LAÍSE ROSADO DE SOUZA

NATURAL VARIATION IN PHYSIOLOGICAL TRAITS IN A LARGE PANEL OF BRAZILIAN *Capsicum chinense* ACCESSIONS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fisiologia Vegetal, para obtenção do título de *Magister Scientiae.*

VIÇOSA
MINAS GERAIS – BRASIL
2013

LAÍSE ROSADO DE SOUZA

NATURAL VARIATION IN PHYSIOLOGICAL TRAITS IN A LARGE PANEL OF BRAZILIAN *Capsicum chinense* ACCESSIONS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fisiologia Vegetal, para obtenção do título de *Magister Scientiae.*

Aprovada: 27 de fevereiro de 2013

Fernando L. Finger

Fernando Luiz Finger

Leonardo Lopes Bhering

Leonardo Lopes Bhering

Adriano Nunes Nesi

Adriano Nunes Nesi
(Adviser)

To my dear family, Odélia, Hélio and my brother Mário,

for being my first teachers in life, for all the love and understanding during this journey.

Acknowledgments

To the Universidade Federal de Viçosa, especially the Departamento de Biologia Vegetal and Programa de Pós-Graduação em Fisiologia Vegetal, for all support, assistance and opportunity.

To Fapemig, for the financial support through the scholarship.

To Professor Adriano, for the orientation, example of dedication, encouragement, friendship and thousands of ideas throughout the execution of this work. Thank you very much!

To the Professors Wagner Araújo, Marcelo Rogalski and Thomas, for all the encouragement and questions during our meetings. To Professor Fábio and all professors of Plant Physiology, for the teachings.

To Professors Leonardo Bhering and Fernando Finger, for their great contribution in reviewing this work.

To Professor Eduardo Gusmão for the first scientific teachings, as well as to Professor Andréa Miyasaka de Almeida and Professor Marco Antonio Oliva.

To the UCP family, especially Rogério Gomide and Carol Müller.

To the friends Izabel, Franklin, Danielle and Paula, and all lab fellows who participated in some way, for their help and friendship during the labwork.

To all those who contributed to this work, my sincere thanks.

To the special friends Nilmara and Alice, for all support and encouragement.

To Cleverson, for the love and friendship.

To Mitson, Catarina and Hugo, for being my family in Viçosa.

To God, for the gift of life.

Sumary

RESUMO

Souza, Laíse Rosado de, M. Sc., Universidade Federal de Viçosa, fevereiro de 2013. **Variação natural de características fisiológicas em um grande painel de acessos de pimentas brasileiras.** Orientador: Adriano Nunes Nesi. Coorientadores: Fábio M. DaMatta e Wagner L. Araújo

A diversidade de acessos dentro de uma mesma espécie tem sido relatada como uma forma alternativa de procurar respostas fisiológicas e metabólicas que podem ter grandes efeitos na regulação do crescimento e produção de biomassa. Este trabalho teve como objetivo investigar características fisiológicas e metabólicas assim como suas interações com o crescimento e a produção de frutos em 49 acessos brasileiros de pimenta (*Capsicum chinense*) geneticamente diversos. Estes diferentes acessos foram cultivados simultaneamente e, assim, grande variação genética foi observado para muitas características. Embora a análise de cada variável individualmente permitiu a formação de até sete grupos distintos através do teste Scott-Knott modificado, como observado para altura de planta, usando o conjunto de dados através de análises multivariadas foi possível separar os 49 acessos em apenas três clusters. Interessantemente os grupos formados por estas análises não seguiram a distribuição geográfica de origem dos genótipos. O primeiro cluster contém dois acessos com os menores valores para altura de planta e taxa de crescimento relativo (TCR). Por outro lado, os mesmos acessos tiveram a maior área foliar específica (AFE), altas taxas de fotossíntese e os maiores conteúdos de nitrato (NO_3). Os genótipos do segundo cluster demonstraram comportamento oposto para os parâmetros de crescimento e NO_3, porém apresentaram padrão indefinido para os parâmetros de trocas gasosas e eficiência do uso da água (EUA), apesar de apresentarem baixos valores de AFE. O terceiro cluster compreende os demais acessos com os mais diversos resultados para as características aqui investigadas. Tomados em conjunto, estes resultados reforçam a idéia de que, embora os acessos pertençam a mesma espécie, eles apresentam mecanismos de adaptação diversos sendo, portanto, uma fonte muito interessante de informações para melhoramento de plantas. Além disso, demonstrou-se que o estabelecimento dos frutos está positivamente correlacionado com fotossíntese. No entanto, a fotossíntese por si só não explica as diferenças em relação ao abortamento de frutos. Análises metabólicas revelaram que malato e fumarato apresentam respostas diferentes em *Capsicum*. Embora fumarato não tenha contribuido individualmente para a separação de grupos, um grande número de correlações significativas foram observadas. Em suma, dados aqui apresentados sugerem fumarato como um importante metabólito possivelmente envolvido na regulação do crescimento e desenvolvimento da parte aérea e dos frutos em *C. chinense*. Além de estender nossos conhecimentos sobre mecanismos e vias biológicas no gênero *Capsicum* o trabalho aqui apresentado destaca a importância de se estudar a variação genética como um meio para entender como a espécie se adapta a diferentes ambientes.

ABSTRACT

Souza, Laíse Rosado de, M. Sc., Universidade Federal de Viçosa, February 2013. **Natural variation in physiological traits in a large panel of brazilian *Capsicum chinense* acessions.** Adviser: Adriano Nunes Nesi. Co-advisers: Fábio Murilo DaMatta e Wagner L. Araújo

Diversity of accessions within the same specie has been reported as an alternative way to search for physiological and metabolic traits that may have large effects on both growth regulation and biomass production. This work aimed to investigate physiological and metabolic traits as well as its interactions with plant growth and fruit production using 49 genetically diverse brazilian pepper accessions of *Capsicum chinense*. These different accessions were grown together and genetic variation was observed for many traits. Although the analysis of each variable individually allowed the formation of up to seven distinct groups, as observed for plant height, working with the whole data set by multivariate analyzes allowed the separation of the 49 accessions in only three clusters. Interestingly, the groups formed by this analysis did not follow the geographical origin of the genotypes. The first cluster contains two accessions with lower plant height and relative growth rate (RGR). On the other hand, the same accessions had the highest specific leaf area (SLA), high photosynthesis rates and have the highest nitrate (NO_3) content. Genotypes in the second cluster demonstrate opposite behavior for growth parameters and $NO_{3,}$ however they had an undefined pattern for gas exchange parameters and water use efficiency (WUE), despite having low values of SLA. The third cluster comprised the other accessions with the most diverse results for the investigated characteristics. Altogether, these results reinforce the idea that, although the accessions belong to the same species, they have diverse adaptation mechanisms being a highly interesting source of information for plant breeders. In addition, it was demonstrated that fruit set is positively correlated with photosynthesis. However, photosynthesis does not explain alone the differences in accession susceptibility to fruit abortion. Metabolite analysis revealed that malate and fumarate had different responses in *Capsicum*. Although fumarate did not individually contribute to the accession group separation, a large number of significant correlations was observed. In summary the results obtained suggest fumarate as an important metabolite that

might be involved in the regulation of both shoot and fruit growth and development of *C. chinense.* Apart from extending our knowledge of biological mechanism and pathways within the *Capsicum* genus the work presented here highlights the importance of studying genetic variation as a mean to understand how a specie adapts to different local environments.

1. Introduction

The pepper species of the genus *Capsicum* are members of the Solanaceae family, together with other crops such as tomatoes, eggplants and potatoes. The genus *Capsicum* comprises a highly diverse group of sweet and hot peppers being the American continent recognized as the center of origin of peppers, more specifically in its tropical and subtropical regions (Reifschneider, 2000). Among the various species of the genus Capsicum, *C. annuum*; *C. bacccatum*; *C. chinense*; *C. frutescens* and *C. pubescens* are commercially cultivated (Rodriguez et al., 1999; Onus and Pickersgill, 2004; Ince et al, 2009).

C. baccatum and *C. chinense* are the most cultivated species for commercial hot peppers in Brazil. This is most likely because these species are well adapted to equatorial and tropical climate conditions having unique flavors for fresh consumption (Lannes et al, 2007). Additionally, the north of Brazil, more specifically the Amazonian region, has been suggested as a probable center of diversity of *C. chinense* species (Pickersgill, 1971).

Due to its large capacity of adaptation to different soil and local climates, *C. chinense* can be grown throughout southern and northern Brazil (Lannes et al, 2007). Interestingly, fruits from *C. chinense* have an enormous variability in terms of size and shape, color and chemical composition (Lannes et al, 2007; Wahyuni et al., 2013). Fruits from this specie, as well as, from other peppers (*Capsicum* spp.) are well known for their ability to cause an intense organoleptic sensation of heat when consumed (Stewart et al., 2005; 2007; Aza-González et al., 2011). Capsaicin and its analogues, collectively called capsaicinoids, are the pungent principle of peppers (Simões et al., 2004; Aza-González et al., 2011), responsible for this sensation

Pepper fruits are also a rich source of metabolites with potential health-promoting properties, such as carotenoids (provitamin A), ascorbic acid (vitamin C), tocopherols (vitamin E), flavonoids and capsaicinoids (Howard and Wildman, 2007; Topuz and Ozdemir, 2007; Wahyuni et al., 2011). Many of these compounds are antioxidants compounds that exert their biological effects through free-radical scavenging, protein binding and interaction with human signal transduction pathways (Edge et al., 1997; Padayatty et al., 2003; Wahyuni et al., 2011).

Wild and domesticated peppers populations represent a genetic material with high potential for use in breeding and biotechnological programs. However, despite their potential, only morphological characterization of accesses has been yet used to study peppers genetic variability for years (Pickersgill, 1977; Bianchetti, 1996; Büttow et al., 2010).

It is important to mention that behind each morphological trait, complex metabolic and physiologic networks must function properly and be finely coordinated for the full plant development. Amongst the factors involved on this interestingly feature, photosynthetic metabolism is possible the most important determinant of biomass production being closely associated with growth rate (Wyżgolik et al., 2008). In this context, plant growth analysis is an explanatory, holistic and integrative approach of interpreting plant form and function (Hunt et al., 2002; Wyżgolik et al., 2008). Furthermore, regulation of plant metabolism is a crucial hallmark enabling the organism to respond to specific demands over the course of its life and on a minute-by-minute basis (Fernie and Klee, 2010).

Despite the high capacity to adapt to completely different environmental conditions (Lannes et al., 2007), virtually nothing is known concerning physiological and metabolic traits related to growth and fruit production in *Capsicum* species. Accordingly natural variation provides a framework to study the adaptation of different traits that define plant growth in relation to simultaneous genetic changes and environmental fluctuations.

To date, relatively few studies have been reported using diversity of accessions, within the same specie, as an alternative way to search for genes having large effects on growth regulation and biomass production (Sulpice et al., 2009; 2010; Ikram et al, 2012; Pyl et al., 2012). Notwithstanding for the model plant Arabidopsis it has been observed that although there is a weak correlation between rosette biomass and individual metabolites, a highly significant correlation is observed when multivariate analysis was used on the entire metabolite profile (Meyer et al., 2007). Altogether these results suggest that part of the genetic variation for biomass affects the balance between resource availability as well as the developmental programs that determine how rapidly metabolites are used for growth (Sulpice et al., 2010). It should be also mention that starch content at the end of the light period is an integrator of the metabolic response in Arabidopsis (Sulpice et al., 2009).

This work aimed to investigate physiological and metabolic traits as well as its interactions with plant growth and fruit production using 49 genetically diverse pepper accessions of *C. chinense*, growing under greenhouse conditions. The results obtained are discussed in the context of the importance of physiological and metabolic parameters to determine both shoot growth and fruit production in *C. chinense*.

2. Material and methods

2.1 Plant materials and experimental conditions

The Horticultural Germplasm Bank (BGH) located at the Federal University of Viçosa (UFV) stores one of the largest collection of *Capsicum* spp. in Brazil. From more than 100 accessions of *C. chinense*, 49 were initially selected for diversity of fruit color, size and shape (Lannes et al., 2007). Seeds of the accessions used in this experiment are additionally available at BGH/UFV.

Seeds were germinated in commercial substrate and seedlings were transplanted after 25 days of sowing to 8 liters pots with a mixture of soil and substrate (2.7:1 w/w) fertilized with 3.5 g of limestone, 3.5 g of superphosphate and 1 kg of manure per kilogram of soil. Plants were grown in a greenhouse located in Viçosa (642 m in altitude, 20°45’ S latitude and 42°51’ W longitude) in South-eastern Brazil. Plants were watered regularly and fertilized weekly with 40 mL of solution containing 5.0 g $(NH_4)_2SO_4$ and 2.5 g KCl per liter.

