University of São Paulo
"Luiz de Queiroz" College of Agriculture

Mutations affecting tomato (*Solanum lycopersicum* L. cv. Micro-Tom) response to salt stress and their physiological meaning

Ariadne Felicio Lopo de Sá

Thesis presented to obtain the degree of Doctor in Science. Area: Plant Physiology and Biochemistry

Piracicaba
2016

Ariadne Felicio Lopo de Sá
Bachelor in Biological Sciences

Mutations affecting tomato (*Solanum lycopersicum* L. cv. Micro-Tom) response to salt stress and their physiological meaning

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **LÁZARO EUSTÁQUIO PEREIRA PERES**

Thesis presented to obtain the degree of Doctor in Science. Area: Plant Physiology and Biochemistry

Piracicaba
2016

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA - DIBD/ESALQ/USP

Sá, Ariadne Felicio Lopo de
Mutations affecting tomato (*Solanum lycopersicum* L. cv. Micro-Tom) response to salt stress and their physiological meaning / Ariadne Felicio Lopo de Sá. - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2016.
85 p. : il.

Tese (Doutorado) - - Escola Superior de Agricultura "Luiz de Queiroz".

1. Ácido abscísico 2. Ácido salicílico 3. Auxina 4. Crescimento vegetativo/reprodutivo 5. Estrigolactona 6. Etileno 7. Giberelina 8. Heterose 9. Hormônios vegetais 10. Indução floral 11. Mutante 12. Produtividade 13. Resistencia 14. Salinidade 15. Senescência 16. Tolerância I. Título

CDD 635.642
S111m

"Permitida a cópia total ou parcial deste documento, desde que citada a fonte – O autor"

DEDICATION

I dedicate this work to people that share their knowledge, hope and glee

to shape a better world

ACKNOWLEDGEMENTS

I thank Jehovah God for giving me strength in the most difficult moments, for watching over me in every adversity, for putting wonderful people in my life who taught me and continue teaching me how to be a better person. I also thank God for the achievements during this period and especially for His love.

I thank my parents, Cida and Antonio, and my brother Bryan for all the love, affection, attention, care, concern, help, education and unconditional support. I think them for always overcoming their limitations to be near me, including the long nonstop travels of 13 hours to visit me. I also thank my brother for being my great friend all the time, for having helped in my experiments and for assisting me with Photoshop.

I am very grateful to Prof. Dr. Lázaro Eustáquio Pereira Peres for his friendship, talks, help, maximum collaboration, efficiency, support, respect and knowledge shared during these four years, in addition to scientific discussions during the coffee breaks. All these actions, along with his guidance, helped me streamline important features not only for my professional formation, but also for increasing my perseverance through adversity.

I thank researchers PhD. Francisco Pérez-Alfocea and PhD. Alfonso Albacete Moreno for discussions about my work and for having provided an environment suited to my experiments. I also thank the other members of the project Root Power: PhD. Cristina Martínez, Cristina Soriano, Ascención and Maria del Puerto.

I thank Dr. Roger Chetelat (Tomato Genetics Resource Center, Davis, USA), Dr. Harry Klee (University of Florida, USA) and Dr. Jonathan Jones (The Sainsbury Laboratory, UK) for the donation of tomato seeds in their original genetic backgrounds.

I thank all the teachers of Esalq for their dedication, teachings and examples transmitted, especially Prof. Dr. Daniel Scherer de Moura for his excellent classes. I also thank Prof. Dr. Paulo Ricardo Camargo e Castro for his supervision and attention during my internship in teaching.

I give thanks to the lab technician Cássia Regina Figueiredo by her friendship, assistance in technical activities and attention.

I thank the staff at the Cebas-CSIC, in particular Antonio, for having carried out the repairs necessary for my experiments in growth chambers. I also thank Noemi Mundo for all the help and assistance provided.

