JONAS RAFAEL VARGAS

PROTEOMIC AND METABOLIC IMPACTS OF THE LACK OF 2-OXOGLUTARATE DEHYDROGENASE E1 SUBUNIT IN *Arabidopsis thaliana*

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

Adviser: Adriano Nunes Nesi

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ABSTRACT

VARGAS, Jonas Rafael, D.Sc., Universidade Federal de Viçosa, April, 2022. **Proteomic and metabolic impacts of the lack of 2-oxoglutarate dehydrogenase E1 subunit in *Arabidopsis thaliana.*** Adviser: Adriano Nunes Nesi.

The enzyme 2-oxoglutarate dehydrogenase (2OGDH) is a tricarboxylic acid cycle enzyme. This enzyme is a multi-enzymatic complex formed by three subunits that together are responsible for catalyzing the conversion of 2-oxoglutarate (2OG) into succinyl-CoA with the release of NADH and CO_2 in the mitochondrial matrix. This enzyme is described as an important point of regulation and link between the metabolism of carbon and nitrogen since in its absence, important processes in the plant such as respiration, photosynthesis and nitrogen assimilation are altered. One way this enzyme exerts control over the metabolism is through the control in the levels of its substrate, 2OG, that is also used for the synthesis of amino acids in the chloroplast. In addition, 2OG plays a role in cell signaling and expression regulation through PII proteins and the 2-oxoglutarate-dependent dioxygenases. In order to help elucidate responses associated with the lack of specific subunits of 2OGDH complex, we set out to analyze the impact caused by the lack of the E1 subunit of the 2OGDH enzyme on the proteome of plants grown under control conditions and also approach the contributions of this enzyme in the response of plants to imposition and recovery of abiotic stress. As a result, we observed that the lack of the E1 subunit of the 2OGDH enzyme is related to a reduction in photosynthetic and photorespiratory metabolisms proteins that may explain the large growth reductions observed in the mutant plants. We also observed that during the period of stress and recovery, the lack of the E1 subunit has a greater impact on the metabolism of roots that respond more quickly to stress, where alternative pathways such as GABA shunt can be activated in order to overcome the lack of this enzyme, and during recovery carbon metabolism is prioritized over nitrogen in the roots.

Keywords: 2-oxoglutarate dehydrogenase. Proteome. Abiotic stress. Respiration. Arabidopsis

RESUMO

VARGAS, Jonas Rafael, D.Sc., Universidade Federal de Viçosa, abril de 2022. **A importância da subunidade E1 da enzima 2-oxoglutarato desidrogenase sobre o proteoma de partes aéreas e metabolismo de plantas inteiras de *Arabidopsis thaliana***. Orientador: Adriano Nunes Nesi.

A enzima 2-oxoglutarato desidrogenase (2OGDH) é uma enzima do ciclo do ácido tricarboxílico. Essa enzima é um complexo multi-enzimático formado por três subunidades que juntas são responsáveis por catalisar a conversão de 2-oxoglutarato (2OG) em succinil-CoA com liberação de NADH e CO_2 na matriz mitocondrial. Esta enzima é descrita como um importante ponto de regulação dos metabolismos de carbono e nitrogênio, pois na sua ausência a respiração, fotossíntese e assimilação de nitrogênio são alterados. Uma das frentes de controle do metabolismo exercido por essa enzima se deve ao fato de que seu substrato, 2OG, também ser utilizado para a síntese de aminoácidos no cloroplasto. Além disso, o 2OG desempenha um papel na sinalização celular e na regulação da expressão através das proteínas PII e das dioxigenases dependentes de 2-oxoglutarato. A fim de ajudar a elucidar as respostas associadas à falta de subunidades específicas do complexo 2OGDH, propusemos analisar o impacto causado pela falta da subunidade E1 dessa enzima no proteoma de plantas cultivadas sob condições de controle, assim como abordamos as contribuições desta enzima na resposta das plantas à imposição e recuperação do estresse abiótico. Como resultado, observamos que a falta da subunidade E1 da enzima 2OGDH está relacionada a uma redução nas proteínas do metabolismo fotossintético e fotorrespiratório que podem explicar as grandes reduções de crescimento observadas nas plantas mutantes. Também observamos que durante o período de estresse e recuperação, a falta da subunidade E1 tem maior impacto no metabolismo das raízes que respondem mais rapidamente ao estresse, onde vias alternativas como o GABA “shunt” podem ser ativadas para suprir a falta desta enzima, e durante a recuperação o metabolismo do carbono é priorizado sobre o nitrogênio nas raízes

Palavras-chave: 2-oxoglutarado desidrogenase. Proteoma. Estresse abiótico. Respiração. Arabidopsis

SUMMARY

GENERAL INTRODUCTION

The respiratory metabolism of plants can be divided into two main cellular compartments, the cytosol and mitochondria (Millar et al., 2011). This metabolism consists in the production of energy, obtained in the form of ATP molecules; and reducing potential, obtained in the form of reduced coenzymes such as Nicotinamide Adenosine Dinucleotide (Phosphate) (NAD(P)H) and Flavin Adenosine Dinucleotide ($FADH_2$). The energy production occurs through four main metabolic pathways, glycolysis, the tricarboxylic acid (TCA) cycle, the mitochondrial electron transport chain (mETC) and oxidative phosphorylation (OXPHOS) (Millar et al., 2011) .

While glycolysis takes place in the cytosol and culminates in the formation of ATP, NADH and pyruvate molecules, most of the ATP and respiratory NAD(P)H is generated in the mitochondria, more specifically in the mitochondrial matrix home of the TCA cycle and in the inner membrane, where the mETC and OXPHOS happen (Plaxton and Podestá, 2006). In the mitochondria, pyruvate from glycolysis is converted into acetyl-CoA by the action of pyruvate dehydrogenase complex, already with the formation of NADH and CO_2. Acetyl-CoA is then metabolized by the TCA cycle where the action of eight different enzymes, namely Citrate Synthase, Aconitase, Isocitrate dehydrogenase, 2-Oxoglutarate (2OG) Dehydrogenase (2OGDH), Succinyl CoA-ligase, Succinate dehydrogenase (or Complex II of mETC), Fumarase and Malate Dehydrogenase convert it to oxaloacetate with the consequent production of NADH and $FADH_2$ (Millar et al., 2011; Nunes-Nesi et al., 2013).

The TCA cycle is described as an important point of respiration metabolism and understanding its regulation and how it is incorporated into metabolism has been an intense subject of research (Nunes-Nesi et al., 2008; Sweetlove et al., 2010; Millar et al., 2011; Araújo et al., 2012a; Igamberdiev and Eprintsev, 2016; Zhang et al., 2017; Igamberdiev, 2020). Despite being described as a cycle, the TCA cycle does not always act in this way (Sweetlove et al., 2010). This is possible because several of its intermediates are important in other metabolic pathways in the plant (Lee et al., 2010; Sweetlove et al., 2010; Nunes-Nesi et al., 2013; Araújo et al., 2014a; Hodges et al., 2016). Among the TCA cycle intermediates that are used in other pathways, 2OG plays an important role because it participates in the carbon metabolism by being converted in the TCA cycle by the 2OGDH to succinyl CoA (Millar et al., 1999), as well as participates in the nitrogen metabolism by being converted into glutamate in the chloroplast by the action of glutamine 2-oxoglutarate aminotransferase (GOGAT) (Hodges, 2002). Considering its versatility, the 2OG is considered a key metabolite that participates in the regulation of carbon and nitrogen metabolisms (Huergo and Dixon, 2015), and is also associated with several other functions in plants such as signaling, hormone synthesis and epigenetics (Araújo et al., 2014a; Nadi et al., 2018).

The 2OG is metabolized in the TCA cycle by the enzyme 2-oxoglutarate dehydrogenase (2OGDH). The 2OGDH is a multienzyme complex composed of three subunits, namely, 2-oxoglutarate dehydrogenase (E1), dihydrolipoyl succinyltransferase (E2) and dihydrolipoyl dehydrogenase (E3) (Millar et al., 1999). This complex has a molecular mass of approximately 2000 kDa, and is formed by a core formed by E2 subunits to which the subunits E1 and E3 are binded (Millar et al., 1999). One single mitochondria is believed to contain around of 181 2OGDH active complexes, that are formed following a stoichiometry of 5:5:2 (subunits E1, E2 and E3) (Fuchs et al., 2020).

The conversion of 2OG into succinil-CoA happens through a series of three reactions (Bunik and Fernie, 2009). The E1 subunit requires the presence of the cofactor thiamine pyrophosphate (TPP) and is responsible for the initial decarboxylation of 2-OG, during which it releases CO_2 and forms a succinyl-TPP molecule. The E2 subunit transfers succinyl to lipoic acid and then to a CoA releasing succinyl-CoA and forming a dihydrolipoic acid which is oxidized by E3 using FAD^+ as a cofactor to form lipoic acid with the consequent reduction of NAD^+ and the production of NADH (Bunik and Fernie, 2009).

The 2OGDH complex has several forms of regulation. This complex can be regulated by the ratios of its products and substrates (Bunik and Fernie, 2009). While higher values of NADH reduce the activity of all subunits, the succinyl-CoA/Acyl-CoA ratio has an effect mainly on the activity of the E2 subunit (Bunik and Fernie, 2009). The energetic status of the cell through the ATP/ADP ratio also exerts direct regulation on the activity of 2OGDH, while ATP reduces the activity of all subunits, ADP increase the activity only of the E1 subunit (Bunik and Fernie, 2009). The binding of the E3 subunit to the whole complex is weak and this subunit is lost during the extraction and purification process, which makes its addition necessary to analyze the activity of the whole complex *in vitro* (Millar et al., 1999). The E2 and E3 subunits have potential for regulation by the mitochondrial thioredoxin system (Bunik and Fernie, 2009; Fonseca-Pereira et al., 2019; Møller et al., 2020). The E2 subunit is also inhibited by the presence of 4-hydroxy-2-nonenal (HNE) (Taylor et al., 2002; Taylor et al., 2004; Taylor et al., 2009).

The reaction catalyzed by the 2OGDH enzyme is described as a branch point in the TCA cycle (Sweetlove et al., 2010; Araújo et al., 2013). This effect occurs because the substrate of this enzyme, the 2OG, can move between different metabolisms (carbon and nitrogen)(Araújo et al., 2014b) and organelles (mitochondria and chloroplasts) (Facchinelli and Weber, 2011; Nunes-Nesi et al., 2020)

Mitochondria and chloroplasts are important organelles present in plant cells. They have their own genome, which is essential for their correct functioning (Bock and Timmis, 2008). However, as they share the same origin, through different endosymbiotic processes, a large part of their genetic material was transferred to the nucleus, which today has great control over both organelles (Nott et al., 2006; Burch-Smith et al., 2011; Garg et al., 2016). These two organelles also participate in the regulation of each other metabolisms (Prihoda et al., 2012; Wang et al., 2020a). This cross talk between organelles can be clearly seen in studies where the alteration of mitochondrial metabolism affects intrinsic chloroplast processes, such as inhibitions/reductions in the levels and activity of TCA cycle enzymes having a direct effect on photosynthetic capacity (Carrari et al., 2003; Nunes-Nesi et al., 2005; Nunes-Nesi et al., 2007; Araújo et al., 2011). Although these crosslinks between mitochondria and chloroplast are already being further studied (Stobbe et al., 2012; Araújo et al., 2014b; Wang et al., 2020b), much is still unclear and need to be understood.

In the current scenario, of major climate change and crop migration due to shifts in rainfall regimes; understanding the alteration in metabolism in the whole cell and plants under stressful conditions has attracted research attention in order to find mechanisms that support the obtention of plants more resistant to abiotic stresses (Obata and Fernie, 2012; Rasmussen et al., 2013; Hodges et al., 2016; Zandalinas et al., 2018; Che-othman et al., 2019; Baath et al., 2020; Cárcamo-Fincheira et al. al., 2021). When exposed to water deficit stress, for example, plants resort to several response mechanisms such as stomatal closure, reduction of growth and photosynthesis, alteration of the proteome, reprogramming of amino acid and respiratory metabolism, accumulation of osmolyte compounds such as sugars and proline, are among the more described(Hummel et al., 2010; Obata and Fernie, 2012; Pires et al., 2016; Pereira et al., 2018; Batista-Silva et al., 2019; Heinemann et al., 2020) Known for its accumulation during periods of drought, proline plays several roles in the metabolism of plants under stress and its accumulation occurs both by the positive regulation of its synthesis and by protein degradation. The proline catabolism takes place in the mitochondrial matrix, and this process results in the donation of electrons to the mitochondrial electron chain, in the release of reducing poise and glutamate in the matrix (Hare and Cress, 1997; Szabados and Savouré, 2010). These glutamate molecules can be used in the nitrogen metabolism, reintroduced in the TCA cycle as succinate (Fait et al., 2007a) or via 2OG after action of the enzyme glutamate dehydrogenase (Schertl and Braun, 2014b).

As already indicated here, the TCA cycle enzymes and their physiological roles have already been described under optimal conditions (Carrari et al., 2003; Fait et al., 2007; Lemaitre et al., 2007; Nunes-Nesi et al., 2007; Studart-Guimarães et al., 2007; Sienkiewicz-Porzucek et al., 2008; Tomaz et al., 2010; Araújo et al., 2011). This list includes the 2OGDH, which had its activity and expression reduced by different methods that confirmed how this enzyme plays an important role in the regulation of TCA and nitrogen metabolism, reducing respiration and photosynthesis as well as altering seed production (Araújo et al., 2008; Araújo et al., 2012c; Condori-Apfata et al., 2019; Condori-Apfata et al., 2021). The 2OGDH has also being described by the importance it plays in the regulation of gene expression (Araújo et al., 2012b; Nadi et al., 2018), which may affect not only nitrogen and carbon metabolism but several others process in the plant (Araújo et al., 2014a).

The contribution of the 2OGDH to the proteome of illuminated leaves has not yet been described, and also, little is known about the importance of this enzyme during the imposition and recovery of abiotic stress on shoots and roots of *Arabidopsis thaliana*. To fill this gap of knowledge and better understand the role of 2OGDH complex in plants, two independent experiments were designed. The results of these experiments are organized into two chapters. The first one describes the role of the E1 subunit of the 2OGDH complex over the proteome of arabidopsis plants and the second describes the importance of the same complex during periods of osmotic stress and release in whole plants of *Arabidopsis thaliana.*

REFERENCES

Araújo WL, Martins AO, Fernie AR, Tohge T (2014a) 2-oxoglutarate: Linking TCA cycle function with amino acid, glucosinolate, flavonoid, alkaloid, and gibberellin biosynthesis. Front Plant Sci **5**: 1–6

Araújo WL, Nunes-Nesi A, Fernie AR (2014b) On the role of plant mitochondrial metabolism and its impact on photosynthesis in both optimal and sub-optimal growth conditions. Photosynth Res **119**: 141–156

Araújo WL, Nunes-Nesi A, Nikoloski Z, Sweetlove LJ, Fernie AR (2012a) Metabolic control and regulation of the tricarboxylic acid cycle in photosynthetic and heterotrophic plant tissues. Plant, Cell Environ **35**: 1–21

Araújo WL, Nunes-Nesi A, Osorio S, Usadel B, Fuentes D, Nagy R, Balbo I, Lehmann M, Studart-Witkowski C, Tohge T, et al (2011) Antisense inhibition of the iron-sulphur subunit of succinate dehydrogenase enhances photosynthesis and growth in tomato via an organic acid-mediated effect on stomatal aperture. Plant Cell **23**: 600–627

Araújo WL, Nunes-Nesi A, Trenkamp S, Bunik VI, Fernie AR (2008) Inhibition of 2-oxoglutarate dehydrogenase in potato tuber suggests the enzyme is limiting for respiration and confirms its importance in nitrogen assimilation. Plant Physiol **148**: 1782–1796

Araújo WL, Tohge T, Nunes-Nesi A, Daloso DM, Nimick M, Krahnert I, Bunik VI, Moorhead GBG, Fernie AR (2012b) Phosphonate analogs of 2-oxoglutarate perturb metabolism and gene expression in illuminated Arabidopsis leave. Front Plant Sci **3**: 1–19

Araújo WL, Tohge T, Osorio S, Lohse M, Balbo I, Krahnert I, Sienkiewicz-Porzucek A, Usadel B, Nunes-Nesi A, Fernie AR (2012c) Antisense inhibition of the 2-oxoglutarate dehydrogenase complex in tomato demonstrates its importance for plant respiration and during leaf senescence and fruit maturation. Plant Cell **24**: 2328–2351

Araújo WL, Trofimova L, Mkrtchyan G, Steinhauser D, Krall L, Graf A, Fernie AR, Bunik VI (2013) On the role of the mitochondrial 2-oxoglutarate dehydrogenase complex in amino acid metabolism. Amino Acids **44**: 683–700

Baath GS, Kakani VG, Gowda PH, Rocateli AC, Northup BK, Singh H, Katta JR (2020) Guar responses to temperature: Estimation of cardinal temperatures and photosynthetic parameters. Ind Crops Prod **145**: 111940

Batista-Silva W, Heinemann B, Rugen N, Nunes-Nesi A, Araújo WL, Braun HP, Hildebrandt TM (2019) The role of amino acid metabolism during abiotic stress release. Plant Cell Environ **42**: 1630–1644

Bock R, Timmis JN (2008) Reconstructing evolution: Gene transfer from plastids to the nucleus. BioEssays **30**: 556–566

Bunik VI, Fernie AR (2009) Metabolic control exerted by the 2-oxoglutarate dehydrogenase reaction: A cross-kingdom comparison of the crossroad between energy production and nitrogen assimilation. Biochem J **422**: 405–421

Burch-Smith TM, Brunkard JO, Choi YG, Zambryski PC (2011) Organelle-nucleus cross-talk regulates plant intercellular communication via plasmodesmata. Proc Natl Acad Sci U S A. doi: 10.1073/pnas.1117226108

Cárcamo-Fincheira P, Reyes-Díaz M, Omena-García RP, Vargas JR, Alvear M, Florez-Sarasa I, Rosado-Souza L, Rengel Z, Fernie AR, Nunes-Nesi A, et al (2021) Metabolomic analyses of highbush blueberry (Vaccinium corymbosum L.) cultivars revealed mechanisms of resistance to aluminum toxicity. Environ Exp Bot. doi: 10.1016/j.envexpbot.2020.104338

Carrari F, Nunes-Nesi A, Gibon Y, Lytovchenko A, Loureiro ME, Fernie AR (2003) Reduced Expression of Aconitase Results in an Enhanced Rate of Photosynthesis and Marked Shifts in Carbon Partitioning in Illuminated Leaves of Wild Species Tomato. Plant Physiol **133**: 1322–1335

Che-othman MH, Jacoby RP, Millar AH, Taylor NL, Taylor NL (2019) Wheat mitochondrial respiration shifts from the tricarboxylic acid cycle to the GABA shunt under salt stress. doi: 10.1111/nph.15713

Condori-Apfata JA, Batista-Silva W, Medeiros DB, Vargas JR, Valente LML, Heyneke E, Pérez-Diaz JL, Fernie AR, Araújo WL, Nunes-Nesi A (2019) The Arabidopsis E1 subunit of the 2-oxoglutarate dehydrogenase complex modulates plant growth and seed production. Plant Mol Biol **101**: 183–202

Condori-Apfata JA, Batista-Silva W, Medeiros DB, Vargas JR, Valente LML, Pérez-Díaz JL, Fernie AR, Araújo WL, Nunes-Nesi A (2021) Downregulation of the E2 Subunit of 2-Oxoglutarate Dehydrogenase Modulates Plant Growth by Impacting Carbon–Nitrogen Metabolism in Arabidopsis thaliana . Plant Cell Physiol **0**: 1–17

Facchinelli F, Weber APM (2011) The metabolite transporters of the plastid envelope: An update. Front Plant Sci. doi: 10.3389/fpls.2011.00050

Fait A, Fromm H, Walter D, Galili G, Fernie AR (2007a) Highway or byway: the metabolic role of the GABA shunt in plants. Trends Plant Sci **13**: 14–19

Fait A, Nunes-nesi A, Carrari F, Studart-guimara C, Fernie AR (2007b) Reduced Expression of Succinyl-Coenzyme A Ligase Can Be Compensated for by Up-Regulation of the g -Aminobutyrate Shunt in Illuminated Tomato Leaves 1 [W]. **145**: 626–639

Fonseca-Pereira P da, Souza PVL, Hou LY, Schwab S, Geigenberger P, Nunes-Nesi A, Timm S, Fernie AR, Thormählen I, Araújo WL, et al (2019) Thioredoxin h2 contributes to the redox regulation of mitochondrial photorespiratory metabolism. Plant Cell Environ **43**: 188–208

Fuchs P, Rugen N, Carrie C, Elsässer M, Finkemeier I, Giese J, Hildebrandt TM, Kühn K, Maurino VG, Ruberti C, et al (2020) Single organelle function and organization as estimated from Arabidopsis mitochondrial proteomics. Plant J **101**: 420–441

Garg S, Zimorski V, Martin WF (2016) Endosymbiotic Theory. Encycl Evol Biol **1**: 511–517

Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul **21**: 79–102

Heinemann B, Künzler P, Eubel H, Braun H-P, Hildebrandt TM (2020) Estimating the number of protein molecules in a plant cell: protein and amino acid homeostasis during drought. Plant Physiol. doi: 10.1093/plphys/kiaa050

Hodges M (2002) Enzyme redundancy and the importance of 2-oxoglutarate in plant ammonium assimilation. J Exp Bot **53**: 905–916

Hodges M, Dellero Y, Keech O, Betti M, Raghavendra AS, Sage R, Zhu XG, Allen DK, Weber APM (2016) Perspectives for a better understanding of the metabolic integration of photorespiration within a complex plant primary metabolism network. J Exp Bot **67**: 3015–3026

Huergo LF, Dixon R (2015) The Emergence of 2-Oxoglutarate as a Master Regulator Metabolite. Microbiol Mol Biol Rev **79**: 419–435

Hummel I, Pantin F, Sulpice R, Piques M, Rolland G, Dauzat M, Christophe A, Pervent M, Bouteillé M, Stitt M, et al (2010) Arabidopsis Plants Acclimate to Water Deficit at Low Cost through Changes of Carbon Usage: An Integrated Perspective Using Growth, Metabolite, Enzyme, and Gene Expression Analysis. Plant Physiol **154**: 357–372

Igamberdiev AU (2020) Citrate valve integrates mitochondria into photosynthetic metabolism. Mitochondrion **52**: 218–230

Igamberdiev AU, Eprintsev AT (2016) Organic acids: The pools of fixed carbon involved in redox regulation and energy balance in higher plants. Front Plant Sci **7**: 1–15

Kaul S, Sharma SS, Mehta IK (2008) Free radical scavenging potential of L-proline: Evidence from in vitro assays. Amino Acids **34**: 315–320

Kishor PBK, Hima Kumari P, Sunita MSL, Sreenivasulu N (2015) Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. Front Plant Sci **6**: 1–17

Lee CP, Eubel H, Millar AH (2010) Diurnal changes in mitochondrial function reveal daily optimization of light and dark respiratory metabolism in Arabidopsis. Mol Cell Proteomics **9**: 2125–2139

Lemaitre T, Urbanczyk-Wochniak E, Flesch V, Bismuth E, Fernie AR, Hodges M (2007) NAD-dependent isocitrate dehydrogenase mutants of Arabidopsis suggest the enzyme is not limiting for nitrogen assimilation. Plant Physiol **144**: 1546–1558

Millar AH, Hill SA, Leaver CJ (1999) Plant mitochondrial 2-oxoglutarate dehydrogenase complex: Purification and characterization in potato. Biochem J **343**: 327–334

Millar AH, Whelan J, Soole KL, Day DA (2011) Organization and Regulation of Mitochondrial Respiration in Plants. Annu Rev Plant Biol **62**: 79–104

Møller IM, Igamberdiev AU, Bykova N V., Finkemeier I, Rasmusson AG, Schwarzländer M (2020) Matrix redox physiology governs the regulation of plant mitochondrial metabolism through posttranslational protein modifications. Plant Cell **32**: 573–594

Nadi R, Mateo-Bonmatí E, Juan-Vicente L, Micol JL (2018) The 2OGD Superfamily: Emerging Functions in Plant Epigenetics and Hormone Metabolism. Mol Plant **11**: 1222–1224

Nott A, Jung HS, Koussevitzky S, Chory J (2006) Plastid-to-nucleus retrograde signaling. Annu Rev Plant Biol **57**: 739–759

Nunes-Nesi A, Araújo WL, Obata T, Fernie AR (2013) Regulation of the mitochondrial tricarboxylic acid cycle. Curr Opin Plant Biol **16**: 335–343

Nunes-Nesi A, Carrari F, Gibon Y, Sulpice R, Lytovchenko A, Fisahn J, Graham J, Ratcliffe RG, Sweetlove LJ, Fernie AR (2007) Deficiency of mitochondrial fumarase activity in tomato plants impairs photosynthesis via an effect on stomatal function. Plant J **50**: 1093–1106

Nunes-Nesi A, Carrari F, Lytovchenko A, Smith AMO, Loureiro ME, Ratcliffe RG, Sweetlove LJ, Fernie AR (2005) Enhanced photosynthetic performance and growth as a consequence of decreasing mitochondrial malate dehydrogenase activity in transgenic tomato plants. Plant Physiol **137**: 611–622

Nunes-Nesi A, Cavalcanti JHF, Fernie AR (2020) Characterization of in vivo function(s) of members of the plant mitochondrial carrier family. Biomolecules **10**: 1–20

Nunes-Nesi A, Sulpice R, Gibon Y, Fernie AR (2008) The enigmatic contribution of mitochondrial function in photosynthesis. J Exp Bot **59**: 1675–1684

Obata T, Fernie AR (2012) The use of metabolomics to dissect plant responses to abiotic stresses. Cell Mol Life Sci **69**: 3225–3243

Pereira LFM, Zanetti S, Silva M de A (2018) Water relations of cassava cultivated under water-deficit levels. Acta Physiol Plant **40**: 1–13

Pires M V., Pereira Júnior AA, Medeiros DB, Daloso DM, Pham PA, Barros KA, Engqvist MKM, Florian A, Krahnert I, Maurino VG, et al (2016) The influence of alternative pathways of respiration that utilize branched-chain amino acids following water shortage in Arabidopsis. Plant Cell Environ **39**: 1304–1319

Plaxton WC, Podestá FE (2006) The functional organization and control of plant respiration. CRC Crit Rev Plant Sci **25**: 159–198

Prihoda J, Tanaka A, De Paula WBM, Allen JF, Tirichine L, Bowler C (2012) Chloroplast-mitochondria cross-talk in diatoms. J Exp Bot **63**: 1543–1557

Rasmussen S, Barah P, Suarez-Rodriguez MC, Bressendorff S, Friis P, Costantino P, Bones AM, Nielsen HB, Mundy J (2013) Transcriptome responses to combinations of stresses in Arabidopsis. Plant Physiol **161**: 1783–1794

Schertl P, Braun HP (2014) Respiratory electron transfer pathways in plant mitochondria. Front Plant Sci **5**: 1–11

Sharma S, Villamor JG, Verslues PE (2011) Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. Plant Physiol **157**: 292–304

Sharma SS, Schat H, Vooijs R (1998) In vitro alleviation of heavy metal-induced enzyme inhibition by proline. Phytochemistry **49**: 1531–1535

Sienkiewicz-Porzucek A, Nunes-Nesi A, Sulpice R, Lisec J, Centeno DC, Carillo P, Leisse A, Urbanczyk-Wochniak E, Fernie AR (2008) Mild reductions in mitochondrial citrate synthase activity result in a compromised nitrate assimilation and reduced leaf pigmentation but have no effect on photosynthetic performance or growth. Plant Physiol **147**: 115–127

Stobbe MD, Houten SM, Van Kampen AHC, Wanders RJA, Moerlan PD (2012) Improving the description of metabolic networks: The TCA cycle as example. FASEB J **26**: 3625–3636

Studart-Guimarães C, Fait A, Nunes-Nesi A, Carrari F, Usadel B, Fernie AR (2007) Reduced expression of succinyl-coenzyme A ligase can be compensated for by up-regulation of the γ-aminobutyrate shunt in illuminated tomato leaves. Plant Physiol **145**: 626–639

Sweetlove LJ, Beard KFM, Nunes-Nesi A, Fernie AR, R. George Ratcliffe (2010) Not just a circle: Flux modes in the plant TCA cycle. Trends Plant Sci **15**: 462–470

Szabados L, Savouré A (2010) Proline: a multifunctional amino acid. Trends Plant Sci **15**: 89–97

Taylor NL, Day DA, Millar AH (2002) Environmental stress causes oxidative damage to plant mitochondria leading to inhibition of glycine decarboxylase. J Biol Chem **277**: 42663–42668

Taylor NL, Heazlewood JL, Day DA, Millar AH (2004) Lipoic Acid-Dependent Oxidative Catabolism of ⎵ -Keto Acids in Mitochondria Provides Evidence for Branched- Chain Amino Acid Catabolism in Arabidopsis 1. **134**: 838–848

Taylor NL, Tan YF, Jacoby RP, Millar AH (2009) Abiotic environmental stress induced changes in the Arabidopsis thaliana chloroplast, mitochondria and peroxisome proteomes. J Proteomics **72**: 367–378

Tomaz T, Bagard M, Pracharoenwattana I, Lindén P, Lee CP, Carroll AJ, Ströher E, Smith SM, Gardeström P, Millar AH (2010) Mitochondrial malate dehydrogenase lowers leaf respiration and alters photorespiration and plant growth in Arabidopsis. Plant Physiol **154**: 1143–1157

Urano K, Maruyama K, Ogata Y, Morishita Y, Takeda M, Sakurai N, Suzuki H, Saito K, Shibata D, Kobayashi M, et al (2009) Characterization of the ABA-regulated global responses to dehydration in Arabidopsis by metabolomics. Plant J **57**: 1065–1078

Viana YA, Garrote-Filho MS, Penha-Silva N (2005) Estabilização de proteínas por osmólitos. Biosci J **21**: 83–88

Wang Y, Selinski J, Mao C, Zhu Y, Berkowitz O, Whelan J (2020a) Linking mitochondrial and chloroplast retrograde signalling in plants. Philos Trans R Soc

B Biol Sci. doi: 10.1098/rstb.2019.0410

Wang Y, Selinski J, Mao C, Zhu Y, Berkowitz O, Whelan J (2020b) Linking mitochondrial and chloroplast retrograde signalling in plants.

Zandalinas SI, Mittler R, Balfagón D, Arbona V, Gómez-Cadenas A (2018) Plant adaptations to the combination of drought and high temperatures. Physiol Plant **162**: 2–12

Zhang Y, Beard KFM, Swart C, Bergmann S, Krahnert I, Nikoloski Z, Graf A, George Ratcliffe R, Sweetlove LJ, Fernie AR, et al (2017) Protein-protein interactions and metabolite channelling in the plant tricarboxylic acid cycle. Nat Commun. doi: 10.1038/ncomms15212

Chapter 1: The lack of E1 subunit of the 2-Oxoglutarate dehydrogenase and its implications over the proteome of illuminated leaves in *Arabidopsis thaliana*

ABSTRACT

VARGAS, Jonas Rafael, D.Sc., Universidade Federal de Viçosa, April, 2022. **The lack of E1 subunit of the 2-Oxoglutarate dehydrogenase and its implications over the proteome of illuminated leaves in *Arabidopsis thaliana***. Adviser: Adriano Nunes Nesi.

Considered a central pathway of the respiratory process the tricarboxylic acid (TCA) cycle is responsible for linking the production of pyruvate in the cytosol with the synthesis of ATP in the mitochondrial matrix through the production of reduced coenzymes. In the TCA cycle the 2-oxoglutarate dehydrogenase (2OGDH) is responsible for converting 2-oxoglutarate (2OG) into succinyl CoA while releasing NADH and CO_2. 2OGDH is composed of three subunits and described as one of the main regulatory points of respiration metabolism. Its inhibition and/or low expression are related to lower photosynthesis, reduced respiration and growth as well as changes in gene expression on leaf and root tissues. For a better understanding of these phenotypes, we evaluated the effects of the lack of 2OGDH E1 subunit over the whole leaf proteome. For that, we analyzed the proteome of *Arabidopsis thaliana* double mutant line, lacking the expression of both genes encoding the E1 subunit of 2OGDH, grown under optimal conditions. The results revealed that the lack of both isoforms of the 2OGDH E1 subunit altered the levels of whole 2OGDH complex, reduced the levels of Aconitase and citrate synthase. The double mutant plants exhibited reductions in the levels of other mitochondrial complexes like the PDH and GDC and increased levels of proteins related to the mitochondrial electron transport chain. We also observed reductions in the levels of proteins related to the glycolysis and photosynthesis. Altogether, the results confirm the importance of the E1 subunit of the 2OGDH enzyme over the proteome and metabolism of illuminated leaves of arabidopsis.

Keywords: 2-oxoglutarate. TCA cycle. Respiration. Proteome. Arabidopsis

INTRODUCTION

The respiratory process in plants is composed of four main pathways. While glycolysis occurs majorly in the cytosol, the tricarboxylic acid (TCA) cycle, the electron transport chain (mETC) and oxidative phosphorylation (OXPHOS) occur in the mitochondrial matrix and in the inner mitochondrial membrane, respectively (Sweetlove et al., 2010). The TCA cycle is described as a central pathway of respiration, as it connects the cytosolic pyruvate production with the ATP synthesis in mitochondria. This cycle is composed of a set of eight enzymes and is described as a central pathway in plant respiration, once it is responsible for the production of organic acids, ATP and reduced coenzymes (NADH and $FADH_2$) in the mitochondrial matrix (Millar et al. , 2011). The TCA cycle is described as a cycle but in many situations it can operate in a non-cyclic mode (Sweetlove et al. , 2010). This is possible because one of its functions is to provide intermediates that serve as carbon skeletons for nitrogen assimilation, as well as for other metabolic pathways, thus acting as a carbon and nitrogen metabolism regulator (Sweetlove et al., 2010; Huergo e Dixon, 2015).

Among the TCA cycle intermediates that can be used in other pathways, acetyl-CoA, fumarate, malate and 2-oxoglutarate (2OG) are the most studied. Acetyl-CoA can be used in the metabolism of flavonoids, alkaloids, fatty acids, isoprenoids and pigments (Araújo et al., 2012a). Fumarate and malate can participate in the glucose synthesis during gluconeogenesis, as well as participate in photosynthesis and act in fruit ripening (Araújo et al., 2012a). 2OG can be exported from the mitochondrial matrix to the plastid and be used in other important metabolic pathways, such as ammonium assimilation and amino acid biosynthesis through the action of glutamine synthetase (GS) and glutamine 2- oxoglutarate -aminotransferase (GOGAT) (Araújo et al., 2012b; Araújo et al., 2014).

2-oxoglutarate is synthesized by the action of isocitrate dehydrogenase which has NAD^+ and $NADP^+$ dependent isoforms that can occur in mitochondria (NAD(P)-IDH) or in the cytosol, chloroplast and peroxisome (NADP-IDH) (Niazi et al., 2019). Despite different synthesis sites within the plant cells, the degradation of 2OG occurs only in the mitochondria by the action of the enzyme 2-oxoglutarate dehydrogenase (2OGDH) which converts it to succinyl-CoA through an oxidative decarboxylation that results in the release of water, CO_2 and NADH (Millar et al., 1999; Schnarrenberger and Martin, 2002; Bunik and Fernie, 2009; Araújo et al., 2012a). This uniqueness and the importance of 2OG in carbon and nitrogen metabolism make this enzyme and its substrate control points of the TCA cycle flux (Araújo et al., 2008; Araújo et al., 2012c; Araújo et al., 2014b).

The 2OGDH complex, involved in the metabolism of 2OG, is composed of three subunits, namely, 2-oxoglutarate dehydrogenase (E1), dihydrolipoyl succinyltransferase (E2) and dihydrolipoyl dehydrogenase (E3) (Millar et al., 1999). This complex has a molecular mass of approximately 2000 kDa and is formed by a core nucleus with around 24 E2 subunits to which homodimers of E3 and E1 subunits are bound (Millar et al., 1999). The E1 subunit requires the presence of the cofactor thiamine pyrophosphate (TPP) and is responsible for the initial decarboxylation of 2OG, during which it releases CO_2 and forms a succinyl-TPP molecule. The E2 subunit transfers succinyl to lipoic acid and then to a CoA releasing succinyl-CoA and forming a dihydrolipoic acid which is oxidized by E3 using FAD^+ as a cofactor to form lipoic acid with consequent reduction of NAD^+ and production of NADH (Millar et ai., 1999) .

