and SCARECROW (SCR) regulate cell fates around quiescent-center (QC) cells promoting *de novo* formative cell divisions. **B,** mobile elements over the diverse root cell-types describe the non-autonomous pathway. Thus, the SHORTROOT (SHR) protein moves from epidermis to endodermis, where SHR activates SCR that will induce the movement of miR165/6, which in turn suppresses the expression of cell differentiation regulators to release the root foraging phase. The JACKDAW (JKD) protein activity around QC cells delimits SHR movement in these cells, whereas the expression of GLABRA2 (GL2), CAPRICE (CPC), and WEREWOLF (WER) genes is regulated by JKD, resulting in mobility of these three genes to originate epidermis. Overall, the non-autonomous pathway sustains root foraging phase (since middle of the phase), vascular tissue formation, and growth. **C,** Hormones are essential to organism development, emerging a hormonal pathway to

orchestrate root developmental phases. Thus, opposite gradients of auxin (AUX) and cytokinin regulate the patterns of cell proliferation and differentiation. In the root tip, maximum levels of AUX with Jasmonates (JA) might induce the transcription factor ETHYLENE RESPONSE FACTOR 109 (ERF109) that in turn will activate QC cells to promote stem cell fates particularly around root tip. Following, the local AUX maximum at pericycle cells activates transcription factors CLASS III HOMEODOMAIN-LEUCINE ZIPPER (HD-ZIP III), where the raise of AUX and HD-ZIPIII causes xylem identity acquisition. In consequence, at the transition zone ERF109 activity is up regulated according with JA and AUX balance, which induce lateral root (LR) formation. Therefore, the hormonal pathway regulates not only both early and later stages of foraging phase, but also entrance to the senescence phase. **D,** Roots exhibit cells with a large variation for ontogeny requiring complex genetic circuits to regulate developmental phases, suggesting a periodic pathway. The condensation of AUXIN RESPONSE FACTOR (ARF) transcription localization to the cytoplasm, where they are inactivated limiting AUX-responsiveness. Similarly, cyclic cell death on epidermal cells of the transition zone results in periodic LR induction. Meantime, the GNOM protein, a vesicle trafficking regulator, is repressed by ARF-GTP ACTIVATING PROTEIN DOMAIN (AGD3), where both proteins regulate the balance between esterification and de-esterification orientating the turnover of pectin-esterification. In the oscillation zone, root clock is established according a competitive network between GNOM and AGD3 that will orientate the functionality of LR clock to promote root foraging phase. Taken together, the periodic pathway seems to regulate overall phases of root development since early until late cell development.