I give thanks to my lab partners of the Hormonal Control of Plant Development Laboratory for their friendship and a lot of other things. I give thanks to Eloisa for the delicious cakes; to Frederico for the discussions; to Guilherme for his help and patience during the tests of tissue culture; to João for helping in the preparation of the trays of hydroponics, to Mateus for the introgression of the mutant *sft* into the MT background and for installing the hydroponic system; to Maísa for her assistance in obtaining balls of expanded polystyrene, to Marcela for the tips on propagation in nutrient medium and for helping collect signatures while I was abroad. I give thanks to all the others who took part of my life at some point in these last four years and contributed for this period to be more productive and edifying: Agustin, Airton, Antoine, Carlos, Diego, Eder, Geraldo, João, Ignacio, Ivan, Lilian, Jonata and Mariana.

I thank my boyfriend Alex D. de Oliveira for being my partner even in the early hours before the contest listening to my class about photosynthesis. I thank him for multiplying exponentially my moments of happiness, because without doubt happiness is to know that I have your love but also loving someone as special as you.

I thank my grandparents Apparecida G. Ortiz and José Roberto Ortiz all their over love, affection and care, for never forgetting about me even when there was an ocean separating us. Thanks for the best breakfasts ever, as well as thanks to her, for always being willing to learn new technologies and for keeping in touch with me and transmitting knowledge about God.

I thank the Marín family: Cristina, Antonia, Jovita *in memorium*, Paco, Lídia, Andres, Maribel and Javí for having hosted me and for being my family in Spain.

I give thanks to Márcia E. Carvalho and Karina G. Ferracioli for being good friends during my doctorate, for being willing to help me and for the support. I also thank for providing doses of optimism and rationality.

I also thank my friends Ana Flávia da Hora, Cristiane Munhoz, Dayane Fabrício Bressan, Daniela Tastch Baptista, Luciane Mendes, Marli Vieira, Tatiane Kim, Neila Moura and Khaloufi Mouna for their friendships, support, help, company, conversations, patience, travels and meals.

I thank Alice Aranda-Peres for being so kind and nice to me, but mostly when my grandpa got sick. Thank you for your kind words. Thank you for the tips before my trip and the wonderful cakes and the meat pie.

I thank all the taxpayers of this country who work hard for a better country and for underpinning all public institutions, including the University of São Paulo.

I also give thanks to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), which granted me scholarships for my studies in the country and abroad (Process: BEX-2014 05/3815), without them I would never have been able to carry out my doctoral course.

EPIGRAPHY

"Life, like a mist, appears for just a day,
Then disappears tomorrow.
If a man should die, can he live again?
Hear the promise God has made:
He will call; The dead will answer.
They shall live at his command.
So have faith, and do not wonder,
For our God can make us stand.
And we shall live forever.
All those asleep who in God's mem'rystay,
From death he will awaken."

John 6:40; 11:11, 43; Jas. 4:14

SUMMARY

RESUMO

Mutações afetando a resposta ao estresse salino em tomateiro (*Solanum lycopersicum* L. cv. Micro-Tom) e seu significado fisiológico