Given the importance of the TCA cycle, several techniques have already been applied to understand the role of its enzymes in mitochondrial and plant metabolism as a whole. These studies revealed that while not all of them affect the photosynthetic process, almost all of them affect nitrogen metabolism. The reduced expression of citrate synthase revealed reduced nitrate assimilation and pigment levels without altering the photosynthesis (Sienkiewicz-Porzucek et al., 2008). Aconitase repression revealed lower TCA cycle flux, higher photosynthesis and higher productivity (Carrari et al., 2003). Mutation in three isoforms of Isocitrate dehydrogenase revealed that this enzyme has little importance in the metabolism, not affecting the activity of the TCA cycle or nitrogen assimilation (Lemaitre et al., 2007). The lack of Succinyl-CoA ligase did not affect respiration and photosynthesis or growth but activated the GABA shunt pathway to maintain the succinate supply for the TCA cycle (Studart-Guimarães et al., 2007) while plants with reduced expression of the succinate dehydrogenase suffered positive changes in photosynthesis due to changes in the provision of organic acids (malate and fumarate) (Araújo et al., 2011). Inhibition of fumarase revealed plants with lower photosynthesis and lower growth due to deficiencies in stomatal control (Nunes-Nesi et al., 2007). Antisense inhibition of mitochondrial malate dehydrogenase in tomatoes resulted in increased chloroplast electron transport chain, increased photosynthesis and growth and reduced respiration rates (Nunes-Nesi et al., 2005), while in arabidopsis the mutation of this enzyme via T-DNA insertion revealed smaller plants, with reduced respiration and photorespiration (Tomaz et al., 2010)

Concerning 2OGDH, extensive work has been carried out to demonstrate the importance of this enzyme. Its inhibition in potato tubers revealed a reduction in the respiratory process as well as its importance in nitrate assimilation and amino acid metabolism, and revealed that the lack of this enzyme lead to a greater flow through the GABA shunt (Araujo et al., 2008). The antisense inhibition of 2OGDH in tomato plants maintained the reduction in the respiratory process, reduced the flow in the TCA cycle, the root growth and the mass accumulation in the shoot as well as accelerated senescence process and did not affect photosynthesis, production of fruits, seeds or germination (Araújo et al., 2012c). The evaluation of plants showing reduced expression of the two genes coding the E1 subunit of the 2OGDH complex in arabidopsis confirmed that 2OGDH is a key step in the regulation of the respiration (Condori-Apfata et al., 2021). This work revealed that these two genes are not redundant, delegating greater importance to gene e1-ogdh1 in somatic tissues and the e1-ogdh2 in reproductive tissues and also revealed the lower expression of the genes e1-ogdh1 and 2 can be related to photosynthesis inhibition (Condori-Apfata et al., 2019). The analysis of the metabolism of plants with low expression of E2 subunit genes, e2-ogdh 1 and 2, revealed redundancy of those genes and also that the lack of this subunit as well as the E1, reduces the activity of the whole complex, perpetuating the negative effect on the respiratory process (Condori-Apfata et al., 2021).

When analyzing the effects of chemical inhibition of 2OGDH in arabidopsis leaves, Araújo et al. (2012b) observed that the reduction in the activity of this enzyme maintains the changes in metabolism of nitrogen and respiration, and also has effects over gene expression. These results raised the hypothesis that the accumulation of 2OG itself, or some downstream metabolite, triggers a signaling cascade that acts, regulating the expression of several different genes. Following this hypothesis, Yue et al. (2018) described that the inhibition of 2OGDH in poplar plants led to the accumulation of GABA and this accumulation changed the expression patterns of several genes involved in different metabolisms, including the synthesis and signaling of hormones important in the growth of adventitious roots.

From all we have described so far, it is clear that the 2OGDH enzyme plays a major role in regulating respiratory and nitrogen metabolism, as it is clear that the lack or inhibition of this enzyme, as well as others in the TCA cycle, trigger reactions that go far beyond mitochondrial metabolism, causing significant changes in photosynthesis, photorespiration, amino acid synthesis, and senescence. Therefore, based in the data collected here, in this work we sought to analyze the effect of the low expression of the two genes that encode the E1 subunit of the 2OGDH enzyme (e1-ogdh1 and 2) over the proteome of *Arabidopsis thaliana* under optimal conditions. Working with a previously described double mutant line (e1-ogdh2.2::1.2) (Condori-Apfata et al., 2019), we report here that the lack of this subunit leads to drastic alterations in the global level of proteins, with significant reductions in the levels of proteins involved in the light reactions of photosynthesis, respiration and mitochondrial steps of photorespiration.

MATERIALS AND METHODS

Plant material

In this study we used plants of *Arabidopsis thaliana* ecotype Columbia (Col-0) as control and a double mutant line, with low expression for both genes coding the E1 subunit of the 2OGDH enzyme (At3g55410 and At5g65750, e1-ogdh1.2::2.2) previously used by Condori-Apfata et al. (2019).

Growth conditions, measurement and harvest

Arabidopsis plants and homozygous double mutant lines were grown for four weeks in pots (200 cm^3) in a Phyto chamber (22–24 ºC, 16 h light, 8 h darkness, 110 µmol photons m^{-2} s^{-1}). Two weeks after sowing, pictures of the plants were taken daily. The pictures were used to measure the rosette area using the ImageJ software (Schneider et al., 2012). For the harvest, whole shoots of four weeks old plants were collected during the middle of the light period, immediately frozen in liquid nitrogen and freeze-dried. Each pot, containing one plant accounts for one experimental unit.

Metabolite analysis

Aliquots of up to 7 mg (± 0,5 mg) of the dry weight of each sample were prepared and used for the Chloroform/methanol extraction. The levels of glucose, fructose, sucrose and starch in the tissues were determined as described by Fernie et al. (2001). Proteins were measured according to Bradford, (1976). Photosynthetic pigments were determined exactly as described before (Porra et al., 1989). The amino acid profile was performed following the protocol described by (Batista-Silva et al., 2019).

Protein extraction and identification

The extraction was performed with freshly prepared SDT Buffer (4% sodium dodecyl sulfate SDS; 0.1M dithiothreitol; 0.1M Tris-HCl, pH 7.6). 500 µL of SDT buffer was added to 5 mg (± 0,5 mg) of freeze-dried plant powder and incubated at 60°C for 30 minutes, 1000 rpm. 400 µL of the extracted supernatant was transferred to new tubes, sonicated for 10 minutes and then centrifuged for 10 minutes at 20000 *g* and room temperature. For the alkylation 30 µL of the sonicated supernatant was transferred to new tubes and added with 7,5 µL of Iodoacetamide (IAA 100 mM stock solution) and incubated for 30 minutes in the dark.

For the binding process, the alkylated samples were added with 2uL of DTT (100 mM, stock solution), 30 µL of the bead stock(prepared with hydrophobic and hydrophilic solids, Sera-Mag Carbolxylat-Modified Beads, GE life sciences, GE44152105050250 and GE24152105050250) and 100% ethanol and then incubated for 10 minutes at 24°C and 1000 rpm, after a spin, the tubes were left to rest in the magnetic rack for 20 minutes, to all the beads to form a pellet that would be gently washed for three times. Then, the clean bead pellets were added with 140 µL of 80% ethanol and transferred to a new low binding protein tube (LBP tube) and left to rest for 2 minutes in the magnetic racks at room temperature. During the next steps, the peptide adjustment and digestion. All the ethanol 80% used to the rinsing was removed and the beads were added with 20 µL of trypsin (1ug per sample) and 40 µL of ammonia bicarbonate (50 mM stock solution) and sonicated for 30 seconds. The trypsin digestion occurred for 18 hours in the dark, at 37°C and 1000 rpm. After this period the tubes were spined off, left to rest in the magnetic rack for 2 minutes and the supernatant was recovered (60 µL), the pelleted beads were rinsed with 60 µl of ammonia bicarbonate (to elute lasting peptides), left to rest in the magnetic rack and the supernatant recovered and pooled together with the previous one adding to a total of 120 µL, that was centrifuged at 20000 *g* for 10 minutes and room temperature. The final eluted was used for peptide quantification and adjusted to 10 µg in a new low protein binding tube and dried for one hour in the speed vacuum. The label free quantitative shot-gun Mass Spectrometry (MS) analyzes, the protein identification via

Max Quant and data processing via Perseus software was performed following the instructions described by Heinemann et al., 2021).

Statistical analyzes

The statistical analyzes were performed with the Student's t-Tests using the Perseus software (Tyanova et al., 2016) for the proteome data, and using MS excel software (Microsoft excel, 2016) for growth and metabolism analyzes. We compared the double mutant against the wild type and the term significant was applied when the values differ from each other considering a *p*-value ≤ 0,05.

RESULTS

In this work, aiming to better understand how the lack of the 2OGDH E1 subunit affects the proteome and metabolism of *Arabidopsis thaliana* plants, we analyzed the proteome and the primary metabolism of 4 weeks old, double mutant plants, with low expression for both genes coding the E1 subunit of the enzyme 2OGDH (e1-ogdh1.2 and e1-ogdh2.2). These plants were cultivated under optimal conditions and harvested in the middle of the light period and here we described the most important results.

The lack of 2OGDH E1 subunit and the effects in primary metabolism of illuminated leaves

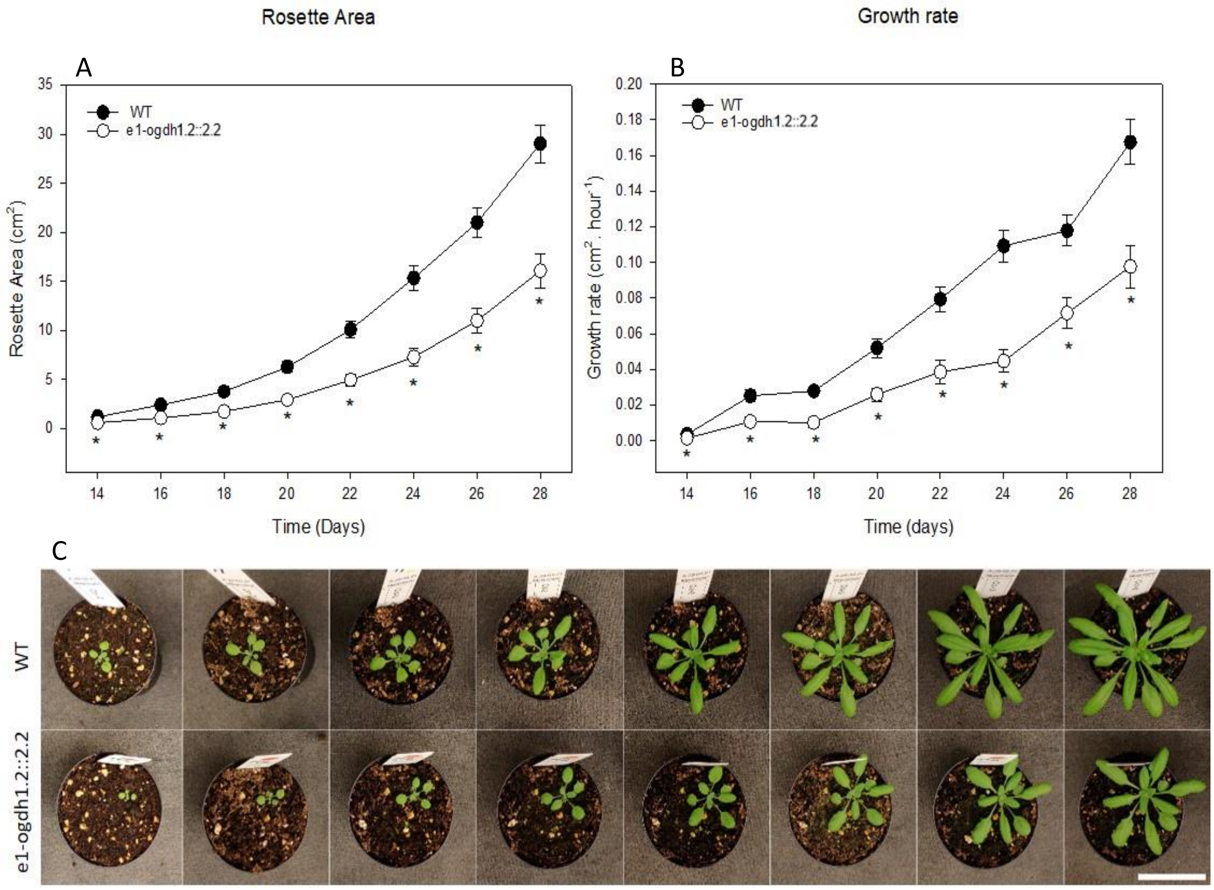

Figure 1: Effect of the lack of the E1 subunit of the enzyme 2-OGDH on the rosette area (A) and the growth rate (B) of wild type (WT) and double mutant arabidopsis plants (e1-ogdh1.2::2.2). Values are means (± standard error). Phenotype of WT and double mutant plants for the E1 subunit of the 2-OGDH enzyme throughout the analyze period, from 14 to 28 days after sowing (C). Asterisks indicated significantly difference between the double mutant line and WT as attested by students t-test ($p \leq 0.05$, n=8). Scale bar= 5 cm.

The lack of the 2OGDH E1 Subunit (e1-ogdh1.2::2.2) led to a strong reduction in the rosette area when compared with the WT plants (Figure 1). Despite the huge difference in the rosette area in the double mutant plants, we found no changes in the total levels of proteins neither in the levels of chlorophylls *a* and *b*, starch or in the levels of the three major sugars (sucrose, fructose and glucose) (Figure 3A, B, C and D). In agreement, the amino acid profile of leaves from these plants indicated that the lack in the expression of both isoforms of E1 subunit led to minor changes over amino acids metabolism. Nevertheless, we observed reductions in the levels of aspartate, asparagine, glutamine and histidine (Figure 4).

The lack of 2OGDH E1 subunit and the effects over the mitochondrial proteome

Given the impact in growth, the importance of the 2OGDH enzyme over the metabolism and over the gene expression previously described by Araújo et al.,

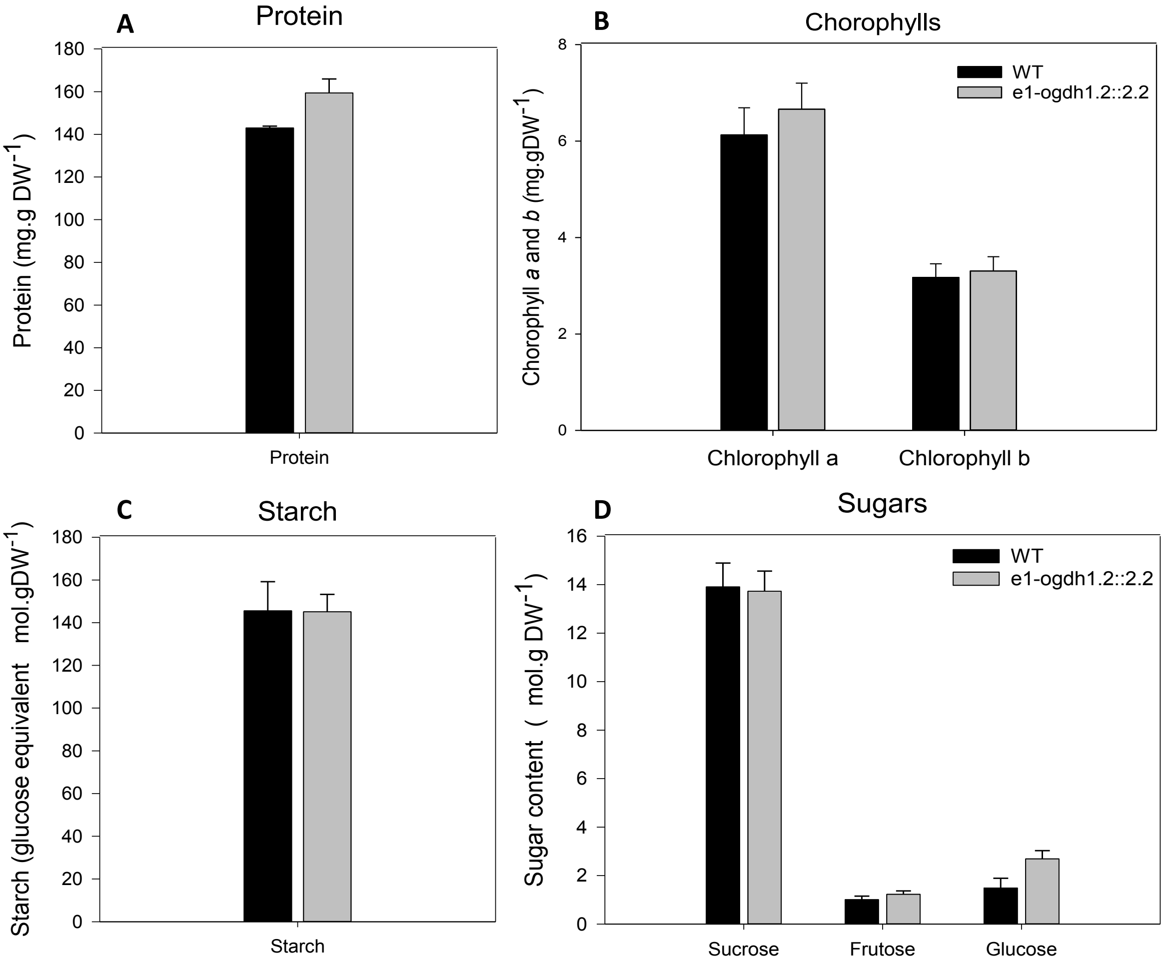

Figure 3: Effect of the lack of the E1 subunit of the enzyme 2-OGDH on levels of Protein (A), Chlorophylls *a* and *b* (B), Starch (C) and the Sugars (D) of wild type (WT) and double mutant arabidopsis plants (e1-ogdh1.2::2.2) harvested in the middle of the light period. Values are means (± standard error). Asterisks indicated significantly difference between the double mutant line and WT as attested by students t-test ($p \leq 0.05$, n=5).

(2012b), we set an experiment to access how the lack of the 2OGDH E1 subunit affect the proteome of arabidopsis plants. In this experiment, we were able to identify a total of 2673 proteins, distributed among 10 cellular sub compartments. 26,9% of the identified proteins were described as cytosolic, 35,6% were from plastids, 7,9% were mitochondrial, 6,4% were proteins from the nucleus, 5.3% from the plasma membrane, 5.3% were found in the apoplast, 3,8% were described as Golgi complex proteins, 3,4% were from the endoplasmic reticulum, 3,0% were peroxisomal proteins, and 2,5% were proteins found in the vacuole. From the whole proteome 24% of the proteins were significantly altered in the double mutant plants and among those alterations in the protein levels, 52% were increased and 48% reduced in the mutant plants when compared with the WT plants (Figure 5A and B).

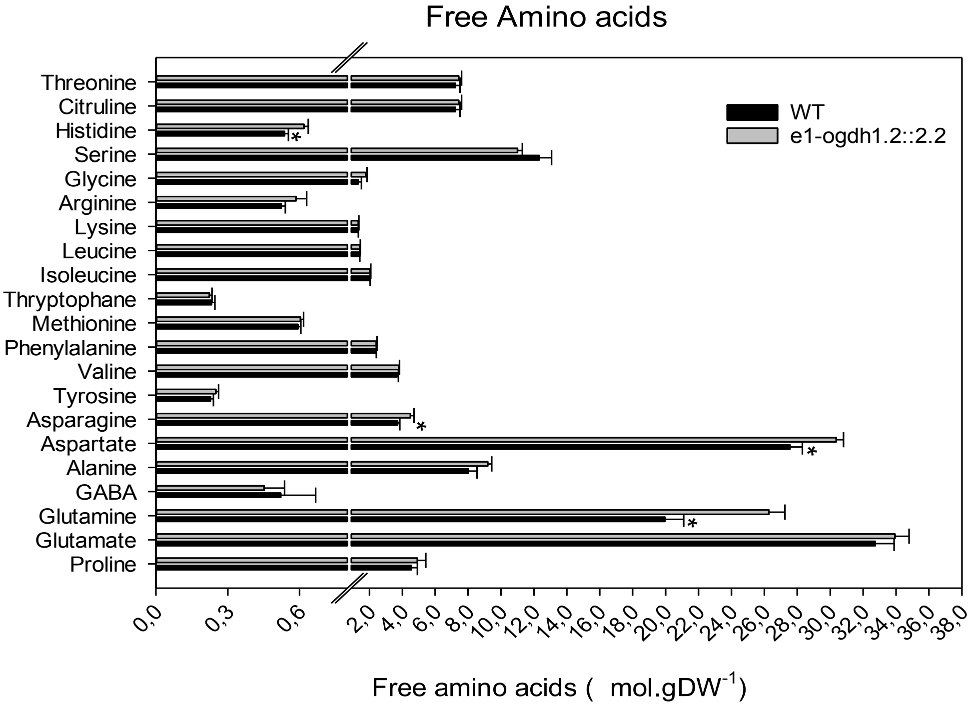

Figure 4: Effect of the lack of the E1 subunit of the enzyme 2-OGDH in the levels of Free amino acids of wild type (WT) and double mutant arabidopsis plants (e1-ogdh1.2::2.2) harvested in the middle of the light period. Values are means (± standard error). Asterisks indicated significantly difference between the double mutant line and WT as attested by students t-test ($p \leq 0.05$, n=5).

In the figure 5C is possible to oversee the negative and positive impact among the significant alterations caused by the lack of the 2OGDH E1 subunit over different metabolisms. We observed that several plant metabolisms were affected by the lack of expression in the isoforms of E1 subunit of the 2OGDH enzyme (Figure 6C). Among the alterations we verified that 23,8% of the changes occurred in proteins involved in the protein's metabolism, 8.5% in the photosynthesis, 5,5% in the amino acid metabolism, 2,3% in the TCA cycle, 2,3% in the mETC and OXPHOS, 1,4% in the glycolysis, and 1,1% in the starch/sucrose metabolism.

A total of 204 mitochondrial proteins were identified in this experiment and a of this total, 54 different proteins related to 12 major metabolisms were significantly affected in this organelle. We observed a reduction in 50% (27) of the significant alterations. All analyzed proteins related to TCA cycle were reduced in the double mutant plants. The mETC and OXPHOS were mainly upregulated in the double mutant (12 proteins out of 14). This major reduction was also observed for the proteins involved in the photorespiration

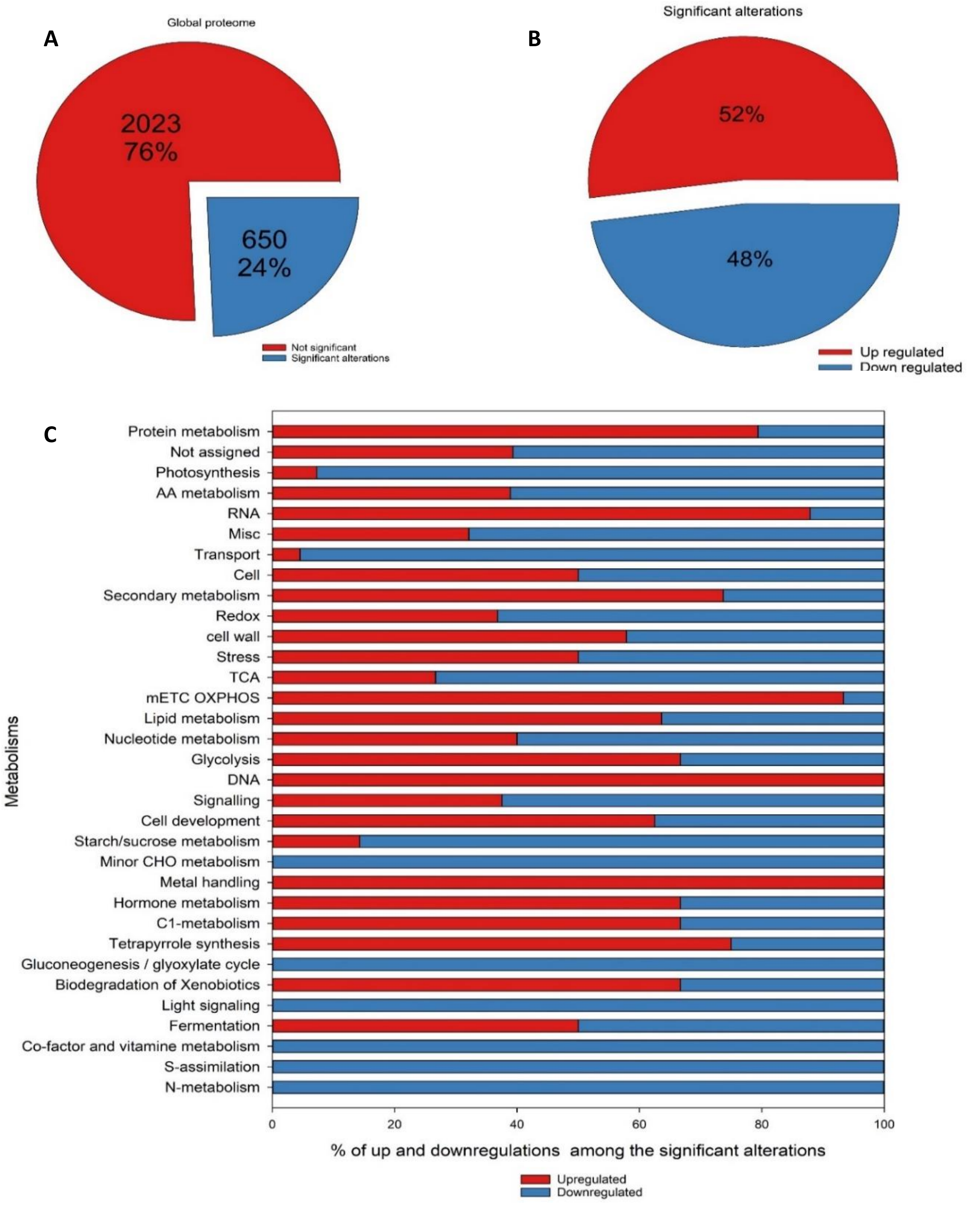

Figure 5: Global alterations in the proteome of arabidopsis plants lacking both genes of the E1 subunit of the 2-OGDH enzyme. (A) Total number of identified proteins and percentage of significant alterations. (B) Percentage of up and downregulated proteins among the significant changes. (C) Percentage of Up and down-regulations among the significant alterations distributed over the different metabolisms ranked from the most (protein metabolism), to the least (N assimilation) affected.

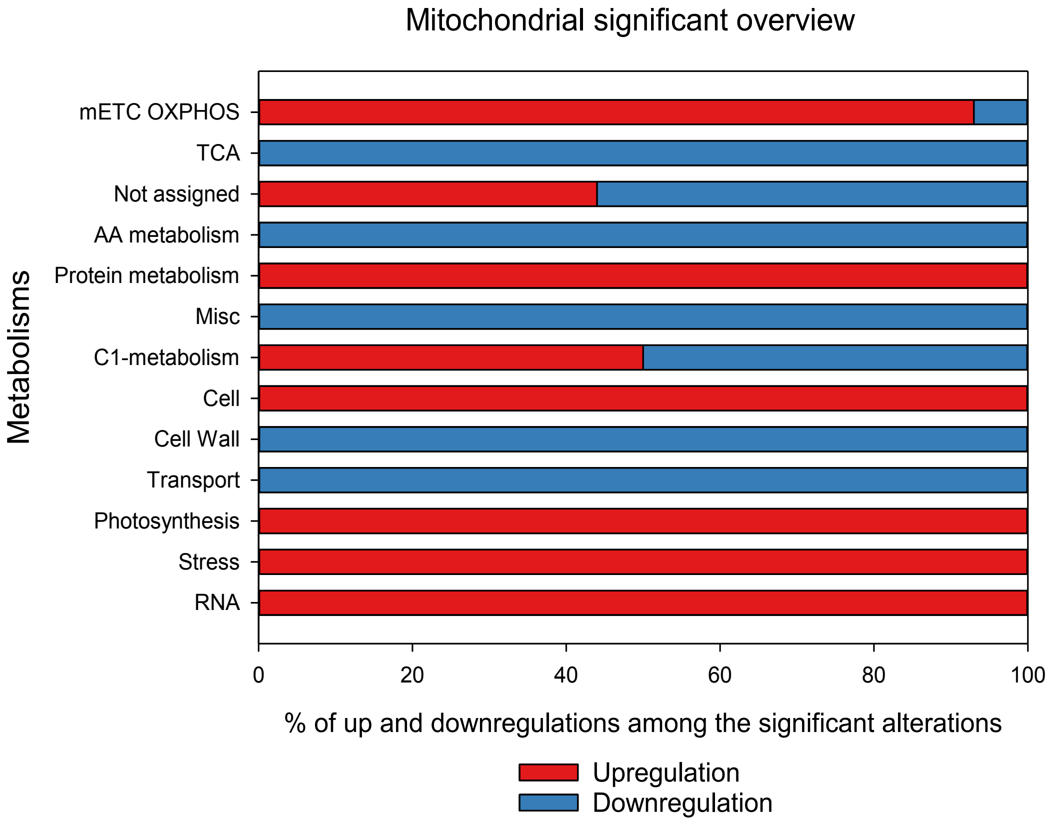

Figure 6: Overview of up and downregulations among the significant alterations of the mitochondrial proteome of arabidopsis plants lacking the expression of both genes of the E1 subunit of the 2-OGDH enzyme (e1-ogdh1.2::2.2).

process in the mitochondria. An overview of these alterations over the mitochondrial metabolism can be seen in the figure 6.

The lack of both genes coding the E1 subunit of the 2OGDH enzyme resulted in a significant reduction for the E1-OGDH1.2 protein and a slightly, but not significant increase for the E1-OGDH2.2 protein (Table 1). The alteration in the levels of the E1 subunit had an impact over the levels of the lipoamide dehydrogenase (At1g48030,mtLPD1), that was significantly down-regulated (Table 1). Other enzymatic complexes present in the mitochondrial matrix that are described for sharing the E3 subunit, like the pyruvate dehydrogenase complex (PDH), and the glycine decarboxylase complex (GDC), also had the amounts of their subunits altered in the mutant plants (Table 1). For the GDC, besides de reduction in the levels of the L protein (E3), we saw a significant reduction in the protein levels for both isoforms of the P protein (genes At2g26080 and At4g33010), for the isoform of the T protein (At1g11860) and for the H1 (At2g35370), while we observed a strong upregulation in the levels of the H2 protein (At2g35120) (Table 1). For the PDH complex we observed a reduction in the levels of two subunits for the subunit E1 (At5g50850 and At1g59900) and a reduction for the subunit E2 of this complex, the dihydrolipoamide acetyltransferase (At1g54220, At3g52200 and At3g13930) (Table 1).

The lack of genes coding to the subunit E1 of the enzyme 2OGDH led to a downregulation of proteins related to the TCA cycle. Beside the reduction described already for the subunits of the 2OGDH complex, we observed a significant reduction for the citrate synthase (At3g44350), and also for the aconitase (At4g26970) (Table 1).

Minor reductions were also observed in the levels of different isoforms of Isocitrate dehydrogenase (At3g09810, At3g17240, At4g35260, At5g03290 and

Table 1: Significant alterations over the mETC/OXPHOS, TCA cycle and photorespiration metabolism of the mitochondrial proteome of arabidopsis plants lacking the expression of both genes of the E1 subunit of the 2-OGDH enzyme (e1-ogdh1.2::2.2). The whole set of significant alterations can be found in the supplemental table 1. Values are Log_2 of means of the relative alterations between the LFQs of double mutant plants versus WT plants n=5. The term significant is applied for the differences with p value ≤ 0,05 as attested by the Student t-Test. (ns) indicate not significant difference against WT.

Metabolism	Gene ID	Description	Log-2 of relative values
Photorespiration	AT4G33010	Glycine decarboxylase P-protein 1	-0,151825
Photorespiration	AT2G26080	Glycine decarboxylase P-protein 2	-0,135743
Photorespiration	AT1G11860	Glycine cleavage T-protein family	-0,127692
Photorespiration	AT2G35370	Glycine decarboxylase complex H	-0,11637
Photorespiration	AT4G37930	Serine transhydroxymethyltransferase 1	-0,157115
Photorespiration	AT2G35120	Glycine cleavage system H protein	2,73298
mETC/OXPHOS	AT3G54110	Plant Uncoupling mitochondrial protein 1	-0,114199
mETC/OXPHOS	AT1G47260	Gamma carbonic anhydrase 2	0,082386
mETC/OXPHOS	AT5G37510	NADH-ubiquinone dehydrogenase	0,0909801
mETC/OXPHOS	ATMG00070	NADH dehydrogenase subunit 9	0,101849
mETC/OXPHOS	AT2G20360	NAD(P)-binding Rossmann-fold superfamily protein	0,129509
mETC/OXPHOS	AT5G66510	Gamma carbonic anhydrase 3	0,135479
mETC/OXPHOS	AT5G08530	51 kDa subunit of complex I	0,149537
mETC/OXPHOS	AT5G52840	NADH-ubiquinone oxidoreductase-like protein	0,17252
mETC/OXPHOS	ATMG00510	NADH dehydrogenase subunit 7	0,207151
mETC/OXPHOS	ATMG00580	NADH dehydrogenase subunit 4	0,210438
mETC/OXPHOS	AT5G13430;AT5G13440	Ubiquinol-cytochrome C reductase iron-sulfur subunit	0,220179
mETC/OXPHOS	ATMG00640	Hydrogen ion transporting ATP synthase rotational mechanism	0,221657
mETC/OXPHOS	AT4G02580	NADH-ubiquinone oxidoreductase 24 kDa subunit	0,256717
mETC/OXPHOS	AT3G48680;AT5G63510	Gamma carbonic anhydrase like 1 ;gamma carbonic anhydrase-like 2	0,26827
mETC/OXPHOS	AT1G80230	Rubredoxin-like superfamily protein	0,320911
TCA	AT3G55410	2-oxoglutarate dehydrogenase E1 component (e1-ogdh1.2)	-4,38101
TCA	AT5G65750	2-oxoglutarate dehydrogenase E1 component (e1-ogdh2.2)	0,112927 (ns)
TCA	AT1G48030	mtLPD1	-0,151704
TCA	AT1G54220	Pyruvate dehydrogenase E2 component	-0,214532
TCA	AT3G52200	Pyruvate dehydrogenase E2 component	-0,189577
TCA	AT4G26970	Aconitase 2	-0,176525
TCA	AT2G44350	Citrate synthase family protein	-0,139264
TCA	AT3G13930	Dihydrolipoamide acetyltransferase long form protein	-0,13517
TCA	AT1G59900	Pyruvate dehydrogenase complex E1 alpha subunit	-0,100887
TCA	AT5G50850	Pyruvate dehydrogenase complex E1 beta subunit	-0,0674248

At5g14590), succinate dehydrogenase (At3g27380, At5g40650 and At5g66760), malate dehydrogenase (At1g53240), the NAD dependent malic enzyme (At2g13560 and At4g00570) (Supplemental table 1). We also observed an increase in the levels of Fumarase 1 (At2g47510) and succinyl-CoA ligase alfa subunit (At5g08300) (Supplemental table 1). Furthermore, the alternative pathways of the TCA cycle like the GABA shunt, were also altered in the double mutant plants, suggested by a significant downregulation for Gaba Transaminase (GabaT, At3g22200) (supplemental table 1).

Several proteins in the mETC and OXPHOS pathway were upregulated in the double mutant plants. In the complex I, we observed increase in the levels of the following proteins: the NADH dehydrogenase subunits 7 (ND7, AtMg00510), 9 (ND9, AtMg00070), and 4 (ND4, AtMg00580); NADH dehydrogenase 1 alpha subcomplex subunit 9 (39kDa subunit, At2g20360), the Gamma carbonic anhydrase 2 and 3 (CAL2 AND CA3, At5g66510 and At3g48680), the NADH dehydrogenase flavoprotein (51kDa subunit, At5g08530) and the 24kDA subunit (At4g02580). In the complex III we observed up-regulation in the levels of the Ubiquinol-cytochrome C reductase iron-sulfur subunit (At5g13440), in the complex IV, the cytochrome oxidase subunit 5 (COX5b2 At1g80230) was also upregulated.

The plant uncoupling mitochondrial protein (UCP1, At3g54110) was the only component of the mETC downregulated in the double mutant plants. Over the photorespiration and C1 metabolism, the protein serine hydroxymenthyltransferase 2 (SHM2, At5g26780) was upregulated while the SHM1 (At4g37930) and the Formate dehydrogenase protein (AT5G14780) were downregulated.

We did not observe alterations in any of the proteins involved in the proline catabolism, neither proline dehydrogenases or the pyrroline-5-carboxilate dehydrogenase were spotted in this experiment. The synthesis final step protein, pyrroline-5-carboxilate reductase was the only spotted in this experiment and it was upregulated, although it was not significantly altered (Supplemental table 1).

The lack of the 2OGDH E1 subunit and the effects over the plastid proteome

As described above, 35,8% of the proteins that were identified in this experiment were from the chloroplast. Among the significant alterations found in the total proteome, 233 proteins were from the chloroplast. From these 233 alterations, 172 proteins were downregulated, and 61 were upregulated. The most impacted metabolisms in this organelle were related with photosynthesis (54 proteins, 23,2%), protein metabolism (27 proteins, 11,6%) and amino acids metabolism (16 proteins, 7,1%) (Figure 7). The overview of negative and positive regulations among the significant alterations over several metabolism is showed in the figure 7 where is possible to see the majority of downregulations that occurred in this organelle.