2.2. Plant biometric growth parameters

The relative growth rate (RGR) was estimated in three-moths-old plant~~s~~ according to Cavatte et al. (2012), throughout plant height measurements over three months, according to the formula (1).

$$\text{RGR (cm cm}^{-1}\text{ dia}^{-1}) = \frac{\text{Ln (final height)} - \text{Ln (initial height)}}{\text{Days}} \qquad (1)$$

The specific leaf area (SLA) was estimated in three-moths-old plants using the dry mass and leaf surface area of three leaves using the formula (2) as described by Cavatte et al. (2012).

$$\text{SLA (m}^2\text{ Kg}^{-1}\text{)} = \frac{\text{Leaf area (m}^2\text{)}}{\text{Dry weight (Kg)}} \qquad (2)$$

When plants reached seven-months-old plants, aerial part were collected and separated in leaves, stem and fruits. The tissues were then oven-dried at 65 ºC until constant weight, after which the dry matter was determined. Due to difficulties on tissue washing and collecting the underground plant biomass was not analyzed. To estimate the percentage of fruit set a random number of flowers on each plant was marked after anthesis and at the end of the experiment the number of marked fruits was counted.

2.3 Measurements of photosynthetic parameters

Gas exchange analyses were performed on the first fully expanded leaf of three-months-old plants using a portable infrared gas analyzer (Li 6400XT, Li-Cor, Lincoln, EUA) equipped with an integrated fluorescence chamber. Light (1000 µmol m^{-2} s^{-1}) was provided by a light-emitting diode with 10% of blue light in order to maximize the stomatal aperture. The measurements were performed on attached leaves from 8:00 to 12:00 hours (solar time) in greenhouse conditions; block temperature was set for 25ºC; CO_2 concentration of 400 µmol mol^{-1} and a flow rate of 500 µmol s^{-1}. The CO_2 concentration and water vapor between the leaf and the reference chamber were automatically matched before the data were recorded. Intrinsic water use efficiency (WUE) was estimate as described by Cavatte et al. (2012). The rates of dark respiration (R_d) were determined from 09:00 to 2:00 hours (solar time) in the same leaf previously used to determine the net carbon assimilation.

2.4 Measurements of chlorophyll fluorescence parameters

In dark adapted leaves minimal (F_0) and maximum fluorescence (F_m) measurements were made in the predawn using light measurement (0.03 µmol m^{-2} s^{-1}) and saturating light pulse (6000 µmol m^{-2} s^{-1}) for 0.8 s, respectively. The minimal fluorescence of light adapted leaves (F_0') and maximum chlorophyll fluorescence (F_m') were determined simultaneously with data collection for photosynthetic parameters. The obtained values were used to determine the maximum photochemical efficiency of PSII (F_v/F_m), actual quantum yield of PSII electron transport (Φ_{PSII}), the coefficients of photochemical quenching (qp) and non-photochemical quenching (NPQ) and the electron transport rate (ETR), as described previously (Schreiber et al., 1994).

2.5 Determination of metabolite levels

Leaf samples were harvested at the middle of light period, using the same leaf previously used to determine the net carbon assimilation. After collecting, the tissue was immediately frozen in liquid nitrogen and stored at -80 ºC, until further analysis. Metabolites extraction was performed by rapid grinding of tissue in liquid nitrogen and immediate addition of the appropriate extraction buffer as described by Gibon et al. (2004). The levels of starch, sucrose, fructose, and glucose in the leaf tissue were determined exactly as described previously (Fernie et al., 2001). Malate and fumarate were determined exactly as detailed by Nunes-Nesi et al. (2007), whilst nitrate (NO_3) and chlorophyll *a* and *b* contents as detailed by Sulpice et al. (2009). Total protein was quantified as in Bradford (1976) adapted for microplate. To quantify the content of total amino acids (AA) protocol described by Gibon et al. (2004) was used.

2.6 Statistical analysis

The experiment was designed in randomized blocks with four replicates. The experimental unit was one plant per pot. Statistical analyzes were performed using the GENES program (Cruz, 2006). All data were subjected to analysis of variance (ANOVA), and the means were grouped by Modified Scott-Knott (Bhering et al., 2008) test at 5% probability. The Pearson linear correlation technique was used for examining the relationships among variables.

Multivariate analyzes were also performed such as canonical variables in all data collected. In order to verify the consistency of the formed groups, Fisher discriminant analysis was also performed. A two-way ANOVA with accessions and environment as main effects and an accession *X* experiment interaction effect was performed to determine the amount of total variation were due to genetic causes using the genotypic determination coefficient (H^2).

3. Results

3.1 Growth related parameters

Plants were measured periodically to assess height and allow the subsequent determination of relative growth rate (RGR). Based on the plant height it was possible to classify statistically the genotypes in seven different groups (Figure 1) using Modified Scott-Knott (Bhering et al., 2008) test at 5% probability. It was observed that plants from the accession a59 are the tallest plants within the population, with average height of 65 cm. The second accession in average height is the a93 (55 cm). The remaining accessions were grouped with height averages between 16 and 50 cm, forming more five groups, being the smallest accessions a55, a56 and a86, with heights lower than 21 cm. As expected for RGR, the same number of accessions groups was formed and the pattern distribution within the groups remained the same observed for height (Figure 1). The accession a59 composes the first group (RGR = 4.12 cm cm^{-1} dia^{-1}, while the accession a93 together with a67 and a94 form the second group (RGR values between 3.81 and 3.96 cm cm^{-1} day^{-1}. Five more groups had values between 2.7 and 3.8 cm cm^{-1} day^{-1}; and the last group is formed by accessions 86 and 56, the same accessions between preseting lower heights.

At the end of the experiment, shoot dry weight (Shoot DW) was evaluated. Surprisingly, the accessions with taller plants and higher RGR were not the accessions with higher shoot dry matter accumulation (Figure 1). The accession a101 presented the highest value of shoot DW (76.52 g), followed by three other groups of accessions with values varying from 23.4 g to 61.6 g.

Only three significantly different groups were formed based on the SLA and interestingly the two accessions with smallest height, a56 and a86, correspond to the accessions with higher SLA (9.74 and 9.28 m^2 Kg^{-1}, respectively). The second group has intermediate values (between 6.78 and 8.34 m^2 Kg^{-1}), containing among others, accession a59, which has the highest height values and RGR. The third group has values between 5.26 and 6.75 m^2 Kg^{-1} and contains the accession a93, the second biggest in height, and a101, the accession with the highest value of shoot DW, among the 23 remaining accessions.

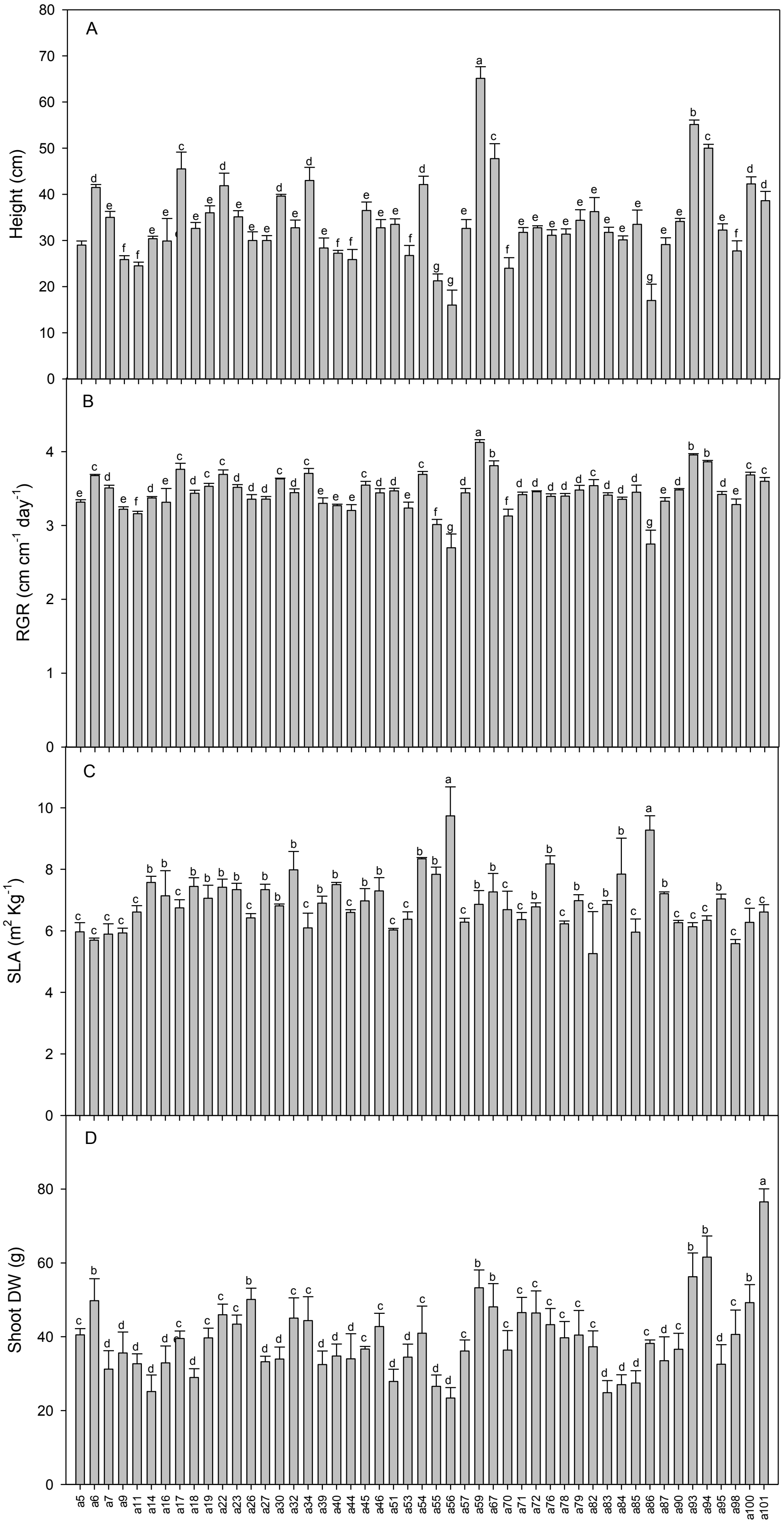

Figure 1. Growth related parameters of 49 *C. chinense* accessions. (A) Plant height; (B) Relative growth rate (RGR); (C) Specific leaf area (SLA); (D) Shoot dry weight (Shoot DW). Values are presented as mean ± SE (n = 4). Different letters represent the groups formed by the Scott-Knot test (p ≤ 0.05).

3.2 Gas exchange and chlorophyll a fluorescence parameters

Net carbon assimilation (*A*) formed three groups (Figure 2). It was observed that 26 accessions displayed higher *A* values (from 19 to 25 μmol CO_2 m^{-2} s^{-1}). In a second group, containing 14 accessions, the values varied from 17 to 19 μmol CO_2 m^{-2} s^{-1}; and last group, with 9 accessions, the values varied between 13 and 16 μmol CO_2 m^{-2} s^{-1}. Less variability was observed for stomatal conductance (g_s) with a formation of only two large groups of accessions, one with 25 accessions, ranging from 300 to 429 mmol H_2O m^{-2} s^{-1} and a second group with values from 130 to 287 mmol H_2O m^{-2} s^{-1}. In good agreement with the results observed for g_s transpiration rates (*E*) also enabled the formation of two groups of accessions (Figure 2). Interestingly, few accessions (a7, a22, a23, a30, a39, a83 and a93) showed low g_s and high *E*. For intrinsic water use efficiency (WUE) three groups were formed: one comprehending accessions with high WUE, varying from 90 to 110 μmol CO_2 mol^{-1} H_2O; a second group of accessions with extremes in 74 and 88 μmol CO_2 mol^{-1} H_2O; and a third group of less efficient genotypes with values between 52 and 70 μmol CO_2 mol^{-1} H_2O.

Alongside photosynthesis, the rates of respiration during the dark period (R_d) are one of the key determinants of crop productivity (Siedow and Day, 2000). Surprisingly, the accessions did not differ regarding the *Rd* (Figure 2).