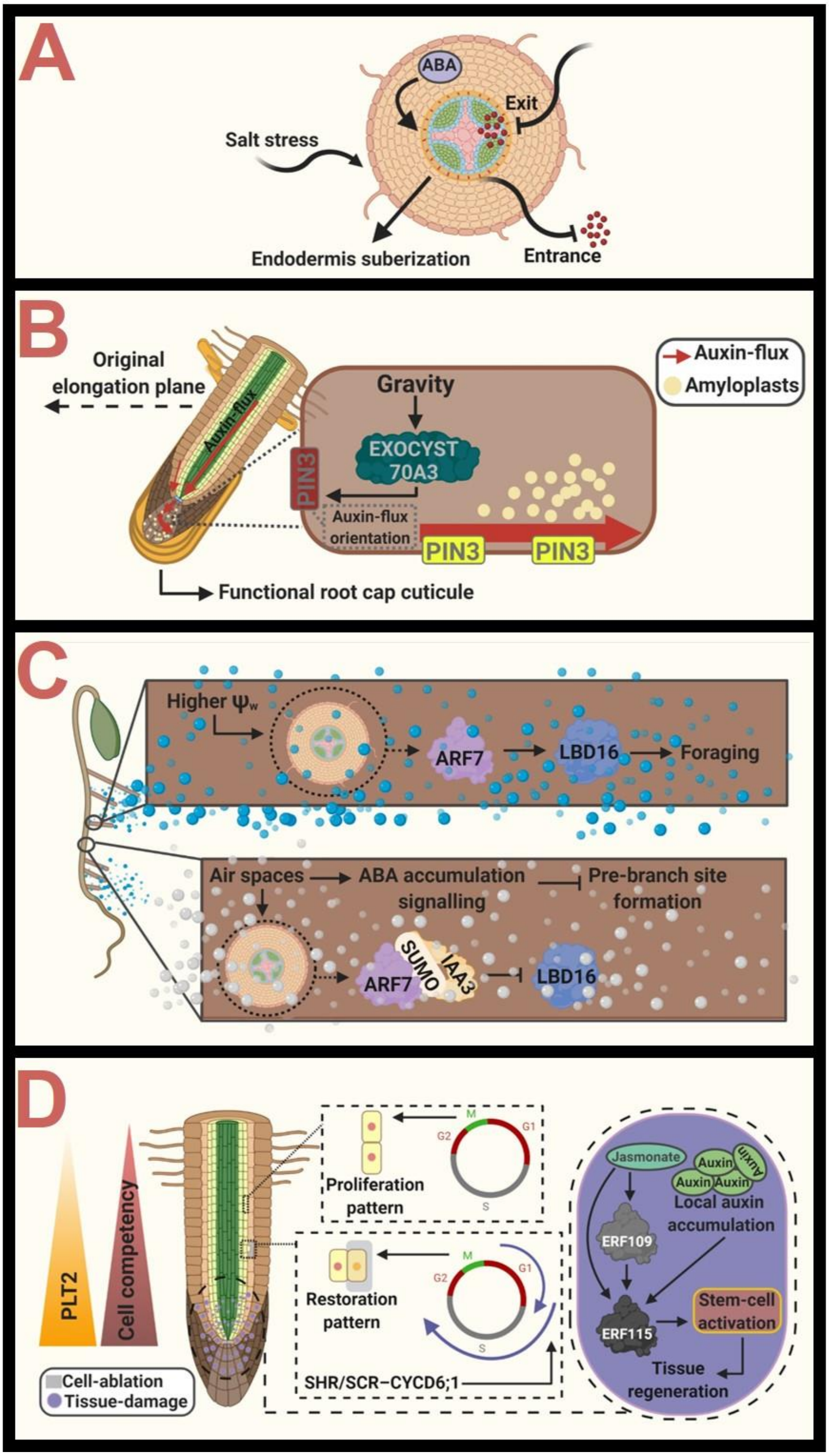

Figure 3. Root developmental phases and plasticity. A, roots growing under natural conditions may experience salt stress. Under these conditions, abscisic acid (ABA) triggers endodermis suberization, which arrests the entrance and exit of substances.