In table 2, we summarized the significant alterations caused in proteins related to the photosynthesis metabolism. We observed significant changes in the levels of proteins related to the light reactions (41) and the Calvin-Benson cycle (13) (Table 2). Among the protein identified as part of the Calvin-Benson cycle, 11 of them were significantly downregulated and two were upregulated. We identified three subunits from Rubisco, two small subunits (At5g38430 and At5g38410) and one large (AtCg00490) and all of them were downregulated in the double mutant plants, but these alterations were not significant when compared to the WT plants (Supplemental table 1). Both isoforms of phosphoglycerate kinase (PGK1 and 2, At3g12780 and At1g56190) were spotted and significantly downregulated in the double mutant plants. The levels of the subunits A and B of the glyceraldeyde-3 phosphate dehydrogenase

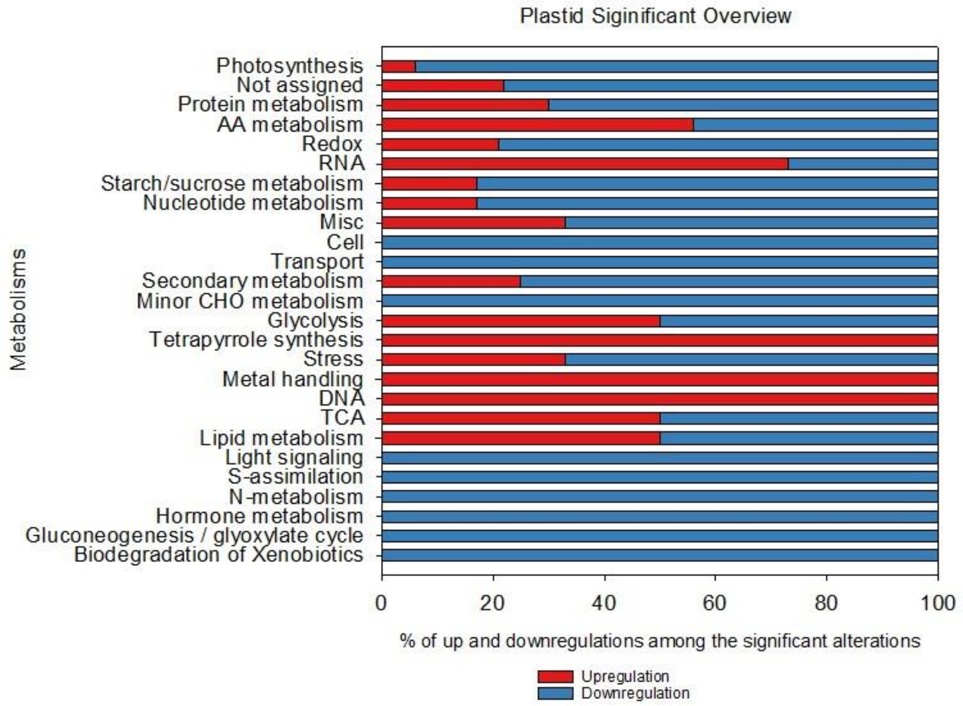

Figure 7: Overview of up and downregulations among the significant alterations of the plastids proteome of arabidopsis plants lacking the expression of both genes of the E1 subunit of the 2-OGDH enzyme (e1-ogdh1.2::2.2).

(GAPA2 and GAPB, At1g12900 and At1g42970) were also significantly reduced. Two isoforms of fructose-6-biphosphate aldolase proteins (FBA1 e 2, At2g21330 and At4g38970) were downregulated in the double mutants. The levels of phosphoribulokinase (PRK, At1g32060), putative ribose-5-phosphate isomerase (RPI, At3g04790) and sedoheptulose-bisphosphatase (SBPase, At3g55800) were all downregulated in the plants lacking the expression of the E1 subunit of the 2OGDH enzyme. The only significant upregulated enzymes involved in the Calvin-Benson cycle were the chaperonin 60 subunits alpha and beta (Cpn60A and B1, AT2G28000 and AT1G55490) (Table 2).

Several proteins related to the light reactions were significantly affected in the double mutant plants as well. The cETC was very much affected. Several proteins found in the photosystems and in complexes of this pathway had their levels reduced in a significantly way. We also spotted not significant alterations in other proteins of this pathway, a total of 49 of which 41 were downregulated (Supplemental table 1). Among the significant alterations in the light reaction related proteins, we observed reductions in the levels of several subunits from the photosystem II like the PsbP-like protein 1

Table 2: Significant alterations over the photosynthesis and redox metabolism of the plastids proteome of arabidopsis plants lacking the expression of both genes of the E1 subunit of the 2-OGDH enzyme (e1-ogdh1.2::2.2). The whole set of significant alterations can be found in the supplememtal table 1. Values are Log_2 of means of the relative alterations between the LFQs of double mutant plants versus WT plants n=5. The term significant is applied for the differences with p value ≤ 0,05. Positive numbers represent upregulation and negative number represent downregulation

Metabolism	Gene ID	Description	Log-2 of relative values
Calvin cycle	AT2G21330	fructose-bisphosphate aldolase 1	-0,122153
Calvin cycle	AT4G38970	fructose-bisphosphate aldolase 2	-0,0945129
Calvin cycle	AT3G54050	high cyclic electron flow 1	-0,0945362
Calvin cycle	AT1G42970	glyceraldehyde-3-phosphate dehydrogenase B subunit	-0,139861
Calvin cycle	AT1G12900	glyceraldehyde 3-phosphate dehydrogenase A subunit 2	-0,10205
Calvin cycle	AT1G56190	Phosphoglycerate kinase family protein	-0,108762
Calvin cycle	AT3G12780	phosphoglycerate kinase 1	-0,106715
Calvin cycle	AT1G32060	phosphoribulokinase	-0,188316
Calvin cycle	AT3G04790	Ribose 5-phosphate isomerase type A protein	-0,201438
Calvin cycle	AT2G39730	rubisco activase	-0,0723587
Calvin cycle	AT2G28000	chaperonin-60alpha	0,161058
Calvin cycle	AT1G55490	chaperonin 60 beta	0,184262
Calvin cycle	AT3G55800	sedoheptulose-bisphosphatase	-0,0724613
Light reaction	ATCG00470	ATP synthase epsilon chain	-0,141209
Light reaction	ATCG00120	ATP synthase subunit alpha	-0,0693233
Light reaction	ATCG00480	ATP synthase subunit beta	-0,0690445
Light reaction	AT4G32260	ATPase F0 complex subunit B/B'	-0,199912
Light reaction	ATCG00130	ATPase F0 complexsubunit B/B'	-0,142494
Light reaction	AT4G04640	ATPase F1 complex gamma subunit protein	-0,141115
Light reaction	ATCG00150	ATPaseF0 complex subunit A protein	-3,41516
Light reaction	AT1G29930	chlorophyll A/B binding protein 1	0,293992
Light reaction	AT1G44575	Chlorophyll A-B binding family protein	-0,146143
Light reaction	AT3G54890	chlorophyll a-b binding protein 6	-0,135818
Light reaction	AT1G20340	Cupredoxin superfamily protein	-0,522002
Light reaction	AT5G66190	ferredoxin-NADP[+]-oxidoreductase 1	-0,136836
Light reaction	AT4G09650	F-type H+-transporting ATPase subunit delta	-0,131621
Light reaction	AT3G08940	light harvesting complex photosystem II	-0,1129
Light reaction	ATCG01110	NAD(P)H dehydrogenase subunit H	-0,160678
Light reaction	ATCG01100	NADH dehydrogenase family protein	-0,122023
Light reaction	ATCG01090	NADPH dehydrogenase	-0,20124
Light reaction	AT5G58260	oxidoreductases acting on NADH or NADPH quinone or similar compound as acceptor	-0,29264
Light reaction	AT4G22890	PGR5-LIKE A	-0,176483
Light reaction	ATCG00540	photosynthetic electron transfer A	-0,0563477
Light reaction	AT4G03280	photosynthetic electron transfer C	-0,100047
Light reaction	ATCG00350	Photosystem I PsaA/PsaB protein	-0,138109
Light reaction	AT4G02770	photosystem I subunit D-1	-0,182108
Light reaction	AT2G20260	photosystem I subunit E-2	-0,214196
Light reaction	AT1G31330	photosystem I subunit F	-0,174319
Light reaction	AT4G12800	photosystem I subunit I	-0,192532
Light reaction	AT1G03600	photosystem II family protein	-0,148532
Light reaction	ATCG00580	photosystem II reaction center protein E	-0,157779
Light reaction	ATCG00430	photosystem II reaction center protein G	-0,139233
Light reaction	AT5G23120	photosystem II stability/assembly factor chloroplast (HCF136)	-0,174235
Light reaction	AT3G50820	photosystem II subunit O-2	-0,161195
Light reaction	AT4G05180	photosystem II subunit Q-2	-0,146431
Light reaction	AT4G21280	photosystem II subunit QA	-0,125943
Light reaction	ATCG00340	Photosystem IPsaA/PsaB protein	-0,145997
Light reaction	AT3G15840	post-illumination chlorophyll fluorescence increase	-0,206913
Light reaction	AT5G66570	PS II oxygen-evolving complex 1	-0,0938622
Light reaction	AT3G55330	PsbP-like protein 1	-0,210085
Light reaction	AT1G14150	PsbQ-like 2	-0,183482
Light reaction	AT1G61520	PSI type III chlorophyll a/b-binding protein	-0,132274
Light reaction	AT4G37925	subunit NDH-M of NAD(P)H:plastoquinone dehydrogenase complex	-0,130475
Light reaction	AT1G77090	thylakoid lumenal protein	-0,233482

(At3g55330), PsbQ-like protein (At1g14150), high chlorophyll fluorescence 136 (HCF136, At5g23120), oxygen evolving complex subunit 33 kda (PsbO2, At3g50820), photosystem II reaction center protein E (PsbE, Atcg00580), Psb27 (At1g03600), photosystem II subunit Q (PsbQ, At4g05180), photosystem II subunit S (PsbS, At1g44575), photosystem II subunit Q1 (PsbQ1, At4g21280), light harvesting complex photosystem II (LhcB4.2, At3g08940), PS II oxygen-evolving complex 1 (PsbO1, At5g66570) and chlorophyll A/B binding protein 1 (CAB1, At1g29930), photosystem II reaction center protein G (PsbG Atcg00430).

Several proteins from photosystem I were also downregulated in the double mutant lines and among then, the significantly altered was photosystem I subunit E-2 (PsaE2, At2g20260), photosystem I subunit I (PsaL, At4g12800), photosystem I subunit D-1 (PsaD-1, At4g02770 photosystem I subunit F (PsaF, At1g31330), Photosystem PsaB protein (PsaB, Atcg00340), Photosystem PsaA protein (PsaA, Atcg00350), chlorophyll a-b binding protein 6 (Lhca1, At3g54890), PSI type III chlorophyll a/b-binding protein (Lhca3, At1g61520).

The final step of the cETC was also affected in the double mutant plants as we can see by the reduced levels of subunits from the ATP synthase like the ATPase F0 complex subunit A protein (ATPI, Atcg00150), ATPase F0 complex subunit B/B (At4g32260), ATPase F0 complex subunit B/B (ATPF, Atcg00130), ATP synthase epsilon chain (ATPE, Atcg00470), ATPase F1 complex gamma subunit protein (ATPC1, At4g04640), F-type H^+-transporting ATPase subunit delta (ATPD, At4g09650), ATP synthase subunit alpha (ATPA, Atcg00120) and the ATP synthase subunit beta (ATPB, Atcg00480). Proteins involved in the cyclic electron flow were also affected in the chloroplast. Proteins like the post-illumination chlorophyll fluorescence increase (At3g15840), NADPH dehydrogenase (Atcg01090), PGR5-LIKE A (At4g22890), NAD(P)H dehydrogenase subunit H (Atcg01110) and NADH dehydrogenase family protein (Atcg01100) were all reduced in the mutant plants. Other intermediates of the cETC were also significantly affected in the mutant plants, Two subunits from the cytochrome b6f, the photosynthetic electron C and A (PGR1, At4g03280 and PETA, Atcg00540); subunit N and M from the NAD(P)Plastoquinone dehydrogenase complex (NDH-N, At5g58260 and NDH-M, At4g37925) and the ferredoxin-NADP[+]-oxidoreductase 1 protein (FNR1, At5g66190) were all downregulated.

The lack of 2OGDH E1 subunit and the effects over different metabolisms

When we investigated into different metabolisms, disregarding the organelle, we observed that the lack of the E1 subunit of the 2OGDH enzyme led to various alterations in a whole plant level. We were able to spot 151 proteins involved in the amino acid metabolism, and among these proteins, 36 had their levels significantly altered, 22 were down and 14 upregulated. The system GS/GOGAT, important regulator of the nitrogen metabolism was affected in the mutant plants. One of the most important isoforms of the cytosolic Glutamine synthetase GS1.3 (At3g17820) was strongly upregulated while the GS1.2 (At1g66200) and the plastids GS2 (At5g35630) was slightly, but significantly downregulated when compared with the WT plants. For GOGAT we observed significant reductions in the levels of one Fd-GOGAT isoform (GLU1, At5g04140) and one NADH-GOGAT (GLT1, At5g53460).

Table 3: Significant alterations in the levels of proteins related to Amino acids metabolism, Glycolysis, Redox metabolism, and transport of arabidopsis plants lacking the expression of both genes of the E1 subunit of the 2-OGDH enzyme (e1-ogdh1.2::2.2). The whole set of significant and not significant alterations can be found in the supplemental table 1 Values are Log_2 of means of the relative alterations between the LFQs of double mutant plants versus WT plants n=5. The term significant is applied for the differences with *p* value ≤ 0,05.

Organelle	Gene ID	Metabolism	Description	Log-2 of relative values
cytosol	AT3G17820	amino acid synthesis	Glutamine synthetase 1.3	2,46076
cytosol	AT1G66200	amino acid synthesis	Glutamine Synthase	-0,242948
plastid	AT5G35630	amino acid synthesis	Gutamine synthetase 2	-0,096233
plastid	AT5G04140	amino acid synthesis	Glutamate synthase 1	-0,156262
plastid	AT5G53460	amino acid synthesis	NADH-dependent Glutamate synthase 1	-0,280892
cytosol	AT2G42600	glycolysis	phosphoenolpyruvate carboxylase 2	-0,14124
cytosol	AT2G24270	glycolysis	aldehyde dehydrogenase 11A3	0,108022
cytosol	AT3G52930	glycolysis	fructose-bisphosphate aldolase	0,11067
cytosol	AT1G20950	glycolysis	Phosphofructokinase family protein	0,159044
cytosol	AT2G29560	glycolysis	cytosolic enolase	0,189132
plastid	AT1G70820	glycolysis plastid branch	phosphoglucomutase putative	-0,169416
plastid	AT4G24620	glycolysis plastid branch	phosphoglucose isomerase 1	-0,102454
plastid	AT3G22960	glycolysis plastid branch	Pyruvate kinase family protein	0,213561
plastid	AT5G52920	glycolysis plastid branch	plastidic pyruvate kinase beta subunit 1	0,282861
plastid	AT2G25080	Redox/ascorbate and glutathione	glutathione peroxidase 1	-0,378577
plastid	AT3G54660	Redox/ascorbate and glutathione	glutathione reductase	-0,155135
plastid	AT5G16710	Redox/ascorbate and glutathione	dehydroascorbate reductase 1	-0,150504
plastid	AT1G63940	Redox/ascorbate and glutathione	monodehydroascorbate reductase 6	-0,105929
plastid	AT2G28190	Redox/dismutases and catalases	copper/zinc superoxide dismutase 2	-1,32068
plastid	AT4G25100	Redox/dismutases and catalases	Fe superoxide dismutase 1	0,650841
plastid	AT5G51100	Redox/dismutases and catalases	Fe superoxide dismutase 2	1,37126
plastid	AT3G27820	Redox/ascorbate and glutathione	monodehydroascorbate reductase 4	-0,131119
plastid	AT5G20140	Redox/heme	SOUL heme-binding family protein	-0,168979
plastid	AT3G11630	Redox/peroxiredoxin	Thioredoxin superfamily protein	-0,0960003
plastid	AT5G16400	Redox/thioredoxin	thioredoxin F2	-1,81142
plastid	AT1G03680	Redox/thioredoxin	thioredoxin M-type 1	-0,104242
plastid	AT4G03520	Redox/thioredoxin	thioredoxin M-type 2	-0,0941891
plastid	AT3G06730	Redox/thioredoxin	Thioredoxin z	0,269162

Several proteins that participate in the glycolysis were affected (Table 3), the phosphoglucomutase (At1g70820), and Glucose-6-phosphate isomerase (PGI1, At4g24620) were downregulated, fructose-bisphosphate aldolase (FBA8, At3g52930), glyceraldehyde-3-phosphate dehydrogenase [NADP(+)] (AT2G24270), Enolase (ENO3, AT2G29560), plastidial pyruvate kinase 1 (PKP1, AT3G22960), plastidial pyruvate kinase 2 (PKP2, AT5G52920) were upregulated while phosphoenolpyruvate carboxylase 2 (PPC2, AT2G42600) was also downregulated (Table 3).

The redox metabolism was also affected in the mutant lines. Glutathione peroxidase 1 (GPX1, At2g25080), glutathione reductase (GR, AT3G54660), Dehydroascorbate reductase 1 (DHAR3, At5g16710), Monodehydroascorbate reductase 4 (MDAR4, At3g27820), Monodehydroascorbate reductase 6 (MDAR6, At1g63940) involved in the ascorbate and glutathione metabolism were all downregulated (Table 3). While the copper/zinc superoxide dismutase 2 (CSD2, At2g28190) was downregulated, the Fe superoxide dismutase 1 (FSD1, At4g25100), and the Fe superoxide dismutase 2 (FSD2, At5g51100) were up regulated. Among the thioredoxin system the Z one (TRXz, AT3G06730) was upregulated while Thioredoxin-dependent peroxiredoxin (2CPA, At3g11630), Thioredoxin F2 (TRXF2, At5g16400), Thioredoxin M-type 1 (THM1, At1g03680) and Thioredoxin superfamily protein (ATGM2, AT4G03520) were downregulated (Table 3).

DISCUSSION

In this work, based on the importance of the 2OGDH enzyme in plant metabolism, and on the already observed impact of the lack of this enzyme on gene expression (Araújo et al., 2012b; Yue et al., 2018), we analyzed the impacts caused by the lack 2OGDH E1 subunit over the whole rosette proteome of arabidopsis plants. The obtained results revealed that the lack of expression of both E1 isoforms generates a phenotype with smaller plants, without large effects on the levels of primary metabolites. Nevertheless, the lack of both isoforms of the E1 subunit led to alterations in the levels of other important complexes in the mitochondrial matrix involved in the respiration and photorespiration. In addition, we also observed that the levels of proteins localized in other organelles and metabolisms such as photosynthesis and amino acid synthesis in plastids and glycolysis in the cytosol were significantly altered in the double mutant plants. Together these results confirm that the lack of the 2OGDH E1 subunit is important in keeping mitochondria proteome homeostasis. The reduction in the E1 subunit is linked to changes in the whole energy metabolism, affecting both the production of assimilates (photosynthesis) and their consumption (respiration), resulting in smaller plants.

The lack of the 2OGDH E1 subunit alters the balance of important mitochondrial enzymatic complexes

The 2OGDH enzyme is actually an enzymatic complex composed of three subunits (Millar et al., 1999). As expected, the reduction in the expression levels of the two genes of the 2OGDH E1 subunit resulted in alterations in the levels of these proteins. Surprisingly, E1-OGDH1 was reduced while E1-OGDH2 had a slight, but no significant increase in its levels (Table 1). As the T-DNA insertion in this gene (e1-ogdh2.2) occurred close to the C-terminal region (Condori-Apfata et al., 2019), this may result in an almost complete transcription and translation of the gene justifying the levels of this protein in the analyzed tissues. It is worth mentioning that even when this isoform is intact, it has low participation in the activity of the whole enzyme complex (Condori-Apfata et al., 2019).

The reduction in the 2OGDH E1 subunit level led to a decrement in other subunits of this complex, including a significant reduction for the E3 subunit (mtLPD1, Table 1). This E3 subunit is shared by different complexes in the mitochondrial matrix, such as pyruvate dehydrogenase (PDH) and glycine decarboxylase (GDC), and the reduction of its levels affected different subunits of this enzymes in a similar way. This observations corroborates with the hypothesis of a metabolon formation between the different TCA and mitochondrial enzymes recently proposed in arabidopsis (Zhang et al., 2017; Zhang et al., 2018). The term metabolon has different interpretations, but it comprehend the association of different proteins in order to channel metabolites in different pathways acting as an important metabolism regulation factor (Sweetlove and Fernie, 2013; Zhang et al., 2017; Obata, 2019; Jacobowitz and Weng, 2020). This connections happen among proteins involved in the same pathway (Zhang et al., 2017), and also among proteins of different metabolisms (Zhang et al., 2018; Obata, 2019), and usually depends on a balanced stoichiometry to work (Fuchs et al., 2020). Therefore, the reduction of one subunit or of a whole enzyme complex like the 2OGDH alters the levels of other enzymes, in order to maintain a stoichiometric balance of these complexes and keep the answer to different demands in the cell.

For GDC, the reduction of the L subunit (E3, mtLPD1) also negatively altered the levels of P, T and an H1 protein isoforms in a negative manner (Table 1). The reduction of these subunits are all linked to the reduction of the photorespiratory process (Bauwe and Kolukisaoglu, 2003; Giuliani et al., 2019; Engel et al., 2007).

Interestingly, the levels of H2 subunit, was increased in plants lacking the 2OGDH E1 subunit (Table 2). The H subunit of GDC plays a coenzyme and substrate role in all GDC reactions interacting with all the other subunits of the GDC complex (Bauwe and Kolukisaoglu, 2003; Wittmiß et al., 2020). Given its connecting role, one can argue that the increase in the H2 subunit happened as a response to the reduction in the subunits P, T and one H1 to keep the balance and functioning of the whole complex.

The lack of the 2OGDH E1 subunit has consequences over the respiration in the mitochondria and cytosol, and over the photosynthesis

Several points of the mitochondrial respiration metabolism, can affect the efficiency of the photosynthesis in the plastids (Nunes-Nesi et al., 2008; Igamberdiev and Bykova, 2018; Igamberdiev, 2020b). The lack of the E1 subunit of the 2OGDH led to a significant reduction in the levels of citrate synthase (CS) and aconitase (ACO) (Table 1). These enzymes participate in the first steps of the TCA cycle (Millar et al., 2011). The reduction in their levels can lead to changes in the balance of intermediates like aconitate, cis and trans-aconitate and negatively affect the citrate and malate valves (Igamberdiev and Bykova, 2018) These valves are very important for the maintenance of the photosynthesis (Nunes-Nesi et al., 2008; Igamberdiev and Bykova, 2018; Igamberdiev, 2020b). Therefore, alteration in their functioning in the double mutant plants can lead to an impairment in photosynthesis. The reductions in the levels of the TCA cycle enzymes, caused by the lack of the 2OGDH E1 subunit, may result in a negative feedback regulation over the proteins involved in the glycolysis and also in the photosynthesis, reducing not only their activity (Wittmiß et al., 2020), but, in the long term, their protein levels (Plaxton and Podestá, 2006) (Table 2-3).

Among all the alterations in the mitochondrial proteome related to the mETC, the only protein downregulated was the decoupling protein 1 (UCP1, Table1). The UCP1 is involved not only in the decoupling of the respiration but also in the exchange of reducing power between the mitochondria and cytosol, through the transport of aspartate and glutamate, and it is co-expressed with the 2OGDH (Monné et al., 2018; Barreto et al., 2020). The mutation of the UCP1 in arabidopsis led to reduced photosynthetic rates through the inhibition of the photorespiration process (Sweetlove et al., 2006). Curiously, we observed significant reductions in important photorespiration proteins like the GDC and SHMT2 (Table 1) and in several proteins related to photosynthesis (Table 2). Altogether, these results help to better understand the mitochondrial responses that are involved in the control of photosynthesis, linking the lack of the E1 subunit of the 2OGDH enzyme to disturbances in the redox metabolism and the mitochondrial photorespiration.

Several proteins related to mETC were affected in response to the lack of the E1 subunit of the 2OGDH enzyme. Proteins that together assemble the complexes I, III and IV had their levels upregulated in the double mutant plants. The light reactions of photosynthesis produce the reducing power (NADPH) used in the Calvin-Benson cycle. Usually the ratio of production and consumption of ATP/NADPH in the chloroplast are balanced in a way that little importation of ATP from the cytosol is required (Voon et al., 2021). The reduction in the levels of proteins related to the light reactions (Table 2) may lead to a higher demand of ATP in the chloroplasts that might be answered by a higher mitochondrial production, also favored by the down-regulation of the UCP1 (Table 1).

In the chloroplast, we observed significant reductions in the proteins involved in the assemble and functioning of the cETC and Calvin-Benson cycle multiprotein complexes (Table 2). The down-regulation of those proteins can be related to a decrement in the photoautotrophic growth, photosynthesis constancy, photoprotection and ATP production (Haldrup et al., 2000; Varotto et al., 2000; Scheller et al., 2001; Yi et al., 2007; de Bianchi et al., 2011; Schöttler et al., 2015; Che et al., 2020; Zancani et al., 2020; Ishikawa et al., 2021). All those responses corroborate with the smaller size of the mutant plants (Figure 1) and together with the down-regulations observed in the redox metabolism (Table 3) may enlighten the mechanisms behind the smaller photosynthesis rates described for the double mutant plants by Condori-Apfata et al. (2019).

This reduction in the chloroplast proteins might be triggered by the down-regulation of TCA cycle enzymes in the mitochondria, once this reduction may lead to a negative feedback regulation of photosynthesis activity (Wittmiß et al., 2020) and, in the long term can be related to a reduction in the protein levels of a pathway (Plaxton and Podestá, 2006). Given that the photorespiration process play a distinct role protecting the cETC from photoinhibition and oxidative stress (Hebbelmann et al., 2012; Shi et al., 2022), the reductions occurred in the levels the GDC and SHAMT in the mitochondria (Table 1), can also be held accountable for the reduction of cETC proteins.

The lack of the 2OGDH E1 subunit has impact over the nitrogen metabolism

The enzymes Glutamine synthetase (GS) and Glutamate synthase (GOGAT) act as gateways between the carbon and nitrogen metabolisms (Lea and Miflin, 2003; Gaufichon et al., 2016; Liao et al., 2022). Almost all of their isoforms were down-regulated but interestingly, one of the GS isoforms, the GS 1.3 was strongly up-regulated in the double mutants. This isoform is cytosolic and described as important for transport during nitrogen remobilization in source tissues (Martin et al., 2006), during protein synthesis and degradation and also during nitrogen shortage (Fujita et al., 2022). The mutation and inhibition of the 2OGDH is usually accompanied by increase in the 2OG levels (Araújo et al., 2008; Araújo et al., 2012b; Yue et al., 2018). This increase in the 2OG would explain the upregulation in the GS levels (Hodges, 2002).

The alterations in the proteome related to amino acid synthesis were accompanied by significant increase in the levels of aspartate, asparagine, glutamine and histidine in the double mutant plants (Figure 4). These amino acids (with exception of histidine) are usually described as the main forms of nitrogen storage and transport across the plant and also as the first products of nitrogen assimilation (Planchet et al., 2011; Hildebrandt et al., 2015; Gaufichon et al., 2016). The increase of these particular amino acids, in plants that are significantly smaller than the WT (Figure 1) together with the increase in the GS may indicate that the lack of the 2OGDH E1 subunit do not affect the nitrogen assimilation itself, but yet by promoting a reduction in growth lead to the amino acid accumulation.

CONCLUSION

The results obtained allowed us to propose new regulatory roles for the E1 subunit of the 2OGDH. First, the reduced levels of the E1 subunit of 2OGDH complex resulted in a down-regulated TCA cycle, PDH and glycolysis, that would explain the smaller respiration rate and the lower growth. In the second regulatory pathway, the lower levels of the 2OGDH E1 subunit led to a reduction in the levels of UCP1 and the GDC complex subunits. These reductions would lead to a decrease in the photorespiration process, and may be connected with the reduction in the levels of the cETC complexes and Calvin-Benson cycle proteins. This impairment in the photosynthesis metabolism might lead to an increase in the demand for mitochondrial ATP explaining the up regulation of the mETC proteins. Both mechanisms would end up leading to smaller plants with lower demand for amino acids and that are so adapted to the alterations in the proteome, that do not show significant alterations in the primary metabolism. More research is necessary to better explain how exactly the E1 subunit of the 2OGDH is linked to the proteome alterations reported in here. However, it is clear, that this enzyme can still be considered of great importance in the metabolism of illuminated leaves.

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REFERENCES

Araújo WL, Martins AO, Fernie AR, Tohge T (2014a) 2-oxoglutarate: Linking TCA cycle function with amino acid, glucosinolate, flavonoid, alkaloid, and gibberellin biosynthesis. Front Plant Sci **5**: 1–6

Araújo WL, Nunes-Nesi A, Fernie AR (2014b) On the role of plant mitochondrial metabolism and its impact on photosynthesis in both optimal and sub-optimal growth conditions. Photosynth Res **119**: 141–156

Araújo WL, Nunes-Nesi A, Nikoloski Z, Sweetlove LJ, Fernie AR (2012a) Metabolic control and regulation of the tricarboxylic acid cycle in photosynthetic and heterotrophic plant tissues. Plant, Cell Environ **35**: 1–21

Araújo WL, Nunes-Nesi A, Osorio S, Usadel B, Fuentes D, Nagy R, Balbo I, Lehmann M, Studart-Witkowski C, Tohge T, et al (2011) Antisense inhibition of the iron-sulphur subunit of succinate dehydrogenase enhances photosynthesis and growth in tomato via an organic acid-mediated effect on stomatal aperture. Plant Cell **23**: 600–627

Araújo WL, Nunes-Nesi A, Trenkamp S, Bunik VI, Fernie AR (2008) Inhibition of 2-oxoglutarate dehydrogenase in potato tuber suggests the enzyme is limiting for respiration and confirms its importance in nitrogen assimilation. Plant Physiol **148**: 1782–1796

Araújo WL, Tohge T, Nunes-Nesi A, Daloso DM, Nimick M, Krahnert I, Bunik VI, Moorhead GBG, Fernie AR (2012b) Phosphonate analogs of 2-oxoglutarate perturb metabolism and gene expression in illuminated Arabidopsis leave. Front Plant Sci **3**: 1–19

Araújo WL, Tohge T, Osorio S, Lohse M, Balbo I, Krahnert I, Sienkiewicz-Porzucek A, Usadel B, Nunes-Nesi A, Fernie AR (2012c) Antisense inhibition of the 2-oxoglutarate dehydrogenase complex in tomato demonstrates its importance for plant respiration and during leaf senescence and fruit maturation. Plant Cell **24**: 2328–2351

Barreto P, Couñago RM, Arruda P (2020) Mitochondrial uncoupling protein-dependent signaling in plant bioenergetics and stress response. Mitochondrion **53**: 109–120

Batista-Silva W, Heinemann B, Rugen N, Nunes-Nesi A, Araújo WL, Braun HP, Hildebrandt TM (2019) The role of amino acid metabolism during abiotic stress release. Plant Cell Environ **42**: 1630–1644

Bauwe H, Kolukisaoglu Ü (2003) Genetic manipulation of glycine decarboxylation. J Exp Bot **54**: 1523–1535

de Bianchi S, Betterle N, Kouril R, Cazzaniga S, Boekema E, Bassi R, Dall'Osto L (2011) Arabidopsis mutants deleted in the light-harvesting protein Lhcb4 have a disrupted photosystem II macrostructure and are defective in photoprotection. Plant Cell **23**: 2659–2679

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72**: 248–254

Bunik VI, Fernie AR (2009) Metabolic control exerted by the 2-oxoglutarate dehydrogenase reaction: A cross-kingdom comparison of the crossroad between energy production and nitrogen assimilation. Biochem J **422**: 405–421

Bykova N V., Keerberg O, Pärnik T, Bauwe H, Gardeström P (2005) Interaction between photorespiration and respiration in transgenic potato plants with antisense reduction in glycine decarboxylase. Planta **222**: 130–140

Carrari F, Nunes-Nesi A, Gibon Y, Lytovchenko A, Loureiro ME, Fernie AR (2003) Reduced Expression of Aconitase Results in an Enhanced Rate of Photosynthesis and Marked Shifts in Carbon Partitioning in Illuminated Leaves of Wild Species Tomato. Plant Physiol **133**: 1322–1335

Carillo P, Gibon Y (2011) PROTOCOL : Extraction and determination proline. Kasetsart J - Nat Sci 43: 146–152

Che Y, Kusama S, Matsui S, Suorsa M, Nakano T, Aro EM, Ifuku K (2020) Arabidopsis PsbP-Like Protein 1 Facilitates the Assembly of the Photosystem II Supercomplexes and Optimizes Plant Fitness under Fluctuating Light. Plant Cell Physiol **61**: 1168–1180

Condori-Apfata JA, Batista-Silva W, Medeiros DB, Vargas JR, Valente LML, Heyneke E, Pérez-Diaz JL, Fernie AR, Araújo WL, Nunes-Nesi A (2019) The Arabidopsis E1 subunit of the 2-oxoglutarate dehydrogenase complex modulates plant growth and seed production. Plant Mol Biol **101**: 183–202

Condori-Apfata JA, Batista-Silva W, Medeiros DB, Vargas JR, Valente LML, Pérez-Díaz JL, Fernie AR, Araújo WL, Nunes-Nesi A (2021) Downregulation of the E2 Subunit of 2-Oxoglutarate Dehydrogenase Modulates Plant Growth by

Impacting Carbon–Nitrogen Metabolism in Arabidopsis thaliana . Plant Cell Physiol **0**: 1–17

Engel N, Van Den Daele K, Kolukisaoglu Ü, Morgenthal K, Weckwerth W, Pärnik T, Keerberg O, Bauwe H (2007) Deletion of glycine decarboxylase in arabidopsis is lethal under nonphotorespiratory conditions. Plant Physiol **144**: 1328–1335

Fernie AR, Roscher A, Ratcli RG, Kruger NJ (2001) Fructose 2 , 6-bisphosphate activates pyrophosphate : fructose-6-phos- phate 1-phosphotransferase and increases triose phosphate to hexose phosphate cycling in heterotrophic cells. Planta **212**: 250–263

Fuchs P, Rugen N, Carrie C, Elsässer M, Finkemeier I, Giese J, Hildebrandt TM, Kühn K, Maurino VG, Ruberti C, et al (2020) Single organelle function and organization as estimated from Arabidopsis mitochondrial proteomics. Plant J **101**: 420–441

Fujita T, Beier MP, Tabuchi-Kobayashi M, Hayatsu Y, Nakamura H, Umetsu-Ohashi T, Sasaki K, Ishiyama K, Murozuka E, Kojima M, et al (2022) Cytosolic Glutamine Synthetase GS1;3 Is Involved in Rice Grain Ripening and Germination. Front Plant Sci. doi: 10.3389/fpls.2022.835835

Gaufichon L, Rothstein SJ, Suzuki A (2016) Asparagine metabolic pathways in arabidopsis. Plant Cell Physiol **57**: 675–689

Giuliani R, Karki S, Covshoff S, Lin H, Coe RA, Koteyeva NK, Quick WP, Caemmerer S Von, Furbank RT, Hibberd JM, et al (2019) Knockdown of glycine decarboxylase complex alters photorespiratory carbon isotope fractionation in Oryza sativa leaves. **70**: 2773–2786

Haldrup A, Simpson DJ, Scheller HV (2000) Down-regulation of the PSI-F subunit of photosystem I (PSI) in Arabidopsis thaliana. The PSI-F subunit is essential for photoautotrophic growth and contributes to antenna function. J Biol Chem **275**: 31211–31218

Hebbelmann I, Selinski J, Wehmeyer C, Goss T, Voss I, Mulo P, Kangasjärvi S, Aro EM, Oelze ML, Dietz KJ, et al (2012) Multiple strategies to prevent oxidative stress in Arabidopsis plants lacking the malate valve enzyme NADP-malate dehydrogenase. J Exp Bot **63**: 1445–1459

Heinemann B, Künzler P, Eubel H, Braun HP, Hildebrandt TM (2021) Estimating the number of protein molecules in a plant cell: protein and amino acid homeostasis during drought. Plant Physiol **185**: 385–404

Hildebrandt TM, Nunes Nesi A, Araújo WL, Braun HP (2015) Amino Acid Catabolism in Plants. Mol Plant **8**: 1563–1579

Hodges M (2002) Enzyme redundancy and the importance of 2-oxoglutarate in plant ammonium assimilation. J Exp Bot **53**: 905–916

Huergo LF, Dixon R (2015) The Emergence of 2-Oxoglutarate as a Master Regulator Metabolite. Microbiol Mol Biol Rev **79**: 419–435

Igamberdiev AU (2020) Citrate valve integrates mitochondria into photosynthetic metabolism. Mitochondrion **52**: 218–230

Igamberdiev AU, Bykova N V. (2018) Role of organic acids in the integration of cellular redox metabolism and mediation of redox signalling in photosynthetic tissues of higher plants. Free Radic Biol Med **122**: 74–85

Ishikawa N, Yokoe Y, Nishimura T, Nakano T, Ifuku K (2021) PsbQ-like protein 3 functions as an assembly factor for the chloroplast nadh dehydrogenase-like complex in arabidopsis. Plant Cell Physiol **61**: 1252–1261

Jacobowitz JR, Weng J-K (2020) Exploring Uncharted Territories of Plant Specialized Metabolism in the Postgenomic Era. Annu Rev Plant Biol **71**: 631–658