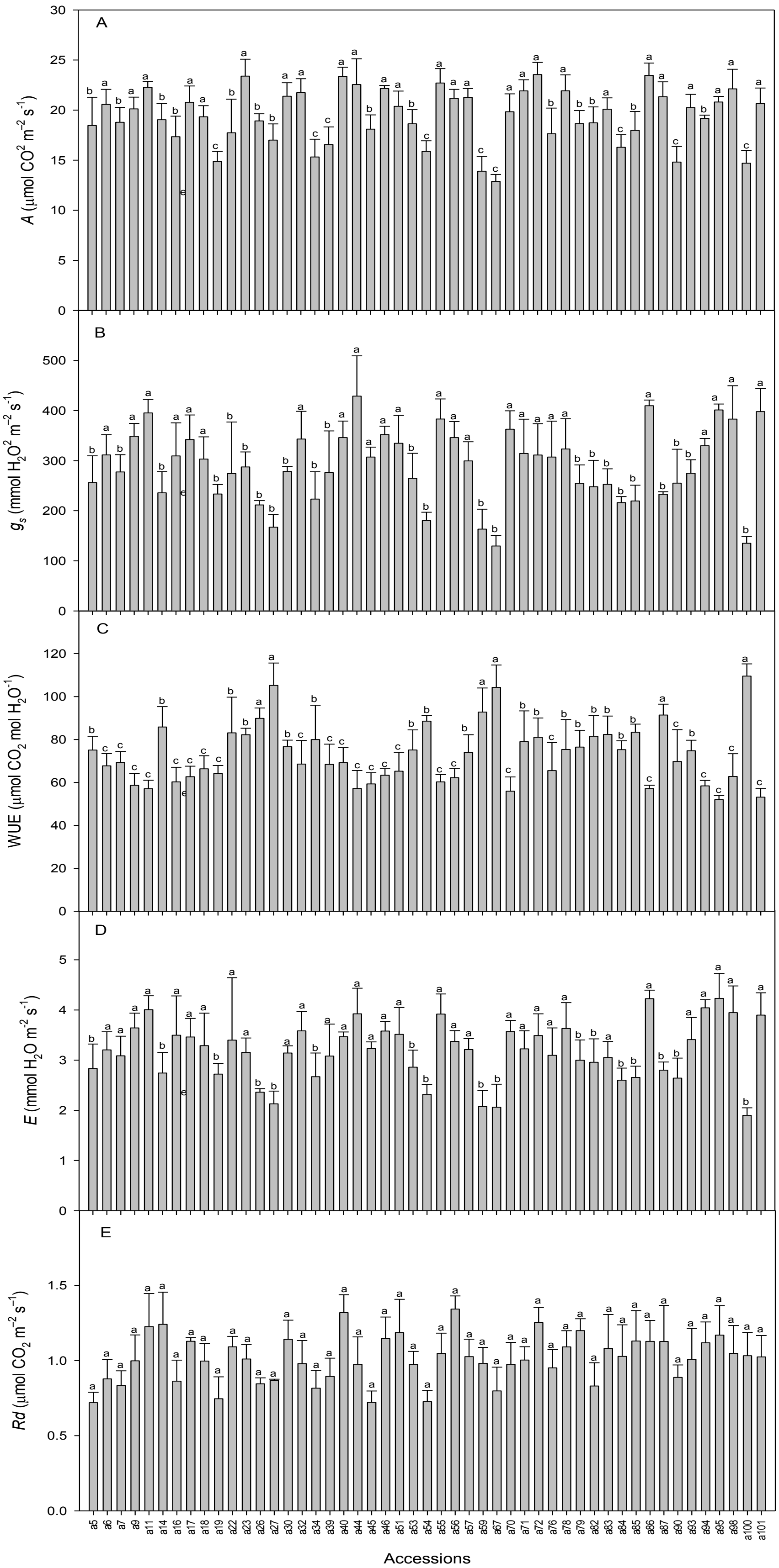

Figure 2. Gas exchange parameters of 49 *C. chinense* accessions. (A) Photosynthesis (*A*); (B) Stomatal conductance (g_s); (C) Intrinsic water use efficiency (WUE); (D) Transpiration rates (*E*); (E) Dark respiration (R_d). Values are presented as mean ± SE (n = 4). Different letters represent the groups formed by the Scott-Knot test ($p \leq 0.05$).

The maximum photochemical efficiency of photosystem II (PSII) represented by the ratio F_v/F_m, also distributed the accessions into three statistically different groups (Figure 3). One comprehending high efficient genotypes, with PSII values between 0.813 and 0.827; an intermediate group with values varying from 0.789 to 0.810, and a less efficient group with values from 0.755 to 0.784. By contrast, the photochemical quenching coefficient (*qP*) allowed the formation of four groups (Figure 3). The first group with values between 0.608 and 0.669; a second group with values between 0.526 and 0.597; the third group between 0.460 and 0.504; and the last group containing only accession a34 with value of 0.358. The coefficient of non-photochemical quenching (*NPQ*) formed only two major groups with values between 1.298 and 1.745 for the first one; and 0.644 and 1.267 for the second group. The electron transport rate (*ETR*) again formed three groups (Figure 3), the first group with values between 144.3 3 and 180.8 containing the largest number of accessions; a second group with values between 128.4 and 142.2; and the last group between 102.6 and 125.0.

When considered together these data demonstrated a high natural variation in fluorescence parameter highlighting the complexity behind these parameter and suggest that caution must be taken when interpreting such dataset.

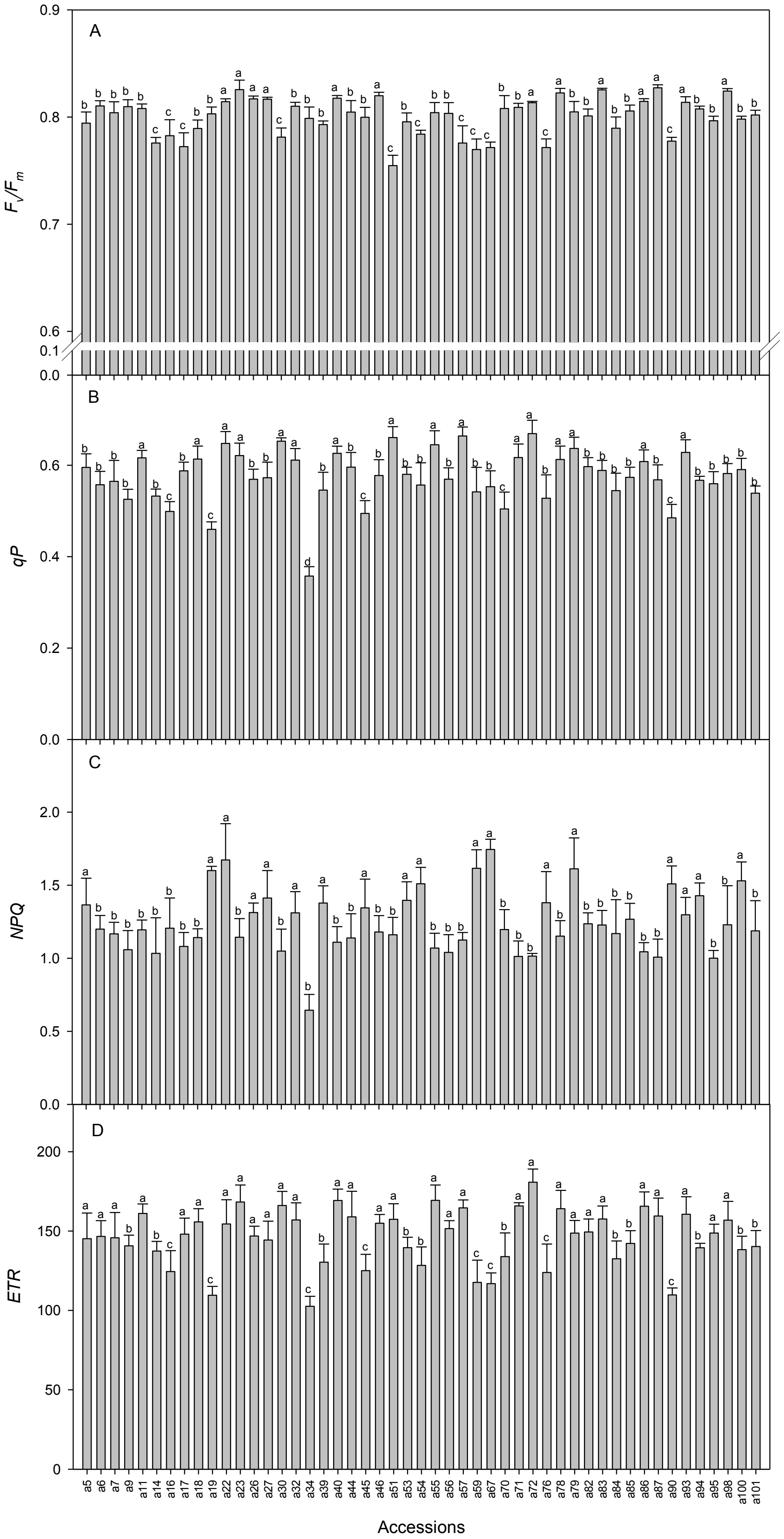

Figure 3. Chlorophyll *a* fluorescence parameters of 49 *C. chinense* accessions. (A) Maximum photochemical efficiency of photosystem II (F_v/F_m); (B) Photochemical quenching coefficient (*qP*); (C) Non-photochemical quenching (*NPQ*); (D) Electron transport rate (*ETR*). Values are presented as mean ± SE (n = 4). Different letters represent the groups formed by the Scott-Knot test ($p \leq 0.05$).

3.3 Metabolic traits associated with natural variability in *C. chinense*

To better understand the variation observed in plant height and growth between accessions, a metabolic characterization was performed. Three different groups of accessions can be observed within the population for Chlorophyll *a* (Figure 4). Interestingly, only two groups were observed for Chlorophyll *b* and the ratio chlorophyll *a/b* (Figure 4). In addition, to obtain more information about carbon availability the levels of sucrose, fructose, glucose and starch were determined. Based on sugars the accessions could be separated into two groups (Figure 5). For glucose and fructose a first small group was formed, highlighting the accessions a11, a22, a26, a45, a55, a84 and 98 that appear in both groups; and a second large group. For sucrose the two groups formed have values between 20.0 and 26.8 µmol g^{-1} FW; and 12.8 and 19.5 µmol g^{-1} FW.

By quantifying starch in the middle of the light period, three groups were separated (Figure 5). A first group containing values from 130.6 and 181.1 µmol g^{-1} FW; a second group with values from 90.8 to 127.8 µmol g^{-1} FW; and the last group containing only the accessions a56 and a83 with values of 43.9 and 70.9 µmol g^{-1} FW respectively.

Surprisingly, since both malate and fumarate have been demonstrated as carbon source related to growth, the level of these two organic acids were determined, very distinct results were observed for malate and fumarate (Figure 5). First, the malate levels allowed the formation of four groups of accessions. The first group containing only two accessions, a34 and a84, with values of 108.7 and 110.7 µmol g^{-1} FW. The other three groups comprehend accessions with values ranging between 29.0 and 85.3 µmol g^{-1} FW. Second and perhaps more surprisingly it was not possible to distinguish the accessions by the amount of fumarate.

Concerning nitrogen metabolism the levels of nitrate (NO_3), total amino acids (AA) and proteins (Figure 6) were determined. It was observed that NO_3 has a very interesting behavior, with separation of accessions into three remarkable groups, the first formed only by accession a56 with 0.95 μmol g^{-1} FW; the second group contains only one accession, a86, with 0.42 μmol g^{-1} FW and the remaining accessions belong to the last group with values between 0.0 and 0.15 μmol g^{-1} FW. Amino acids and total proteins formed only two major groups (Figure 6).

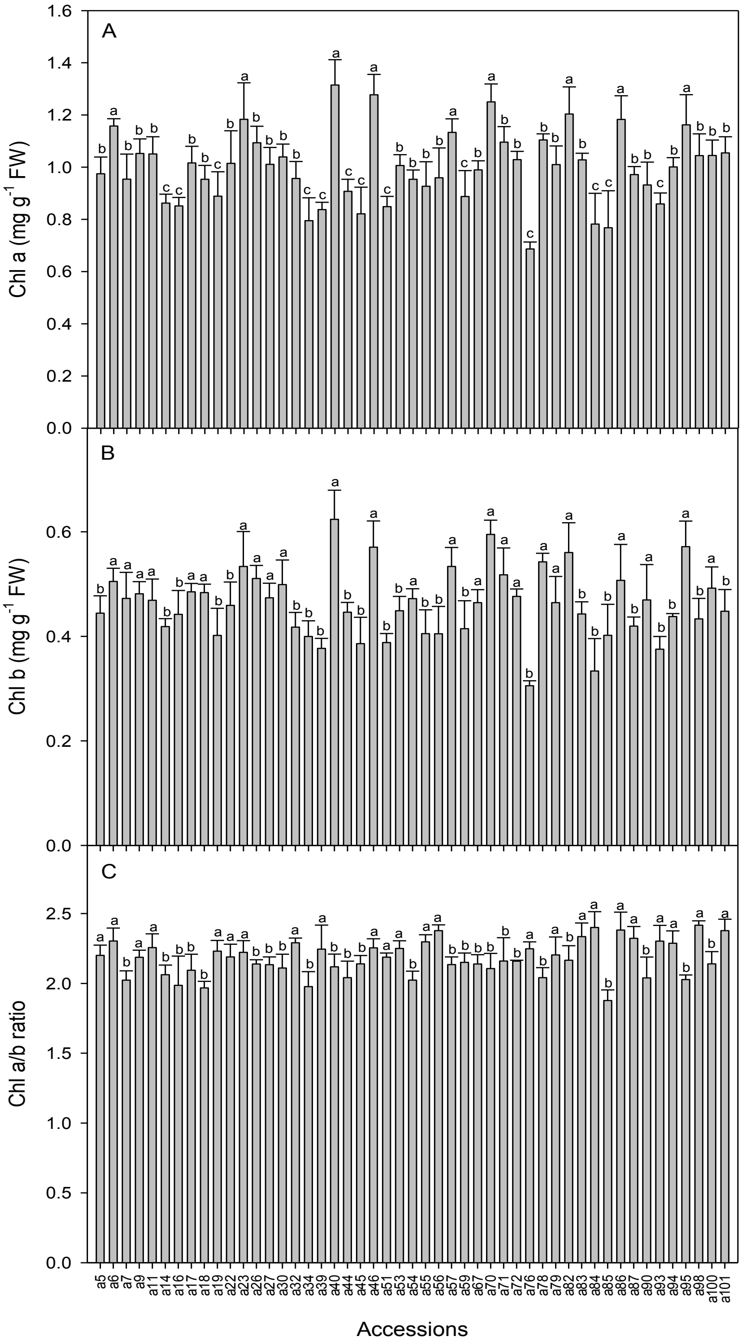

Figure 4. Pigment content of 49 *C. chinense* accessions. (A) Chlorophyll a (Chl a); (B) Chlorophyll b (Chl b); (C) chlorophyll a/b ratio (Chl a/b ratio)). Values are presented as mean ± SE (n = 4). Different letters represent the groups formed by the Scott-Knot test ($p \leq 0.05$).