A salinidade é um desafio para a produtividade agrícola, uma vez que plantas expostas à salinidade tem o crescimento vegetativo e reprodutivo reduzido devido aos efeitos adversos de íons específicos no metabolismo e nas relações hídricas. A fim de lidar com a salinidade, as plantas desempenham mecanismos fisiológicos baseados em três principais características: *i*) relações fonte-dreno; *ii*) alocação de reservas e *iii*) alterações nos níveis endógenos de hormônios. Nesse trabalho, investigamos a relação entre os processos de desenvolvimento e de regulação hormonal com a resposta à salinidade. Para tanto foram usados genótipos de tomateiro com alteração em diferentes vias de desenvolvimento e de produção ou sinalização de hormônios vegetais. Os seguintes genótipos foram usados: *Galapagos dwarf* (*Gdw*), *Lanata* (*Ln*), *lutescent* (*l*), *single flower truss* (*sft*), *sft heterozygous* (*sft/+*), *diageotropica* (*dgt*), *entire* (*e*), *Never ripe* (*Nr*), *epinastic* (*epi*), *procera* (*pro*), *notabilis* (*not*), *anti sense Dioxigenase cloroplastídica de carotenoide 7* (*35S::asCCD7*) e *Salicilato hidroxilase* (*35S::nahG*). Entre os genótipos de desenvolvimento estudados, *sft* e *l*, relacionados à menor indução floral e senescência respectivamente, foram os menos afetados quando expostos à salinidade. O genótipo *l* acumulou maior biomassa e área foliar, apesar de ser considerado deletério devido à senescência precoce. As plantas heterozigotas, *sft/+,* cuja maior produtividade foi recentemente relacionada a um melhor balanço vegetativo/reprodutivo, alteraram esse balanço sob salinidade e reduziram sua produtividade mais que o controle MT sob estresse salino. Na análise dos genótipos com alteração hormonais foram observados quatro tipos de respostas à salinidade: *i*) elevado crescimento da parte aérea, apesar da razão Na:K ser alta no genótipo *CCD7* cujo transgene induz deficiência de estrigolactona e excessiva ramificação; *ii*) elevado crescimento e acúmulo reduzido de Na nos tecidos (devido provavelmente a diluição) apresentada pelo mutante de resposta constitutiva a auxina *e; iii*) o oposto da resposta anterior foi apresentado pelo mutante pouco sensível à auxina , *dg*t; *iv*) inibição do crescimento combinado com nível reduzido de Na e alto acúmulo de K apresentada pelo mutante *not* que produz menos ácido abscísico. Considerados em conjunto, os resultados apresentaram temas para novos mecanismos de desenvolvimento, como a promoção moderada de senescência e do crescimento vegetativo além dos desbalanços hormonais, para serem explorados na busca de culturas resistentes ao estresse salino.

Palavras-chave: Ácido abscísico; Ácido salicílico; Auxina; Crescimento vegetativo/reprodutivo; Estrigolactona; Etileno; Giberelina; Heterose; Hormônios vegetais; Indução floral; Mutante; Produtividade; Resistência; Salinidade; Senescência; Tolerância

ABSTRACT

Mutations affecting tomato (*Solanum lycopersicum* L. cv. Micro-Tom) response to salt stress and their physiological meaning

Salinity is a challenge for crop productivity. Hence, plants exposed to saline environments reduce their vegetative and reproductive growth due to adverse effects of specific ions on metabolism and water relations. In order to cope with salinity, plants display physiological mechanisms based on three main aspects: *i*) source-sink relationships, *ii*) resource allocation and *iii*) alterations in endogenous hormone levels. The roles of developmental and hormonal mechanisms in salt response were investigated here. We employed mutants and transgenic tomato plants affecting different aspects of plant development and hormone response in the same genetic background (cultivar Micro-Tom). The following genotypes were used: *Galapagos dwarf* (*Gdw*), *Lanata* (*Ln*), *lutescent* (*l*), *single flower truss* (*sft*), *sft heterozygous* (*sft/+*), *diageotropica* (*dgt*), *entire* (*e*), *Never ripe* (*Nr*), *epinastic* (*epi*), *procera* (*pro*), *notabilis* (*not*), *anti sense Chloroplastic carotenoid cleavage dioxygenase 7* (*35S::asCCD7*) and *Salicylate hydroxylase* (*35S::nahG*). Among the developmental genotypes studied, *sft* and *l*, involved in flower induction and senescence, respectively, were less affected when exposed to salt stress. Although *l* is considered deleterious due to its precocious senescence, it presented greater shoot biomass and leaf area during salinity. The heterozygous *sft/+*, whose high productivity was recently linked to an improved vegetative-to-reproductive balance, changed this balance and lowered its yield more than the control MT upon salt treatment. In the analysis of genotypes affecting hormonal status/signaling four kinds of salt responses among the genotypes were observed: *i*) High shoot growth in spite of high Na:K ratio presented by the strigolactone deficient and high branching *CCD7* transgene; *ii*) High shoot growth and reduced accumulation of Na in tissues (probably due to dilution) presented by the auxin constitutive response *e* mutant; *iii*) The opposite response observed in "*ii*" presented by the low auxin sensitivity *dgt* mutant and *iv*) growth inhibition combined with reduced levels of Na and higher accumulation of K presented by the *not* mutant, which produces less ABA. Taken together, the results presented here points to novel developmental mechanisms, such as the promotion of moderate senescence and vegetative growth, and hormonal imbalances to be explored in the pursuing of crops resistant to salt stress.