Lea PJ, Miflin BJ (2003) Glutamate synthase and the synthesis of glutamate in plants. Plant Physiol Biochem **41**: 555–564

Lemaitre T, Urbanczyk-Wochniak E, Flesch V, Bismuth E, Fernie AR, Hodges M (2007) NAD-dependent isocitrate dehydrogenase mutants of Arabidopsis suggest the enzyme is not limiting for nitrogen assimilation. Plant Physiol **144**: 1546–1558

Liao H-S, Chung Y-H, Hsieh M-H (2022) Glutamate: A multifunctional amino acid in plants. Plant Sci **318**: 111238

Martin A, Lee J, Kichey T, Gerentes D, Zivy M, Tatout C, Fre´de´ric Dubois, Thierry Balliau B, Davanture M ne, The´re` se Terce-Laforgue, a Isabelle Quillere, Gallais MC, et al (2006) Two Cytosolic Glutamine Synthetase Isoforms of Maize Are Specifically Involved in the Control of Grain Production. **18**: 3252–3274

Millar AH, Hill SA, Leaver CJ (1999) Plant mitochondrial 2-oxoglutarate dehydrogenase complex: Purification and characterization in potato. Biochem J **343**: 327–334

Millar AH, Whelan J, Soole KL, Day DA (2011a) Organization and Regulation of Mitochondrial Respiration in Plants. Annu Rev Plant Biol **62**: 79–104

Millar AH, Whelan J, Soole KL, Day DA (2011b) Organization and Regulation of Mitochondrial Respiration in Plants. Annu Rev Plant Biol **62**: 79–104

Monné M, Daddabbo L, Gagneul D, Obata T, Hielscher B, Palmieri L, Miniero DV, Fernie AR, Weber APM, Palmieri F (2018) Uncoupling proteins 1 and 2 (UCP1 and UCP2) fromArabidopsis thaliana are mitochondrial transporters of aspartate, glutamate, and dicarboxylates. J Biol Chem **293**: 4213–4227

Niazi AK, Bariat L, Riondet C, Carapito C, Mhamdi A, Noctor G, Reichheld JP (2019) Cytosolic isocitrate dehydrogenase from arabidopsis thaliana is regulated by glutathionylation. Antioxidants. doi: 10.3390/antiox8010016

Nunes-Nesi A, Carrari F, Gibon Y, Sulpice R, Lytovchenko A, Fisahn J, Graham J, Ratcliffe RG, Sweetlove LJ, Fernie AR (2007) Deficiency of mitochondrial fumarase activity in tomato plants impairs photosynthesis via an effect on stomatal function. Plant J **50**: 1093–1106

Nunes-Nesi A, Carrari F, Lytovchenko A, Smith AMO, Loureiro ME, Ratcliffe RG, Sweetlove LJ, Fernie AR (2005) Enhanced photosynthetic performance and growth as a consequence of decreasing mitochondrial malate dehydrogenase activity in transgenic tomato plants. Plant Physiol **137**: 611–622

Nunes-Nesi A, Sulpice R, Gibon Y, Fernie AR (2008) The enigmatic contribution of mitochondrial function in photosynthesis. J Exp Bot **59**: 1675–1684

Obata T (2019) Metabolons in plant primary and secondary metabolism. Phytochem Rev **18**: 1483–1507

Planchet E, Rannou O, Ricoult C, Boutet-Mercey S, Maia-Grondard A, Limami AM (2011) Nitrogen metabolism responses to water deficit act through both abscisic acid (ABA)-dependent and independent pathways in Medicago truncatula during post-germination. J Exp Bot **62**: 605–615

Plaxton WC, Podestá FE (2006) The functional organization and control of plant respiration. CRC Crit Rev Plant Sci **25**: 159–198

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta - Bioenerg **975**: 384–394

Scheller HV, Jensen PE, Haldrup A, Lunde C, Knoetzel J (2001) Role of subunits in eukaryotic Photosystem I. Biochim Biophys Acta - Bioenerg **1507**: 41–60

Schnarrenberger C, Martin W (2002) Evolution of the enzymes of the citric acid cycle and the glyoxylate cycle of higher plants: A case study of endosymbiotic gene transfer. Eur J Biochem **269**: 868–883

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods **9**: 671–675

Schöttler MA, Tóth SZ, Boulouis A, Kahlau S (2015) Photosynthetic complex stoichiometry dynamics in higher plants: Biogenesis, function, and turnover of ATP synthase and the cytochrome b6f complex. J Exp Bot **66**: 2373–2400

Shi Q, Sun H, Timm S, Zhang S, Huang W (2022) Photorespiration Alleviates Photoinhibition of Photosystem I under Fluctuating Light in Tomato. Plants. doi: 10.3390/plants11020195

Sienkiewicz-Porzucek A, Nunes-Nesi A, Sulpice R, Lisec J, Centeno DC, Carillo P, Leisse A, Urbanczyk-Wochniak E, Fernie AR (2008) Mild reductions in mitochondrial citrate synthase activity result in a compromised nitrate assimilation and reduced leaf pigmentation but have no effect on photosynthetic performance or growth. Plant Physiol **147**: 115–127

Studart-Guimarães C, Fait A, Nunes-Nesi A, Carrari F, Usadel B, Fernie AR (2007) Reduced expression of succinyl-coenzyme A ligase can be compensated for by up-regulation of the γ-aminobutyrate shunt in illuminated tomato leaves. Plant Physiol **145**: 626–639

Sweetlove LJ, Beard KFM, Nunes-Nesi A, Fernie AR, R. George Ratcliffe (2010) Not just a circle: Flux modes in the plant TCA cycle. Trends Plant Sci **15**: 462–470

Sweetlove LJ, Fernie AR (2013) The Spatial Organization of Metabolism Within the Plant Cell. Annu Rev Plant Biol **64**: 723–746

Sweetlove LJ, Lytovchenko A, Morgan M, Nunes-nesi A, Taylor NL, Baxter CJ,

Eickmeier I, Fernie AR (2006) Mitochondrial uncoupling protein is required for efficient photosynthesis. **103**: 3–8

Tomaz T, Bagard M, Pracharoenwattana I, Lindén P, Lee CP, Carroll AJ, Ströher E, Smith SM, Gardeström P, Millar AH (2010) Mitochondrial malate dehydrogenase lowers leaf respiration and alters photorespiration and plant growth in Arabidopsis. Plant Physiol **154**: 1143–1157

Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, Mann M, Cox J (2016) The Perseus computational platform for comprehensive analysis of (prote)omics data. Nat Methods **13**: 731–740

Varotto C, Pesaresi P, Meurer J, Oelmüller R, Steiner-Lange S, Salamini F, Leister D (2000) Disruption of the Arabidopsis photosystem I gene psaE1 affects photosynthesis and impairs growth. Plant J **22**: 115–124

Voon CP, Law Y-S, Guan X, Lim S-L, Xu Z, Chu W-T, Zhang R, Sun F, Labs M, Leister D, et al (2021) Modulating the activities of chloroplasts and mitochondria promotes ATP production and plant growth. bioRxiv 2020.09.09.289967

Wittmiß M, Mikkat S, Hagemann M, Bauwe H (2020) Stoichiometry of two plant glycine decarboxylase complexes and comparison with a cyanobacterial glycine cleavage system. 801–813

Yi X, Hargett SR, Liu H, Frankel LK, Bricker TM (2007) The PsbP Protein Is Required for Photosystem II Complex Assembly / Stability and Photoautotrophy in Arabidopsis thaliana *. J Biol Chem **282**: 24833–24841

Yue J, Du C, Ji J, Xie T, Chen W, Chang E, Chen L, Jiang Z, Shi S (2018) Inhibition of α-ketoglutarate dehydrogenase activity affects adventitious root growth in poplar via changes in GABA shunt. Planta **248**: 963–979

Zancani M, Braidot E, Filippi A, Lippe G (2020) Structural and functional properties of plant mitochondrial F-ATP synthase. Mitochondrion **53**: 178–193

Zhang Y, Beard KFM, Swart C, Bergmann S, Krahnert I, Nikoloski Z, Graf A, George Ratcliffe R, Sweetlove LJ, Fernie AR, et al (2017) Protein-protein interactions and metabolite channelling in the plant tricarboxylic acid cycle. Nat Commun. doi: 10.1038/ncomms15212

Zhang Y, Swart C, Alseekh S, Scossa F, Jiang L, Obata T, Graf A, Fernie AR (2018) The extra-pathway interactome of the TCA cycle: Expected and unexpected metabolic interactions. Plant Physiol **177**: 966–979

SUPPLEMENTAL MATERIAL

Supplemental table 1: Complete set of proteome results from double mutant plants lacking both genes for the E1 subunit of 2-OGDH enzyme and Wild type plants. (+) indicates that alterations were significant when compared with the values of the Wild Type as attested by the students *t*-Test p≤0,05. Values are log2 of the relative difference between the double mutant plants and the WT measurements, when negative indicates downregulation and when positive indicates upregulation.

Gene ID			**Gene ID**			**Gene ID**			**Gene ID**			**Gene ID**		
AT3G55410	+	-4,381	AT5G40450	+	-0,391	AT2G30930	+	-0,271	AT2G20230	+	-0,236	AT1G47128	+	-0,209
AT4G20760	+	-3,686	AT4G15530	+	-0,381	AT4G12880	+	-0,271	AT1G72160	+	-0,235	AT3G14990	+	-0,207
ATCG00150	+	-3,415	AT2G25080	+	-0,379	AT3G56290	+	-0,269	AT1G77090	+	-0,233	AT3G15840	+	-0,207
AT5G16400	+	-1,811	AT3G14067	+	-0,377	AT1G74090	+	-0,263	AT2G45960	+	-0,232	AT1G57770	+	-0,205
AT4G39120	+	-1,613	AT1G69840	+	-0,370	AT3G17410	+	-0,263	AT5G39830	+	-0,231	AT4G38680	+	-0,205
AT3G11820	+	-1,364	AT1G21680	+	-0,368	AT1G10360	+	-0,263	AT3G10060	+	-0,230	AT1G06410	+	-0,204
AT2G28190	+	-1,321	AT3G01670	+	-0,362	AT5G57350	+	-0,263	AT2G44920	+	-0,226	AT3G04790	+	-0,201
AT4G15940	+	-1,219	AT3G10740	+	-0,349	AT3G59870	+	-0,262	AT4G19170	+	-0,226	ATCG01090	+	-0,201
AT3G13060	+	-1,001	AT1G72150	+	-0,344	AT2G39010	+	-0,255	AT5G43750	+	-0,225	AT1G52220	+	-0,201
AT5G49360	+	-0,925	AT3G44880	+	-0,338	AT3G52840	+	-0,253	AT5G53490	+	-0,224	AT4G32260	+	-0,200
AT3G13560	+	-0,715	AT5G48790	+	-0,331	AT2G26570	+	-0,252	AT4G17090	+	-0,222	AT3G01480	+	-0,196
AT5G43780	+	-0,669	AT4G38810	+	-0,331	AT2G36390	+	-0,251	AT5G12470	+	-0,222	AT3G16850	+	-0,196
AT2G45180	+	-0,628	AT1G72610	+	-0,327	AT3G48420	+	-0,249	AT4G14070	+	-0,221	AT3G48140	+	-0,196
AT5G14780	+	-0,625	AT1G54100	+	-0,323	AT4G20260	+	-0,248	AT3G07720	+	-0,219	AT4G12800	+	-0,193
AT5G62720	+	-0,623	AT4G39640	+	-0,321	AT1G31190	+	-0,247	AT4G05090	+	-0,219	AT2G32240	+	-0,192
AT4G21650	+	-0,589	AT3G53420	+	-0,300	AT1G32080	+	-0,247	AT1G51110	+	-0,218	AT4G30920	+	-0,191
AT3G13750	+	-0,536	AT2G05310	+	-0,296	AT1G55160	+	-0,246	AT4G26690	+	-0,217	AT1G01790	+	-0,190
AT1G20340	+	-0,522	AT5G58260	+	-0,293	AT4G35230	+	-0,246	AT5G63620	+	-0,216	AT3G52200	+	-0,190
AT5G52540	+	-0,507	AT3G23400	+	-0,291	AT1G08550	+	-0,244	AT1G54220	+	-0,215	AT2G21620	+	-0,189
AT1G12780	+	-0,500	AT5G46860	+	-0,289	AT5G06580	+	-0,243	AT2G20260	+	-0,214	AT1G65260	+	-0,189
AT3G01680	+	-0,452	AT3G15980	+	-0,286	AT1G66200	+	-0,243	AT5G35170	+	-0,213	AT1G32060	+	-0,188
AT2G17840	+	-0,440	AT1G72640	+	-0,282	AT5G20935	+	-0,241	AT5G20830	+	-0,211	AT3G18820	+	-0,188
AT4G12720	+	-0,439	AT5G53460	+	-0,281	AT5G35100	+	-0,241	AT1G18170	+	-0,211	AT1G04530	+	-0,187
AT5G16990	+	-0,421	AT5G19860	+	-0,275	AT4G25450	+	-0,240	AT3G55330	+	-0,210	AT4G37800	+	-0,185

AT4G23400	+	-0,418	AT5G60360	+	-0,273	AT1G53000	+	-0,239	AT5G11720	+	-0,210	AT1G14150	+	-0,183
AT2G33530	+	-0,183	AT4G27700	+	-0,161	AT4G21580	+	-0,145	AT1G61520	+	-0,132	AT5G64290	+	-0,116
AT3G17020	+	-0,183	AT2G23600	+	-0,160	AT3G48720	+	-0,145	AT2G30170	+	-0,132	AT5G08380	+	-0,114
AT4G02770	+	-0,182	AT4G17170	+	-0,159	AT2G26930	+	-0,145	AT5G17520	+	-0,132	AT3G54110	+	-0,114
AT5G45170	+	-0,181	AT4G31530	+	-0,159	AT5G04360	+	-0,144	AT1G48420	+	-0,132	AT4G18810	+	-0,113
AT3G05350	+	-0,181	ATCG00670	+	-0,158	AT5G36700	+	-0,144	AT4G09650	+	-0,132	AT3G08940	+	-0,113
AT5G58070	+	-0,180	ATCG00580	+	-0,158	AT3G06510	+	-0,143	AT3G27820	+	-0,131	AT4G17300	+	-0,111
AT1G66970	+	-0,179	AT4G37930	+	-0,157	AT3G26070	+	-0,143	AT4G37925	+	-0,130	AT1G52510	+	-0,111
AT3G23570	+	-0,179	AT5G63310	+	-0,157	AT3G18890	+	-0,143	AT4G37000	+	-0,129	AT1G14000	+	-0,111
AT4G31390	+	-0,178	AT5G04140	+	-0,156	AT1G12410	+	-0,143	AT2G20890	+	-0,129	AT1G09340	+	-0,110
AT4G26970	+	-0,177	12950;AT5G1	+	-0,156	ATCG00130	+	-0,142	AT3G55260	+	-0,128	AT1G56190	+	-0,109
AT4G22890	+	-0,176	AT4G29590	+	-0,155	AT5G17380	+	-0,141	AT3G14420	+	-0,128	AT1G49240	+	-0,108
AT1G30360	+	-0,176	AT3G54660	+	-0,155	AT2G42600	+	-0,141	AT1G11860	+	-0,128	AT1G16460	+	-0,108
AT1G55480	+	-0,176	AT5G51970	+	-0,155	ATCG00470	+	-0,141	AT1G15980	+	-0,127	AT4G11150	+	-0,108
AT3G63140	+	-0,175	AT5G10860	+	-0,154	AT4G04640	+	-0,141	AT5G34850	+	-0,127	AT1G48600	+	-0,107
AT1G31330	+	-0,174	AT2G41560	+	-0,153	AT1G42970	+	-0,140	AT4G21280	+	-0,126	AT3G12780	+	-0,107
AT5G23120	+	-0,174	AT1G79870	+	-0,152	AT2G44350	+	-0,139	AT4G12060	+	-0,126	AT2G04039	+	-0,107
AT2G47390	+	-0,173	AT1G67280	+	-0,152	ATCG00430	+	-0,139	AT3G58730	+	-0,125	AT3G63520	+	-0,107
AT3G61220	+	-0,171	AT4G33010	+	-0,152	AT3G20320	+	-0,139	AT5G09660	+	-0,125	AT1G63940	+	-0,106
AT4G15545	+	-0,171	AT1G48030	+	-0,152	ATCG00350	+	-0,138	AT5G13030	+	-0,124	AT2G24200	+	-0,106
AT5G42765	+	-0,171	AT1G19910	+	-0,151	AT2G24820	+	-0,138	AT4G01050	+	-0,123	AT2G41790	+	-0,105
AT1G70820	+	-0,169	AT5G16710	+	-0,151	AT3G27925	+	-0,137	AT1G63770	+	-0,123	AT4G35250	+	-0,105
AT5G20140	+	-0,169	AT2G47730	+	-0,150	AT5G66190	+	-0,137	AT5G49970	+	-0,122	AT1G03680	+	-0,104
AT4G16370	+	-0,168	AT1G03600	+	-0,149	AT3G54640	+	-0,137	AT2G21330	+	-0,122	AT1G80380	+	-0,104
AT5G47840	+	-0,166	AT5G12860	+	-0,148	AT3G54890	+	-0,136	ATCG01100	+	-0,122	AT1G42550	+	-0,103
AT1G16720	+	-0,166	AT3G62110	+	-0,148	AT2G26080	+	-0,136	AT1G56500	+	-0,120	AT4G24620	+	-0,102
AT3G17810	+	-0,165	AT4G05180	+	-0,146	AT3G13930	+	-0,135	AT5G21430	+	-0,120	AT2G35490	+	-0,102
AT3G19480	+	-0,162	AT3G63540	+	-0,146	AT2G26340	+	-0,135	AT1G54780	+	-0,118	AT1G12900	+	-0,102
AT3G50820	+	-0,161	AT1G44575	+	-0,146	AT5G04740	+	-0,134	AT5G19220	+	-0,118	AT3G15090	+	-0,102

ATCG01110	+	-0,161	ATCG00340	+	-0,146	AT5G53850	+	-0,132	AT2G35370	+	-0,116	AT3G22200	+	-0,102
AT1G65590	+	-0,101	AT3G55800	+	-0,072	AT4G14030	+	0,099	AT3G29360	+	0,122	AT3G49910	+	0,146
AT1G59900	+	-0,101	AT2G39730	+	-0,072	AT4G34110	+	0,101	AT5G11950	+	0,123	AT2G30050	+	0,147
AT3G61260	+	-0,100	AT3G46780	+	-0,072	AT3G11710	+	0,102	AT3G06650	+	0,125	AT3G09630	+	0,147
AT4G03280	+	-0,100	AT2G19940	+	-0,070	ATMG00070	+	0,102	AT4G18480	+	0,126	AT1G71220	+	0,147
AT3G12290	+	-0,099	ATCG00120	+	-0,069	AT1G13060	+	0,102	AT3G11830	+	0,127	AT3G09200	+	0,148
AT1G01800	+	-0,099	ATCG00480	+	-0,069	AT2G44160	+	0,103	AT5G15200	+	0,127	AT1G03860	+	0,149
AT1G16880	+	-0,098	AT5G50850	+	-0,067	AT2G17360	+	0,106	AT3G48690	+	0,127	AT5G08530	+	0,150
AT3G28710	+	-0,097	AT1G20260	+	-0,066	AT1G52360	+	0,107	AT5G02500	+	0,128	AT2G18020	+	0,150
AT5G35630	+	-0,096	AT5G58330	+	-0,060	AT5G11170	+	0,108	AT3G62870	+	0,128	AT2G27710	+	0,151
AT3G11630	+	-0,096	ATCG00540	+	-0,056	AT2G24270	+	0,108	AT2G19520	+	0,129	AT3G49010	+	0,153
AT2G13360	+	-0,096	AT5G13980	+	-0,054	AT3G04830	+	0,110	AT2G20360	+	0,130	AT3G10690	+	0,153
AT1G50250	+	-0,096	AT3G59970	+	0,052	AT3G48930	+	0,111	AT4G17600	+	0,130	AT5G09510	+	0,154
AT3G54050	+	-0,095	AT1G29900	+	0,055	AT3G52930	+	0,111	AT2G19730	+	0,131	AT2G34480	+	0,154
AT4G38970	+	-0,095	AT3G23990	+	0,068	AT3G58610	+	0,111	AT2G44640	+	0,131	AT5G35160	+	0,154
AT4G03520	+	-0,094	AT3G49560	+	0,073	AT1G30580	+	0,113	AT4G02230	+	0,133	AT4G35790	+	0,156
AT5G66570	+	-0,094	AT5G11880	+	0,076	AT1G30630	+	0,114	AT2G39020	+	0,133	AT5G28540	+	0,156
AT3G16910	+	-0,092	AT4G10320	+	0,076	AT4G39280	+	0,115	AT1G64550	+	0,135	AT2G04390	+	0,156
AT3G19170	+	-0,091	AT1G47260	+	0,082	AT1G08200	+	0,115	AT5G66510	+	0,135	AT4G02930	+	0,158
AT3G42050	+	-0,090	AT2G27020	+	0,083	AT1G45000	+	0,115	AT3G56130	+	0,136	AT3G57290	+	0,159
AT3G08920	+	-0,089	AT3G02090	+	0,086	AT3G29185	+	0,117	AT3G18190	+	0,137	AT4G14800	+	0,159
AT1G70410	+	-0,087	AT1G57720	+	0,087	AT3G54470	+	0,117	AT1G56070	+	0,137	AT4G02510	+	0,159
AT5G65620	+	-0,085	AT2G34040	+	0,088	AT2G29550	+	0,117	AT1G80410	+	0,138	AT1G20950	+	0,159
AT4G12420	+	-0,084	AT2G31670	+	0,090	AT5G35530	+	0,117	AT2G42740	+	0,138	AT5G49460	+	0,159
AT1G17100	+	-0,082	AT5G37510	+	0,091	AT1G75280	+	0,118	AT3G16640	+	0,140	AT5G24690	+	0,160
AT5G19760	+	-0,080	AT2G35040	+	0,092	AT1G04170	+	0,118	AT5G20890	+	0,140	AT2G37190	+	0,160
AT4G35090	+	-0,080	72730;AT3G1	+	0,094	AT3G63170	+	0,119	AT3G20050	+	0,140	AT2G28000	+	0,161
AT2G21530	+	-0,079	AT3G52560	+	0,094	AT1G74050	+	0,119	AT3G20790	+	0,140	AT5G20290	+	0,162
AT1G78900	+	-0,078	AT5G30510	+	0,097	AT5G04430	+	0,120	AT5G45950	+	0,141	AT5G52650	+	0,162

AT3G14415	+	-0,076	AT5G42390	+	0,099	AT3G09840	+	0,122	AT1G49760	+	0,143	AT3G55360	+	0,162
AT5G66680	+	0,163	AT1G50430	+	0,178	AT2G18040	+	0,193	AT2G45710	+	0,217	AT2G39960	+	0,244
AT1G72370	+	0,164	AT5G10840	+	0,178	AT3G17840	+	0,193	AT4G39330	+	0,217	AT5G45930	+	0,246
AT1G07320	+	0,164	AT3G01120	+	0,178	AT2G39990	+	0,194	AT5G19510	+	0,217	AT3G07110	+	0,249
AT3G58460	+	0,166	AT1G33120	+	0,180	AT1G10670	+	0,194	AT1G09200	+	0,218	AT3G23300	+	0,249
AT5G02870	+	0,166	AT1G17880	+	0,180	AT4G16720	+	0,195	AT3G56150	+	0,219	AT2G30490	+	0,250
AT4G39680	+	0,167	AT1G09100	+	0,180	AT2G39460	+	0,196	AT5G13430	+	0,220	AT2G31610	+	0,250
AT3G17390	+	0,167	AT1G01090	+	0,181	AT3G62530	+	0,196	ATMG00640	+	0,222	AT3G07100	+	0,250
AT2G37270	+	0,167	AT3G11510	+	0,184	AT1G26630	+	0,196	AT3G04920	+	0,224	AT5G61170	+	0,252
AT1G04820	+	0,168	AT3G24830	+	0,184	AT1G16350	+	0,198	AT1G29470	+	0,225	AT4G26110	+	0,252
AT4G02450	+	0,168	AT4G31120	+	0,184	AT5G60790	+	0,199	AT5G02740	+	0,226	AT2G44040	+	0,254
AT5G02450	+	0,168	AT1G55490	+	0,184	AT3G44330	+	0,201	AT3G23940	+	0,226	AT4G02610	+	0,256
AT4G27500	+	0,168	AT1G11650	+	0,184	AT1G11840	+	0,202	AT3G13470	+	0,226	AT4G02580	+	0,257
AT1G07370	+	0,168	AT2G04030	+	0,185	AT3G54400	+	0,204	AT5G02530	+	0,229	AT3G05560	+	0,257
AT5G46430	+	0,168	AT5G07350	+	0,186	AT2G14880	+	0,206	AT1G43190	+	0,230	AT1G20620	+	0,259
AT3G04840	+	0,169	AT3G56460	+	0,186	AT2G24940	+	0,206	AT3G49720	+	0,230	AT5G14030	+	0,261
AT5G47210	+	0,169	AT4G24510	+	0,187	AT2G32720	+	0,207	AT3G46740	+	0,230	AT1G56340	+	0,263
AT3G25520	+	0,170	AT2G23350	+	0,187	ATMG00510	+	0,207	AT1G70770	+	0,232	AT4G15560	+	0,264
AT2G31490	+	0,171	AT4G36250	+	0,188	ATMG00580	+	0,210	AT1G66580	+	0,233	AT3G16370	+	0,264
AT1G62390	+	0,172	AT1G66240	+	0,188	AT5G55660	+	0,211	AT1G30230	+	0,233	AT1G10840	+	0,264
AT3G02530	+	0,172	AT4G34670	+	0,188	AT5G14800	+	0,211	AT1G01320	+	0,234	AT1G24510	+	0,265
AT5G52840	+	0,173	AT3G03960	+	0,189	AT2G21580	+	0,212	AT2G01720	+	0,234	AT3G56070	+	0,267
AT5G56680	+	0,173	AT2G29560	+	0,189	AT1G67680	+	0,212	AT2G22125	+	0,235	AT3G48680	+	0,268
AT1G64790	+	0,175	AT4G37910	+	0,189	AT1G03330	+	0,212	AT5G11560	+	0,235	AT3G06730	+	0,269
AT1G04480	+	0,175	AT5G16510	+	0,190	AT3G22960	+	0,214	AT3G04550	+	0,236	AT1G14320	+	0,270
AT3G09500	+	0,176	AT1G51690	+	0,190	AT3G48170	+	0,214	AT4G36130	+	0,238	AT3G48560	+	0,271
AT1G43170	+	0,176	AT4G18100	+	0,190	AT5G13630	+	0,216	AT2G09990	+	0,242	AT3G14600	+	0,272
AT4G28440	+	0,176	AT2G01470	+	0,190	AT2G21660	+	0,216	AT1G20330	+	0,243	AT3G18740	+	0,273
AT4G34870	+	0,177	AT5G27850	+	0,191	AT2G41840	+	0,216	AT1G74560	+	0,243	AT3G54090	+	0,277

AT1G18080	+	0,177	AT5G20920	+	0,191	AT4G39200	+	0,217	AT1G36160	+	0,244	AT3G27570		-0,821
AT1G63660	+	0,282	AT4G00400	+	0,337	AT5G48880	+	0,457	AT5G55940	+	1,879	AT3G53610		-0,816
AT5G52920	+	0,283	AT1G54410	+	0,343	AT1G30530	+	0,469	AT3G17820	+	2,461	AT4G23180		-0,780
AT5G55730	+	0,287	AT3G05590	+	0,344	AT5G65020	+	0,475	AT2G35120	+	2,733	AT3G57260		-0,769
AT1G53840	+	0,291	AT5G03740	+	0,348	AT1G48920	+	0,481	AT4G31340	+	0,330	AT3G23390		-0,756
AT2G21160	+	0,291	AT5G51110	+	0,355	AT5G01600	+	0,483	AT4G31340	+	0,330	AT1G53670		-0,749
AT1G56110	+	0,293	AT1G22780	+	0,361	AT2G26250	+	0,502	ATCG00170	+	0,280	AT1G31160		-0,745
AT1G29930	+	0,294	AT1G04430	+	0,367	AT3G53460	+	0,510	AT5G05270	+	1,820	AT5G08060		-0,719
AT3G52580	+	0,296	AT3G23820	+	0,368	AT5G15490	+	0,513	AT2G32060		-3,074	AT4G01883		-0,706
AT1G01100	+	0,296	AT5G26000	+	0,368	AT1G06000	+	0,560	AT5G02160		-2,048	AT3G43520		-0,703
AT1G02150	+	0,297	AT4G21960	+	0,370	AT5G16290	+	0,590	AT1G20810		-1,944	AT3G48530		-0,701
AT1G09760	+	0,298	AT1G74850	+	0,374	AT4G39040	+	0,597	AT4G37200		-1,869	AT1G09560		-0,672
AT4G28080	+	0,299	ATCG00740	+	0,380	ATCG00190	+	0,607	AT5G25610		-1,579	AT1G13280		-0,667
AT2G40300	+	0,299	AT1G14410	+	0,381	AT4G25100	+	0,651	AT1G14880		-1,397	AT1G71040		-0,653
AT1G58380	+	0,302	AT5G41970	+	0,385	AT3G04260	+	0,684	ATCG00065		-1,396	AT3G54600		-0,650
AT4G19210	+	0,306	AT3G03980	+	0,387	AT3G48870	+	0,697	AT4G38690		-1,294	AT3G57020		-0,647
AT4G10480	+	0,307	AT5G05170	+	0,389	AT4G36910	+	0,765	AT3G58990		-1,226	AT5G11450		-0,640
AT5G41520	+	0,307	AT3G05060	+	0,390	AT5G08640	+	0,799	AT1G54290		-1,202	AT5G47760		-0,615
AT5G49720	+	0,308	AT5G46580	+	0,394	AT5G16070	+	0,906	AT1G55260		-1,130	AT2G30260		-0,596
AT5G26780	+	0,308	AT3G56090	+	0,402	AT5G47010	+	1,034	ATCG01050		-1,095	AT5G52310		-0,584
AT1G06550	+	0,310	AT1G74270	+	0,403	AT3G16400	+	1,050	AT5G58770		-1,036	AT2G32450		-0,582
AT2G27720	+	0,314	AT1G22410	+	0,404	AT1G12010	+	1,094	AT1G32050		-1,033	AT4G05420		-0,580
AT3G26900	+	0,318	AT5G25980	+	0,411	AT1G75680	+	1,164	AT5G49760		-1,017	AT1G52780		-0,580
AT4G10450	+	0,318	AT4G16390	+	0,415	AT4G36220	+	1,286	AT1G76850		-0,992	AT4G01610		-0,575
AT1G78570	+	0,319	AT3G55120	+	0,418	AT5G51100	+	1,371	AT2G39930		-0,948	AT5G18100		-0,573
AT1G80230	+	0,321	AT4G13840	+	0,435	AT4G27080	+	1,384	AT1G09830		-0,944	AT1G49820		-0,568
AT3G19820	+	0,321	AT3G57150	+	0,435	AT1G06760	+	1,394	AT3G54900		-0,899	AT2G33380		-0,563
AT3G02560	+	0,325	AT3G59010	+	0,441	AT3G13460	+	1,695	AT1G21100		-0,885	AT5G04900		-0,560
AT4G13850	+	0,326	AT4G25630	+	0,448	AT3G53710	+	1,712	AT3G10850		-0,835	AT1G20580		-0,558

AT2G05100	-0,552	AT1G47830	-0,419	AT5G52560	-0,300	AT2G40600	-0,234	AT5G48480	-0,203
AT4G14100	-0,546	AT2G30520	-0,416	AT2G36580	-0,299	AT5G51570	-0,231	AT5G52970	-0,202
AT3G63130	-0,539	AT1G03400	-0,401	AT1G02920	-0,298	AT1G18590	-0,231	AT1G32470	-0,201
AT1G75040	-0,537	AT1G21250	-0,394	AT5G03350	-0,291	ATCG00560	-0,230	AT2G23610	-0,200
AT2G43790	-0,535	AT5G21100	-0,384	AT3G01660	-0,287	AT5G64380	-0,226	AT3G27020	-0,199
AT3G51460	-0,528	AT1G73430	-0,382	AT5G57040	-0,284	AT5G63800	-0,226	AT4G29160	-0,199
AT1G07700	-0,523	AT1G12250	-0,378	AT1G30380	-0,279	AT3G02830	-0,224	AT5G13490	-0,198
AT3G01290	-0,518	AT2G03750	-0,373	AT1G78915	-0,278	AT5G14250	-0,224	AT1G54500	-0,196
AT4G19100	-0,509	AT1G78150	-0,363	AT1G11580	-0,277	AT1G71950	-0,223	AT2G04690	-0,195
AT1G35420	-0,504	AT4G21105	-0,355	AT1G19580	-0,272	AT5G22580	-0,222	AT1G22710	-0,194
AT5G39730	-0,494	AT3G02110	-0,353	AT2G29690	-0,270	AT1G22530	-0,221	AT4G20830	-0,194
AT4G14680	-0,477	AT5G57890	-0,344	AT4G32150	-0,266	AT3G26520	-0,220	AT3G48990	-0,192
AT4G36530	-0,476	AT1G61800	-0,343	AT5G11420	-0,266	AT5G43830	-0,217	AT5G57700	-0,188
AT1G01470	-0,471	AT3G01520	-0,343	AT1G20630	-0,266	AT4G12830	-0,217	AT5G54960	-0,188
AT2G35450	-0,464	AT2G32480	-0,340	AT1G55805	-0,264	AT4G30580	-0,214	AT3G29350	-0,188
AT3G21790	-0,459	AT4G34180	-0,336	AT3G13720	-0,262	AT1G21500	-0,214	AT1G08160	-0,188
AT1G78620	-0,458	AT1G26460	-0,336	AT2G43180	-0,260	AT5G19140	-0,214	AT2G02560	-0,186
AT1G75220	-0,456	AT4G33670	-0,330	AT1G53430	-0,258	AT4G03200	-0,213	AT4G18760	-0,186
AT3G17930	-0,453	AT1G45145	-0,330	AT2G40800	-0,258	AT5G22830	-0,213	AT1G53280	-0,186
AT4G12290	-0,451	AT1G03350	-0,326	AT2G36835	-0,257	AT1G78830	-0,213	AT3G15640	-0,185
AT5G65840	-0,448	AT3G55760	-0,326	AT4G34980	-0,257	AT5G66530	-0,213	AT1G13560	-0,184
AT3G07160	-0,448	AT5G37600	-0,326	AT3G61430	-0,257	AT5G18170	-0,208	AT3G04610	-0,183
AT2G20270	-0,436	AT2G41040	-0,325	AT5G67560	-0,256	AT4G23890	-0,208	AT1G68830	-0,183
AT4G26410	-0,435	AT1G08450	-0,314	AT5G27560	-0,256	AT1G76180	-0,207	AT1G63680	-0,182
AT5G65760	-0,434	AT2G41950	-0,308	AT5G65010	-0,255	AT2G02930	-0,207	AT5G17230	-0,182
AT5G04710	-0,434	AT3G62600	-0,306	AT4G37640	-0,244	AT4G38460	-0,207	AT5G11150	-0,181
AT4G02840	-0,431	AT4G28740	-0,306	AT2G39470	-0,243	AT1G04620	-0,205	AT2G01140	-0,179
AT1G20440	-0,430	AT1G78820	-0,305	AT5G63420	-0,242	AT1G49750	-0,204	AT1G18060	-0,178
AT5G65940	-0,424	AT2G25450	-0,300	AT4G32330	-0,235	AT1G74640	-0,203	AT5G38430	-0,178