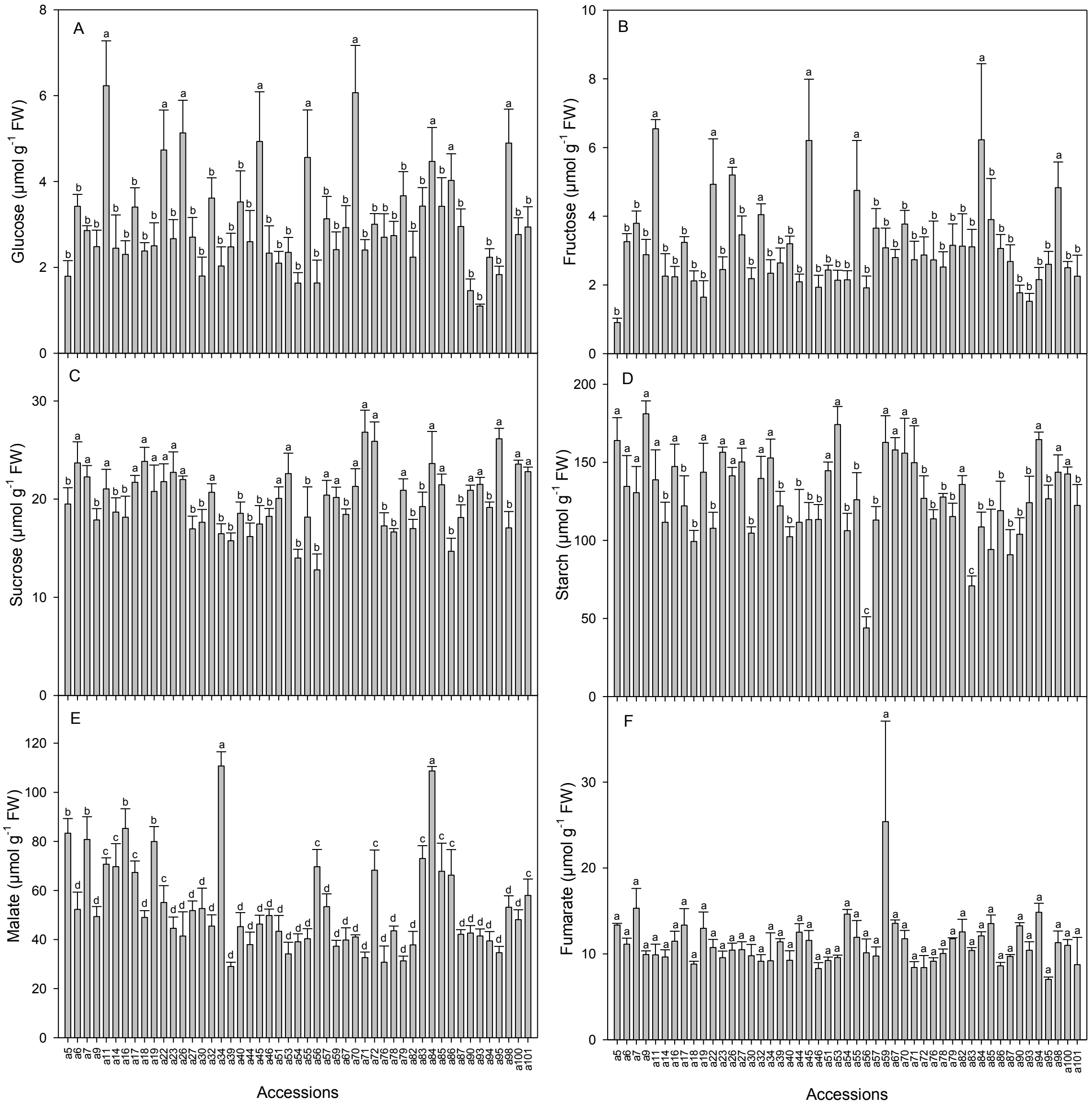

Figure 5. Metabolic traits in leaves of plants from 49 *C. chinense* accessions, harvested at the middle of the light period. (A) Glucose; (B) Fructose; (C) Sucrose; (D) Starch; (E) Malate; (F) Fumarate. Values are presented as mean ± SE (n = 4). Different letters represent the groups formed by the Scott-Knot test (p ≤ 0.05).

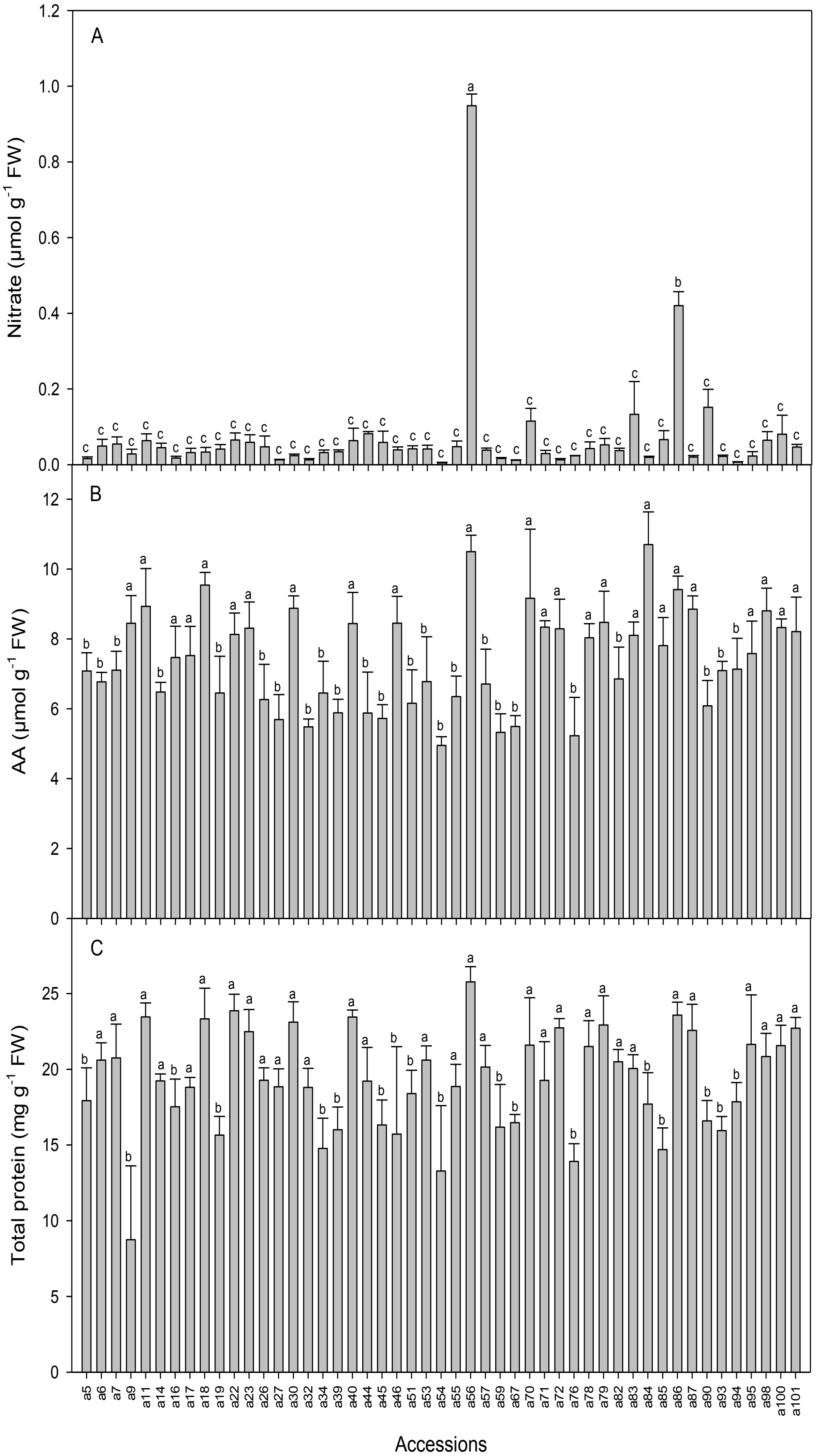

Figure 6. Nitrogen containing compounds in leaves of plants from 49 *C. chinense* accessions, harvested at. middle of the light period. (A) Nitrate (NO_3); (B) Amino acid (AA); (C) Total protein. Values are presented as mean ± SE (n = 4). Different letters represent the groups formed by the Scott-Knot test ($p \leq 0.05$).

3.4 Fruit parameters

Analyzing the total fruit fresh weight (Total fruit FW) the accessions could be separated into three groups (Figure 7). The first group has values ranging from 222.16 to 301.01 g per plant containing the accessions: a22, a40, a78, a83, a82, a85 and a98. The two other groups have values between 25.8 and 197.3 g per plant. For total fruit dry weight two groups of accessions were observed. The first with values between 25.55 and 43.68 g per plant containing the accessions with higher fresh weights listed above and others; and a second group with values between 10.1 and 24.9 g per plant.

The total number of fruits was also evaluated to allow calculation of fresh and dry weight per fruit (Figure 7). The accession a78 appears with the highest fresh weight per fruit with a value of 13.89 g; three other groups have values between 0.52 and 9.80 g. The dry weight per fruit formed only three groups with values from 0.14 to 2.03 g.

Fruit set was calculated from the estimated abortion of flowers. Based on this trait two groups of genotypes are observed (Figure 7). The first group fits amongst others, the accessions a26, a72 and a101, accessions responsible for the formation of the first group with the highest number of fruits. The second group has fruiting percentages between 10.55 and 36.31 %.