B, roots exhibit a protective structure composed by cutin polyesters (orange caps) that protect meristem at early development, whereas the structure also protects lateral roots during the foraging phase. In consonance, gravitropism exhibits a remarkable effect on EXOCYST70A3 protein activity that in turn modulates PIN3 transporter, which will re-orientate auxin flux to shift original elongation plane of root. Similarly, gravity mediates changes in amyloplast patterns on root apex cell-group populations, altering auxin flow orientation according with PIN2 transporter activity that results in root elongation plane modification. **C,** variations on water potential (Ψw) induce distinct developmental patterns in roots. Higher Ψw promotes AUXIN RESPONSE FACTOR 7 (ARF7) optimal activity that induces LATERAL ORGAN BOUNDARIES DOMAIN 16 (LBD16) gene, promoting the root foraging phase. Contrary, regions around rhizosphere with spaces rich in air enhance SUMOylation of ARF7 (SUMO) improving their interaction with IAA3, which repress LBD16 expression arresting root foraging. In these spaces, ABA accumulation impairs cell identity acquisition blocking pre-branch site formation. **D,** cell ablation and tissue damage are repaired according with particular events. Over the root regions, convergent gradients for transcription factors PLETHORA (PLT) and cell competency for regeneration are observed, where the maximum levels of both are found around quiescent-center (QC) cells. Under optimal conditions for growth, PLT might regulate formative cell proliferation patterns (common cell cycle with four phases G1, Synthesis (S), G2 and Mitosis (M)) while the stem cell status re-activation requires the re-establishment of PLT's expression gradient (restoration pattern) converging to gradient of cell competency for regeneration at damaged site. Thus, restoration pattern shows a divergent cell division plane for proliferation pattern, in which cell cycle progression is accelerated according with the activation of SHORTROOT (SHR), SCRECROW (SCR), and CYCLIN D6;1 (CYCD6;1). This acceleration reflects from a shorter time of occurrence for cell cycle phases (G1, S, G2 and M). Consequently, tissue-damage (purple circles) activates a hormonal circuit to orchestrate tissue repair. Jasmonates might induce directly ERF115 protein that will regulate stem-cell activation and tissue regeneration, and/or jasmonate induce ETHYLENE RESPONSE FACTOR (ERF) 109 and ERF115, which promote the last steps for tissue regeneration. In consonance, local auxin accumulation at damage sites promote the expression ERF115, orientating the stem-cell activation and lately tissue regeneration.