AT2G45820	-0,178	AT1G80030	-0,158	AT3G19010	-0,143	AT5G59420	-0,132	AT3G53970	-0,117
AT1G22940	-0,174	AT2G01350	-0,158	AT5G24300	-0,143	AT1G09020	-0,131	AT4G34240	-0,117
AT2G16430	-0,173	AT3G04880	-0,157	AT3G54360	-0,142	AT2G44490	-0,131	AT1G64740	-0,117
AT2G27290	-0,172	AT3G58010	-0,157	AT3G09810	-0,142	AT4G34120	-0,131	AT4G28660	-0,117
AT1G06650	-0,172	AT2G02500	-0,157	AT3G09580	-0,141	AT5G57110	-0,131	AT4G26530	-0,116
AT3G01390	-0,172	AT4G32770	-0,157	AT3G56310	-0,141	AT5G50210	-0,130	AT3G18420	-0,116
AT4G29350	-0,171	AT5G01650	-0,156	AT4G16130	-0,140	AT2G17390	-0,130	AT5G06320	-0,116
AT4G39150	-0,171	AT1G67700	-0,156	AT1G80460	-0,139	AT3G26570	-0,130	AT3G02880	-0,116
AT3G11945	-0,169	AT1G11870	-0,156	AT3G42170	-0,139	AT5G37360	-0,129	AT1G74960	-0,115
AT4G18370	-0,169	AT5G40890	-0,155	AT1G18450	-0,138	AT2G37170	-0,129	AT3G24170	-0,115
AT3G62820	-0,168	AT5G41790	-0,154	AT1G53580	-0,138	AT4G20410	-0,129	AT5G66040	-0,114
AT5G35970	-0,168	AT1G74030	-0,154	AT5G41670	-0,138	AT2G47400	-0,129	AT1G17290	-0,114
AT5G43600	-0,166	AT1G51805	-0,152	AT1G77590	-0,138	AT5G38520	-0,128	AT4G27520	-0,114
AT2G36145	-0,165	AT5G46290	-0,152	AT1G21350	-0,138	AT1G29700	-0,128	AT5G21105	-0,114
AT5G04830	-0,165	AT4G17830	-0,152	AT5G64040	-0,137	AT2G33830	-0,127	AT2G45060	-0,113
AT2G22480	-0,164	AT4G04020	-0,151	AT1G20450	-0,137	AT5G38410	-0,126	AT3G52500	-0,113
AT3G61540	-0,164	AT5G03880	-0,150	AT4G15510	-0,137	AT2G43945	-0,124	AT5G49030	-0,112
AT3G44380	-0,164	AT4G39710	-0,149	AT1G18640	-0,136	AT4G35260	-0,124	AT5G32470	-0,112
AT5G18570	-0,164	AT5G01010	-0,148	AT4G16830	-0,136	AT3G47930	-0,124	AT1G65960	-0,112
AT5G47860	-0,163	AT2G30060	-0,146	AT5G24165	-0,136	AT1G20225	-0,124	AT5G13120	-0,111
AT5G01920	-0,163	AT4G22240	-0,146	AT4G29670	-0,135	AT4G27270	-0,124	AT1G79440	-0,111
AT3G53130	-0,163	AT3G44860	-0,146	AT1G63500	-0,134	AT3G14290	-0,120	AT3G23600	-0,111
AT2G22450	-0,162	AT1G70580	-0,146	AT1G23740	-0,134	AT5G44070	-0,120	AT4G20890	-0,111
AT2G39850	-0,161	AT4G11600	-0,145	AT1G51360	-0,134	AT3G23490	-0,120	AT5G55070	-0,110
AT2G17695	-0,160	AT4G34150	-0,145	AT5G65430	-0,134	AT5G06690	-0,119	AT4G13670	-0,110
AT4G26630	-0,160	AT4G33640	-0,144	AT5G03660	-0,133	AT1G77490	-0,119	AT2G41220	-0,110
AT2G21385	-0,159	AT3G53990	-0,144	AT4G09620	-0,132	AT3G28200	-0,119	ATCG00420	-0,110
AT1G74880	-0,159	AT3G25690	-0,144	AT5G49555	-0,132	AT2G46820	-0,118	AT5G03650	-0,109
AT1G45474	-0,159	AT3G52950	-0,144	AT5G45280	-0,132	AT1G21440	-0,118	AT3G15110	-0,109

AT1G60070	-0,109	AT1G65930	-0,102	AT5G09700	-0,095	AT3G02730	-0,089	AT5G54310	-0,083
AT5G53580	-0,109	AT5G01530	-0,101	AT2G36460	-0,095	AT4G11260	-0,089	AT4G34490	-0,082
AT4G23600	-0,108	AT3G05900	-0,101	AT1G04420	-0,095	AT2G33255	-0,088	AT4G24930	-0,082
AT2G31790	-0,108	ATCG00020	-0,100	AT2G44050	-0,095	AT5G48230	-0,088	AT5G19550	-0,082
AT4G13360	-0,108	AT2G03440	-0,100	AT2G41680	-0,094	AT4G02530	-0,088	AT3G24430	-0,082
AT2G37540	-0,108	AT4G24220	-0,100	AT1G07140	-0,094	AT1G66130	-0,088	AT5G06530	-0,082
AT4G29130	-0,108	AT4G23940	-0,100	AT2G34470	-0,094	AT4G14570	-0,087	AT1G06690	-0,082
AT3G10920	-0,107	AT1G10760	-0,100	AT4G02620	-0,094	AT3G27890	-0,087	AT3G46060	-0,081
AT1G15340	-0,107	AT3G02720	-0,100	AT2G43560	-0,094	AT5G65720	-0,087	AT1G76450	-0,081
AT2G47910	-0,107	AT2G41530	-0,100	AT2G45470	-0,093	AT1G13320	-0,087	AT2G07707	-0,081
AT2G20760	-0,106	AT1G79690	-0,099	AT5G64030	-0,093	AT5G58270	-0,087	AT4G04610	-0,081
AT5G23740	-0,106	AT2G21280	-0,099	AT4G15550	-0,093	AT5G46800	-0,086	AT1G23900	-0,080
AT1G62640	-0,106	AT4G00430	-0,099	AT4G16143	-0,092	ATCG00720	-0,086	AT5G58140	-0,080
AT2G25070	-0,105	AT4G34920	-0,099	AT5G03290	-0,092	AT1G12080	-0,086	AT5G01750	-0,080
AT4G25650	-0,105	AT3G52750	-0,098	AT2G47710	-0,092	AT2G34310	-0,085	AT5G27380	-0,080
AT5G16010	-0,105	AT4G26910	-0,098	AT3G56650	-0,092	AT3G57560	-0,085	AT1G79500	-0,079
AT3G02540	-0,105	AT1G07110	-0,098	AT4G13340	-0,092	ATCG00270	-0,085	AT1G55670	-0,079
AT1G20020	-0,105	AT3G15520	-0,098	AT4G25370	-0,092	AT3G06720	-0,085	AT5G67030	-0,079
AT3G20810	-0,104	AT2G20630	-0,097	AT1G53240	-0,092	AT1G76140	-0,085	AT4G39080	-0,079
AT1G73990	-0,104	AT1G73030	-0,097	AT2G15620	-0,092	AT1G08110	-0,084	AT1G64970	-0,079
AT5G55610	-0,104	AT1G04040	-0,096	AT5G16660	-0,091	AT5G27670	-0,084	AT3G58500	-0,079
AT4G22310	-0,104	AT3G01310	-0,096	AT5G66760	-0,091	AT3G01500	-0,084	AT5G14260	-0,078
AT3G47470	-0,103	AT3G23920	-0,096	AT3G57090	-0,091	AT5G46110	-0,084	AT5G21060	-0,078
AT1G06430	-0,103	AT2G34460	-0,096	AT4G10060	-0,091	AT2G05830	-0,084	AT4G33760	-0,078
AT5G51820	-0,103	AT3G61440	-0,096	AT4G25900	-0,090	AT5G36880	-0,083	AT2G03550	-0,078
AT3G57410	-0,102	AT2G17265	-0,096	AT1G04690	-0,090	AT5G17710	-0,083	AT3G27300	-0,077
AT5G39590	-0,102	AT1G12230	-0,096	AT3G46100	-0,090	AT4G00860	-0,083	AT5G45750	-0,077
AT1G61790	-0,102	AT3G49870	-0,095	AT4G28220	-0,090	AT1G11750	-0,083	AT5G11330	-0,077
AT4G23650	-0,102	AT2G28900	-0,095	AT4G20440	-0,089	AT2G10940	-0,083	AT3G52960	-0,077

AT3G48000	-0,077	AT1G34000	-0,071	AT4G09720	-0,065	AT3G26720	-0,058	AT2G33845	-0,053
AT1G03870	-0,077	AT1G06680	-0,071	AT1G13080	-0,065	AT2G26670	-0,058	AT4G10340	-0,053
AT1G11660	-0,077	AT1G32220	-0,071	AT2G38740	-0,065	AT5G14740	-0,058	AT4G24830	-0,053
AT4G01150	-0,077	AT1G70730	-0,070	AT2G19860	-0,065	AT2G27600	-0,058	AT5G42220	-0,052
AT5G38660	-0,076	AT5G10730	-0,070	AT4G23100	-0,064	AT4G23570	-0,057	AT1G22450	-0,052
AT3G25800	-0,076	AT4G09010	-0,070	AT2G16950	-0,064	AT3G55400	-0,057	AT5G17560	-0,052
AT1G64190	-0,076	AT2G27680	-0,070	AT5G48960	-0,064	AT1G22280	-0,057	AT3G56190	-0,052
AT1G12050	-0,075	AT5G48220	-0,070	AT1G68010	-0,063	AT2G37500	-0,057	AT1G10630	-0,052
AT4G09020	-0,074	AT1G64770	-0,070	AT2G42590	-0,063	AT5G59880	-0,057	AT5G13280	-0,051
AT4G21210	-0,074	AT5G15450	-0,070	AT2G21170	-0,063	AT3G60340	-0,057	AT3G20330	-0,051
AT2G45990	-0,074	AT1G07250	-0,069	AT2G39770	-0,062	AT5G65220	-0,057	AT1G23310	-0,051
AT3G08030	-0,074	AT2G41740	-0,068	AT4G04340	-0,062	AT3G01280	-0,057	AT4G35360	-0,051
AT3G14310	-0,074	AT1G55450	-0,068	AT1G09430	-0,062	AT5G55160	-0,057	AT2G35840	-0,051
AT5G43470	-0,074	AT3G57280	-0,068	AT1G04410	-0,062	AT1G20010	-0,056	AT3G12145	-0,050
AT3G15360	-0,073	AT1G67090	-0,067	AT4G17870	-0,062	AT5G49810	-0,056	AT5G48930	-0,050
AT1G29530	-0,073	AT4G14210	-0,067	AT3G59780	-0,061	AT1G11910	-0,056	AT1G03630	-0,050
AT2G29700	-0,073	AT4G03110	-0,067	AT4G14710	-0,061	AT4G25130	-0,056	AT2G35410	-0,050
AT3G17240	-0,073	AT4G34740	-0,067	AT5G17310	-0,061	AT3G44620	-0,056	AT1G20190	-0,049
AT3G19420	-0,073	AT2G30620	-0,067	AT3G26650	-0,061	AT2G33150	-0,056	AT1G74100	-0,049
AT1G24180	-0,073	AT1G23190	-0,067	AT5G19370	-0,060	AT2G37660	-0,056	AT1G49670	-0,049
AT3G18780	-0,073	AT3G01510	-0,066	AT1G09010	-0,060	AT4G33500	-0,056	AT5G18420	-0,049
AT4G15110	-0,073	AT5G25460	-0,066	AT4G35830	-0,060	AT5G66120	-0,055	AT3G01440	-0,049
AT5G10470	-0,073	AT4G00490	-0,066	AT1G07360	-0,060	AT1G62750	-0,054	AT3G24480	-0,049
AT5G57440	-0,073	AT1G71500	-0,066	AT1G43560	-0,059	AT2G22360	-0,053	AT2G38170	-0,048
AT4G03080	-0,072	AT5G12040	-0,066	AT3G21110	-0,059	AT5G22800	-0,053	AT1G29850	-0,048
AT4G35100	-0,072	AT5G57655	-0,066	AT5G54270	-0,059	AT1G03310	-0,053	AT4G34090	-0,048
AT2G44210	-0,072	AT5G57030	-0,065	AT3G08590	-0,059	AT1G48830	-0,053	AT4G33220	-0,048
AT5G67500	-0,071	AT4G36190	-0,065	AT2G38280	-0,058	AT5G05740	-0,053	AT1G12240	-0,048
AT5G24490	-0,071	AT4G17100	-0,065	AT2G28605	-0,058	AT2G44610	-0,053	AT5G02240	-0,048

AT4G24780	-0,047	AT4G33350	-0,043	AT3G27380	-0,039	AT2G18230	-0,035	AT3G25150	-0,031
AT5G57020	-0,047	AT3G10520	-0,043	AT2G25840	-0,039	AT5G08300	-0,035	AT1G16080	-0,030
AT5G14200	-0,047	AT2G27860	-0,042	AT5G56260	-0,039	AT5G36160	-0,035	AT4G25080	-0,030
AT5G08570	-0,047	AT1G59870	-0,042	AT4G30310	-0,039	AT3G01910	-0,035	AT1G79560	-0,030
AT1G15130	-0,047	AT2G30950	-0,042	AT1G29670	-0,038	AT1G35720	-0,034	AT5G67385	-0,030
AT1G48860	-0,047	AT2G20990	-0,042	AT3G25530	-0,038	AT1G31910	-0,034	AT3G25230	-0,030
AT2G42220	-0,047	AT5G16840	-0,042	AT1G79040	-0,038	AT1G31180	-0,034	AT5G09650	-0,029
AT3G62030	-0,046	AT1G78380	-0,042	AT3G11930	-0,038	AT4G39730	-0,033	AT3G19710	-0,029
AT3G02660	-0,046	AT5G63570	-0,042	AT3G43300	-0,038	AT1G24100	-0,033	AT1G02910	-0,029
AT5G16590	-0,046	AT2G07050	-0,042	AT2G42130	-0,038	AT5G07020	-0,033	AT1G71695	-0,029
AT3G13235	-0,046	AT1G27970	-0,041	AT4G24800	-0,038	AT3G57050	-0,033	AT3G03100	-0,029
AT3G18060	-0,045	AT2G17200	-0,041	AT1G60780	-0,037	AT2G19760	-0,033	ATCG00820	-0,029
ATCG00280	-0,045	AT2G20340	-0,041	AT4G29260	-0,037	AT4G25050	-0,033	AT5G55280	-0,029
AT1G43670	-0,045	AT5G10920	-0,041	AT4G39690	-0,037	AT5G48180	-0,033	AT5G09810	-0,028
AT2G42690	-0,045	AT1G79550	-0,041	AT3G24590	-0,037	AT4G24770	-0,032	AT1G74910	-0,028
AT3G54960	-0,045	AT3G15020	-0,041	AT1G03220	-0,037	AT3G53110	-0,032	AT3G15730	-0,027
AT1G21380	-0,045	AT1G15820	-0,041	AT1G17650	-0,036	AT5G22620	-0,032	AT1G76400	-0,027
AT4G28750	-0,045	AT5G59870	-0,040	AT4G27070	-0,036	AT2G42770	-0,032	AT3G22890	-0,027
AT2G04160	-0,044	AT1G08980	-0,040	AT4G01800	-0,036	AT3G19450	-0,032	AT4G35450	-0,027
AT3G63410	-0,044	AT5G48300	-0,040	AT4G23630	-0,036	AT5G23060	-0,032	AT2G05070	-0,027
AT2G20610	-0,044	AT4G00630	-0,040	AT5G38470	-0,036	AT3G20680	-0,032	AT3G08510	-0,027
AT2G34430	-0,044	AT3G52880	-0,039	ATCG00680	-0,036	AT3G54440	-0,031	AT5G11520	-0,027
AT5G60600	-0,044	AT2G30860	-0,039	AT5G42740	-0,036	AT5G14590	-0,031	AT1G02560	-0,027
AT2G35780	-0,044	AT5G11490	-0,039	AT4G23850	-0,036	AT5G40370	-0,031	AT5G47110	-0,026
AT5G03900	-0,043	AT2G17972	-0,039	AT5G14040	-0,035	AT4G21660	-0,031	AT1G69830	-0,026
AT5G42270	-0,043	AT3G02360	-0,039	AT4G30160	-0,035	AT4G00570	-0,031	AT3G52990	-0,026
AT3G14840	-0,043	AT1G22700	-0,039	AT5G56350	-0,035	AT5G45390	-0,031	AT1G79930	-0,026
AT5G10450	-0,043	AT1G78060	-0,039	AT5G50950	-0,035	AT5G39510	-0,031	AT2G35010	-0,025
AT2G40100	-0,043	AT1G13020	-0,039	AT3G05000	-0,035	AT3G43540	-0,031	AT3G60750	-0,025

AT5G44650	-0,025	AT1G37130	-0,022	AT5G17530	-0,017	AT4G03560	-0,013	AT2G38230	-0,008
AT2G29290	-0,025	AT3G11780	-0,022	AT2G20420	-0,017	AT3G61050	-0,013	AT3G19760	-0,008
AT5G61410	-0,024	AT5G53140	-0,022	AT5G08590	-0,017	AT2G38550	-0,013	AT3G10670	-0,007
AT4G08850	-0,024	ATCG00520	-0,021	AT4G20850	-0,017	AT5G06290	-0,013	AT3G52380	-0,007
AT1G73060	-0,024	AT5G62790	-0,021	AT5G66090	-0,016	AT2G30200	-0,012	AT4G39970	-0,007
AT2G36250	-0,024	AT3G29320	-0,021	AT3G18860	-0,016	AT2G04700	-0,012	AT1G35160	-0,006
AT5G14220	-0,024	AT1G01080	-0,021	AT3G06050	-0,016	AT4G19880	-0,012	AT1G77510	-0,006
AT5G42790	-0,024	AT3G07430	-0,021	AT5G36170	-0,016	AT5G24400	-0,012	AT1G19150	-0,006
AT2G40840	-0,024	AT1G80560	-0,020	AT4G30010	-0,016	AT2G05710	-0,012	AT3G12490	-0,006
AT2G31170	-0,024	AT3G26060	-0,020	AT5G16880	-0,015	AT2G27030	-0,012	AT4G13010	-0,006
AT4G33090	-0,024	AT2G22780	-0,020	AT2G43910	-0,015	AT1G09130	-0,011	AT2G43750	-0,005
AT3G08740	-0,024	AT5G20280	-0,020	AT5G08410	-0,015	AT4G12730	-0,011	AT5G11810	-0,005
AT4G12980	-0,024	ATCG00490	-0,020	AT1G52740	-0,015	AT1G43690	-0,011	AT5G36210	-0,005
AT1G67350	-0,023	AT3G10380	-0,020	AT3G55010	-0,015	AT3G51160	-0,011	AT3G58140	-0,005
AT3G60450	-0,023	AT5G67360	-0,020	AT4G10120	-0,014	AT3G59920	-0,011	AT5G63980	-0,004
AT3G61470	-0,023	AT1G74920	-0,019	AT3G63260	-0,014	AT3G20000	-0,011	AT5G57850	-0,004
AT3G63160	-0,023	AT1G18270	-0,019	AT5G11770	-0,014	AT2G29450	-0,011	AT3G57030	-0,004
AT5G57330	-0,023	AT5G13510	-0,019	AT5G23010	-0,014	AT2G13560	-0,010	AT5G06600	-0,004
AT3G55040	-0,023	AT5G13420	-0,019	AT1G62660	-0,014	AT3G60190	-0,010	AT3G02420	-0,004
AT5G61510	-0,023	AT5G44720	-0,019	AT3G11170	-0,014	AT3G03250	-0,010	AT5G13640	-0,004
AT1G23410	-0,023	AT1G50450	-0,019	AT4G20360	-0,014	AT1G32440	-0,010	AT4G13430	-0,004
AT2G40060	-0,022	AT3G27430	-0,019	AT5G39410	-0,014	AT5G18660	-0,010	AT1G72880	-0,004
AT1G51710	-0,022	AT4G16060	-0,019	AT5G20080	-0,013	AT1G79340	-0,010	AT1G04750	-0,003
AT3G16520	-0,022	AT1G29250	-0,019	AT3G26710	-0,013	AT3G47070	-0,010	AT5G41210	-0,003
AT4G01100	-0,022	AT1G50320	-0,018	AT4G09000	-0,013	AT2G18960	-0,009	AT1G76080	-0,003
AT1G15140	-0,022	AT1G28140	-0,018	AT1G27770	-0,013	AT2G42910	-0,009	AT3G11130	-0,003
AT4G09670	-0,022	AT5G11670	-0,018	AT5G08540	-0,013	AT1G73110	-0,008	AT4G36760	-0,002
AT2G38670	-0,022	AT5G42960	-0,018	AT3G10350	-0,013	AT5G16970	-0,008	AT5G09590	-0,002
AT1G07440	-0,022	AT4G04350	-0,017	AT2G04842	-0,013	AT1G63970	-0,008	AT5G44340	-0,002

AT4G28706		-0,001	AT1G48520		0,003	AT3G47520		0,008	AT5G22780		0,013	AT3G51420		0,017
AT1G25350		-0,001	AT4G38220		0,004	AT1G69740		0,008	AT1G30690		0,013	AT3G63490		0,018
AT2G31390		-0,001	AT1G13440		0,004	AT2G44530		0,008	AT3G13870		0,013	AT2G17980		0,018
AT4G24550		-0,001	AT2G05990		0,004	AT3G14210		0,008	AT5G54750		0,014	AT3G12800		0,018
AT5G53480		-0,001	AT5G62140		0,004	AT1G11930		0,008	AT2G44100		0,014	AT4G13590		0,018
AT1G31230		0,000	AT3G55440		0,004	AT3G17210		0,009	AT5G17770		0,014	AT2G38040		0,019
AT1G73600		0,000	AT2G37620		0,004	AT5G28840		0,009	AT1G56050		0,014	AT3G63190		0,019
AT2G18710		0,000	AT3G06860		0,004	AT1G54520		0,009	AT1G62180		0,014	AT1G79230		0,019
AT4G29220		0,000	AT1G79210		0,005	AT3G02630		0,009	AT1G24610		0,014	AT2G24020		0,020
AT1G03475		0,000	AT5G04590		0,005	AT1G80600		0,009	AT3G07390		0,014	AT3G57890		0,020
AT1G09780		0,001	AT5G09900		0,005	AT4G04040		0,009	AT2G21390		0,015	AT3G02230		0,020
AT3G22110		0,001	AT1G66670		0,005	AT1G21065		0,010	AT4G30190		0,015	AT1G06290		0,020
AT1G22920		0,001	AT5G17170		0,005	AT1G26110		0,010	AT3G07670		0,015	AT3G08530		0,020
AT1G66430		0,001	AT3G27830		0,006	AT5G06060		0,010	AT3G62910		0,015	AT1G31730		0,020
AT5G63890		0,001	AT2G45740		0,006	AT1G07920		0,010	AT2G28760		0,015	AT1G16890		0,021
AT3G45780		0,002	AT2G47510		0,006	AT5G26570		0,010	AT4G16155		0,015	AT4G33360		0,021
AT5G62810		0,002	AT1G15730		0,006	AT1G01960		0,010	AT3G60820		0,016	AT3G25920		0,021
AT1G07890		0,002	AT4G33650		0,006	AT2G33210		0,010	AT5G57490		0,016	AT2G43160		0,022
AT3G47860		0,002	AT3G25660		0,007	AT1G48350		0,011	AT3G54540		0,016	AT1G62820		0,022
AT4G26900		0,002	AT4G35630		0,007	AT3G16000		0,011	AT5G14120		0,016	AT3G04870		0,022
AT4G05160		0,002	AT1G32160		0,007	AT1G70890		0,011	AT1G53310		0,016	AT1G22300		0,022
AT1G72910		0,002	AT3G06580		0,007	AT1G65540		0,011	AT3G09740		0,017	AT2G17340		0,022
AT4G04720		0,002	AT3G23700		0,007	AT3G17970		0,011	AT5G01410		0,017	AT1G06530		0,023
AT1G09630		0,002	AT3G54210		0,007	AT5G63400		0,011	AT2G16600		0,017	AT3G01340		0,023
AT1G12840		0,003	AT4G23670		0,007	AT2G32080		0,012	AT5G28020		0,017	AT5G08160		0,023
AT1G19920		0,003	AT5G06870		0,008	AT2G24420		0,012	AT5G50920		0,017	AT5G62670		0,023
AT1G18070		0,003	AT5G43940		0,008	AT1G03160		0,012	AT3G53900		0,017	AT2G42810		0,023
AT3G47960		0,003	AT4G13720		0,008	AT4G36940		0,013	AT3G11700		0,017	AT4G16500		0,023
AT5G20010		0,003	AT2G22250		0,008	AT5G02790		0,013	AT2G45790		0,017	AT3G26740		0,023

AT4G33030	0,023	AT5G27540	0,028	AT1G15690	0,033	AT4G37300	0,037	AT1G56450	0,043
AT3G53180	0,024	AT5G20720	0,029	AT1G35620	0,033	AT5G10160	0,037	AT5G08670	0,043
AT2G38270	0,024	AT4G22670	0,029	AT2G38700	0,033	AT2G44060	0,037	AT1G79920	0,043
AT4G24750	0,024	AT2G35800	0,029	AT3G07770	0,033	AT5G33320	0,037	AT1G78370	0,043
AT2G14260	0,024	AT1G27450	0,029	AT2G30695	0,033	AT2G30870	0,038	AT3G46970	0,043
AT5G07460	0,024	AT1G51980	0,029	AT1G14980	0,033	AT3G02780	0,038	AT2G40765	0,043
AT1G47420	0,025	AT1G04510	0,029	AT5G19820	0,033	AT5G58490	0,038	AT2G20580	0,044
AT4G37980	0,025	ATCG00160	0,029	AT5G48810	0,034	AT3G62700	0,038	AT2G32730	0,044
AT1G44170	0,025	AT3G43980	0,029	AT3G09440	0,034	AT1G74260	0,038	AT2G31570	0,044
AT3G45190	0,025	AT3G10940	0,029	AT4G19006	0,034	AT1G07660	0,038	AT3G59760	0,045
AT2G30110	0,025	AT5G53620	0,030	AT5G07440	0,034	AT5G16050	0,038	AT1G12270	0,045
AT1G60710	0,025	AT1G62480	0,030	AT5G66720	0,034	AT2G45810	0,039	ATCG01060	0,045
AT3G20820	0,025	AT5G13450	0,030	AT2G18510	0,034	AT2G30970	0,039	AT5G57870	0,045
AT1G48850	0,026	AT2G23070	0,030	AT4G13770	0,034	AT1G78680	0,039	AT3G48750	0,045
AT3G46430	0,026	AT4G34350	0,030	AT4G13510	0,035	AT5G10360	0,039	AT4G04770	0,045
AT3G48730	0,026	AT1G09070	0,030	AT5G37830	0,035	AT1G68560	0,040	AT1G43890	0,045
AT5G16760	0,027	AT4G30530	0,030	AT4G24280	0,035	AT5G22880	0,040	AT4G27000	0,045
AT2G05840	0,027	AT2G25830	0,031	AT4G34260	0,035	AT4G13780	0,040	AT2G22990	0,046
AT2G43030	0,027	AT3G61870	0,031	AT5G60980	0,035	AT2G40290	0,040	AT1G20200	0,046
AT5G64050	0,027	AT2G34420	0,031	AT1G47550	0,035	AT3G44890	0,041	AT3G52300	0,046
AT3G13300	0,027	AT1G49970	0,031	AT5G44020	0,035	AT2G32920	0,041	AT1G15750	0,046
AT1G33810	0,027	AT4G34050	0,031	AT4G14880	0,035	AT5G40810	0,041	AT1G10510	0,046
AT3G20630	0,028	AT4G00100	0,031	AT1G62740	0,036	AT5G37850	0,041	AT1G51680	0,046
AT3G13120	0,028	AT5G64860	0,031	AT4G29840	0,037	AT2G32520	0,042	AT2G46650	0,046
AT4G31300	0,028	AT5G19770	0,032	AT5G08650	0,037	AT1G29150	0,042	ATCG00360	0,047
AT4G14870	0,028	AT4G31990	0,032	AT3G08580	0,037	AT1G53850	0,042	AT3G63460	0,047
AT1G13270	0,028	AT1G14610	0,032	AT2G21250	0,037	AT4G02080	0,042	AT4G08520	0,047
AT1G55860	0,028	AT1G53750	0,032	AT3G19340	0,037	AT5G05730	0,043	AT5G52520	0,047
AT1G12310	0,028	AT1G20960	0,032	AT5G12250	0,037	AT2G43950	0,043	AT2G28800	0,048

AT1G70330	0,048	AT5G15090	0,053	AT5G23140	0,057	AT1G75350	0,062	AT3G03780	0,068
AT3G58510	0,048	AT1G78630	0,053	AT1G78300	0,057	AT4G31180	0,062	AT1G21720	0,068
AT4G08900	0,048	AT5G39570	0,053	AT5G26710	0,057	AT3G54840	0,062	AT5G58290	0,068
AT2G29630	0,048	AT4G19410	0,053	AT3G24503	0,058	AT4G22010	0,063	AT3G02870	0,068
AT5G05780	0,049	AT5G45420	0,053	AT1G19570	0,058	AT4G26500	0,063	AT1G32990	0,068
AT1G09620	0,049	AT3G25860	0,053	AT1G50670	0,058	AT5G56030	0,063	AT2G03510	0,069
AT1G24360	0,049	AT2G43710	0,054	AT4G29120	0,058	AT1G07770	0,064	AT2G47940	0,069
ATCG00900	0,049	AT5G14660	0,054	AT1G74070	0,058	AT5G08280	0,064	AT2G33800	0,069
AT5G13410	0,049	AT4G29060	0,054	AT3G10950	0,059	AT5G23890	0,064	ATCG01130	0,069
AT3G18080	0,049	AT1G65980	0,054	AT1G54270	0,059	AT1G53210	0,065	AT4G33110	0,069
AT4G17530	0,049	AT5G64350	0,054	AT1G29880	0,059	AT5G62530	0,065	AT3G62120	0,069
AT4G11380	0,050	AT4G37550	0,054	AT1G20840	0,059	AT4G05390	0,065	AT2G45770	0,070
AT2G35830	0,050	AT4G18440	0,054	AT1G15930	0,059	AT5G47030	0,065	AT4G34450	0,070
AT4G17560	0,050	AT5G49650	0,054	AT5G23540	0,059	AT1G59610	0,065	AT1G29310	0,070
AT2G40660	0,050	AT3G46830	0,054	AT3G10370	0,060	AT2G36530	0,065	AT1G35680	0,070
AT2G16660	0,050	AT4G17040	0,054	AT1G53520	0,060	AT1G06220	0,065	AT1G14030	0,070
AT3G57610	0,051	AT2G06850	0,054	AT4G24190	0,060	AT3G08943	0,065	AT4G36810	0,070
AT5G42980	0,051	AT5G27390	0,054	AT4G01690	0,060	AT4G31490	0,066	AT2G36410	0,071
AT1G29660	0,051	AT1G08640	0,055	AT3G07010	0,061	AT5G04230	0,066	AT3G44110	0,071
AT3G59980	0,051	AT5G38480	0,055	AT2G17420	0,061	AT4G39090	0,066	AT1G75950	0,071
AT4G34200	0,051	AT2G01250	0,056	AT1G75270	0,061	AT1G32900	0,066	AT3G04940	0,072
AT4G29010	0,052	AT2G47450	0,056	AT1G09640	0,061	AT5G40770	0,067	AT5G14910	0,072
AT2G05920	0,052	AT1G50900	0,057	AT3G56240	0,061	AT1G10370	0,067	AT4G22930	0,072
AT1G14810	0,052	AT3G53580	0,057	AT1G02930	0,062	AT1G52730	0,067	AT3G28860	0,072
AT3G51820	0,052	AT1G01560	0,057	AT5G36890	0,062	AT4G12650	0,067	AT4G20960	0,072
AT5G49910	0,052	AT5G42890	0,057	AT2G46520	0,062	AT2G33450	0,067	AT1G50480	0,073
AT1G09210	0,053	AT1G23820	0,057	AT2G21870	0,062	AT4G33680	0,067	AT4G14160	0,073
AT3G12390	0,053	AT4G11010	0,057	AT1G60420	0,062	AT5G13850	0,068	AT2G40490	0,073
AT3G17940	0,053	AT5G56950	0,057	AT5G19990	0,062	AT3G14930	0,068	ATCG00800	0,073

AT1G08490	0,073	AT3G12260	0,077	AT5G25780	0,084	AT1G18500	0,090	AT5G63840	0,098
AT1G70070	0,073	AT4G27585	0,077	AT4G20980	0,084	AT1G26880	0,090	AT2G25670	0,098
AT1G50200	0,073	AT1G80480	0,077	AT4G16760	0,084	AT5G55220	0,090	AT1G67730	0,100
AT1G27130	0,073	AT1G76680	0,077	ATCG00830	0,084	AT5G17920	0,090	AT2G43090	0,100
AT3G52150	0,074	AT3G48110	0,077	AT5G26830	0,084	AT2G19480	0,091	AT5G64940	0,100
AT3G51140	0,074	AT1G17220	0,078	AT5G35620	0,085	AT4G04910	0,091	AT4G13930	0,100
AT3G07480	0,074	AT5G13650	0,078	AT4G38630	0,085	AT4G39260	0,091	AT4G05530	0,100
AT5G53170	0,074	AT3G27740	0,079	AT3G09820	0,085	AT3G04600	0,091	AT4G22690	0,101
AT1G74470	0,074	AT1G14830	0,079	AT3G52180	0,085	AT1G36390	0,091	AT1G02080	0,101
AT1G08520	0,074	AT1G74040	0,079	AT1G63000	0,085	AT5G62890	0,091	AT1G02780	0,101
AT3G55610	0,074	AT1G02500	0,079	AT3G51260	0,085	AT2G01970	0,092	AT5G61790	0,102
AT5G03940	0,074	AT1G11430	0,080	AT5G12140	0,085	AT3G14110	0,092	AT2G37220	0,102
AT1G76810	0,075	AT5G03630	0,080	AT3G05280	0,086	AT5G42570	0,092	AT2G44650	0,102
AT5G42080	0,075	AT3G51840	0,080	AT3G06483	0,086	AT4G35000	0,092	AT5G50250	0,102
AT1G50370	0,075	AT5G19940	0,080	AT5G05010	0,086	AT1G05190	0,093	ATCG00710	0,103
AT1G12800	0,075	ATCG00500	0,080	AT1G17890	0,086	AT3G02200	0,093	AT1G62020	0,103
AT3G15190	0,075	AT2G43460	0,081	AT2G26900	0,087	AT3G14390	0,093	AT2G33040	0,103
AT4G32520	0,075	AT2G20690	0,081	AT3G49680	0,087	AT1G09750	0,093	AT4G33250	0,103
AT5G62690	0,076	AT2G28470	0,081	AT3G20390	0,088	AT2G45240	0,093	AT2G27730	0,103
AT2G27450	0,076	AT4G09320	0,081	AT5G45620	0,088	AT2G04350	0,094	AT5G14320	0,103
AT3G24550	0,076	AT3G56910	0,082	AT1G32500	0,088	AT3G02760	0,094	AT3G44320	0,103
AT1G54080	0,076	AT3G09680	0,082	AT3G60240	0,088	AT4G24820	0,094	AT4G24920	0,104
AT5G55480	0,076	AT4G26300	0,082	AT2G47970	0,088	AT3G62360	0,094	AT3G18410	0,104
AT5G03040	0,076	AT5G11710	0,082	ATCG00380	0,088	AT1G74970	0,094	AT1G80300	0,104
AT1G41830	0,076	AT4G33510	0,083	AT5G17990	0,089	AT5G35360	0,095	AT2G31810	0,104
AT5G16715	0,076	AT5G15650	0,083	AT3G44310	0,089	AT5G16130	0,095	AT3G12110	0,104
AT4G01310	0,076	AT2G20140	0,084	AT2G25970	0,089	AT2G44120	0,095	AT5G60160	0,104
AT3G05970	0,076	AT5G66420	0,084	AT4G34700	0,089	AT3G55250	0,097	AT4G01900	0,105
ATCG00810	0,077	AT4G19710	0,084	AT3G21750	0,089	AT5G47770	0,098	AT1G79850	0,105

AT5G35590	0,105	AT5G07030	0,113	AT1G25490	0,120	AT1G49660	0,126	AT3G28940	0,135
AT5G20630	0,106	AT3G13490	0,114	AT2G06050	0,120	AT3G11910	0,127	AT3G05910	0,136
AT5G16620	0,106	AT2G36880	0,114	AT1G23290	0,120	AT1G62380	0,127	AT1G13110	0,136
AT1G06950	0,106	AT1G05350	0,114	AT5G54770	0,120	AT1G48610	0,127	AT1G20510	0,136
AT2G47240	0,106	AT1G76160	0,115	AT5G02940	0,120	AT4G29410	0,127	ATCG00770	0,136
AT1G66680	0,106	AT1G32200	0,115	AT1G06190	0,121	AT5G47190	0,128	AT3G50270	0,136
AT1G70940	0,106	AT4G16660	0,115	AT5G42970	0,121	AT3G01780	0,128	AT1G16700	0,137
AT4G09040	0,107	AT5G03345	0,115	AT4G10300	0,121	AT5G39740	0,128	AT1G33600	0,137
AT5G42020	0,107	AT5G27470	0,115	AT4G31700	0,121	AT4G31500	0,129	AT1G79750	0,137
AT4G27090	0,107	AT5G03300	0,116	AT3G13860	0,121	AT4G39660	0,129	AT2G42680	0,137
AT1G21750	0,107	AT3G03890	0,116	ATCG00750	0,122	AT5G42650	0,129	AT5G28500	0,137
AT4G38740	0,107	AT1G71920	0,116	AT4G00620	0,122	AT5G20500	0,129	AT5G61780	0,138
AT3G43800	0,107	ATCG00780	0,116	AT5G35790	0,122	AT3G06350	0,130	AT3G57330	0,138
AT5G40950	0,108	AT3G05420	0,116	AT4G34950	0,123	AT3G08010	0,130	AT5G56010	0,139
AT5G25754	0,108	AT5G23900	0,117	AT5G58250	0,123	AT2G24060	0,131	AT4G39960	0,141
AT2G42210	0,108	AT4G01850	0,117	AT5G55230	0,123	AT5G62390	0,132	AT3G53520	0,141
AT4G08870	0,108	AT1G09795	0,117	AT1G62780	0,124	AT3G10270	0,133	ATCG00790	0,141
AT1G73230	0,108	AT3G51800	0,118	AT1G35580	0,124	AT3G04120	0,133	AT5G52240	0,142
AT1G20050	0,108	AT1G17050	0,118	ATCG01120	0,124	AT1G29350	0,133	AT2G47840	0,142
AT5G59950	0,110	AT1G09590	0,118	AT4G28030	0,124	AT1G49140	0,133	AT4G17520	0,142
AT5G08080	0,110	AT5G10010	0,118	AT4G13940	0,124	AT5G43330	0,133	AT2G46170	0,142
AT3G03710	0,110	AT1G34430	0,118	AT1G54350	0,124	AT5G19620	0,133	AT1G01910	0,142
AT5G08740	0,111	AT4G21150	0,119	AT4G34620	0,125	AT1G66260	0,134	AT1G27400	0,143
AT5G17020	0,112	AT5G08050	0,119	AT1G60990	0,125	AT5G14060	0,134	AT1G75330	0,143
AT1G78850	0,112	AT4G13200	0,119	AT4G11420	0,126	AT1G60000	0,134	AT5G60640	0,143
AT1G26560	0,112	AT1G64520	0,119	AT4G14040	0,126	AT5G63860	0,134	AT1G31812	0,144
AT1G12920	0,113	AT5G54430	0,120	AT2G07360	0,126	AT5G61970	0,134	AT1G10290	0,144
AT5G65750	0,113	AT3G56490	0,120	AT4G38350	0,126	AT3G09300	0,134	AT2G47470	0,144
AT5G36230	0,113	AT1G64510	0,120	AT2G22240	0,126	AT1G70310	0,135	AT3G26450	0,145