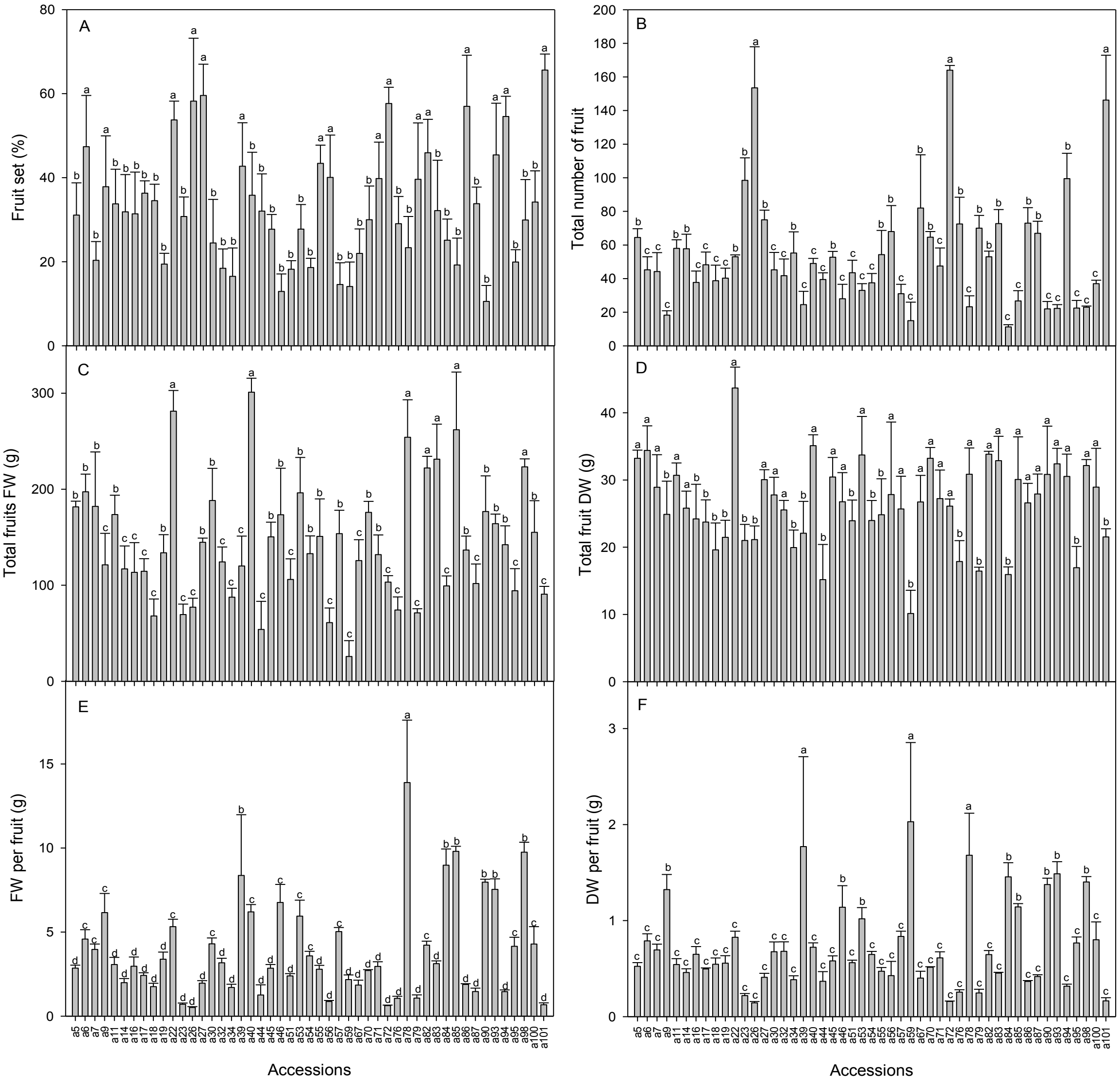

Figure 7. Fruit related parameters of 49 *C. chinense* accessions. (A) Fruit set; (B) Total number of fruits; (C) Total fruit fresh weight (Total fruit FW); (D) Total fruit dry weight (Total fruit DW); (E) Fresh weight per fruit (FW per fruit); (F) Dry weight per fruit (DW per fruit). Values are presented as mean ± SE (n = 4). Different letters represent the groups formed by the Scott-Knot test ($p \leq 0.05$).

3.5 Pearson linear correlation analyzes

To better assess the level of linear association between the variables obtained for the population (Chen and Popovic, 2003) correlation analyses were performed for all 33 variables studied, which resulted in 547 different combinations. Between the correlations statistically significant observed, 102 were positive and 61 negative. Among the significant ones, some were quite obvious, as the positive correlation between height and growth rate (r = 0.97);

fruit set and total number of fruits (r = 0.63); *A* and g_s conductance (r = 0.77), as well as *A* and *ETR* (r = 0.87). On the other hand, very interesting correlations were observed such, as the ones related to fumarate. For a better visualization of interactions between variables from the Person correlation coefficient, a heat map was created, where each square represents the correlation coefficient in a color scale (Figure 8).

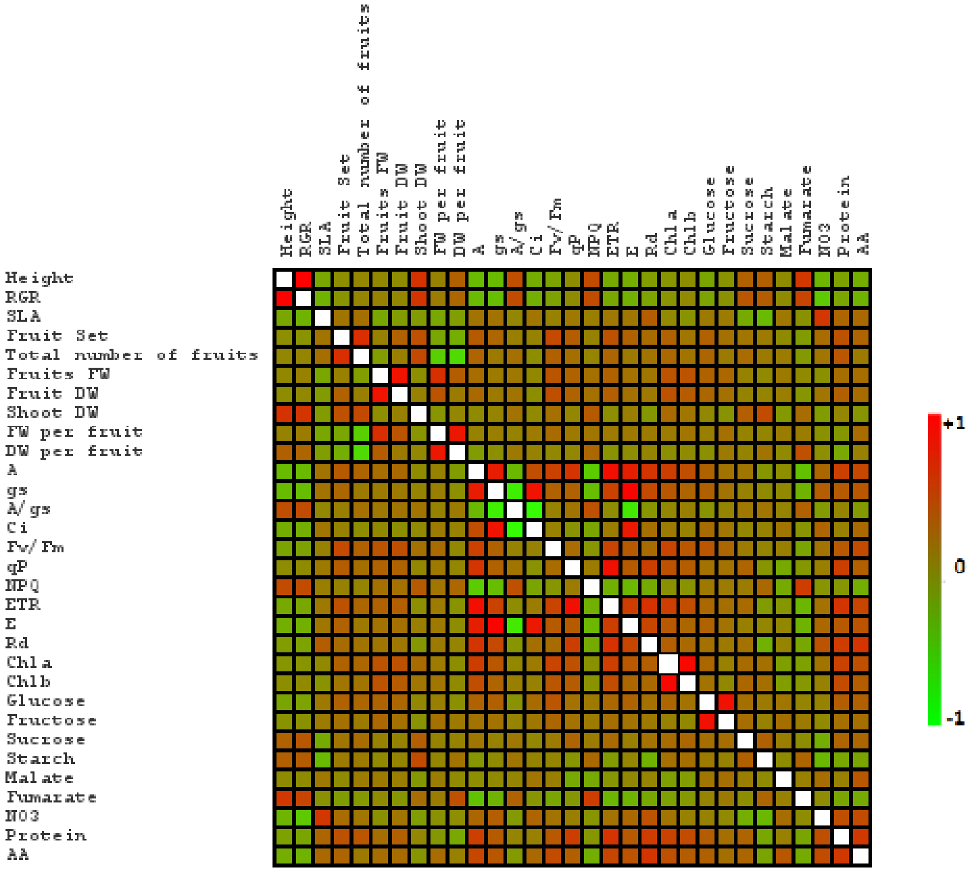

Figure 8. Heat map for Person analysis correlation. Each square represents the correlation coefficient in a color scale, where red, green and brown represent positive, negative and non-significant correlations, respectively. Abbreviations: Relative growth rate (RGR); Specific leaf area (SLA); Shoot dry weight (Shoot DW); Photosynthesis (*A*); Stomatal conductance (g_s); Intrinsic water use efficiency (WUE); Transpiration rates (*E*); Dark respiration (R_d). Maximum photochemical efficiency of photosystem II (F_v/F_m); Photochemical quenching coefficient (*qP*); Non-photochemical quenching (*NPQ*); Electron transport rate (ETR); Chlorophyll *a* (Chl *a*); Chlorophyll *b* (Chl *b*); Chlorophyll *a/b* ratio (Chl *a/b* ratio); Nitrate (NO_3); Amino acid (AA).

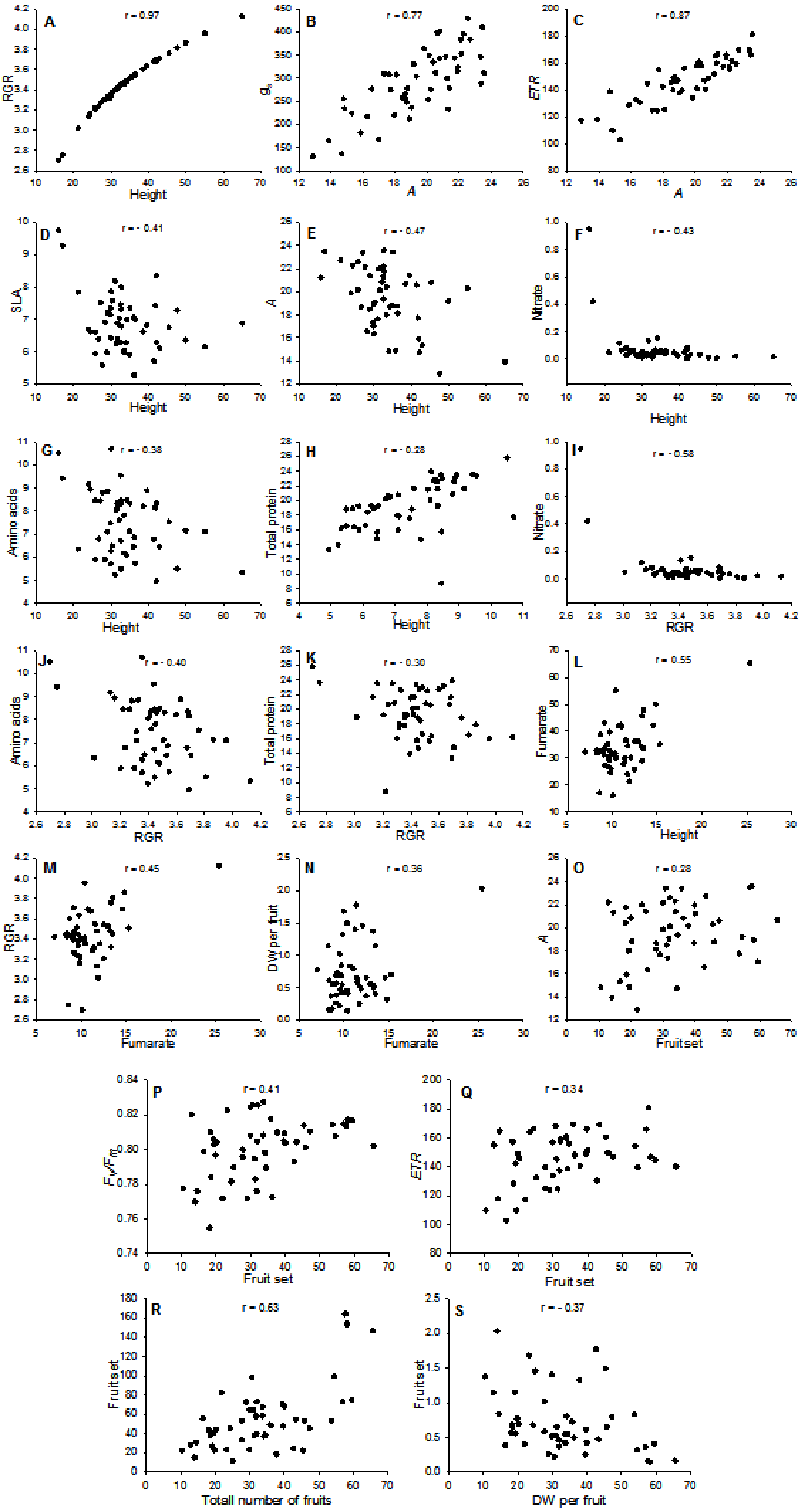

Figure 9. Scatter plots of correlations Pearson coefficients (r) between different parameters of 49 *C. chinense* accessions. Abbreviations: Relative growth rate (RGR); Specific leaf area (SLA); Photosynthesis (*A*); Stomatal conductance (g_s); Maximum photochemical efficiency of photosystem II (F_v/F_m); Electron transport rate (*ETR*); Dry weight (DW).

3.6 Canonical variables analysis

When there is interdependence within a set of variables, as in this case, the Pearson correlations do not adequately exploit this interdependence, which can be obtained through multivariate analysis (Rencher, 2002). The canonical variable (CV) analysis reduced the 24 variables associated with growth parameters (except shoot dry weight which was evaluated at the end of the experiment), gas exchange, fluorescence and metabolites, in three main CV. The first CV is responsible for explaining 27.5% of the variance, the second 18.9% and by analyzing the third CV is possible to explain a total of 56.1% of the total variation.

Through a visual analysis of the dispersion of accessions on CV analysis three clusters were formed (Figure 10). The first cluster contains the accessions a56 and a86, the accessions with lower height and RGR. On the other hand, these same accessions have the highest SLA and higher values of NO_3, AA and total protein. The second cluster comprises the accessions a59, a93 and a94, the largest height values and RGR. These three accessions are among the ones with lower NO_3 contents and have no defined standard for gas exchange parameters. The third cluster comprising the 44 additional accessions present the genotypes with have the most diverse results for all the characteristics studied. The clusters formation was confirmed through Fisher discriminant analysis 100% for the consistency of the three groups was obtained (data not shown).