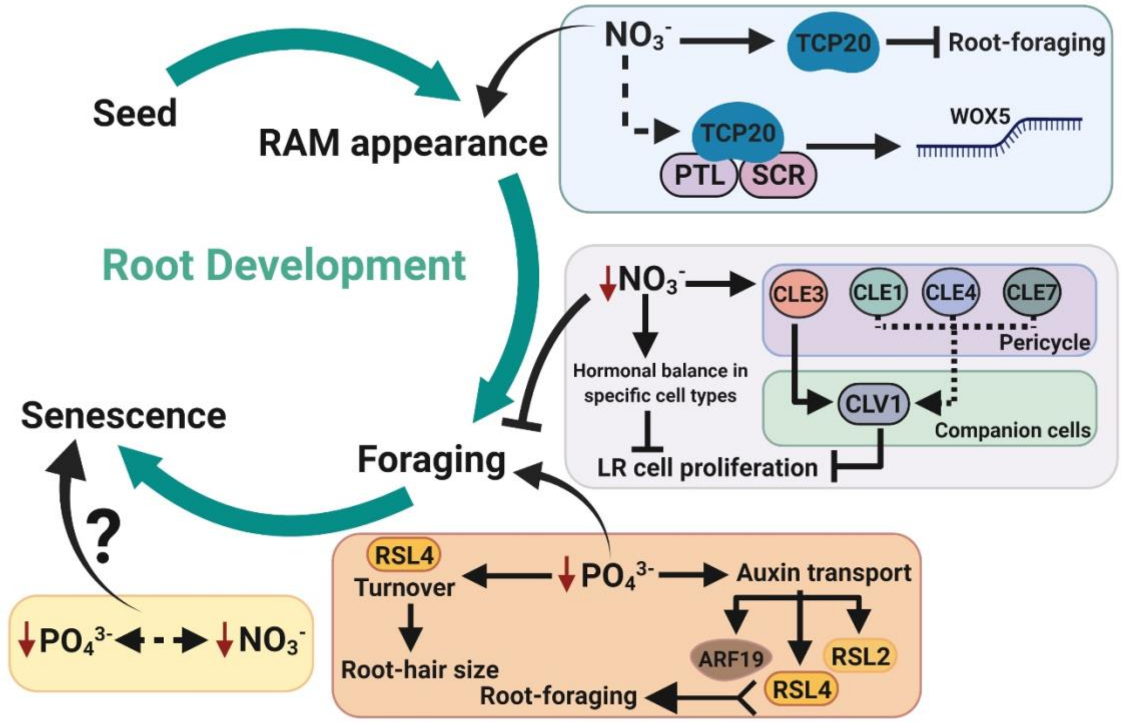

Figure 4. Nutritional checkpoints governing root developmental phase transitions. Nitrate (NO_3) regulates the activity of transcription factor TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR1-20 (TCP20), which arrests the entrance to root foraging phase. Since TCP20 interacts with PLETHORA (PLT) and SCARECROW (SCR) to regulated transcripts like WUSCHEL-RELATED HOMEOBOX 5 (WOX5), which we suggest a NO_3^- role (dotted arrow) to regulate transition of embryonic to root apical meristem (RAM) appearance phase. Low levels of NO_3^- drive the preferential gene expression of CLE (CLAVATA3/ESR-related) 1, 3 and 7 peptides in pericycle cells while CLAVATA1 (CLV1) protein are located in the phloem companion cells, which represses cell proliferation at lateral roots (LR). The precise interactions among hormones (auxin, cytokinin, and abscisic acid) and NO_3^- arrest LR development. Collectively, NO_3^- seems regulate the early developmental root phases, arresting cell differentiation and root foraging phase. Phosphate (PO_4) limitations improve synthesis/degradation of the transcription factor ROOT HAIR DEFECTIVE 6-LIKE 4 (RSL4), in which protein turnover determines the size of root hairs. Still, these conditions trigger the auxin transport from root apex to differentiation zone, where the transcription factors AUXIN RESPONSE FACTOR 19 (ARF19), RSL2, and RSL4 are induced to promote root branch development and foraging on soil layers. Therefore, phosphorous seems to promote and orientate the progression by foraging

phase. Like the general life rule, roots experience aging culminating in senescence phase. The molecular mechanisms programming nutritional checkpoints able to mediate root senescence phase remain poorly documented.

General Conclusions

The results presented within this thesis were mainly focused on the regulation of biological clocks and its implications on crop domestication and plant responsiveness to environmental conditions. The experimental evidence discussed here indicates that biological clocks were synchronized across a single plant during tomato domestication, which apparently cannot be an isolated example of synchronization in domesticated species. This is most likely due to the observed similarities between circadian rhythms on cotyledons of domesticated tomato, while these are rather contrasting in the wild species *Solanum pimpinellifolium*. Furthermore, source-sink relationships were most likely acquired during tomato domestication, highlighting a highly precise metabolic regulation across plant sections. In addition, genetic manipulation of domestication-related genes on *S. pimpinellifolium* synchronized the development and physiology of multiplexed lines, resembling the development of the domesticated tomato. Altogether, these findings support the notion that the relationship between biological clocks have the potential to improve crop yield whereas simultaneously maintaining the resilience of wild species to environmental adversities. We further demonstrated that watering tomato during different periods of the day can result in a tailored development, arresting or inducing the flowering. In terms of fruit production, the best period to water plants is near evening, although watering in the morning can increase leaf production. It seems reasonable to assume, therefore, that watering in different periods of the day can be important not only to improve fruit production in tomato but it can also enhance leaf production, which may be specially interesting in species where leaves are the organ of agronomic importance.