AT2G42520	0,145	AT2G04520	0,157	AT4G34830	0,175	AT5G18230	0,194	AT1G20090	0,217
AT1G58290	0,145	AT4G30210	0,157	AT1G27390	0,175	AT5G47870	0,194	AT1G71820	0,218
AT1G23180	0,146	AT1G27090	0,159	AT2G36070	0,176	AT5G42950	0,194	AT5G47690	0,219
AT5G63680	0,146	AT3G58750	0,159	AT1G08360	0,176	AT1G13930	0,195	AT5G64300	0,219
AT3G49120	0,146	AT1G47200	0,160	AT4G15000	0,176	ATCG00660	0,195	AT3G25760	0,219
AT3G56940	0,147	AT5G64460	0,160	ATCG00840	0,176	AT3G49470	0,195	AT1G72550	0,221
AT5G26360	0,147	AT1G35670	0,162	AT3G27240	0,177	AT5G53560	0,196	AT5G21930	0,221
AT3G45770	0,147	AT5G10480	0,162	AT3G49430	0,177	AT1G52340	0,197	AT4G38710	0,221
AT1G21670	0,148	AT5G23920	0,162	AT4G08920	0,177	AT5G10540	0,197	AT2G26740	0,221
AT3G48890	0,149	AT5G13780	0,162	AT3G18035	0,179	AT3G08610	0,197	AT3G47650	0,222
AT5G06140	0,149	AT4G02570	0,163	AT4G08390	0,179	AT3G47370	0,200	AT5G26667	0,222
AT2G02740	0,149	AT5G22650	0,164	AT5G20950	0,180	AT5G63810	0,201	AT4G37040	0,222
AT1G19360	0,149	AT1G31800	0,164	AT4G38800	0,181	AT2G18110	0,202	AT4G00810	0,223
AT3G15356	0,149	AT1G48900	0,164	AT4G27320	0,181	AT5G41950	0,204	AT5G59250	0,223
ATCG00650	0,149	AT1G79600	0,165	AT1G04640	0,182	AT4G26870	0,204	AT1G76010	0,223
AT4G04950	0,150	AT2G22170	0,166	AT3G27160	0,183	AT1G68530	0,205	AT1G31690	0,224
AT5G42190	0,150	AT3G07680	0,166	AT4G26780	0,183	AT1G07670	0,206	AT5G25265	0,229
AT5G22510	0,151	AT2G24765	0,167	AT3G45030	0,184	AT4G30950	0,206	AT4G36690	0,233
AT1G64650	0,151	AT5G24650	0,167	AT3G02520	0,185	AT5G53530	0,209	AT1G21630	0,235
AT5G54160	0,154	AT1G32400	0,169	AT2G37550	0,186	AT3G45140	0,210	AT3G55620	0,237
AT3G44340	0,154	AT2G38750	0,170	AT3G53560	0,187	AT2G27530	0,212	AT5G46790	0,237
AT1G04630	0,154	AT2G46280	0,170	AT3G01060	0,189	AT3G11730	0,212	AT1G80360	0,238
AT1G77670	0,154	AT5G03160	0,170	AT1G15290	0,190	AT1G26850	0,214	AT3G23810	0,239
AT3G02080	0,155	AT3G26420	0,171	AT5G19460	0,190	AT2G30390	0,214	AT3G47800	0,239
AT1G50380	0,155	AT4G12590	0,173	AT1G65270	0,191	AT2G36620	0,214	AT3G26290	0,242
AT3G21865	0,155	AT1G48480	0,173	AT5G28050	0,191	AT4G16990	0,215	AT4G37870	0,243
AT5G19690	0,156	AT1G33590	0,174	AT4G27440	0,191	AT4G13350	0,216	AT3G50590	0,243
AT1G09310	0,156	AT1G03230	0,174	AT1G06460	0,192	AT3G16140	0,216	AT1G12000	0,244
AT2G17870	0,157	AT5G62350	0,175	AT1G80070	0,192	AT3G61820	0,216	AT3G53870	0,246

AT1G58080		0,247	AT5G54600		0,280	AT4G32470		0,326	AT1G36310		0,404	AT4G24090		0,476
AT5G46750		0,247	AT5G27700		0,283	AT1G30400		0,327	AT4G36750		0,405	AT5G58220		0,476
AT4G17770		0,248	AT3G18130		0,284	AT1G77060		0,328	AT3G51550		0,409	AT2G45300		0,477
AT1G18540		0,248	AT2G39080		0,285	AT3G16470		0,328	AT1G01050		0,409	AT2G03390		0,484
AT3G01180		0,249	AT1G19670		0,288	AT4G14890		0,328	AT1G26340		0,409	AT1G24050		0,484
AT3G52140		0,253	AT1G52380		0,290	AT1G11360		0,334	AT1G17470		0,410	AT5G42850		0,490
AT2G31750		0,253	AT1G26770		0,290	AT1G28200		0,340	AT3G25770		0,411	AT2G04400		0,491
AT1G69200		0,258	AT5G65730		0,290	AT1G33040		0,342	AT4G18930		0,413	AT2G34590		0,492
AT5G26742		0,258	AT2G42530		0,296	AT1G16410		0,344	AT5G42130		0,415	AT1G63610		0,497
AT1G14650		0,259	AT5G56500		0,297	AT1G11720		0,347	AT3G62940		0,416	AT4G30720		0,498
AT4G11820		0,259	AT1G49650		0,300	AT1G69530		0,348	AT5G36120		0,419	AT1G70610		0,499
AT3G56340		0,259	AT2G21410		0,301	AT1G53500		0,349	AT5G42480		0,419	AT2G17190		0,503
AT3G59020		0,259	AT5G15970		0,302	AT2G33470		0,352	AT5G20070		0,423	AT5G10560		0,506
AT5G38830		0,260	AT2G44310		0,302	AT5G04280		0,353	AT5G64580		0,427	AT5G24020		0,513
AT2G40510		0,261	AT1G05010		0,303	AT4G09350		0,353	AT1G48620		0,427	AT5G54900		0,513
AT1G73180		0,265	AT3G11560		0,303	AT3G07460		0,354	AT1G15950		0,429	AT3G13772		0,516
AT1G59990		0,265	AT2G21600		0,303	AT1G69410		0,358	AT4G35850		0,430	AT1G23860		0,516
AT2G37860		0,267	ATCG01010		0,303	AT5G65810		0,359	AT4G39520		0,434	AT5G17050		0,524
AT3G53890		0,269	AT2G14170		0,305	AT1G24020		0,360	AT1G17720		0,435	AT1G52400		0,530
AT5G42240		0,272	AT1G76550		0,306	ATCG00860		0,363	AT2G25430		0,440	AT2G43100		0,532
AT1G02280		0,272	AT5G22640		0,306	AT2G25800		0,367	AT4G39860		0,446	AT5G02120		0,534
AT2G39800		0,272	AT4G01897		0,307	AT2G44230		0,374	AT1G10430		0,447	AT2G37790		0,535
AT4G14230		0,273	AT5G65780		0,310	AT3G11250		0,375	AT2G19740		0,456	AT5G12940		0,542
AT3G16530		0,273	AT2G26590		0,314	AT1G45201		0,376	AT1G74690		0,458	AT2G25870		0,546
AT3G04650		0,274	AT1G23130		0,316	AT2G31660		0,380	AT4G27560		0,459	AT1G01300		0,546
AT1G09270		0,277	AT2G25110		0,316	AT2G26230		0,382	AT1G08470		0,463	AT1G08250		0,546
AT5G13710		0,277	AT1G79830		0,319	AT5G66470		0,384	AT4G39460		0,466	AT4G19120		0,551
AT4G38550		0,278	AT5G38530		0,321	AT2G18740		0,392	AT3G47560		0,467	AT4G29810		0,555
AT5G47700		0,279	AT1G22430		0,324	AT1G64980		0,403	AT5G63030		0,475	AT1G36730		0,580

AT1G48630		0,583	AT4G10840		0,701	AT5G08260		0,877	AT2G04780		1,872			
AT1G44446		0,587	AT2G40380		0,703	AT3G48500		0,884						
AT2G14720		0,598	AT1G06820		0,706	AT2G35500		0,895						
AT1G66070		0,603	AT1G18730		0,709	AT1G17160		0,923						
AT2G34160		0,606	AT3G28290		0,709	AT2G38140		0,937						
AT1G65010		0,607	AT1G75990		0,711	AT4G25550		0,958						
AT1G58270		0,614	AT1G05520		0,712	AT2G17630		0,990						
AT4G17890		0,617	AT4G36945		0,712	AT3G59400		1,022						
AT5G23820		0,617	AT4G12440		0,714	AT3G12080		1,023						
AT2G38770		0,619	AT1G51570		0,726	AT5G23210		1,058						
ATMG00060		0,625	AT5G25100		0,727	AT3G16240		1,058						
AT4G35460		0,627	AT3G16480		0,733	AT4G02340		1,092						
AT2G29340		0,629	AT2G43970		0,735	AT3G32930		1,093						
AT1G78870		0,634	AT4G39980		0,744	AT1G51100		1,097						
AT2G30410		0,635	AT2G20530		0,753	AT3G28270		1,163						
AT3G44100		0,636	AT2G34640		0,771	AT1G09770		1,165						
AT3G12050		0,642	AT5G16150		0,772	AT5G12370		1,194						
AT3G11400		0,652	AT1G20220		0,772	AT3G11940		1,220						
AT2G25060		0,654	AT5G44130		0,786	AT1G10590		1,253						
AT5G23310		0,659	AT1G42960		0,806	AT4G16450		1,343						
AT1G52670		0,663	AT3G16780		0,810	AT4G10000		1,363						
AT2G47650		0,671	AT4G24350		0,811	AT3G18680		1,435						
AT4G20130		0,675	AT3G11070		0,812	AT3G25220		1,454						
AT1G60550		0,676	AT1G01620		0,830	AT3G11770		1,502						
AT3G46940		0,677	AT1G08480		0,864	AT5G47930		1,518						
AT1G64090		0,693	AT2G07727		0,866	ATCG00730		1,554						
AT1G19740		0,695	AT2G22230		0,867	AT4G21860		1,579						
AT3G10410		0,700	AT1G29790		0,868	AT5G23040		1,583						
AT1G53260		0,701	AT3G18490		0,872	AT5G57290		1,686						

Chapter 2: The role of E1 subunit of 2-oxoglutarate dehydrogenase complex in response to osmotic stress and recovery period in plants of *Arabidopsis thaliana*

ABSTRACT

VARGAS, Jonas Rafael, D.Sc., Universidade Federal de Viçosa, April, 2022. **The role of E1 subunit of 2-oxoglutarate dehydrogenase complex in response to osmotic stress and recovery period in plants of *Arabidopsis thaliana***. Adviser: Adriano Nunes Nesi.

To deal with periods of water limitation, plants make use of different mechanisms. Among them stomatal closure and proline accumulation. After the stress period, during the recovery phase, the excess of proline is degraded releasing glutamate into the mitochondrial matrix. Glutamate can then be either (i) degraded, releasing ammonium for assimilation and *de novo* amino acid synthesis, (ii) metabolized by the gamma-aminobutyric acid (GABA) shunt pathway and reintroduced in the tricarboxylic acid (TCA) cycle as succinate, or (iii) oxidized by glutamate dehydrogenase being converted to 2-oxoglutarate (2OG) which is metabolized by 2-oxoglutarare dehydrogenase (2OGDH). Given the expected role of this enzyme in the metabolic alterations during the establishment and recovery period of osmotic stress, we investigated the importance of specific E1 subunit isoforms of 2OGDH complex in plants of *Arabidopsis thaliana*. For this aim, we evaluated plants from single T-DNA insertion lines of e1-ogdh1.2 and e1-ogdh2.2 genes and a double mutant line. Interestingly, the rosette area was affected by the lack of E1 subunit and the osmotic stress symptoms were more pronounced in the mutant plants. Furthermore, we observed more remarkable alterations in the root metabolite levels than in shoots from the mutant plants, and larger changes in the levels of TCA cycle intermediates during the imposition and recovery period of abiotic stress in this tissue. The double mutant plants struggled to degrade proline in the first 24 hours of recovery and the excess of glutamate resulting from the degradation of proline was preferentially diverted to the TCA cycle during the first 24 hours of recovery. These results suggest that E1 subunit of the 2OGDH play a distinctive role in proline degradation in the first 24 hours of recovery and also, that this subunit is required for plants to cope with periods of osmotic stress.

Keywords: Arabidopsis. 2OGDH. Osmotic stress. Proline. Metabolism

INTRODUCTION

The respiration process in plants is composed of four main pathways, the glycolysis in the cytosol, the tricarboxylic acid (TCA) cycle in the mitochondrial matrix and the electron transport chain (mETC) and oxidative phosphorylation (OXPHOS) in the inner mitochondrial membrane (Sweetlove et al., 2010). The TCA cycle is composed of a set of eight enzymes and it is described as a central pathway in plant respiration, since it is responsible for the production of important organic acids, ATP and reduction of NAD^+ (Millar et al., 2011). Although it is generally described as a cycle, in several situations TCA cycle can operate in a non-cyclic mode (Sweetlove et al., 2010). This non-cyclic mode of the TCA cycle allows that intermediates of the cycle can be used in different pathways such as, provision of carbon skeletons for the assimilation of nitrogen as well as for other metabolic pathways (Sweetlove et al., 2010; Huergo and Dixon, 2015). Also related to the TCA cycle, both during cyclic and non-cyclic modes are the formation of metabolons (Zhang et al., 2017; Obata, 2019). Metabolons are described as the association of different proteins, in order to channel substrates, favor specific flows and directions of the metabolism and also improve the regulation and control of some key reactions (Obata, 2019). The enzyme 2OGDH by itself is considered a metabolon because is a multienzyme complex (Obata, 2019). This enzyme is described for their possible interaction with the Succinil-CoA ligase or with the Isocitrate dehydrogenase (IDH) (Zhang et al., 2018). The IDH, interacts with Aconitase, and citrate synthase in order to better control the efficiency of these reactions (Zhang et al., 2017; Zhang et al., 2018; Obata, 2019). In this way favoring the flow of the TCA cycle in specific directions (Zhang et al., 2017)

Among the TCA cycle intermediates that can be used in other pathways, acetyl-CoA, fumarate, malate, and 2-oxoglutarate (2OG) are the most studied ones. Acetyl-CoA can be used in the metabolism of flavonoids, alkaloids, fatty acids, isoprenoids and pigments (Araújo et al., 2012a). Fumarate and malate can participate in the synthesis of glucose during the gluconeogenesis as well as in the photosynthesis and fruit ripening (Araújo et al., 2012a). The NAD-dependent IDH, through an oxidative decarboxylation of isocitrate, produces 2OG in a reaction that also results in NADH (Lemaitre et al., 2007). 2 oxoglutarate is then used as a substrate for the multienzymatic complex 2 oxoglutarate dehydrogenase (2OGDH) that converts it into succinyl-CoA and release of NADH through another oxidative decarboxylation (Bunik and Fernie, 2009). Alternatively 2-OG can be exported from the mitochondrial matrix to the cytosol via specific transporters that coupled this exchange with the transport of other dicarboxylic (DICs) and tricarboxylic acids (DTCs) such as citrate and also with succinate and fumarate (SFCs) (Etienne et al., 2013; Toleco et al., 2020). This 2OG then, is imported to the chloroplasts by the action of plastid dicarboxylic transporters (DiTs) (Facchinelli and Weber, 2011), where it can be used in other major metabolic pathways such as ammonium assimilation and amino acid biosynthesis by the action of glutamine synthetase (GS) and Glutamate synthase (GOGAT) (Araújo et al., 2014b).

The 2OGDH complex is composed of three subunits, namely 2-oxoglutarate dehydrogenase (E1), dihydrolipoyl succinyltransferase (E2) and dihydrolipoyl dehydrogenase (E3) (Millar et al., 1999). This complex has a molecular mass of approximately 2000 kDa, and it is formed by the union of a core with about 24 E2 subunits to which E3 and E1 homodimers join (Millar et al., 1999). Under control conditions one mitochondria contains around of 181 2-OGDH active complexes, that are formed following a stoichiometry of 5:5:2 (subunits E1, E2 and E3) (Fuchs et al., 2020). Together this complex accounts for 1% of the whole mitochondrial matrix protein volume (Fuchs et al., 2020). The E1 subunit requires the presence of the cofactor thiamine pyrophosphate (TPP) and is responsible for the initial decarboxylation of 2-OG in which it releases CO_2 and forms a succinyl-TPP molecule. The E2 subunit transfers the succinyl to lipoic acid and then to a CoA releasing succinyl-CoA and forming a dihydrolipoic acid that is oxidized by E3 using FAD^+ as a cofactor to form lipoic acid with consequent reduction of NAD^+ and production of NADH (Millar et al., 1999).

Basically the biological importance of all TCA cycle enzymes have been described under optimal conditions (Carrari et al., 2003; Nunes-Nesi et al., 2005; Fait et al., 2007b; Lemaitre et al., 2007; Nunes-Nesi et al., 2007; Araújo et al., 2008; Sienkiewicz-Porzucek et al., 2008; Araújo et al., 2012b; Sienkiewicz-porzucek et al., 2012; Yue et al., 2018). However, the physiological roles of these enzymes during periods of stress and recovery is not well understood. During periods of abiotic stresses displayed by salinity, alkalinity, drought, ionic and osmotic stress, that reduces the water availability in plant tissues, the non-cyclic flows of the TCA cycle may be increased in order to meet the demand for 2-OG in the assimilation of ammonium resulted from the photorespiration or protein degradation (Sweetlove et al., 2002; Atkin and Macherel, 2009; Jacoby et al., 2011). These stress conditions are also related to different forms of oxidative stress (Hayat et al., 2012), that may lead to a reduction of the 2-OGDH activity by the presence of lipid catabolism intermediates such as 4-hydroxy-2-nonenal (HNE), that act as inhibitors of the E2 subunit (Taylor et al., 2002; Taylor et al., 2004; Taylor et al., 2009). These conditions can activate certain alternative pathways such as the GABA shunt (Fait et al., 2007a), that is responsible for supplying the TCA cycle demand for succinate through the action of glutamate decarboxylase (GAD), GABA transaminase (GABA-T) and succinate-semialdehyde dehydrogenase (SSDH). This shunt is able to establish an alternative pathway to the reactions performed by 2-OGDH and Succinyl-CoA ligase within the TCA cycle (Fait et al., 2007a). Another metabolic response to abiotic stress condicions is an accumulation of compatible solutes, a group of molecules known by their capacity of being accumulated without altering cellular homeostasis (Hayat et al., 2012; Obata and Fernie, 2012; Liang et al., 2013). Among other things, these metabolites are involved in protein stabilization and can be classified into three categories: polyalcohol (e.g., glycerol and sorbitol), sugars (e.g., sucrose and trehalose), amino acids and their derivatives (e.g., proline, glycine, and betaines) (Viana et al., 2005).

Proline accumulates in *Arabidopsis thaliana* when exposed to abiotic stresses conditions, that reduces the free water status of the plant (Székely et al., 2008; Verbruggen and Hermans, 2008; Hummel et al., 2010b; Obata and Fernie, 2012; Pál et al., 2018; Furlan et al., 2020). This osmolyte can act by reducing the tissue water potential where it accumulates in order to avoid water loss by the plant (Hayat et al., 2012; Obata and Fernie, 2012; Liang et al., 2013). Besides, proline may act in different ways during stress establishment, such as chemical chaperone (Arakawa and Timasheff, 1985; Islam et al., 2009), heavy metal chelator (Kaul et al., 2008), and ROS quenching (Kaul et al., 2008). Since it is synthesized in chloroplasts and degraded in mitochondria, proline metabolism may be important to maintain the $NAD(P)^+/NAD(P)H$ ratio within these organelles maintaining the redox homeostasis of the cell (Liang et al., 2013).

Proline can be produced by proteins degradation, due to abiotic stress conditions as well as *de novo* synthesis (Hayat et al., 2012). During the *de novo* synthesis proline is synthesized by two major enzymes pyrroline-5-carboxylate synthase (P5CS) and pyrroline-5-carboxylate reductase (P5CR). P5CS has two isoforms being one found in the chloroplast and another in the cytosol. These enzymes are responsible for converting glutamate to glutamate-semialdehyde, which spontaneously releases a H_2O molecule and is converted to delta1-pyrroline-5-carboxylate. This intermediate is then converted by P5CR into proline. Proline synthesis can occur in the cytosol or chloroplasts and results in the oxidation of NADH and NADPH molecules. In addition, proline can be synthesized by the action of ornithine aminotransferase (OAT) in the mitochondria matrix (Liang et al., 2013; Cabassa-Hourton et al., 2016a).

Degradation of proline takes place in the mitochondrial matrix and it occurs through the enzymes proline dehydrogenase (ProDH) and pyrroline-5-carboxylate dehydrogenase (P5CDH). Proline dehydrogenase presents two isoforms to ProDH1 and 2 (Schertl et al., 2014). While ProDH 1 is mitochondrial and more abundant, ProDH 2 is almost non-expressed and found most frequently in vascular tissues (Schertl et al., 2014). Proline dehydrogenase converts proline to delta1-pyrroline-5-carboxylate, which is spontaneously converted to semialdehyde glutamate which is converted by P5CDH into glutamate (Schertl et al., 2014). The glutamate formed from the proline degradation can be used by different pathways: (i) it can be transported from the cytosol to the chloroplast where it is used in nitrogen assimilation; (ii) glutamate might remain in the mitochondrial matrix and then is metabolized via GABA shunt returning to the TCA cycle as succinate; (iii) additionally, still in the matrix, glutamate can be converted to 2OG by the action of glutamate dehydrogenase (GDH-NADH) and thus return to the TCA cycle to be converted into succinyl-CoA by 2OGDH (Fait et al., 2007a; Araújo et al., 2012b; Schertl et al., 2014). Proline degradation that takes place in the mitochondrial matrix, ultimately can result in an increase of ATP production (Hildebrandt et al., 2015; Batista-Silva et al., 2019). This can happen either by the production of reduced coenzymes in the mitochondrial matrix (NAD(P)H) (Hebbelmann et al., 2012; Caretto et al., 2015; Hildebrandt et al., 2015; Batista-Silva et al., 2019), by the release of electrons direct in the mitochondrial electrons transport chain (Cabassa-Hourton et al., 2016b; Launay et al., 2019) and also, by an increase in carbon skeletons used in the TCA cycle, once the final product of its degradation, glutamate, is converted into 2OG (Schertl and Braun, 2014a; Hildebrandt et al., 2015).

Recently, characterization of *A. thaliana* mutant plants with lower expression of the genes encoding the E1 subunit the 2-OGDH (At5g65750 and At3g55410) cultivated in optimal conditions, revealed that these genes have important roles in modulating the primary metabolism of plants and its development, and that the lack of these genes results in an impaired respiration and photosynthesis rate (Condori-Apfata et al., 2019). It was demonstrated also that the two different isoforms for the E1 subunit are not redundant playing different physiological roles in plant development (Condori-Apfata et al.; 2019). The lack of the gene e1-ogdh1 resulted in a significant reduction in the whole complex activity, smaller rosette area, smaller root, and in plants that took longer to produce inflorescences (Condori-Apfata et al., 2019). The mutant lines for the gene e1-ogdh2 displayed higher rosette area, dry weight and number of leaves, and the lack of this gene resulted in early production of inflorescences but had no effect over the activity of the whole complex (Condori-Apfata et al., 2019).

After all the aforementioned results, it is clear that the TCA cycle, and more importantly, the 2OGDH enzyme plays a regulatory role over the metabolism of plants. Here, we sought out to better understand what is the role of the E1 subunit of the 2OGDH enzyme during implementation and recovery of osmotic stress in shoots and roots of *A. thaliana*. We also aimed to answer questions: (i) is there any connection between the 2-OGDH enzyme and proline accumulation/degradation in tissues of *A. thaliana* undergoing osmotic stress and (ii) among the nitrogen and carbon metabolism, which one is favored during the osmotic stress imposition and release? Based on the results obtained in this study, we verified the importance of the E1 subunit of 2-OGDH for the metabolic alterations necessary for osmotic stress responses and during the recovery period.

MATERIAL AND METHODS

Plant material

In this study, T-DNA insertion lines for the genes encoding the two isoforms of the E1 subunit of the enzyme 2-OGDH, obtained from the Salk collection (Salk Institute for Biological Studies, La Jolla, USA) were used. For the *e1-ogdh1* gene (At3g55410), the line e1-ogdh1.2 (Salk_072343) was selected and for the *e1-ogdh2* gene (At5g65750), the line e1-ogdh2.2 (Salk_055824) was selected end the lines were characterized as described by Condori-Apfata et al. (2019). We also used a double mutant line obtained through the crossing of the two single lines, also previously described in the same study. Plants from homozygous knockout single lines and double mutant lines were used in the experiments. The gene expression levels of the target genes in the single and double mutant lines as well as the activity of the whole complex in the single mutant lines were previously described (Condori-Apfata et al. (2019).

Growth conditions and stress application

Seeds from *A. thaliana* (ecotype Columbia 0) and homozygous mutant lines were sterilized and let in the dark at 4ºC for four days. To the germination the seeds were transferred to a hydroponic system stablished by Monte-Bello et al. (2018), with half strength MS medium (Murashige and Skoog, 1962) without sucrose and 0.8% Agar. The seeds were germinated under neutral day conditions (12h light / 12h dark) with an irradiance of 150 µmol photons m^{-2} s^{-1}, 22°C in light and 20°C in dark, with a relative humidity of 60%. Fifteen days later, the seedlings were transferred to new hydroponic system tip boxes without the solid MS medium, only with the liquid MS medium (350 mL each box). The seedlings were grown for additional fifteen days under the same conditions where they were germinated. Around sixteen seedlings were used per tip box. Each hydroponic tip box contained plants of only one genotype and constituted one experimental unit.

When the plants were four weeks old, the osmotic stress treatment was applied. For that, the half strength MS solution without sucrose, was replaced with new medium added with polyethylene-glycol 6000 (PEG). The amount of PEG applied (202,13 $g.L^{-1}$), was determined following recommendations previously established by (Villela et al., 1991), to obtain a water potential of -0,5 MPa. The plants were kept under this condition for 48 hours and then, where transferred to new medium without PEG in order to recover for 96 hours.

The stress application and recovery started in the middle of the light period and all the harvests also took place in the middle of the light period. In total seven harvesting points were used (Figure 1). One before stress (control), two during the stress conditions, after 24 and 48 hours, then four during the recovery period, after 24, 48, 72 and 96 hours of recovery. Both shoots and roots samples were separately harvested in order to obtain enough fresh material. Each sample were formed by six independent plants. The material was snap frozen in liquid nitrogen, and kept in ultrafreezer until further analyses. Samples of two different rounds of experiments were evaluated all together.

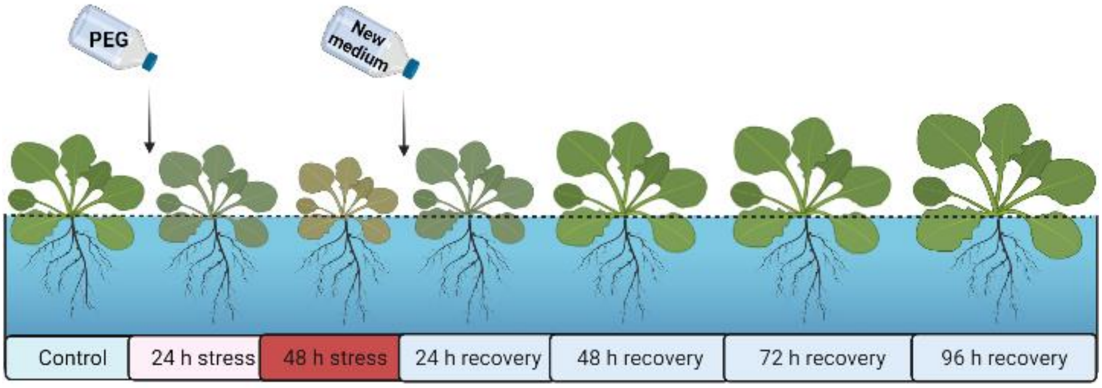

Figure 1: Representative diagram of the hydroponic system indicating the moment of application of the osmotic stress treatment with Polyethyleneglycol 6000 (PEG), and recovery (New medium) as well as the points of shoot and root harvest

Metabolite analysis

The levels of glucose, fructose, sucrose and starch in the tissues were determined as described by Fernie et al. (2001). The levels of malate and fumarate were determined as described previously by Nunes-Nesi et al. (2007). Proteins were measured according with (Bradford, 1976)and amino acids were quantified following protocol described by Gibon et al. (2004). Photosynthetic pigments were determined exactly as described before (Porra et al., 1989).

For metabolite profiling, samples from leaves and roots, harvested at the middle of the light period was used for the Chloroform/methanol extraction. And the analyze was performed by gas chromatography–mass spectrometry (GC–MS) according to the protocol described by Lisec et al. (2006). Peaks were manually annotated, and ion intensity was determined with the aid of TagFinder software (Luedemann et al., 2012), using a reference library from the Golm Metabolome Database (Kopka et al., 2005) and following the recommended reporting format for metabolic profiling (Fernie et al., 2011).

Root growth experiment

To access the effect of different proline concentrations on the root growth, the seeds were sterilized and kept in the dark at 4ºC for four days. After that, the seeds were transferred to round plates with half strength MS media without sucrose to germinate for five days under photoperiod of 12 h light / 12 h dark, with an irradiance of 150 µmol photons m^{-2} s^{-1}, 22°C in light and 20°C in dark, with a relative humidity of 60%. After this period, the seedlings were transferred to square plates (5 plants/genotype/plate) containing half strength MS media without sucrose, supplemented with different concentrations of proline (0, 50, 100, 250 and 500 uM). The plates were kept in the same growing conditions that they germinated and the root growth was measured each day for the following 15 days. After that, the plates were scanned and the total root growth was measured using the ImageJ program.

Establishment of Arabidopsis cell suspension and proline treatment

For the establishment of *A. thaliana* cell suspensions we followed protocol previously described (Cavalcanti et al., 2017) with minor modifications. Briefly, WT (Col-0) and double mutant (e1-ogdh1.2::2.2) seeds were surface sterilized and kept for four days at 4°C in the dark in microtubes with autoclaved deionized water for imbibition. After this period the seeds were transferred to plates containing half-strength MS medium (pH 5.7) and 0.8% (w/v) agar, and then germinated under long-day conditions (16 h light/8 h dark) at 100 µmol photons m^{-2} s^{-1}. After germination, ten days old seedlings were cut into small pieces (3 cm^2) and laid out on plates with gamborgs medium (B5 medium; Gamborg et al., 1968) containing (0.316% (w/v) B5 mixture (g5893, sigma-Aldrich), 0.75 g (w/v) agar, 3% sucrose (w/v), 0.0001% (w/v) 2,4-dichloropenoxyacetic acid and 0.00001% kinetin (w/v), pH 5.7 (KOH) and then stored in darkness for three weeks for callus induction. Afterwards, the calluses were transferred into liquid B5 medium (B5 medium without agarose) for establishing a cell suspension culture. Cells were cultivated in 250-mL flasks containing 100 mL of medium at 25°C and 90 rpm in the dark. The cell suspensions were transferred into fresh B5 medium every seven days.

For the respiration measurements with and without proline, we established two treatments, (i) B5 medium without sucrose, and (ii) B5 medium without sucrose plus 50 mM of proline. This proline concentration was chosen following the established by Schertl et al. (2014). Seven days old WT and double mutant cell suspensions were transferred into new flasks, containing fresh B5 medium without sucrose and to flasks with B5 medium without sucrose supplemented with 50 mM proline. The treatments ware applied 24 hours before the respiration measurements. The respiration measurements were performed as described below.

Respiration measurements

The respiration measurements for the roots were performed in an oxygen electrode (Hansatech, Kings Lynn, Norfolk, UK) containing 2 ml buffer solution (10 mM MES-KOH; 50 mM HEPES; 0,2 mM $CaCl_2$; 100 mM Sucrose and pH 6.5;) about 30 mg of fresh root tissue were harvested, sliced and used to the measurements. The root material was latter used to quantify the protein amount (Bradford, 1976).

The cell culture respiration measurements were performed in an oxygraph respirometer (Oroboros, Innsbruck, Austria) at 25°C. The respiration measurements were performed using a fresh B5 medium matching the treatment characteristic (without sucrose and without sucrose plus 50 mM proline). Each measurement took about 20 minutes to deplete the chambers oxygen, the first five minutes were used to stabilization and the next 10 were used to the measurements.

Statistical analysis

All the experiments were set up in a completely randomized design. For the osmotic stress and recovery experiments, each tip box contained 15 plants of each genotype, and constituted an experimental unit. At each harvesting point, *ca*. five plants were harvested to assemble one biological sample of the root and the shoot. We collected four replicates of each genotype in each harvest point. In the germination assay, five independent plates of each treatment were analyzed, and for the cell culture respiration three independent flasks of each treatment were analyzed with technical duplicates, for the root respiration measurements six biological samples (assembled with *ca*. six independent plants) were analyzed. Statistical analysis was performed with ANOVA, followed by Tukey test using a value of $p \leq 0.05$, performed with R 4.1.1 R Core team (2019). R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

RESULTS

In this work, we set the goal to understand the importance of the E1 subunit of the 2-OGDH enzyme in plants of arabidopsis under periods of osmotic stress imposition and recovery, and evaluate the association between this enzyme and the proline, carbon and nitrogen metabolisms. We perform a set of experiments and used the harvested material to perform different metabolic analyzes and here we describe our major results.

The lack of 2OGDH E1 subunit and the growth-related responses to osmotic stress and recovery

We used the stress symptoms on rosette area as a visible way to evaluate the stress perception and impact in the studied genotypes throughout the osmotic stress and recovery experiment. We observed that the lack of the E1 subunit led to plants more sensitive to the stress (Figure 2A). Both single and double mutant plants suffered a significant reduction in the rosette area after 24 hours of stress when compared with the WT plants. While all genotypes were able to recover from the period of stress, the

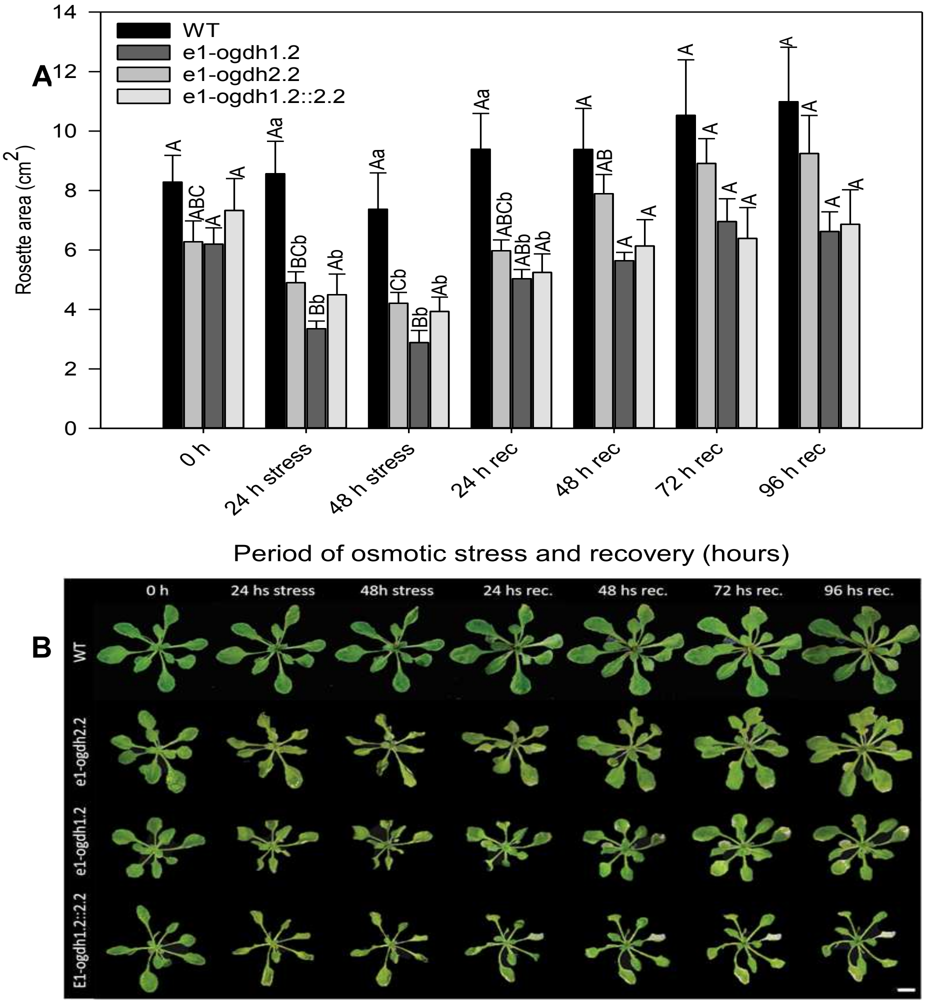

Figure 2: Effect of the lack of the E1 subunit of the enzyme 2-OGDH on the rosette area (A) and shoot phenotype (B) of wild type (WT), single mutants (e1-ogdh1.2 and e1-ogdh2.2) and double mutant arabidopsis plants (e1-ogdh1.2::2.2) during the period of osmotic stress and recovery. Values are means (± standard error). Capital letters compare the same genotype in the different points of stress and recovery throughout the experiment. Small letters compares the different genotypes in the same point of stress or recovery. Means followed by different differs significantly from each other as determined by Tukey test ($p \leq 0.05$, n=4). White bar= 1cm

mutant lines took longer, with significant differences even after 24 hours of recovery (Figure 2A and B).