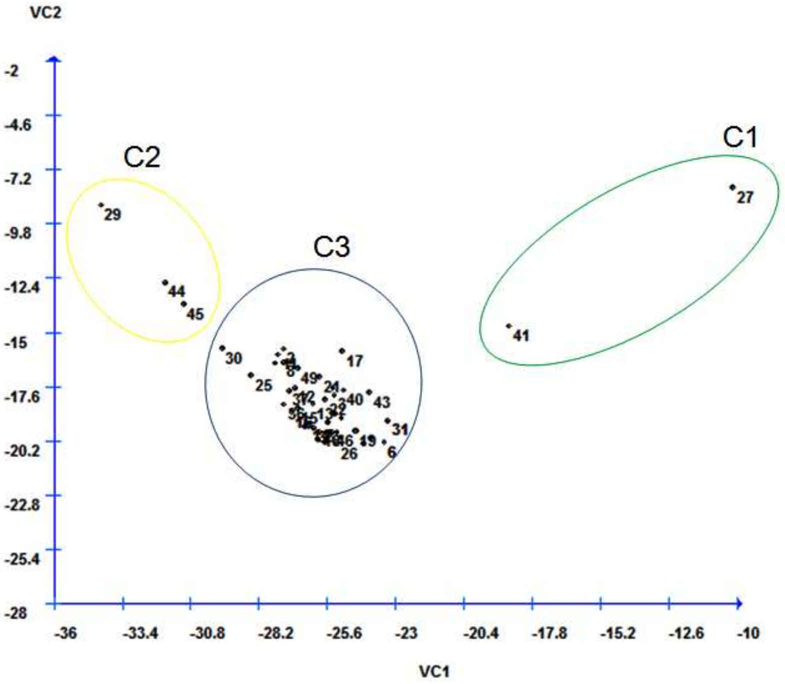

Figure 10. Canonical variables analysis of 49 *C. chinense* accessions. Groups formed based on scores obtained for the 49 accessions regarding the canonical variable 1 (VC1) and canonical variable 2 (VC2). Accessions are identified with sequential numbers as follows: 1-a5; 2-a6; 3-a7; 4-a9; 5-a11; 6-a14; 7-a16; 8-a17; 9-a18; 10-a19; 11-a22; 12-a23; 13-a26; 14-a27; 15-a30; 16-a32; 17-a34; 18-a39; 19-a40; 20-a44; 21-a45; 22-a46; 23-a51; 24-a53; 25-a54; 26-a55; 27-a56; 28-a57; 29-a59; 30-a67; 31-a70; 32-a71; 33-a72; 34-a76; 35-a78; 36-a79; 37-a82; 38-a83; 39-a84; 40-a85; 41-a86; 42-a87; 43-a90; 44-a93; 45-a94; 46-a95; 47-a98; 48-a100; 49-a101. Abbreviations: Accession (a), Cluster 1 (C1), Cluster 2 (C2) and Cluster 3 (C3).

3.7 Two-way ANOVA

In order to verify the amount of total variation that can be attributed to genetic causes, a second experiment was performed and a two-way ANOVA with accessions and environment as main effects and an accession *versus* experiment interaction effect was performed. The genotypic determination coefficient (H^2) presented by the two-way ANOVA suggest that, with the exception of the Chl *a/b* ratio, morphological and metabolic traits have genetic variation around 90% and 60%. By sharp contrast, the fluorescence parameters are greatly influenced by the environment (Figure 11).

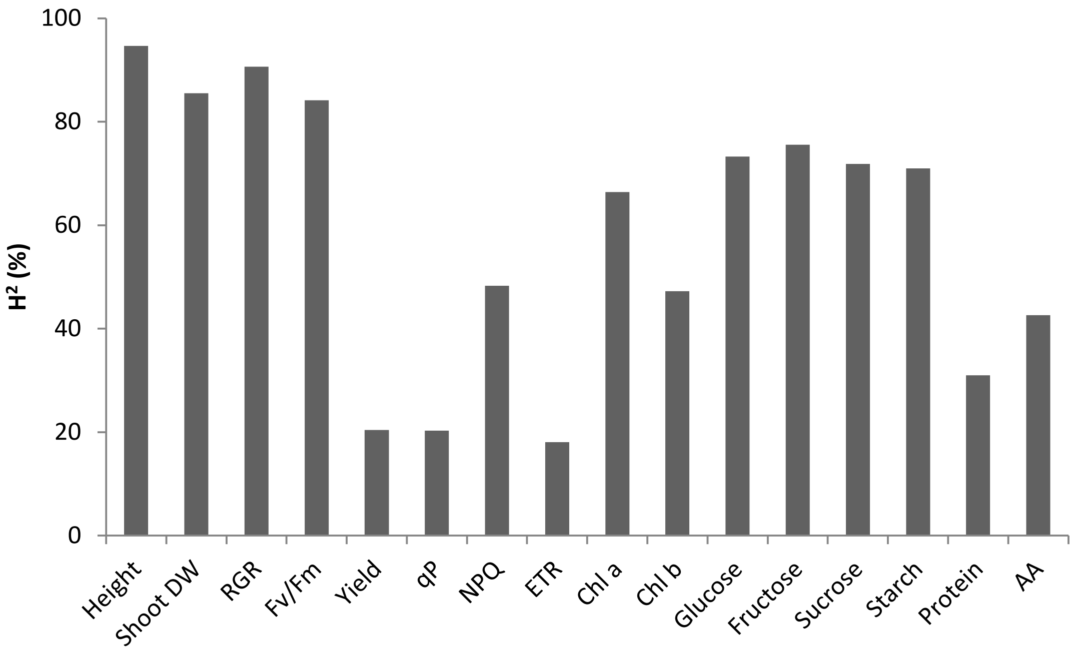

Figure 11. Genotypic determination coefficient (H^2, %) presented by the two-way ANOVA in GENES program (Cruz, 2006).for the variables studied in two distinct experiments. Abbreviations: Shoot dry weight (Shoot DW); Relative growth rate (RGR); Maximum photochemical efficiency of photosystem II (F_v/F_m); Photochemical quenching coefficient (*qP*); Non-photochemical quenching (*NPQ*); Electron transport rate (*ETR*); Chlorophyll a (Chl a); Chlorophyll b (Chl b); Amino acids (AA).

4. Discussion

Land plants are characterized by considerable genetic differences, even between genotypes of the same species, which can reflect in phenotypic variation (Keurentjes and Sulpice, 2009). It is important to mention that the natural genetic variation is probably the most important basic resource for plant biology and naturally occurring genetic variation is extensively observed for most species (Koornneef et al., 2004). This natural variation can be further used to uncover correlations between parameters across large sets of genotypes, which may provide insights into the structure of physiological, metabolic, or regulatory networks (Poorter et al., 2005; Croos et al., 2006; Keurentjes and Sulpice, 2009). This study investigated the natural variation among 49 Brazilian accessions of *Capsicum chinense* in relation of physiological and metabolic parameters in order to better understand how they interact to each other and with other related parameters to both plant growth and fruit production. Altogether, the results presented here suggests that there is not only a huge diversity between the accessions in terms of fruit related traits (Lannes et al., 2007), but also in plant growth and leaf physiological and metabolic terms.

The *Capsicum* accessions used here were collected throughout the Brazilian territory, being 21 collected in the North, 10 in the Northeast, 14 in the Central, 3 in the Southeast region and one of unknown origin (Lannes et al., 2007). Interestingly, the clusters formed by multivariate analysis did not show any correlation with the genotypes geographical distribution. The anthropogenic selection pressure for desired fruit features is certainly one of the factors that most contributed to physiological and metabolic diversity within this species, which justify the grouping of genotypes from distinct regions.

We grow these different *C. chinense* accessions together and observed genetic variation for many traits. Although the analysis of each variable individually allowed the formation of up to seven groups as observed for plant height (Figure 1), when working with the complete data set by CV analysis, the 49 accessions separated in only three clusters (Figure 10). Interestingly, while looking at each cluster in details, the first contains accessions belonging to the group with lower plant height (a56 and a86), which are the same accessions that form the group with lower RGR (Figure 1), as expected, given that these variables have high and positive correlation (Figure 9).

The same accessions form the group with the highest SLA, which contradicts the high rates of photosynthesis. Since these plants exhibited high SLA they were likely to have less chloroplasts per unit leaf surface, and thereby a lower *A* on an area basis is expected (DaMatta et al. 2001). However, this information is contradicted again a by negative correlation between height and SLA and *A* in these accessions (Figure 9). These genotypes also have high g_s and *E*, coupled with high *A* resulted in low values of WUE. Since WUE is a quantitative measurement of how much biomass or yield is produced over a growing season, normalized with the amount of water used up in the process (Sinclair et al., 1984), this lower efficiency for carbon fixation in relation to water loss, probably also contributed to their low values for height and shoot dry weight.

Interestingly, a more extensive vegetative growth was found in some *Capsicum* accessions that display highly branched and reduced apical dominance (data not shown). It is important to note that these traits are important because accessions are normally selected for increased reproductive and reduced vegetative growth (Paran and van der Knaap, 2007), especially when the purpose is to select ornamental plants. The plant size reduction is an interesting focus in these programs because results in the ease of plant care, coupled with reduction in space and nutrient requirements.

The accessions, 56 and 86, have the highest values of NO_3, consequently also have high AA and total protein content. It is also worth noting that the plant height and RGR correlated negatively with the amount of NO_3, AA and total protein (Figure 8). A high NO_3 absorption or availability enables AA and proteins to be synthesized in order to meet the plant metabolic demand, which results in weak but significant and positive correlations between NO_3 and AA, and NO_3 and protein (Figure 8). Using introgression lines populations, it has been shown that rosette biomass in *Arabidopsis* (Meyer et al., 2007) and fruit yield in tomato (Schauer et al., 2006) correlate negatively with pool of metabolites. A highly significant correlation was obtained when multivariate analysis was used to study rosette biomass and the entire metabolite profile, while the correlation was very weak for individual metabolites (Meyer et al., 2007).

Both growth and vegetative development are highly dependent on the interaction between carbon and nitrogen metabolism (Nunes-Nesi et al., 2010; Noctor et al., 2007). In order to assimilate nitrogen, it is essential to provide carbon skeletons (Hachiya et al, 2007; Piques et al., 2009), which results in a negative correlation of NO_3 with sucrose and starch (Figure 8). Since recent studies using isotopic double-labelling ($^{13}C/^{15}N$) and nuclear magnetic resonance analyses have indicated that the carbon skeletons used for day N-assimilation originate from stored organic acids made during the night period (Foyer et al. 2011; Gauthier et al., 2010).

The accessions a59, a93 and a94 form the second cluster, these genotypes demonstrate opposite behavior for growth parameters and NO_3 when compared with the first cluster, however they have no defined standard for gas exchange parameters and WUE (Figure 2), despite having low values of SLA (Figure 1).

These genotypes have the greatest height values and, as expected high RGR and relatively high shoot DW values. Interestingly, plant height correlated negatively with variables related to gas exchange (A, g_s, E) and fluorescence (F_v/F_m, ETR) (Figure 9). Given the high degree of conservation of the structural components of the light reactions among land plants, as determined by chlorophyll fluorescence parameters, there is almost no natural variation in basic photosynthetic parameters among different accessions of the model plant *Arabidopsis* (Leister, 2012). Thus, even with the intense selection of genotypes with desirable characteristics, the correlation with the photosynthetic parameters would remain low, since structural components of the light reactions of photosynthesis are most likely not susceptible to improvement by conventional breeding (Leister, 2012).

The RGR behaved similarly to plant height, with emphasis on the mild negative correlation with *Rd* (Figure 8). Plants obtain the required energy for growth from ATP produced through mitochondrial respiration and photosynthesis, given the broad number of associations between respiration and photosynthesis (Nunes-Nesi et al., 2011). In good agreement recent studies suggest that plant growth is driven not only by the availability of carbohydrates and other central metabolites, but by a much more complex interaction between metabolites and environmental conditions (Sulpice et al., 2009; 2010; Meyer et al., 2007; Osorio et al, 2012).

In terms of nitrogen metabolism, the three accesses in the second cluster are among the lower values of NO_3 content, which contributes to have lower levels of total AA and protein (Figure 6). The capacity to store NO_3 correlated with the tolerance to low nitrogen (North et al.,2009), which is a very interesting characteristic to be explored since nitrogen is a major limiting factor of plant growth in the field (Foyer et al., 2011) and its deficiency induces morphological and physiological parameters modifications (Ciompi et al, 1999).

The third and final cluster comprises the other 44 accessions. They have the most diverse results for the characteristics studied, and accession a101, for example, has intermediate values for height, RGR and SLA. High values for *A* and g_s and a low WUE, however this accession has the highest value for shoot DW probably due to the greater branching observed (data not shown). These results reinforce the idea that, although the accessions belong to the same species, they have diverse adaptation mechanisms being a highly interesting source to study characteristics for breeding programs.

When analyzing organic acids we observed that malate and fumarate, had quite different responses, probably resulting from the different functions performed by these components in this species. Malate has important roles as photosynthetic intermediate in CAM and C4 plants, being also an essential storage carbon molecule and as intermediate of the tricarboxylic acid cycle in all plant species (Fernie and Martinoia, 2009). Furthermore, malate has been suggested as a pH regulator and exhibits partial control over the efficacy of nutrient uptake (Fernie and Martinoia, 2009) and over stomatal function (Fernie and Martinoia, 2009; Araújo et al., 2011). In addition, several recent evidences suggest that malate and fumarate plays an important function as regulators of stomatal function (Nunes-Nesi et al., 2007; Araújo et al., 2011).