The second section of this thesis was largely focused on understanding aluminum (Al) tolerance in plants. It was verified that the currently accepted mechanism that explains Al tolerance missed largely interactions among distinct pathways involved in Al responses. Not surprisingly, this could have been ultimately the reason why several studies failed in improving the Al tolerance of crops. We proposed an interactive Al tolerance mechanism based on the parallel regulation of the mitochondrial organic acids and DNA checkpoints since those compounds would neutralize Al while DNA checkpoint regulators would allow the maintenance of proper cell divisions. By further looking at this interaction, we began to search for candidates that could regulate mitochondrial metabolism and DNA checkpoints in parallel. Our results revealed that photoperiod is a likely regulator of both processes. Al tolerance is inherently related to the sensitivity of root growth to day length variations, once longer day can induce elevated levels of polyploid cells in roots, increasing the occurrence of endoreduplication and further reducing cell divisions. Furthermore, photoperiod mitigates specifically the impacts of Al toxicity as revealed by the fact that genotoxic drugs known to induce endoreduplication did not have their impacts mitigated under shorter days. We also observed that genes associated with Al sensitivity and endoreduplication were induced only under long days, whereas the genetic variability for these genes seems to have been selected according to day length. We concluded that photoperiod is likely a pivotal environmental factor shaping Al tolerance across the plant kingdom. In the last section of this thesis, we turned our attention to the root developmental program, which was largely missed over the last decades. We posit the existence of specific developmental phases for roots, and that these phases are regulated following at least four pathways promoting root phase changes. Meanwhile, root plasticity is dynamically rewired in most cases without shoot involvement, whereas nutritional checkpoints modulate phase changes over the root lifecycle. Therefore, we expect that root developmental phases will be better understood in the next years, either confirming or refuting our main hypotheses for this singular developmental program. Altogether, the complex synchronization of endogenous cues to environmental fluctuations seems to be a promising approach to generate crop ideotypes, which are likely to be cultivated in the next decades, reducing yield instability and sensitivity to abiotic stresses. Further work is clearly required to establish the regulatory mechanisms involved in the responses described here. The further development and combination of many approaches (as performed here) will additionally allow a fuller description of the metabolic status of a plant in a fluctuating environment. Although the usage of these techniques requires substantial financial investment, it is likely to bring returns in the form of an improved ability to carry out rational engineering of plants to cope with stress conditions with minimal to no yield penalty.

Appendix I

Additional publications

1. Batista-Silva, W., Oliveira, A.C., Martins, A.O., **Siqueira, J.A.**, Rodrigues-Salvador, A., Omena-Garcia, R.P., Medeiros, D.B., Peres, L.E.P., Ribeiro, D.M., Zsögön, A., Fernie, A.R., Nunes-Nesi, A., Araújo, W.L. (2022) Reduced auxin signalling through the cyclophilin DIAGEOTROPICA impacts tomato fruit development and ripening. **Journal of Experimental Botany**, *in press*, doi:10.1093/jxb/erac143.

2. **Siqueira, J.A.**, Oliveira, H.O., Nunes-Nesi, A., Araújo, W.L. (2021) Guard-cells regulation: Pulling the Strings Behind the Scenes. **Trends in Plant Science** 26, 1093–1095**.**

3. Nascimento, V.L., Pereira, A.M., **Siqueira, J.A.**, Pereira, A.S., Silva V.F., Costa, L.C., Ribeiro, D.M., Zsögön. A., Nunes-Nesi, A., W.L. (2021) Exogenous ethylene reduces growth via changes in central metabolism and cell wall composition in tomato (*Solanum lycopersicum*). **Journal of Plant Physiology 263**:153460.

4. Leal-Pereira, G., **Siqueira, J.A.**, Batista-Silva, W., Nunes-Nesi, A., Araújo, W.L. (2021) Boron: More Than an Essential Element for Land Plants? **Frontiers Plant Science 11**:610307.

5. Barros, J.A.S., **Siqueira, J.A.**, Cavalcanti, J.H.F., Araújo, W.L., Avin-Wittenberg, T. (2020) Multifaceted roles of autophagy in modulating lipid metabolism. **Trends in Plant Science 25**, 1141–1153.

6. **Siqueira, J.A.**, Barros, J.A.S., Dal-Bianco, M., Martins, S.C.V., Magalhães, P.C., Ribeiro, D.M., DaMatta, F.M., Araújo, W.L., Ribeiro, C. (2020) Metabolic and physiological adjustments of maize leaves in response to aluminum stress. **Theoretical and Experimental Plant Physiology 32**, 133–145.