The lack of the of 2OGDH E1 subunit and the responses on carbon metabolism to osmotic stress and recovery

We also analyzed the impact of osmotic stress and recovery in the carbon metabolism of plants deficient in the expression of isoforms of E1 subunit of 2-OGDH by quantifying the levels of starch and the three major soluble sugars (glucose, fructose and sucrose). The levels of starch were significantly reduced in all tissues and genotypes (Figure 3A-B). In roots, we observed reduced amount of starch in the mutant lines and the e1-ogdh2.2 was the only mutant line with significantly lower levels when compared to the WT (Figure 3C).

All analyzed sugars (except for fructose in the root, Figure 4F) accumulated significantly during the period of osmotic stress in both shoots and roots and then reduced during the recovery period (Figure 4). Although the answer to the osmotic stress treatment was similar among our genotypes, the patterns of accumulation was different.

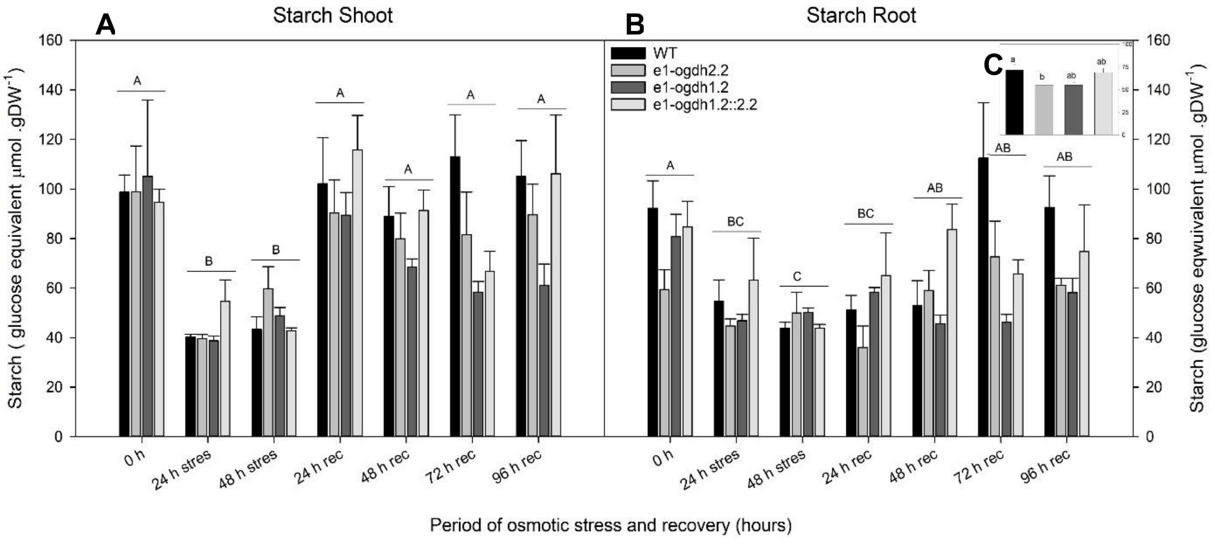

Figure 3: Effect of the lack of the E1 subunit of the enzyme 2-OGDH on the starch levels of the shoot (A) and root (B) of wild type (WT), single mutant (e1-ogdh1.2 and e1-ogdh2.2) and double mutant arabidopsis plants (e1-ogdh1.2::2.2) during the period of osmotic stress and recovery. The detail (C) highlights the effect of the mutation alone over the starch levels in the roots. Values are means (± standard error). Capital letters compare the effect of the stress and recovery throughout the experiment when there was no interaction between the genotype and the stress conditions. Small letters compares the different genotypes. Means followed by different letters differs significantly from each other as determined by Tukey test ($p \leq 0.05$, n=16 for A and B, and 28 for C).

The highest levels of sucrose and glucose were reached in shoots after 48 hours of stress (Figure 4A-C). The highest levels of sucrose in the roots happened afte hours of stress (Figure 4B). After 48 hours of stress, the levels of sucrose were accumulated 9, 8, 6 and 5 times in the shoot (Figure 4A), and 2.1, 1.6, 1.6 and 1.2 times in the roots (WT, e1-ogdh2.2, e1-ogdh1.2, and e1-ogdh1.2::2.2 respectively, Figure 4B). In the roots, after 24 hours of stress an accumulation of 1.9, 2.1, 2.2, and 1.9 folds of sucrose were observed (Figure 4B). Also in the roots, we observed lower global levels of sucrose in the mutant lines being significant lower to the single mutant line for the gene e1-ogdh1 (Figure 4G)

All mutant lines showed significantly lower levels of glucose than the WT plants after 48 hours of stress in the shoots (Figure 4C). At this point, when compared with the control period, the WT plants exhibited an increase of 18 fold in the levels of glucose against 8, 4 and 8 fold for the mutant lines (e1-ogdh 2.2, e1-ogdh1.2 and e1-ogdh1.2::2.2 respectively, Figure 4C). In addition, we observed significant differences between the glucose levels in the shoots after 48 hours of recovery (e1-ogdh2.2 significantly lower than the double mutant, Figure 4C), and 96 hours of recovery (e1-ogdh1.2 significantly lower than WT, Figure 4C). The glucose levels in the roots were significantly higher than the WT for the single mutant line for the gene e1-ogdh1 under control conditions (Figure 4D). The higher levels of glucose in the roots occurred after 24 hours of stress (Figure 4D). We also observed differences between the double mutant line and the WT after 72hs of recovery (Figure 4D)

The highest levels of fructose in shoots were observed after 24 hours of stress (Figure 4E). At this time point, the double mutant line displayed the lowest levels of fructose among all the genotypes and concomitantly, the lowest increase when compared with the 0 hours of stress (4 fold against 6 for WT and e1-ogdh2.2 and 5 fold for e1-ogdh1.2, Figure 4E). Interestingly, after 48 hours of stress, while the double mutant line kept its higher fructose levels, the other genotypes went through a reduction, and the e1-ogdh1.2 line displayed significantly lower fructose contents at this point (Figure 4E). In the roots, the levels of fructose were reduced during water deficit period and remained low during the recovery (Figure 4F).

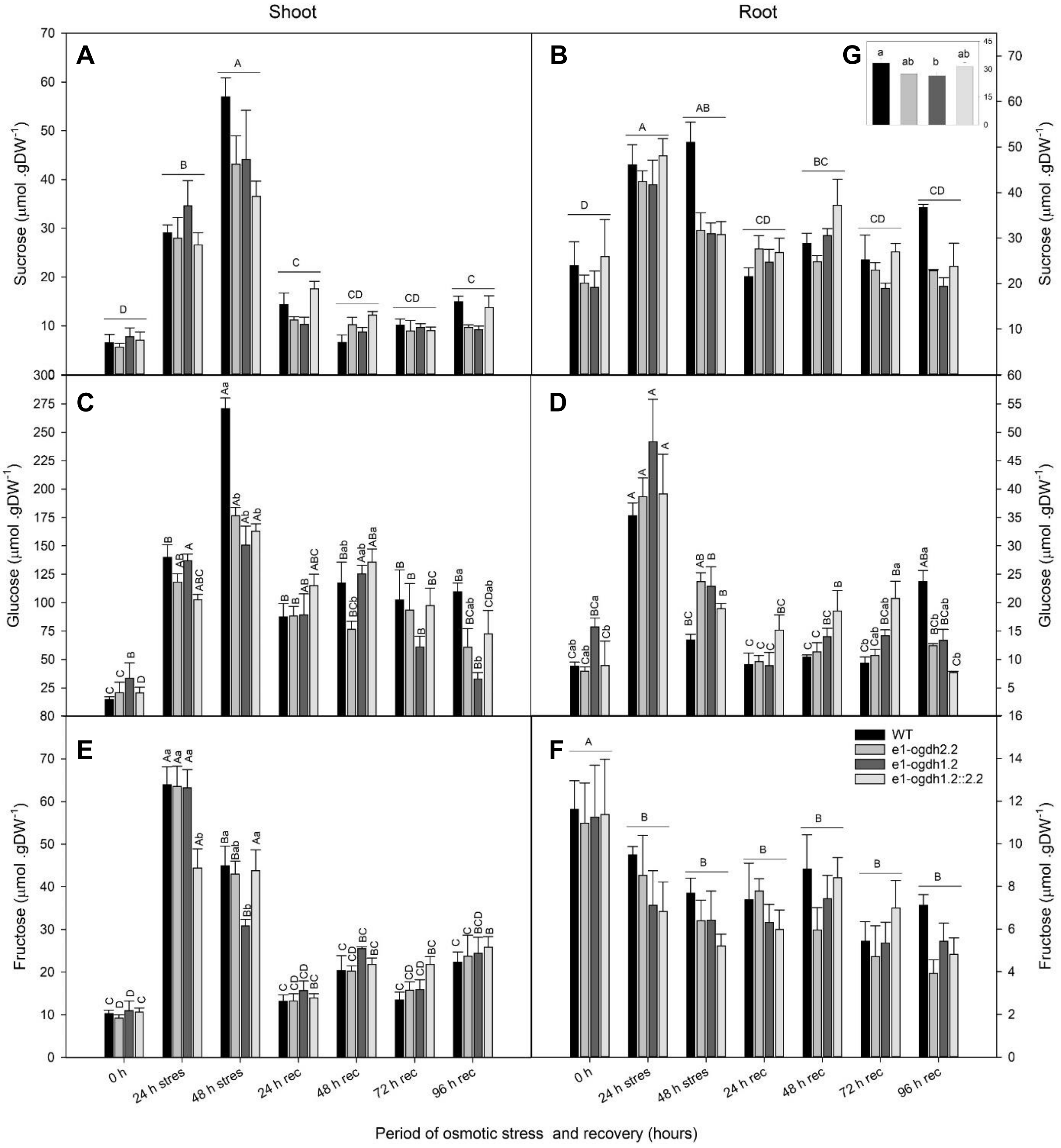

Figure 4: Effect of the lack of the E1 subunit of the enzyme 2-OGDH on the levels of sucrose, glucose and fructose in the shoot (A, C and E) and root (B, D, F and G) of wild type (WT), single mutant (e1-ogdh1.2 and e1-ogdh2.2) and double mutant arabidopsis plants (e1-ogdh1.2::2.2) harvested in the middle od the light period, during imposition of osmotic stress and recovery. The detail (G) highlights the effect of the mutation alone over the sucrose levels in the roots. Values are means (± standard error). Capital letters on A, B and F compare the effect of the stress and recovery throughout the experiment when there was no interaction between the genotype and the stress. Small letters on G, compares the different genotypes. Capital letters on C, D and E, compares the same genotype in the different points of stress and recovery throughout the experiment while the small letters compares the different genotypes in the same point of stress or recovery. Means followed by different letters differs significantly from each other as determined by Tukey test ($p \leq 0.05$, n=4 for C, D and E, 16 for A, B and F, and 28 for G).

The lack of the of 2OGDH E1 subunit and the responses on nitrogen metab to osmotic stress and recovery

To access the impact of the mutation E1 subunit of 2-OGDH isoforms in response to the osmotic stress and also during the recovery period over the nitrogen metabolism, we analyzed the levels of chlorophylls, free amino acids and proteins. The levels of chlorophyll *a* and *b* were reduced during the stress period and were not recovered after the 96 hours recovery period (Figure 5A-B). We did not observe interaction between genotypes and the stress treatment but, globally, the double mutant line (e1-ogdh1.2::2.2) showed significantly lower levels of these pigments (Figure 5C-D). During the experiment, both shoots and roots displayed a reduction on their protein levels (Figure 6A-B). In shoot tissues, no significant interaction between genotypes and treatments were observed, however, we spotted a significant reduction for all genotypes after 48 hours of stress (Figure 6A). Also in the shoots, the protein levels throughout the experiment for the double mutant line and single mutant line e1-ogdh1.2 was significantly lower than the WT (Figure 6G).

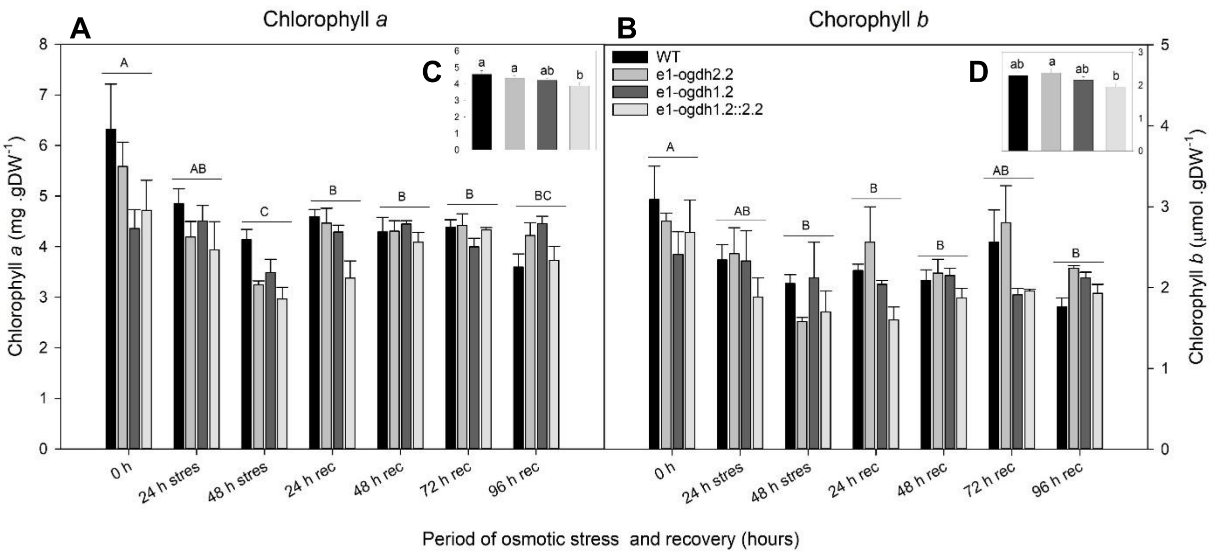

Figure 5: Effect of the lack of the E1 subunit of the enzyme 2-OGDH on the chlorophyll *a* (A) and *b* (B) of wild type (WT), single mutant (e1-ogdh1.2 and e1-ogdh2.2) and double mutant arabidopsis plants (e1-ogdh1.2::2.2) harvested during in the middle of the light period, during imposition of osmotic stress and recovery. The details (C and D) highlight the effect of the mutation alone over the chlorophyll *a* and *b*. Values are means (± standard error). Capital letters compares the effect of the stress and recovery throughout the experiment when there was no interaction between the genotype and the stress conditions. Small letters compare the different genotypes. Means followed by different letters differs significantly from each other as determined by Tukey test ($p \leq 0.05$, n=16 for A and B, and

In root tissues, we observed a drastic reduction in the protein levels for the WT after 24 hours of osmotic stress (70%) (Figure 6B). After 24 hours, WT and the single mutant lines for E1-OGDH2.2 and 1.2 reached the lower levels of protein (Figure 6B). The single mutant lines, kept these lower levels after 48 hours of stress while the WT plants showed a significant increase, reaching levels only 46% lower than the control protein levels (Figure 6B). The double mutant roots followed a different pattern, exhibiting a reduction on the protein levels of 30% with 24 hours and reaching its lower level after 48 hours of stress (a reduction of 48%, Figure 6B). During the rec period, both shoots and roots displayed recovery of the protein levels. The mutant lines displayed an average of 99% of recovery, against 85% for WT in the shoots (Figure 6A). In the roots, an average of recovery of 100% for mutant lines and 75% for WT was observed (Figure 6B).

The total free amino acid levels also were altered throughout the experiment for all the genotypes. We did not observe significant interaction between genotypes and the period of osmotic stress or recovery (Figure 6C-D). Both shoots and roots suffered elevation of its pool of total free amino acids levels. While the shoots reached its maximum level after 24 hours of stress and maintained these high levels until 48 hours of recovery (Figure 6C). The roots only reached the maximum levels of free amino acids with 24 hours of recovery and displayed the lowest levels after 96 hours (Figure 6D). The plants from double mutant line exhibited higher levels of free amino acids then the other genotypes in the roots (Figure 6H).

As already mentioned here, proline is usually accumulated during periods of abiotic stresses that leads to water scarcity. Because of that, proline can be used as a marker of the level of stress sensed by the plants. All genotypes accumulate proline following the same pattern for both shoots and roots but the root levels were around 50% smaller than the one found in the shoots (Figure 6E-F). During the recovery, while the WT and single mutant lines displayed a significant reduction in the proline levels after 24 hours, the double mutant line still exhibited higher levels of this amino acid both in the shoot as in the roots (Figure 6E-F). After 24 hours of recovery, the roots of the double mutant line showed a reduction in the levels of proline, although, this reduction was smaller than the other genotypes (40 % against, 72 % of the WT and 61 % of e1-ogdh2.2 and 1.2; Figure 6F).

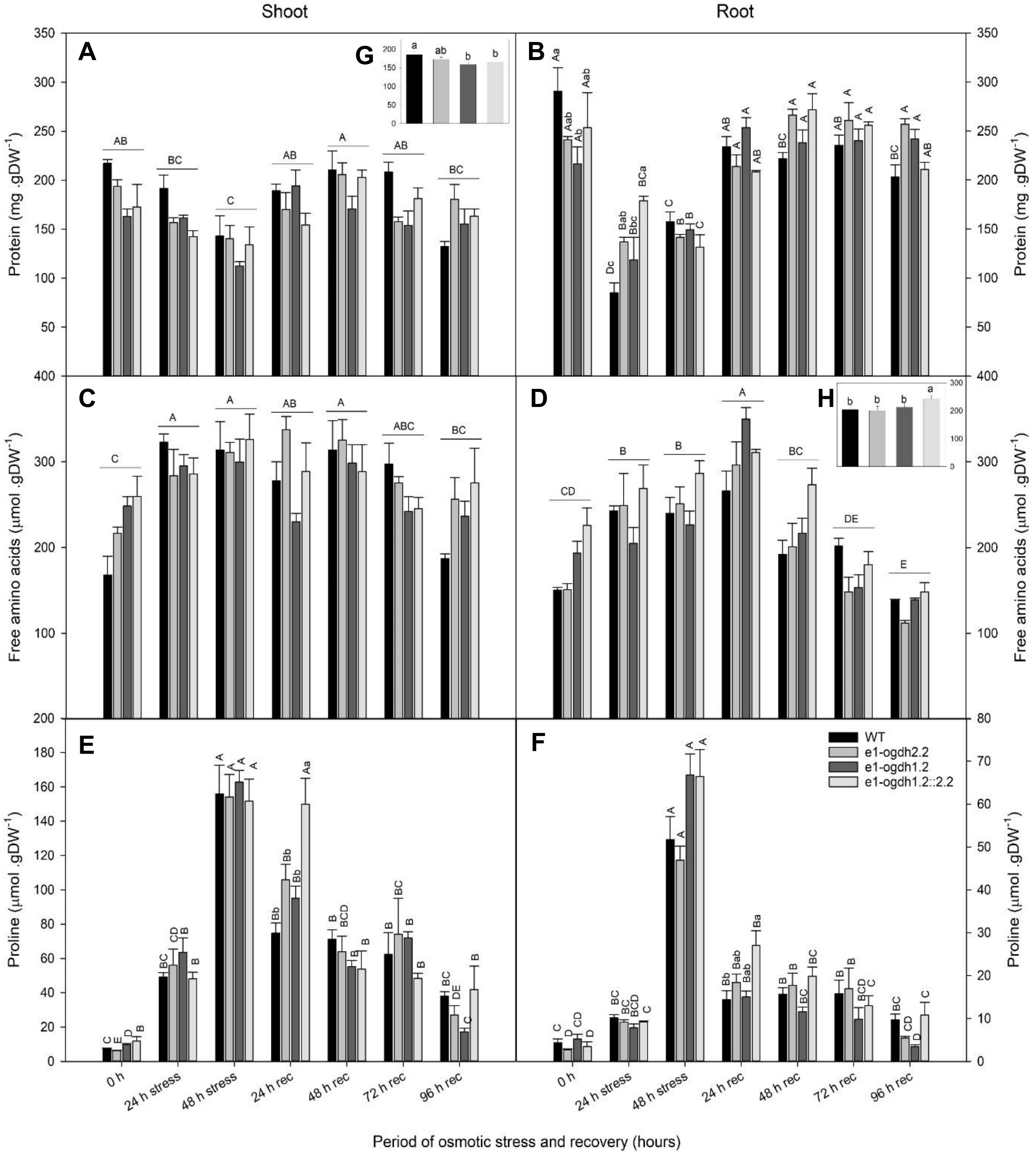

Figure 6: Effect of the lack of the E1 subunit of the enzyme 2-OGDH on the levels of protein, free amino acids and proline in the shoot (A, C, E and G) and root (B, D, F and H) of wild type (WT), single mutant (e1-ogdh1.2 and e1-ogdh2.2) and double mutant arabidopsis plants (e1-ogdh1.2::2.2) harvested in the middle of the light period, during imposition of osmotic stress and recovery. The details (G and H) highlight the effect of the mutation alone on the proteins and free amino acids levels of the shoot and roots respectively). Values are means (± standard error). The capital letters in A, C and D, compares the effect of the stress and recovery throughout the experiment when there was no interaction between the genotype and the stress conditions. Capital letters in B, E and F, compares the same genotype in the different points of stress and recovery throughout the experiment while the small letters compares the different genotypes in the same point of stress or recovery. The small letters in G and H compares the different genotypes disregarding the stress treatment. Means followed by different letters differs significantly from each other as determined by Tukey test ($p \leq 0.05$, n=16 for A, C and D and 16 for B, E and F and 28 for G and H).

The lack of the 2OGDH E1 subunit and responses to osmotic stress and recovery on the metabolite profile

In order to improve our understanding over the role of the E1 subunit of the 2-OGDH enzyme during establishment and recovery from osmotic stress we further analyzed the metabolite profile of shoots and roots tissues from all four genotypes. The first major observation was that the lack of the E1 subunit led to greater impacts in the root then in the shoot metabolism (Figure 7A-B). We were able to analyze de levels of 21 amino acids in the shoots, of which 11 reduced during the period of stress while 10 increased, with no significant differences between genotypes. We were also able to analyze the levels of 15 organic acids in the shoot of which eight accumulated during the stress and seven were reduced. In the same period, in the shoots, we observed significant difference for the levels of trans-caffeic acid in both 24 and 48 hours of stress (Figure 7A).

In the shoots, all detected sugars accumulated during the stress period, but the only ones affected by the lack of expression of the E1-OGDH isoforms were raffinose, fucose and galactinol (Figure 7A). When compared with the WT, the raffinose levels were lower in the single mutant line for *e1-ogdh1.2* gene after 24 hours, and for the double mutant line (e1-ogdh1.2::2.2) after 48 hours. The levels of fucose were lower in the double mutant line after 48 hours of stress. The levels of galactinol were lower after 48 hours of stress for the single line e1-ogdh2.2 and the double mutant line (Figure 7A).

In shoots, during the recovery period, while six amino acids accumulated, 15 were decreased (Figure 7A). Although not affected by the lack of the E1 subunit of the 2OGDH, the levels of GABA were influenced by the stress and showed significant accumulation during the recovery (Supplemental table 2). After 96 hours the homoserine levels were lower in the double mutant line when compared with the other genotypes (Figure 10). The organic acids in the shoots were also not much affected by the lack of the E1 subunit of the 2OGDH complex (Figure 7A). After 96 hours of recovery the levels of succinate were lower for the single mutant for *e1-ogdh1.2* gene and for the double mutant line e1-ogdh1.2::2.2. After 72 hours of recovery the levels of shikimate were higher in the double mutant lines and after 96 hours of recovery the

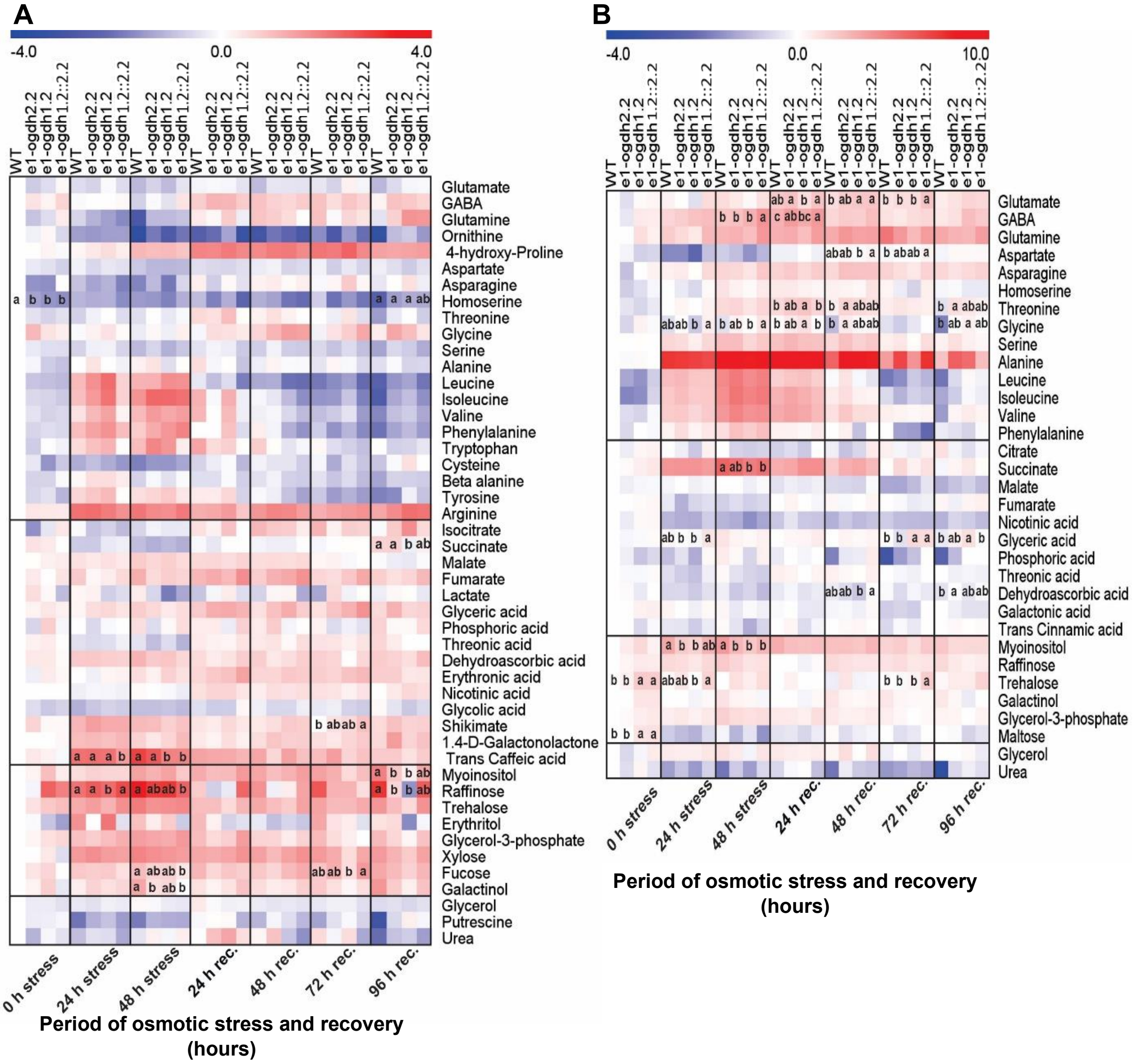

Figure 7: Effect of the lack of the E1 subunit of the enzyme 2-OGDH on the metabolic profile of the shoot (A), and roots (B) of wild type (WT), single mutant (e1-ogdh1.2 and e1-ogdh2.2) and double mutant arabidopsis plants (e1-ogdh1.2::2.2) harvested in the middle of the light period, during imposition of osmotic stress and recovery. Values are relative $\log_2$ transformed values of signal intensities that were normalized with respect to the mean response calculated for the WT in the 0 hours of stress. Blue means reduction, and red means accumulation. Small letters compare the different genotypes in the same point of stress or recovery (only significant interactions are shown). Means followed by different letters differs significantly from each other as determined by Tukey test ($p \leq 0.05$, n=4).

levels of myo-inositol and raffinose were lower for the two single lines e1-odgh1.2 and 2.2, when compared to the WT level (Figure 7).

In the roots we were able to analyze the levels of 14 amino acids of which 13 accumulated during the stress period against one that was reduced (aspartate; Figure

7B). During the stress period, we observed that the lack of the E1 subunit of the 2-OGDH exerted effect on glycine after 24 and 48hours, and GABA after 48 hours. The levels of these two amino acids at this points were higher for the double mutant lines than the WT (Figure 7B). Over the organic acids, during the stress period, nine were reduced and two accumulated. After 48 hours of osmotic stress, an increase on the levels of succinate was observed (Figure 7B). The levels of succinate at this point was lower for the single mutant line for the e1-ogdh1.2 and double mutant line (e1-ogdh1.2::2.2). After 24 hours of stress the levels of glyceric acid higher in the double mutants. For the sugars, among the seven different ones we were able to analyze in the roots, six accumulated while one, was reduced (maltose, Figure 7B). We observed effect of the mutation over the levels of trehalose and maltose in the control conditions, both sugar levels at 0 hours of stress were higher in the double mutant line and for the single mutant line for the gene e1-ogdh1. After 24 hours of stress the levels of myo-inositol and trehalose were also affected. The single mutant lines (e1-ohdh1.2 and 2.2) displayed the lower levels of myo-inositol. For trehalose after 24 hours of stress, the single mutant line for the gene e1-ogdh1 showed the lowest level while the double mutant line showed the highest ones among the genotypes (Figure 7B). After 48 hours of stress, all the mutant lines, both single and double mutant, showed lower levels of myoinositol when compared with the WT plants (Figure 7B).

During the recovery period, we were able to spot significant differences caused by the lack of the E1 subunit of the 2-OGDH enzyme on the amino acids profile. The levels of glutamate were kept elevated during the whole recovery (Figure 7B). The levels of this amino acid were higher for the double mutant and single mutant for the gene e1-ogdh2 after 24 hours of recovery and for the double mutant and the single mutant line for the gene e1-ogdh1 after 48 hours of recovery. After 72 hours of recovery the levels of glutamate were higher only in the double mutant line (Figure 7B). After 24 hours of recovery, the double mutant plants displayed the higher levels of GABA while the WT showed the lowest levels among the genotypes. The double mutant plants also presented the highest levels of aspartate after 48 and 72 hours of recovery.

In the roots the levels of threonine were also affected during the recovery. After 24 hours, the single mutant line for the gene e1-ogdh1 showed the highest levels of this amino acid (Figure 7B). After 48 and 96 hours of recovery, the highest levels of threonine were observed in the single mutant for the gene e1-ogdh2 and the lower in the WT plants (Figure 7B). The levels of glycine were accumulated after 24 hours of recovery and the highest values were found in the single mutant line for the gene e1-ogdh1 (Figure 7B). After 48 hours of recovery while the WT had the lowest levels of glycine, the highest values were found in the single mutant line for the gene e1-ogdh2 (Figure 7B). After 96 hours of recovery, the highest values of glycine were displayed by the single mutant line for the gene e1-ogdh1 and the lowest levels, again, were found in the WT (Figure 7B).

During the recovery period, the majority of the organic acids analyzed in the roots, either had their levels returned to the values close to the control condition or reduced. Succinate was the only organic acid analyzed in the root that displayed increase in their levels during the first 48 hours of recovery, but this alteration happened to all four genotypes. After 72 hours of recovery, the double mutant and single mutant for gene e1-ogdh1 displayed the highest values of glycerate (Figure 7B). After 96 hours the highest value for glycerate was found in the single mutant line for the gene e1-ogdh1.

The levels of dehydroascorbate in the roots were mainly reduced during the recovery (Figure 7B). After 48 hours, the highest levels were found in the double mutant and the lowest in the single mutant line for the gene e1-ogdh1 (Figure 7B). After 96 hours the highest level of dehydroascorbate was found in the single mutant for the gene e1-ogdh2 (Figure 7B).

During the recovery period, the sugars in the roots either returned to values close to control or increased (Figure 7B). The only significant difference was observed for the trehalose after 72 hours of recovery. The levels of this sugar was higher for the double mutant line (Figure 7B).

Effect of proline over the root growth and respiration of plants lacking the of the 2OGDH E1 subunit

As aforementioned, the proline degradation takes place in the mitochondria matrix and it can result in an increase of 2OG in this organelle (Szabados and Savouré, 2010; Hildebrandt, 2018). The double mutant line, was the only genotype that displayed altered pattern of reduction in the proline levels during the first 24 hours of recovery (Figure 6E-F). These results led us to believe that the complete lack of E1 subunit might be linked with an inhibition of proline degradation.

In order to access the possible connection between the 2OGDH enzyme and the proline metabolism we performed a set of experiments using this amino acid as an energy source for the plants (Figure 8C). We started with a root growth experiment (Figure 8A). This experiment confirmed the phenotype previously described for the root growth of the single mutant lines, smaller to e1-ogdh1.2 and not affected in the e1-ogdh2.2 (Figure 8A; 0 uM) (Condori-Apfata et al., 2019). The root growth assay, also revealed that the double mutant plants (e1-ogdh2.2::1.2) displays a strong phenotype under control conditions with significantly smaller roots than the WT plants (Figure 8A; 0 uM).

The root growth experiment, also allowed us to observe that increasing proline concentrations (50, 100 and 250 uM) are capable of revert the roots phenotype observed for the single mutant line for the gene e1-ogdh1 (Figure 8A) and double mutant line (e1-ogdh1.2::2.2; Figure 8A). There was no difference in the root growth of the single mutant line for the gene e1-ogdh1 and the double mutant, between 250 and 500 uM of proline (Figure 8A). While the single mutant line for gene e1-ogdh2 did not respond to any proline concentration tested (e1-ogdh2.2, Figure 8A), the WT only responded to the highest concentration used (500 uM, Figure 8A).