Although fumarate did not individual contribute to the accession group separation, a large number of significant correlations is observed, for example, the positive correlation between this metabolite and growth parameters (Figure 9) suggesting the importance of this metabolite in *C. chinense*. In addition recent studies demonstrated that fumarate accumulates in Arabidopsis shoots under low gibberellin (GA) regimes. Additionally it has been demonstrated that adding gibberellic acid to plants under low GA regimes (paclobutrazol treatment) completely rescued their growth and concomitantly decreased the fumarate content (Ribeiro et al., 2012a, b). Thus, it is possible that greater fumarate availability observed in *Capsicum* accessions, together with a lower respiratory rate, would result in an increase in growth.

It has been reported that starch content at the end of the day integrates many other metabolic traits and is negatively correlated with biomass in *Arabidopsis thaliana* (Sulpice et al., 2009). Interestingly in *C. chinense* fumarate levels seems to correlate positively with growth related parameters (Figure 9). However negative correlation between starch and AA, protein and SLA suggests that part of the carbon stored is probably being invested in photosynthetic machinery. Fact of great importance since half or more of the total protein in leaves is part of the photosynthetic machinery (Farquhar et al., 2001; Zhu et al., 2007) allowing quickly adaptive responses to changes in environmental conditions.

In the present study it was demonstrated that fruit set is positively correlated with *A* and other photosynthesis related factors, such as F_v/F_m and *ETR* (Figure 9). It has been shown that photosynthetic rates are related to pepper flower abortion (Aloni et al., 1996) however, it does not explain alone the differences in cultivar susceptibility (Aloni et al., 1996; Turner and Wien, 1994). Thus, suggesting that other factors are also involved in the metabolism targeted to the fruit. The carbon allocation to the flower and its metabolism within the flower is probably more important for fruit retention than photosynthesis is (Aloni et al., 1996).

Additionally, it was observed that the fruit set in *C. chinense* accessions correlates positively to total number of fruit and negatively to the fruit size (Figure 9). Although it has been claimed that hormones are the regulators of fruit-set, it is also well recognized that source–sink relationship is also highly correlated (Valantin-Morison et al., 2006; Kang et al., 2011). In pepper, a positive correlation has been previously observed between the number of fruits successfully ripened and source–sink ratio (Marcelis et al., 2004), and the threshold for fruit-set is cultivar-dependent (Wubs et al., 2009).

It is interesting to note in the present study that fruit dry weight correlates positively with leaf fumarate levels (Figure 9), suggesting that at least in *C. chinense*, fumarate is not only involved vegetative growth regulation, but also in fruit development. Recently, it was demonstrated that malate is important in the regulation of starch metabolism in tomato fruit (Centeno et al., 2011). Additionally, malate levels are highly correlated to genes associated with starch and cell wall pathways as well as protein degradation (Osorio et al., 2012). Thus, the present data set suggest fumarate as an important metabolite that might be involved in the regulation of shoot and fruit growth and development of *C. chinense*.

Despite the fact the most of the growth and metabolic parameters are supposed to be highly influenced by environment factors, the results suggested that morphological and metabolic traits, such as plant height, RGR and starch, have much of its total variation associated with genetic factor, whereas the fluorescence parameters were greatly influenced by the environment in the present study (Figure 11).

5. Conclusions

The results reinforce the idea that, although the accessions belonging to the same species, they exhibit great natural variability of physiological and metabolic characters being a very interesting source for future research.

It was demonstrated that the establishment of fruits is positively correlated with photosynthesis and other related factors. However, photosynthesis alone does not explain the differences in establishing fruit.

Moreover fumarate appears as a candidate metabolite for the regulation of shoot growth and fruit in *Capsicum chinense*.

6. References

Aloni B, Karni L, Zaidman Z, Schaffer AA (1996) Changes of carbohydrates in pepper (*Capsicum annuum* L.) flowers in relation to their abscission under different shading regimes. Ann Bot 78: 163-168.

Araújo WL, Nunes-Nesi A, Osorio S, Usadel B, Fuentes D, Nagy R, Balbo I, Lehmann M, Studart-Witkowski C, Tohge T, Martinoia E, Jordana X, DaMatta FM, Fernie AR (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.

Aza-González CG, Núnez-Palenius H, Ochoa-Alejo N (2011) Molecular biology of capsaicinoids biosynthesis in chili pepper (*Capsicum* spp.). Plant Cell Rep 30: 695-706.

Bhering LL, Cruz CD, Vasconcelos ES, Ferreira A, Rezende Jr MFR (2008) Alternative methodology for Scott Knott test. Crop Breed Appl Biotechnol 8: 9-16.

Bianchetti LB (1996) Aspectos morfológicos, ecológicos e biogeográficos de dez táxons de *Capsicum* (Solanaceae) ocorrentes no Brasil. 174p. Dissertação (Mestrado em Botânica) - Departamento de Botânica do Instituto de Ciências Biológicas. Universidade de Brasília. Brasília. Brasil.

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

Büttow MV, Barbieri RL, Neitzke RS, Heiden G, Carvalho FIF (2010) Genetic diversity in peppers and sweet peppers of Embrapa Clima Temperado genebank. Ciênc Rural 40: 1264-1269.

Cavatte PC, Oliveira AAG, Morais LE, Martins SCV, Sanglard LMVP, DaMatta FM (2012) Could shading reduce the negative impacts of drought on coffee? A morphophysiological analysis. Physiol Plant 144: 111-122.

Centeno DC, Osorio S, Nunes-Nesi A, Bertolo AL, Carneiro RT, Araujo WL, Steinhauser MC, Michalska J, Rohrmann J, Geigenberger P, Oliver SN, Stitt M, Carrari F, Rose JK, Fernie AR (2011) Malate plays a crucial role in starch metabolism, ripening, and soluble solid content of tomato fruit and affects postharvest softening. Plant Cell 23: 162-184

Chen JI, Popovich K (2003) Understanding customer relationship (CRM) People, process and technology. Business Process Manag J 9: 672-688.

Ciompi S, Gentili E, Guidi L, Soldatini GF (1999) The effect of nitrogen deficiency on leaf gas exchange and chlorophyll fluorescence parameters in sunflower. Plant Sci 118: 177-184.

Cross JM, von Korff M, Altmann T, Bartzetko L, Sulpice R, Gibon Y, Palacios N, Stitt M (2006) Variation of enzyme activities and metabolite levels in 24

Arabidopsis accessions growing in carbon-limited conditions. Plant Physiol 142: 1574-1588.

Cruz CD (2006) Programa Genes: Análise multivariada e simulação. Editora UFV. Viçosa, MG. 175p.

DaMatta FM, Loos RA, Rodrigues R, Barros RS (2001) Actual and potential photosynthetic rates of tropical crop species. Braz J Plant Physiol 13: 24–32

DaMatta FM, Loos RA, Silva EA, Loureiro ME (2002) Limitations to photosynthesis in *Coffea canephora* as a result of nitrogen and water availability. J Plant Physiol 159: 975-981.

Edge R, McGarvey DJ, Truscott TG (1997). The carotenoids as anti-oxidants – a review. J Photochem Photobiol B 41: 189-200.

Farquhar GD, von Caemmerer S, Berry JA (2001) Models of photosynthesis. Plant Physiol 125: 42-45.

Fernie AR and Klee HJ (2011) The use of natural genetic diversity in the understanding of metabolic organization and regulation. Front Plant Sci 2: 59.

Fernie AR, Martinoia E (2009) Malate. Jack of all trades or master of a few? Phytochemistry 70: 828-832.

Fernie AR, Roessner U, Trethewey RN, Willmitzer L (2001) The contribution of plastidial phosphoglucomutase to the control of starch synthesis within the potato tuber. Planta 213: 418-426.

Fisher RA (1935) The logic of inductive inference. J Roy Stat Soc 98: 39-54. In: Cruz CD, Ferreira FM, Pessoni LA (2011) Biometria aplicada ao estudo da diversidade genética. 1 ed. 620p. Visconde do Rio Branco, MG, Brasil.

Foyer CH, Noctor G, Hodges M (2011) Respiration and nitrogen assimilation: targeting mitochondria-associated metabolism as a means to enhance nitrogen use efficiency. J Exp Bot 62: 1467-1482.

Gauthier P, Bligny R, Gout E, Mahé A, Nogués S, Hodges M,Tcherkez G. (2010) *In folio* isotopic tracing demonstrates that nitrogen assimilation into glutamate is mostly independent from current CO_2 assimilation in illuminated leaves of *Brassica napus*. New Phytol 185: 988-999.

Gibon Y, Blaesing OE, Hannemann J, Carillo P, Hoehne M, Hendriks JHM, Palacios N, Cross J, Selbig J, Stitt M (2004) A robot-based platform to measure multiple enzyme activities in *Arabidopsis* using a set of cycling assays: comparison of changes of enzyme activities and transcript levels during diurnal cycles and in prolonged darkness. Plant Cell 16: 3304-3325.

Hachiya T, Terashima I, Noguchi K (2007) Increase in respiratory cost at high growth temperature is attributed to high protein turnover cost in *Petunia x hybrida* petals. Plant Cell Environ 30: 1269-1283.

Hair JF (1998) Multivariate data analysis. Upper Saddle River: Prentice Hall 1: 730.

Howard LR, Wildman REC (2007) Antioxidant vitamin and phytochemical content of fresh and processed pepper fruit (*Capsicum annuum*). In: Wildman, R.E.C. (Ed.), Handbook of Nutraceuticals and Functional Foods. CRC Press 2000, eBook ISBN: 978-1-4200-3669-5; DOI: 10.1201/9781420036695.ch13

Hunt R, Causton DR, Shipley B, Askew AP (2002). a modern tool for classical plant growth analysis. Ann Bot 90: 485-488.

Ikram S, Bedu M, Daniel-Vedele F, Chaillou S, Chardon F (2012) Natural variation of *Arabidopsis* response to nitrogen availability. J Exp Bot 63: 91-105.

Ince AG, Karaca M, Onus AN (2009) Development and utilization of diagnostic DAMD-PCR markers for *Capsicum* accessions. Genet Resour Crop Ev 56: 211-221.

Kang M, Yang L, Zhang B, de Reffye P (2011) Correlation between dynamic tomato fruit-set and source-sink ratio: a common relationship for different plant densities and seasons? Ann Bot 107: 805-815

Keurentjes JJ, Sulpice R (2009) The role of natural variation in dissecting genetic regulation of primary metabolism. Plant Signal Behav 4: 244-246.

Keurentjes JJB, Fu J, de Vos CH, Lommen A, Hall RD, Bino RJ, van der Plas LHW, Jansen RC, Vreugdenhil D, Koornneef M (2006) The genetics of plant metabolism. Nat Genet 38: 842-849.

Keurentjes JJB, Sulpice R, Gibon Y, Fu J, Koornneef M, Stitt M, Vreugdenhil D (2008) Integrative analyses of genetic variation in enzyme activities of primary carbohydrate metabolism reveal distinct modes of regulation in *Arabidopsis thaliana*. Genome Biol 9: R129

Koornneef M, Alonso-Blanco C, Vreugdenhil D (2004) Naturally occurring genetic variation in *Arabidopsis thaliana*. Annu Rev Plant Biol 55: 141-172.

Lannes SD, Finger FL, Schuelter AR, Casali VWD (2007) Growth and quality of Brazilian accessions of *Capsicum chinense* fruits. Sci Hort 112: 266-270.

Leister D (2012) How can the light reactions of photosynthesis be improved in plants? Front Plant Sci 3: 199.

Lima ALS, DaMatta FM, Pinheiro HA, Totola MR, Loureiro ME (2002) Photochemical responses and oxidative stress in two clones of *Coffea canephora* under water deficit conditions. Environ Exp Bot 47: 239-247.

Luz FJF (2007) Caracterizações morfológica e molecular de acessos de pimenta (*Capsicum chinense* Jaqc.) Dissertação (Doutorado em Produção Vegetal) 81p - Universidade Estadual Paulista – UNESP - Faculdade de Ciências Agrárias e Veterinárias do Campus de Jaboticabal – UNESP. Jaboticabal. São Paulo. Brasil.

Marcelis LFM, Heuvelink E, Hofman-Eijer LRB (2004) Flower and fruit abortion in sweet pepper in relation to source and sink strength. J Exp Bot 55: 2261-2268.

Meyer RC, Steinfath M, Lisec J, Becher M, Witucka-Wall H, Törjék O, Fiehn O, Eckardt A, Willmitzer L, Selbig J, Altmann T (2007) The metabolic signature related to high plant growth rate in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 104: 4759-4764.

Noctor G, Dutilleul C, Lelarge C, Prioul J, De Paepe R, Foyer C (2007) The role of the mitochondrial electron transport chain in photosynthesis, stress responses, and the integration of carbon-nitrogen metabolism. Photosynth Res 91: 259-259.

North KA, Ehlting B, Koprivova A, Rennenberg H, Kopriva S (2009) Natural variation in Arabidopsis adaptation to growth at low nitrogen conditions. Plant Physiol Biochem 47: 912-918.