The lack of the E1 subunit of the 2OGDH enzyme, and its inhibition are usually described together with reduction in plant size and respiration (Araújo et al., 2012c; Condori-Apfata et al., 2019). The smaller roots observed in the single mutant lines for

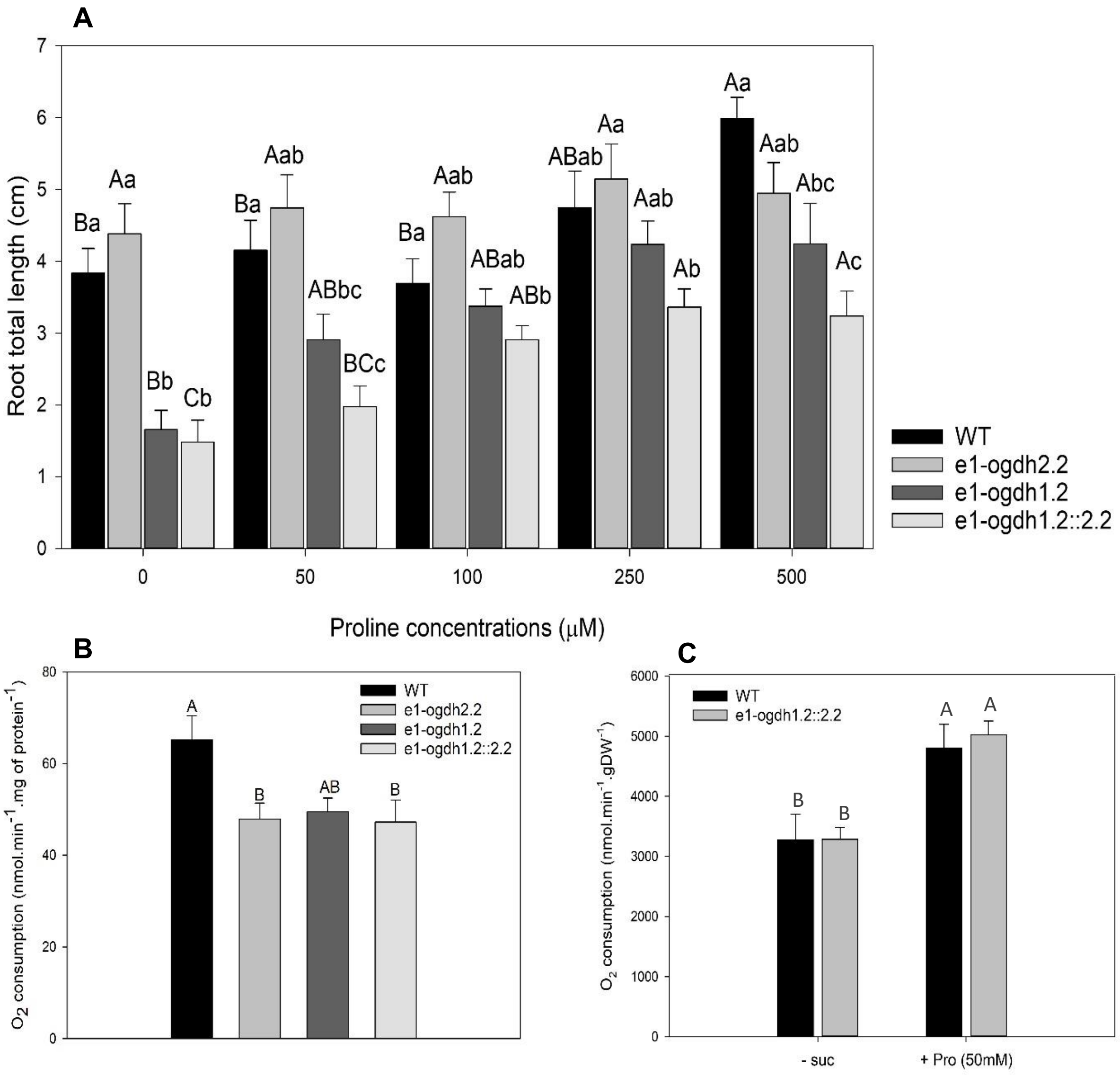

Figure 8: Effect of different proline concentrations over the root growth (A) and respiration of the roots (B) of single mutant (e1-ogdh1.2 and e1-ogdh2.2) and double mutant arabidopsis plants (e1-ogdh1.2::2.2). Respiration. Effect of proline (50 mM) over the cell culture respiration of wild type (WT) and double mutant arabidopsis plants (e1-ogdh1.2::2.2) lacking both genes of the e1 subunit of the 2-OGDH enzyme (C). Values are means (± standard error). The capital letters in “A” compares the same genotype response to the different proline concentrations while the small letters compares the different genotypes responses to the same proline concentration. For both A, B and C, means followed by different letters differs significantly from each other as determined by Tukey test ($p \leq 0.05$, n=4 for A and 6 for B and C).

gene e1-ogdh1 and double mutant plants led us to question if this phenotype could be happening due to an also smaller, root respiration rate. The roots respiration experiment, revealed that both the single and double mutant lines, show reduced respiration rate when compared to the WT roots (Figure 8B) what might help to explain the lower growth observed in the mutant plants.

Given that the increased concentrations of proline were able to revert the smaller roots phenotype (Figure 8A), we wondered if this amino acid could also revert the lower respiration rate we found for the mutant plants (Figure 8B). To answer this question, we used cell suspensions of WT and the double mutant (e1-ogdh1.2::2,2).

We selected the double mutant for the E1 subunit of the 2OGDH enzyme, once it was the only genotype that displayed signs of struggle to reduce the levels of proline during the first 24 hours of recovery from osmotic stress. The respiration assay, revealed that both genotypes used (WT and e1-ogdh1.2::2.2) are capable of metabolize proline and also that this process increase the respiration in a significant amount for both genotypes (Figure 8C).

DISCUSSION

In this work we aimed the understanding of the importance of the E1 subunit of the 2OGDH enzyme during periods of osmotic stress and recovery from this condition. We hypothesized that the lack of E1 subunit of the 2OGDH complex alters the degradation of proline accumulated during the stress in the recovery period. Furthermore, we evaluated if carbon and nitrogen metabolisms would be altered by the lack of this enzyme, under the same conditions. Although the importance of this enzyme has already been described in autotrophic (Araújo et al., 2012c; Araújo et al., 2012b; Condori-Apfata et al., 2019; Condori-Apfata et al., 2021) and heterotrophic tissues (Araújo et al., 2008) under optimal growth conditions, here, we report its role in a whole plant level and under an abiotic stress condition. We observed that while the shoot tissues are able to cope with the lack of the E1 subunit during periods of stress establishment and recovery, the greatest impact is observed on root metabolism. In the following sections we discuss the main metabolic implications of the lack of the E1 subunit of the 2OGDG enzyme during the establishment of osmotic stress and the recovery period.

The lack of the E1 subunit of the 2OGDH complex results in higher sensitivity to osmotic stress

Reduction in growth is described as one of the first responses in periods of abiotic stress that limit water availability (Hummel et al., 2010b; Muller et al., 2011).

The lack of the E1 subunit of the 2OGDH complex in plants exposed to osmotic stress conditions led to a significant reduction in rosette area when compared to WT plants (Figure 2A-B). This result suggest that the E1 subunit is linked to phenotypes more sensitive to osmotic stress. This growth reduction occurs while the photosynthesis is still active, and this might explain the sugar accumulation in response to abiotic stresses (Hummel et al., 2010b; Muller et al., 2011; Pantin et al., 2013). The lower accumulation of sugars (Figure 4C and E, and figure 7A), in the plants lacking the E1 subunit might be related with the higher sensitivity observed for these plants. In addition, the low accumulation of sugar in the mutant plants might be explained by the lower photosynthesis described for plants lacking the E1 subunit of the 2OGDH enzyme (Condori-Apfata et al., 2019). Therefore, while the sugar accumulation still happens, this mechanism is less intense in the mutant lines, especially in the double mutants and leads to higher stress sensitivity.

The lack of the E1 subunit of the 2OGDH is efficiently bypassed in autotrophic tissues during periods of osmotic stress and recovery

Our results clearly indicate that the lack of the E1 subunit of the 2OGDH complex does not lead to remarkable alterations in the metabolites of shoot tissues during periods of osmotic stress and recovery. Despite the importance assigned for this enzyme over the respiration, photosynthesis, nitrogen metabolism and senescence in optimal conditions (Araújo et al., 2012b; Araújo et al., 2012c; Condori-Apfata et al., 2019; Condori-Apfata et al., 2021), the impairments caused by the lack of the subunit E1 are somehow bypassed by the plant metabolism in autotrophic tissues in response to stress and recovery situations.

Although most of the changes in metabolite levels were not affected by the mutation in the E1 subunit of the 2OGDH complex, several stress responses were observed in the shoot tissues. The reduced levels of chlorophyll *a* and *b* (Figure 5A and B) together with the lower levels of starch in the shoots (Figure 3A) during the stress period, indicate an adaptation and reduction in the photosynthetic process (Chaves et al., 2002; Chaves et al., 2009; Hummel et al., 2010). The reduction in the level of proteins (Figure 6A) and the accumulation of amino acids (Figure 6B) corroborates with the reduction in growth already described here (Hummel et al., 2010b; Muller et al., 2011) and also with a remobilization of nitrogen in response to stress (Hildebrandt, 2018; Batista-Silva et al., 2019; Heinemann et al., 2021).

In response to stress, several amino acids had their levels altered (Figure 7A). The reduction in ornithine, (Figure 7A, supplemental table 2), might be linked to the accumulation of arginine (Funck et al., 2008; Anwar et al., 2015). In plants, arginine is a precursor of nitric oxide, an important molecule that acts in signaling and ROS scavenging during periods of osmotic stress (Arasimowicz and Floryszak-Wieczorek, 2007; Funck et al., 2008; Alavi et al., 2014; Planchais et al., 2014; Anwar et al., 2015). During periods of stress, some alternative pathways of energy may be activated in order to balance the ATP generation in the mitochondria and among then we can cite the ETF/ETFQO pathway (Araújo et al., 2011b). This pathway is responsible for the degradation of branch chain amino acids (Araújo et al., 2011b), and the significant accumulation in the levels of isoleucine, leucine and valine (Figure 7A, and Supplementary table 2) usually are described as indicative of its activation (Pires et al., 2016; Batista-Silva et al., 2019).

In the stress period, the alteration in the levels of organic acids in the shoots, indicate an accumulation of malate and fumarate (Figure 7A and Supplementary table 2). These organic acids play important roles during periods of stress once they are related not only to the respiration (Araújo et al., 2012a), but also in the redox metabolisms (Zhao et al., 2020b) and the stomatal control (Atkin and Macherel, 2009; Daloso et al., 2017; Zhao et al., 2018; Lawson and Matthews, 2020; Zhao et al., 2020a; Zhao et al., 2020b).

During recovery, after 24 and 48 hours of, the increase in protein (Figure 6A), the reduction of free amino acids (Figure 6C), sugars (Figure 4A,C and E) and increase of asparagine and glutamine (Figure 7A and supplementary table 2), may indicate the restart of growth (Figure 2), and the reboot of nitrogen metabolism once asparagine and glutamine are the main amino acids used for nitrogen transport (Hildebrandt et al., 2015; Gaufichon et al., 2016).

During recovery, the accumulation of 4-hydroxyproline in the shoots (Figure 7A) may indicate that part of the proline accumulated during stress is not being all degraded but yet, reincorporated into the growth metabolism, once 4-hydroxyproline is described as a component of the cell wall and important for its synthesis, expansion and adaptation to periods of stress and recovery (Kishor et al., 2015). The accumulation of GABA observed in the in the shoots during recovery (Figure 7A and supplementary table 2) allows us to infer that part of the proline is being degraded and being reinserted in the TCA cycle through GABA shunt (Fait et al., 2007a; Hildebrandt et al., 2015; Hildebrandt, 2018). Revealing that this pathway has more importance during the recovery than during the stress imposition.

The lack of the E1 subunit of the 2OGDH complex alter root metabolism of under osmotic stress conditions

The metabolite profile of plants lacking the E1 subunit of the 2OGDH enzyme, revealed a greater impact on the metabolism of roots. We also observed that under stress conditions, the root metabolism is amended in order to favor the supply of TCA cycle intermediates. When exposed to stress conditions that lead the plant to water scarcity, the root tissues usually keeps growing (Hsiao and Xu, 2000; Ghosh and Xu, 2014; Kim et al., 2020) to escape the stress condition in the search for water (Kim et al., 2020). In this tissue, after 24 hours of osmotic stress, we observed an accumulation of glucose and sucrose (Figure 4B-D), and a reduction in fructose levels (Figure 4F). The fructose probably continued to be used as a substrate in glycolysis given its importance to roots metabolism under stress conditions (Valifard et al., 2021). After 48 hours of stress, the levels of sucrose and glucose decrease along with fructose (Figure 4B, D and F). This sugar oxidation might lead to a sudden increased flow into the respiratory process that may elevate the 2OG levels in the mitochondrial matrix. Thus explaining the increase in the GABA levels after 48 hours of stress, increase that was significantly higher to the double mutant line (e1-ogdh1.2::2.2, Figure 7B). This increase in GABA for all genotypes in the root may be linked to the lack of the E1 subunit, and may also have been exacerbated by the inhibition of the E2 subunit of the 2OGDH activity by the presence of lipid catabolism intermediates, such as 4-hydroxy-2-nonena (HNE) (Millar and Leaver, 2000; Taylor et al., 2002; Taylor et al., 2009). Interestingly the presence of HNE is also related to an inhibition of the Alternative oxidase (AOX) (Winger et al., 2005) and an activation of the mitochondrial Uncoupling protein (UCP). In this setting, the mitochondrial electron transport chain would keep a consumption of reduced coenzymes allowing the function of the TCA cycle dehydrogenases during the period of stress.

During the recovery, after 24 hours, the proline accumulated in the root during the stress, started to be degraded (Figure 6F). The proline degradation in the roots can be related to the increase of glutamate that occurred for all genotypes (Figure 7B) (Launay et al., 2019). This increase in root glutamate was not uniform, being higher to the mutant lines (Figure 7B) and it was accompanied by an increase in the GABA levels which, interestingly, were also higher for the mutant plants (Figure 7A). Based on this result, one can argue that the glutamate generated in mitochondria of roots during the first 24 hours of recovery remains in this organelle for entry into the TCA cycle. Thus, demonstrating again, how important the TCA cycle is during periods of stress and recovery (Matsunami et al., 2020). During the recovery points, the glutamate levels remained high with significant higher values for the double mutant plants (Figure 7B) and GABA levels also remained high but without significance between genotypes. The activation of the GABA shunt under stress conditions corroborates with the control data described for Araújo et al. (2008b) when working with inhibition of 2OGDH in heterotrophic tissues and also with the importance of this pathway under stress conditions (Hijaz and Killiny, 2019).

The lack of the 2OGDH E1 subunit delays proline degradation and this amino acid boost root growth of E1-2OGDH mutants

The inhibition and lack of the 2OGDH in plants is associated with an increase in the levels of 2OG and reduction in the whole 2OGDH activity (Araújo et al., 2008; Araújo et al., 2012c; Condori-Apfata et al., 2019). During the recovery period, the lack of E1 subunit of the 2OGDH complex in the double mutant plants resulted in an impaired proline degradation during the first 24 hours in both tissues (Figure 6E-F). Together with the GABA accumulation in the roots (Figure 7B), this fact suggest that the lack of the E1 Subunit has some kind of regulation role over the proline metabolism, possibly through the accumulation of 2OG and glutamate (Figure 7B), reducing the proline degradation in the first hours of recovery. The levels of proline were reduced during the recovery period (Figure 6E-F) and taking in consideration the increase of root growth (Figure 8A) and respiration rates with supplementation of proline (Figure 8C), it is possible that this delay in degradation is also caused by a higher level of stress suffered by these plants, once they can metabolize this amino acid.

CONCLUSION

In this work we found out that during the first hours of recovery, the lack of E1 subunit of 2OGDH has negative effects over proline degradation once the impairment in the 2OG metabolism by the TCA pathway, lead to an accumulation of glutamate that might exert a negative feedback over the proline catabolism enzymes. We also demonstrated that the lack of the E1 subunit of the 2OGDH enzyme does not have a great effect on the shoot of plants during the period of imposition and recovery from osmotic stress. Its lacking might be surpassed by alternative pathways like the GABA shunt or the glyoxylate cycle and other known mechanisms activated in response to stress. Conversely, in root tissues, the lack of this enzyme allowed us to establish how the TCA cycle is important and prioritized during the periods of stress and recovery over the nitrogen metabolism, while in the shoots both metabolisms seem to recover in a similar way.

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REFERENCES

Alavi SMN, Arvin MJ, Kalantari KM (2014) Salicylic acid and nitric oxide alleviate osmotic stress in wheat (Triticum aestivum L.) seedlings. J Plant Interact **9**: 683–688

Anwar S, Inselsbacher E, Grundler FMW, Hofmann J (2015) Arginine metabolism of Arabidopsis thaliana is modulated by Heterodera schachtii infection. Nematology **17**: 1027–1043

Arakawa T, Timasheff SN (1985) The stabilization of proteins by osmolytes. Biophys J **47**: 411–414

Arasimowicz M, Floryszak-Wieczorek J (2007) Nitric oxide as a bioactive signalling molecule in plant stress responses. Plant Sci **172**: 876–887

Araújo WL, Nunes-Nesi A, Fernie AR (2014) On the role of plant mitochondrial metabolism and its impact on photosynthesis in both optimal and sub-optimal growth conditions. Photosynth Res **119**: 141–156

Araújo WL, Nunes-Nesi A, Nikoloski Z, Sweetlove LJ, Fernie AR (2012a) Metabolic control and regulation of the tricarboxylic acid cycle in photosynthetic and heterotrophic plant tissues. Plant, Cell Environ **35**: 1–21

Araújo WL, Nunes-Nesi A, Trenkamp S, Bunik VI, Fernie AR (2008) Inhibition of 2-oxoglutarate dehydrogenase in potato tuber suggests the enzyme is limiting for respiration and confirms its importance in nitrogen assimilation. Plant Physiol **148**: 1782–1796

Araújo WL, Tohge T, Ishizaki K, Leaver CJ, Fernie AR (2011) Protein degradation - an alternative respiratory substrate for stressed plants. Trends Plant Sci **16**: 489–498

Araújo WL, Tohge T, Nunes-Nesi A, Daloso DM, Nimick M, Krahnert I, Bunik VI, Moorhead GBG, Fernie AR (2012b) Phosphonate analogs of 2-oxoglutarate perturb metabolism and gene expression in illuminated Arabidopsis leave. Front Plant Sci **3**: 1–19

Araújo WL, Tohge T, Osorio S, Lohse M, Balbo I, Krahnert I, Sienkiewicz-Porzucek A, Usadel B, Nunes-Nesi A, Fernie AR (2012c) Antisense inhibition of the 2-oxoglutarate dehydrogenase complex in tomato demonstrates its importance for plant respiration and during leaf senescence and fruit maturation. Plant Cell **24**: 2328–2351

Atkin OK, Macherel D (2009) The crucial role of plant mitochondria in orchestrating drought tolerance. Ann Bot **103**: 581–597

Batista-Silva W, Heinemann B, Rugen N, Nunes-Nesi A, Araújo WL, Braun HP, Hildebrandt TM (2019) The role of amino acid metabolism during abiotic stress release. Plant Cell Environ **42**: 1630–1644

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72**: 248–254

Bunik VI, Fernie AR (2009) Metabolic control exerted by the 2-oxoglutarate dehydrogenase reaction: A cross-kingdom comparison of the crossroad between energy production and nitrogen assimilation. Biochem J **422**: 405–421

Cabassa-Hourton C, Schertl P, Bordenave-Jacquemin M, Saadallah K, Guivarc'h A, Lebreton S, Planchais S, Klodmann J, Eubel H, Crilat E, et al (2016a) Proteomic and functional analysis of proline dehydrogenase 1 link proline catabolism to mitochondrial electron transport in Arabidopsis thaliana. Biochem J **473**: 2623–2634

Cabassa-Hourton Ć, Schertl P, Marianne BJ, Saadallah K, Guivarc'h A, Lebreton S, Planchais Ś, Klodmann J, Eubel H, Crilat E, et al (2016b) Proteomic and functional analysis of proline dehydrogenase 1 link proline catabolism to mitochondrial electron transport in Arabidopsis thaliana. Biochem J **473**: 2623–2634

Caretto S, Linsalata V, Colella G, Mita G, Lattanzio V (2015) Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. Int J Mol Sci **16**: 26378–26394

Carillo P, Gibon Y (2011) PROTOCOL : Extraction and determination proline. Kasetsart J - Nat Sci 43: 146–152

Carrari F, Nunes-Nesi A, Gibon Y, Lytovchenko A, Loureiro ME, Fernie AR (2003) Reduced Expression of Aconitase Results in an Enhanced Rate of Photosynthesis and Marked Shifts in Carbon Partitioning in Illuminated Leaves of Wild Species

Tomato. Plant Physiol **133**: 1322–1335

Cavalcanti JHF, Quinhones CGS, Schertl P, Brito DS, Eubel H, Hildebrandt T, Nunes-Nesi A, Braun HP, Araújo WL (2017) Differential impact of amino ac on OXPHOS system activity following carbohydrate starvation in Arabidopsis suspensions. Physiol Plant **161**: 451–467

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Ann Bot **103**: 551–560

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osório ML, Carvalho I, Faria T, Pinheiro C (2002) How plants cope with water stress in the field. Photosynthesis and growth. Ann Bot **89**: 907–916

Condori-Apfata JA, Batista-Silva W, Medeiros DB, Vargas JR, Valente LML, Heyneke E, Pérez-Diaz JL, Fernie AR, Araújo WL, Nunes-Nesi A (2019) The Arabidopsis E1 subunit of the 2-oxoglutarate dehydrogenase complex modulates plant growth and seed production. Plant Mol Biol **101**: 183–202

Condori-Apfata JA, Batista-Silva W, Medeiros DB, Vargas JR, Valente LML, Pérez-Díaz JL, Fernie AR, Araújo WL, Nunes-Nesi A (2021) Downregulation of the E2 Subunit of 2-Oxoglutarate Dehydrogenase Modulates Plant Growth by Impacting Carbon–Nitrogen Metabolism in Arabidopsis thaliana . Plant Cell Physiol **0**: 1–17

Daloso DM, Medeiros DB, dos Anjos L, Yoshida T, Araújo WL, Fernie AR (2017) Metabolism within the specialized guard cells of plants. New Phytol **216**: 1018–1033

Etienne A, Génard M, Lobit P, Mbeguié-A-Mbéguié D, Bugaud C (2013) What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. J Exp Bot **64**: 1451–1469

Facchinelli F, Weber APM (2011) The metabolite transporters of the plastid envelope: An update. Front Plant Sci. doi: 10.3389/fpls.2011.00050

Fait A, Fromm H, Walter D, Galili G, Fernie AR (2007a) Highway or byway: the metabolic role of the GABA shunt in plants. Trends Plant Sci **13**: 14–19

Fait A, Nunes-nesi A, Carrari F, Studart-guimara C, Fernie AR (2007b) Reduced Expression of Succinyl-Coenzyme A Ligase Can Be Compensated for by Up-Regulation of the g -Aminobutyrate Shunt in Illuminated Tomato Leaves 1 [W]. **145**: 626–639

Fernie AR, Aharoni A, Willmitzer L, Stitt M (2011) Recommendations for Reporting Metabolite Data. **23**: 2477–2482

Fernie AR, Roscher A, Ratcli RG, Kruger NJ (2001) Fructose 2 , 6-bisphosphate activates pyrophosphate : fructose-6-phos- phate 1-phosphotransferase and increases triose phosphate to hexose phosphate cycling in heterotrophic cells. Planta **212**: 250–263

Fuchs P, Rugen N, Carrie C, Elsässer M, Finkemeier I, Giese J, Hildebrandt TM, Kühn K, Maurino VG, Ruberti C, et al (2020) Single organelle function and organization as estimated from Arabidopsis mitochondrial proteomics. Plant J **101**:

420–441

Funck D, Stadelhofer B, Koch W (2008) Ornithine-δ-aminotransferase is essential for Arginine Catabolism but not for Proline Biosynthesis. BMC Plant Biol **8**: 1–14

Furlan AL, Bianucci E, Giordano W, Castro S, Becker DF (2020) Proline metab dynamics and implications in drought tolerance of peanut plants. Plant Physiol Biochem **151**: 566–578

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res **50**: 151–158

Gaufichon L, Rothstein SJ, Suzuki A (2016) Asparagine metabolic pathways in arabidopsis. Plant Cell Physiol **57**: 675–689

Ghosh D, Xu J (2014) Abiotic stress responses in plant roots: A proteomics perspective. Front Plant Sci **5**: 1–13

Gibon Y, Bläsing OE, Palacios-Rojas N, Pankovic D, Hendriks JHM, Fisahn J, Höhne M, Günther M, Stitt M (2004) Adjustment of diurnal starch turnover to short days: depletion of sugar during the night leads to a temporary inhibition of carbohydrate utilization, accumulation of sugars and post-translational activation of ADP-glucose pyrophosphorylase in the following light period. Plant J **39**: 847–862

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A (2012) Role of Proline Under Changing Enviromental. Plant Signal Behav **7**: 1456–1466

Hebbelmann I, Selinski J, Wehmeyer C, Goss T, Voss I, Mulo P, Kangasjärvi S, Aro EM, Oelze ML, Dietz KJ, et al (2012) Multiple strategies to prevent oxidative stress in Arabidopsis plants lacking the malate valve enzyme NADP-malate dehydrogenase. J Exp Bot **63**: 1445–1459

Heinemann B, Künzler P, Eubel H, Braun HP, Hildebrandt TM (2021) Estimating the number of protein molecules in a plant cell: protein and amino acid homeostasis during drought. Plant Physiol **185**: 385–404

Hijaz F, Killiny N (2019) Exogenous GABA is quickly metabolized to succinic acid and fed into the plant TCA cycle. https://doi.org/101080/1559232420191573096 **14**: 1–3

Hildebrandt TM (2018) Synthesis versus degradation: directions of amino acid metabolism during Arabidopsis abiotic stress response. Plant Mol Biol **98**: 121–135

Hildebrandt TM, Nunes Nesi A, Araújo WL, Braun HP (2015) Amino Acid Catabolism in Plants. Mol Plant **8**: 1563–1579

Hsiao TC, Xu L (2000) Sensitivity of growth of roots versus leaves to water stress : biophysical analysis and relation to water transport. **51**: 1595–1616

Huergo LF, Dixon R (2015) The Emergence of 2-Oxoglutarate as a Master Regulator Metabolite. Microbiol Mol Biol Rev **79**: 419–435

Hummel I, Pantin F, Sulpice R, Piques M, Rolland G, Dauzat M, Christophe A, Pervent M, Bouteillé M, Stitt M, et al (2010) Arabidopsis plants acclimate to water deficit at low cost through changes of carbon usage: An integrated perspective using growth, metabolite, enzyme, and gene expression analysis. Plant Physiol **154**: 357–372

Islam MM, Hoque MA, Okuma E, Jannat R, Banu MNA, Jahan MS, Nakamura Y, Murata Y (2009) Proline and glycinebetaine confer cadmium tolerance on tobacco bright yellow-2 cells by increasing scorbate-glutathione cycle enzyme activities. Biosci Biotechnol Biochem **73**: 2320–2323

Jacoby RP, Taylor NL, Millar AH (2011) The role of mitochondrial respiration in salinity tolerance. Trends Plant Sci **16**: 614–623

Kaul S, Sharma SS, Mehta IK (2008) Free radical scavenging potential of L-proline: Evidence from in vitro assays. Amino Acids **34**: 315–320

Kim Y, Chung YS, Lee E, Tripathi P, Heo S, Kim KH (2020) Root response to drought stress in rice (Oryza sativa L.). Int J Mol Sci **21**: 12–14

Kishor PBK, Hima Kumari P, Sunita MSL, Sreenivasulu N (2015) Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. Front Plant Sci **6**: 1–17

Kopka J, Schauer N, Krueger S, Birkemeyer C, Usadel B, Bergmüller E, Dörmann P, Weckwerth W, Gibon Y, Stitt M, et al (2005) GMD@CSB.DB : the Golm Metabolome Database. Bioinformatics **21**: 1635–1638

Launay A, Cabassa-Hourton C, Eubel H, Maldiney R, Guivarc'h A, Crilat E, Planchais S, Lacoste J, Bordenave-Jacquemin M, Clément G, et al (2019) Proline oxidation fuels mitochondrial respiration during dark-induced leaf senescence in Arabidopsis thaliana. J Exp Bot **70**: 6203–6214

Lawson T, Matthews J (2020) Guard Cell Metabolism and Stomatal Function. Annu Rev Plant Biol **71**: 273–302

Lemaitre T, Urbanczyk-Wochniak E, Flesch V, Bismuth E, Fernie AR, Hodges M (2007) NAD-dependent isocitrate dehydrogenase mutants of Arabidopsis suggest the enzyme is not limiting for nitrogen assimilation. Plant Physiol **144**: 1546–1558

Liang X, Zhang L, Natarajan SK, Becker DF (2013) Proline mechanisms of stress survival. Antioxidants Redox Signal **19**: 998–1011

Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR (2006) Gas chromatography mass spectrometry – based metabolite profiling in plants. Nat Protoc **1**: 387–396

Luedemann A, Von Malotky L, Erban A, Kopka J (2012) TagFinder: preprocessing software for the fingerprinting and the profiling of gas chromatography-mass spectrometry based metabolome analyses. Methods Mol Biol **860**: 255–286

Matsunami M, Toyofuku K, Kimura N, Ogawa A (2020) Osmotic Stress Leads to Significant Changes in Rice Root Metabolic Profiles between Tolerant and.

Millar AH, Hill SA, Leaver CJ (1999) Plant mitochondrial 2-oxoglutarate dehydrogenase complex: Purification and characterization in potato. Biochem J **343**: 327–334

Millar AH, Leaver CJ (2000) The cytotoxic lipid peroxidation product, 4-hydroxy-2-

nonenal, specifically inhibits decarboxylating dehydrogenases in the matrix of plant mitochondria. FEBS Lett **481**: 117–121

Millar AH, Whelan J, Soole KL, Day DA (2011) Organization and Regulation of Mitochondrial Respiration in Plants. Annu Rev Plant Biol **62**: 79–104

Monte-Bello CC, Araujo EF, Martins MCM, Mafra V, da Silva VCH, Celente ▾, Caldana C (2018) A flexible low cost hydroponic system for assessing plant responses to small molecules in sterile conditions. J Vis Exp **2018**: 1–9

Muller B, Pantin F, Génard M, Turc O, Freixes S, Piques M, Gibon Y (2011) Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. J Exp Bot **62**: 1715–1729

Murashige T, Skoog F (1962) a Revised Medium for Rapid Growth and Bio Assays With Tobacco Tissue Cultures. Physiol Plant **15**: 473–497

Nunes-Nesi A, Carrari F, Gibon Y, Sulpice R, Lytovchenko A, Fisahn J, Graham J, Ratcliffe RG, Sweetlove LJ, Fernie AR (2007) Deficiency of mitochondrial fumarase activity in tomato plants impairs photosynthesis via an effect on stomatal function. Plant J **50**: 1093–1106

Nunes-Nesi A, Carrari F, Lytovchenko A, Smith AMO, Loureiro ME, Ratcliffe RG, Sweetlove LJ, Fernie AR (2005) Enhanced photosynthetic performance and growth as a consequence of decreasing mitochondrial malate dehydrogenase activity in transgenic tomato plants. Plant Physiol **137**: 611–622

Obata T (2019) Metabolons in plant primary and secondary metabolism. Phytochem Rev **18**: 1483–1507

Obata T, Fernie AR (2012) The use of metabolomics to dissect plant responses to abiotic stresses. Cell Mol Life Sci **69**: 3225–3243

Pál M, Tajti J, Szalai G, Peeva V, Végh B, Janda T (2018) Interaction of polyamines, abscisic acid and proline under osmotic stress in the leaves of wheat plants. Sci Rep **8**: 1–12

Pantin F, Fanciullino AL, Massonnet C, Dauzat M, Simonneau T, Muller B (2013) Buffering growth variations against water deficits through timely carbon usage. Front Plant Sci **4**: 1–11

Pires M V., Pereira Júnior AA, Medeiros DB, Daloso DM, Pham PA, Barros KA, Engqvist MKM, Florian A, Krahnert I, Maurino VG, et al (2016) The influence of alternative pathways of respiration that utilize branched-chain amino acids following water shortage in Arabidopsis. Plant Cell Environ **39**: 1304–1319

Planchais S, Cabassa C, Toka I, Justin AM, Renou JP, Savouré A, Carol P (2014) BASIC AMINO ACID CARRIER 2 gene expression modulates arginine and urea content and stress recovery in Arabidopsis leaves. Front Plant Sci. doi: 10.3389/fpls.2014.00330

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta -

Bioenerg **975**: 384–394

Schertl P, Braun H-P (2014) Respiratory electron transfer pathways in plant mitochondria. Front Plant Sci **5**: 1–11

Schertl P, Cabassa C, Saadallah K, Bordenave M, Savouré A, Braun HP (20 Biochemical characterization of proline dehydrogenase in Arabidop mitochondria. FEBS J **281**: 2794–2804

Sienkiewicz-Porzucek A, Nunes-Nesi A, Sulpice R, Lisec J, Centeno DC, Carillo P, Leisse A, Urbanczyk-Wochniak E, Fernie AR (2008) Mild reductions in mitochondrial citrate synthase activity result in a compromised nitrate assimilation and reduced leaf pigmentation but have no effect on photosynthetic performance or growth. Plant Physiol **147**: 115–127

Sienkiewicz-porzucek A, Usadel B, Nunes-nesi A, Fernie AR (2012) Antisense Inhibition of the 2-Oxoglutarate Dehydrogenase Complex in Tomato Demonstrates Its Importance for Plant Respiration and during Leaf Senescence and Fruit Maturation. **24**: 2328–2351

Sweetlove LJ, Beard KFM, Nunes-Nesi A, Fernie AR, R. George Ratcliffe (2010) Not just a circle: Flux modes in the plant TCA cycle. Trends Plant Sci **15**: 462–470

Sweetlove LJ, Heazlewood JL, Herald V, Holtzapffel R, Day DA, Leaver CJ, Millar AH (2002) The impact of oxidative stress on Arabidopsis mitochondria. 891–904

Szabados L, Savouré A (2010) Proline: a multifunctional amino acid. Trends Plant Sci **15**: 89–97

Székely G, Ábrahám E, Cséplo Á, Rigó G, Zsigmond L, Csiszár J, Ayaydin F, Strizhov N, Jásik J, Schmelzer E, et al (2008) Duplicated P5CS genes of Arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. Plant J **53**: 11–28

Taylor NL, Day DA, Millar AH (2002) Environmental stress causes oxidative damage to plant mitochondria leading to inhibition of glycine decarboxylase. J Biol Chem **277**: 42663–42668

Taylor NL, Heazlewood JL, Day DA, Millar AH (2004) Lipoic Acid-Dependent Oxidative Catabolism of ␣ -Keto Acids in Mitochondria Provides Evidence for Branched- Chain Amino Acid Catabolism in Arabidopsis 1. **134**: 838–848

Taylor NL, Tan YF, Jacoby RP, Millar AH (2009) Abiotic environmental stress induced changes in the Arabidopsis thaliana chloroplast, mitochondria and peroxisome proteomes. J Proteomics **72**: 367–378

Toleco MR, Naake T, Zhang Y, Heazlewood JL, Fernie AR (2020) Plant mitochondrial carriers: Molecular gatekeepers that help to regulate plant central carbon metabolism. Plants. doi: 10.3390/plants9010117

Valifard M, Le Hir R, Müller J, Scheuring D, Neuhaus HE, Pommerrenig B (2021) Vacuolar fructose transporter SWEET17 is critical for root development and drought tolerance. Plant Physiol **187**: 2716–2730

Verbruggen N, Hermans C (2008) Proline accumulation in plants: A review. Amino Acids **35**: 753–759

Villela FA, Doni Filho L, Sequeira EL (1991) Tabela de potencial osmótico em função da concentração de polietileno glicol 6.000 e da temperatura. Pesqui Agropecuária Bras **26**: 1957–1968

Winger AM, Millar AH, Day DA (2005) Sensitivity of plant mitochondrial term oxidases to the lipid peroxidation product 4-hydroxy-2-nonenal (HNE). Bioche… **387**: 865

Yue J, Du C, Ji J, Xie T, Chen W, Chang E, Chen L, Jiang Z, Shi S (2018) Inhibition of α-ketoglutarate dehydrogenase activity affects adventitious root growth in poplar via changes in GABA shunt. Planta **248**: 963–979

Zhang Y, Beard KFM, Swart C, Bergmann S, Krahnert I, Nikoloski Z, Graf A, George Ratcliffe R, Sweetlove LJ, Fernie AR, et al (2017) Protein-protein interactions and metabolite channelling in the plant tricarboxylic acid cycle. Nat Commun. doi: 10.1038/ncomms15212

Zhang Y, Swart C, Alseekh S, Scossa F, Jiang L, Obata T, Graf A, Fernie AR (2018) The extra-pathway interactome of the TCA cycle: Expected and unexpected metabolic interactions. Plant Physiol **177**: 966–979

Zhao C, Zhang H, Song C, Zhu J, Shabala S (2020a) Mechanisms of Plant Responses and Adaptation to Soil Salinity. Innov **1**: 100017

Zhao Y, Luo L, Xu J, Xin P, Guo H, Wu J, Bai L, Wang G (2018) Malate transported from chloroplast to mitochondrion triggers production of ROS and PCD in Arabidopsis thaliana. Cell Res. doi: 10.1038/s41422-018-0024-8

Zhao Y, Yu H, Zhou J, Smith SM (2020b) Malate Circulation : Linking Chloroplast Metabolism to Mitochondrial ROS. Trends Plant Sci **xx**: 1–9

GENERAL CONCLUSION

At the end of our work, the data collected and analyzed led us to conclude that under control conditions, the lack of the 2OGDH enzyme is related to reduction in photosynthetic capacity, by reducing the level of several proteins involved in this metabolism. This complex also has great importance over the respiratory process and the lack of its subunits might lead to a great reduction in plant growth. When exposed to stress situations, the lack of the E1 subunit is perceived differently between roots and shoots. The lack of the E1 subunit of the 2OGDH is much more noticed in heterotrophic tissues, while during stress, alternative pathways such as GABA shunt are strongly increased to circumvent this deficiency. The lack of the two genes of the E1 subunit generates plants more sensitive to stress, and with greater difficulties in recovery. In the roots, during recovery, the carbon metabolism seems to be favored to the detriment of nitrogen, while in the shoot both metabolisms recovered in a similar way.