Nunes-Nesi A, Araújo WL, Fernie AR (2011) Targeting mitochondrial metabolism and machinery as a means to enhance photosynthesis. Plant Physiol 155: 101-107.

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 AM, 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, Fernie AR, Stitt M (2010) Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. Mol Plant 3: 973-996.

Onus AN, Pickersgill B (2004) Unilateral incompatibility in *Capsicum* (*Solanaceae*): occurrence and taxonomic distribution. Ann Bot 94: 289-295.

Osorio S, Alba R, Nikoloski Z, Kochevenko A, Fernie AR, Giovannoni JJ (2012) Integrative comparative analyses of transcript and metabolite profiles from pepper and tomato ripening and development stages uncovers species-specific patterns of network regulatory behavior. Plant Physiol 159: 1713-1729.

Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, Chen S, Corpe C, Dutta A, Dutta SK, Levine M (2003) Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Nutr 22: 18-35.

Paran I, van der Knaap E (2007) Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. J Exp Bot 58: 3841-3852.

Pickersgill B (1971) Relationships between weedy and cultivated forms in some species of chilli peppers (genus Capsicum). Evolution 25: 683-691.

Pickersgill B (1977) Chromossomes and evolution in *Capsicum*. In: Pochard, E. (Ed) Capsicum 77. INRA Montfavet-Avignon, France 27-37.

Piques M, Schulze WX, Hohne M, Usadel B, Gibon Y, Rohwer J, Stitt M. (2009) Ribosome and transcript copy numbers, polysome occupancy and enzyme dynamics in Arabidopsis. Mol Syst Biol 5: 314.

Poorter H, van Rijn CP, Vanhala TK, Verhoeven KJ, de Jong YE, Stam P, Lambers H (2005) A genetic analysis of relative growth rate and underlying components in *Hordeum spontaneum*. Oecologia 142: 360-377.

Pyl E-T, Piques M, Ivakov A, Schulze W, Ishihara H, Stitt M, Sulpice R (2012) Metabolism and growth in *Arabidopsis* depend on the daytime temperature but are temperature-compensated against cool nights. Plant Cell 24: 2443-2469.

Reifschneider FJB (2000) *Capsicum* – Pimentas e Pimentões no Brasil. Embrapa Comunicação para Transferência de Tecnologia / Embrapa Hortaliças. Brasília, p.113.

Reifschneider FJB, Ribeiro CSC (2004) Embrapa Hortaliças. Sistemas de Produção, 4 ISSN 1678 - Versão Eletrônica Dezembro/2004. Available on: http://www.cnph.embrapa.br/sistprod/pimenta/index.htm

Rencher A (2002) Methods of multivariate analysis. 2.ed. New York: John Wiley & Son.

Ribeiro DM, Araújo WL, Fernie AR, Schippers JH, Mueller-Roeber B (2012a) Translatome and metabolome effects triggered by gibberellins during rosette growth in *Arabidopsis*. J Exp Bot 63: 2769-2786

Ribeiro DM, Araújo WL, Fernie AR, Schippers JH, Mueller-Roeber B (2012b) Action of gibberellins on growth and metabolism of *Arabidopsis* plants associated with high concentration of carbon dioxide. Plant Physiol 160: 1781-1794.

Rodriguez JM, Berke T, Engle L, Nienhuis J (1999) Variation among and within *Capsicum* species revealed by RAPD markers. Theor Appl Genet 99: 147-156.

Schreiber U, Bilger W, Neubauer C (1994) Chlorophyll fluorescence as a nonintrusive indicator of rapid assessment of in vivo photosynthesis. In: Schulze E-D M, Caldwell MM (eds) Ecophysiology of Photosynthesis. Springer-Verlag, Berlin pp 49-70.

Siedow JN, Day DA (2000) Respiration and photorespiration. Bioch Mol Bio Plant 56: 676-728

Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR (2004) Farmacognosia: da planta ao medicamento. 5 ed. Editora da UFRGS / Editora da UFSC, 1102p.

Sinclair TR, Tanner CB, Bennett JM (1984) Water-use efficiency in crop production. Bio Science 34: 36-40

Silva FMO (2012) O uso de linhas de introgressão para a compreensão de variações da fotossíntese e respiração em tomate. Dissertação (Mestrado em

Fisiologia Vegetal) 65p – Universidade Federal de Viçosa – UFV. Viçosa, Minas Gerais, Brasil.

Stewart C Jr, Kang BC, Liu K, Mazourek M, Moore SL, Yoo EY, Kim BD, Paran I, Jahn MM (2005) The Pun1 gene for pungency in pepper encodes a putative acyltransferase. Plant J 42: 675-688.

Stewart C Jr, Mazourek M, Stellari GM, O'Connell M, Jahn M (2007). Genetic control of pungency in *Capsicum chinense* via the Pun1 locus. J Exp Bot 58: 979-991.

Sulpice R, Pyl ET, Ishihara H, Trenkamp S, Steinfath M, Witucka-Wall H, Gibon Y, Usadel B, Poree F, Piques MC, Von Korff M, Steinhauser MC, Keurentjes JJ, Guenther M, Hoehne M, Selbig J, Fernie AR, Altmann T, Stitt M (2009) Starch as a major integrator in the regulation of plant growth. Proc Natl Acad Sci USA 106: 10348-10353.

Sulpice R, Trenkamp S, Steinfath M, Usadel B, Gibon Y, Witucka-Wall H, Pyl ET, Tschoep H, Steinhauser MC, Guenther M, Hoehne M, Rohwer JM, Altmann T, Fernie AR, Stitt M (2010) Network analysis of enzyme activities and metabolite levels and their relationship to biomass in a large panel of *Arabidopsis* accessions. Plant Cell 22: 2872-2893.

Sulpice R, Tschoep H, von Korff M, Büssis D, Usadel B, Höhne M, Witucka-Wall H, Altmann T, Stitt M, Gibon Y (2007). Description and applications of a rapid and sensitive non-radioactive microplate-based assay for maximum and initial activity of d-ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant Cell Environ 30: 1163-1175.

Topuz A, Ozdemir F (2007) Assessment of carotenoids, capsaicinoids and ascorbic acid composition of some selected pepper cultivars (*Capsicum annuum* L.) grown in Turkey. J Food Compos Anal 20: 596-602.

Turner AD, Wien HC. 1994. Dry matter assimilation and partitioning in pepper cultivars differing in susceptibility to stress-induced bud and flower abscission. Ann Bot 73: 617-622.

Valantin-Morison M, Vaissière BE, Gary C, Robin P (2006) Source–sink balance affects reproductive development and fruit quality in cantaloupe (Cucumis melo L.). J Hortic Sci Biotech 81: 105–117.

Wahyuni Y, Ballester AR, Sudarmonowati E, Bino RJ, Bovy AG (2011) Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: Variation in health-related compounds and implications for breeding. Phytochemistry 72: 1358-1370.

Wahyuni Y, Ballester AR, Tikunov Y, de Vos RC, Pelgrom KT, Maharijaya A, Sudarmonowati E, Bino RJ, Bovy AG (2013) Metabolomics and molecular marker analysis to explore pepper (*Capsicum* sp.) biodiversity. Metabolomics 9: 130-144.

Ward JH, Jr (1963) Hierarchical grouping to optimize an objective function. J Am Statist Asso 58: 236-244.

Wubs AM, Ma Y, Heuvelink E, Marcelis LFM (2009) Genetic differences in fruit-set patterns are determined by differences in fruit sink strength and a source: sink threshold for fruit set. Ann Bot 104: 957-964.

Wyżgolik G, Nawara J, Leja M (2008) Photosynthesis and some growth parameters of sweet pepper grown under different light conditions. Sodininkyste ir Daržininkyste 27: 93-98.

Zhu XG, de Sturler E, Long SP (2007) Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: A numerical simulation using an evolutionary algorithm. Plant Physiol 145: 513-526.

Supplementary material

Table 1 - Summary of analysis of variance (ANOVA) in a randomized block design for all variables evaluated in 49 C. chinense accessions. Abbreviations: Relative growth rate (RGR); Specific leaf area (SLA); Dry weight (DW); Fresh Weight (FW); Photosynthesis (A); Stomatal conductance (gs); Intrinsic water use efficiency (WUE); Internal CO_2 concentration (Ci); Maximum photochemical efficiency of photosystem II (Fv/Fm); effective photochemical efficiency of photosystem II (YII); Photochemical quenching coefficient (qP); Non-photochemical quenching (NPQ); Electron transport rate (ETR); Transpiration rates (E); Dark respiration (Rd); Chlorophyll a (Chl a); Chlorophyll b (Chl b); Chlorophyll a/b ratio (Chl a/b ratio); Nitrate (NO_3); Amino acid (AA).

		Quadrados Médios				
FV	GL	Height	RGR	SLA	Fruit Set	Total number of fruits
Blocos	3	28.8861	0.0431	1.7672	240.1148	145.4286
Tratamentos	48	318.8339 **	0.2709 **	3.1028 **	755.9542 **	4383.5515 **
Resíduo	144	15.5892	0.0179	0.6785	240.8848	441.0113
Mínimo		10.00	2.30	1.22	3.23	3.00
Máximo		72.50	4.23	11.27	94.74	203.00
Média		33.91	3.44	6.89	33.24	54.11
CV(%)		11.64	3.89	11.96	46.70	38.81

		Quadrados Médios				
FV	GL	Fruits FW	Fruit DW	Shoot DW	FW per fruit	DW per fruit
Blocos	3	18044.4157	220.7931	1890.0393	3.4435	0.0211
Tratamentos	48	14961.5111 **	157.7003 **	403.2952 **	33.2954 **	0.8045 **
Resíduo	144	2222.3877	56.1009	60.0701	2.9487	0.1697
Mínimo		8.10	6.21	12.76	0.45	0.12
Máximo		405.75	58.77	97.38	20.12	4.53
Média		143.62	26.43	39.15	3.85	0.72
CV(%)		32.82	28.34	19.80	44.61	57.40

		Quadrados Médios				
FV	**GL**	**A**	**g_s**	**WUE**	**Ci**	**Fv/Fm**
Blocos	3	172.3044	0.1010	1953.5658	3478.6716	0.0010
Tratamentos	48	29.8124 **	0.0208 **	749.9803 **	1914.7250 **	0.0012 **
Resíduo	144	6.1147	0.0063	235.2056	583.8000	0.0002
Mínimo		9.25	0.08	41.52	167.69	0.73
Máximo		27.34	0.55	131.52	310.88	0.85
Média		19.40	0.29	73.02	259.41	0.80
CV(%)		12.75	27.31	21.00	9.31	1.78

		Quadrados Médios				
FV	**GL**	**YII**	**qP**	**NPQ**	**ETR**	**E**
Blocos	3	0.0399	0.0515	1.2000	7659.2946	11.8478
Tratamentos	48	0.0065 **	0.0134 **	0.1800 **	1249.1303 **	1.3358 **
Resíduo	144	0.0013	0.0023	0.0547	253.0271	0.4700
Mínimo		0.20	0.33	0.35	89.35	1.24
Máximo		0.45	0.73	2.28	197.63	6.73
Média		0.33	0.58	1.24	146.04	3.18
CV(%)		10.86	8.29	18.82	10.89	21.55

		Quadrados Médios				
FV	**GL**	**Rd**	**Chla**	**Chlb**	**Chl a/b**	**Glucose**
Blocos	3	0.3912	0.0940	0.0416	0.1156	1.6141
Tratamentos	48	0.0974 ns	0.0750 **	0.0170 **	0.0634 **	5.2674 **
Resíduo	144	0.0751	0.0209	0.0057	0.0319	1.2926
Mínimo		0.33	0.47	0.18	1.62	0.69
Máximo		1.84	1.58	0.79	2.76	8.83
Média		1.01	1.00	0.46	2.18	3.01
CV(%)		27.08	14.49	16.32	8.20	37.78

		Quadrados Médios				
FV	GL	Fructose	Sucrose	Starch	Malate	Fumarate
Blocos	3	2.1009	18.5209	658.6333	251.4199	14.2120
Tratamentos	48	6.0496 **	38.6300 **	2768.7655 **	1433.7311 **	31.6063 **
Resíduo	144	1.8262	9.0154	697.8206	123.6884	16.7596
Mínimo		0.62	9.18	21.35	11.41	0.30
Máximo		12.85	33.43	204.41	122.23	60.54
Média		3.06	19.85	127.64	53.39	11.13
CV(%)		44.14	15.12	20.70	20.83	36.78

		Quadrados Médios		
FV	GL	NO3	Protein	AA
Blocos	3	0.0010	24.7459	0.6433
Tratamentos	48	0.0613 **	45.5529 **	7.8137 **
Resíduo	144	0.0022	15.5439	2.4576
Mínimo		0.00	-1.35	2.24
Máximo		1.02	28.45	13.37
Média		0.06	19.30	7.43
CV(%)		74.12	20.42	21.09

*** and ** significant at 1 and 5% probability, respectively, by F test and ns not significant, by F test.**