Keywords: Abscisic acid; Auxin; Ethylene; Flower induction; Gibberellin; Heterosis; Mutant; Plant hormones; Productivity; Resistance; Salicylic acid; Salinity; Senescence; Strigolactone; Tolerance; Vegetative-to-reproductive growth

1 INTRODUCTION

Salinity was probably not a problem at the beginning of agriculture about 12,000 year ago (SNIR et al., 2015), but it emerged with the sophistication of plant cultivation and the demand to obtain more food due to rapid population growth. Hence, salinity is mainly a side effect of irrigation, one of the most revolutionary techniques invented at Sumerian´s times to improve productivity (VARGAS; GALLEGOS, 1990). There are archeological evidences that salinity gradually reduced the yield of the salt-sensitive wheat, which was replaced by the salt-tolerant barley in Mesopotamia (modern Iraq and part of Syria, Iran and Kuwait). However, after seven centuries, the ongoing salinization also decreased the average yield of barley (GOLDSMITH; HILDYARD, 1984) leading to the decline of the Mesopotamia civilization (JACOBSEN; ADAMS, 1958). At present times, 20% of the 230 millions hectares irrigated around the world are in salinization process, decreasing the productivity and preventing the cultivation of several crops (FAO, 2005), which is a challenge to our own civilization.

Plants grown under salinity show impaired of water and nutrient, limited stomatal conductance, increased production of reactive oxygen species, ionic imbalance and altered enzyme activity. All these events restrain plant growth and, in extreme conditions, cause plant death. In order to cope with salinity, plants exhibit complex responses that involve biochemical, physiological and morphological mechanisms.

Plant hormones are considered the major players in salt response. Thus hormones are involved in the control of various mechanisms of acclimation to adverse environments, extending their effects from the operation of ion channels to the control of organ formation and differentiation (FAHAD et al., 2015; RYU; CHO, 2015).

In general, salinization induces hormone status alterations that mediate the rapid inhibition of shoot growth and the preferential partitioning of biomass to roots, increasing survival and maintaining plant growth, although in a lower rate (ALBACETE et al., 2008). This novel source-sink relation leads to yield reduction. As reported by Pérez-Alfocea et al. (2010), salt injury and shoot development is not directly limited by carbohydrate availability but by the changes in distribution and use of assimilates. Therefore, it is expected that plants with different architectures have different degrees of resistance to salinity.

The plant architecture is determined by meristematic activity, which in tomato is partially regulated by the rate of *SINGLE FLOWER TRUSS* (*SFT*) and *SELF PRUNING* (*SP*) regulatory genes. The *SFT* gene is considered the long-sought florigenic signal that induces

flowering, while *SP* inhibits the action of *SFT*, being required to maintain the vegetative stage (MOLINERO-ROSALES et al., 2004; SHALIT et al., 2009). Together, *SFT* and *SP* affect many aspects of plant architecture, such as the development and the identity of meristems, leaves and flowers initiation, sympodium formation, and radial expansion of the stem (SHALIT et al., 2009). However, the effect of *sft* dosage alteration in salt response remains unknown.

In the last two decades, the understanding of the molecular mechanisms involved in salinity resistance broadened with the use of transgenic and mutant plants, especially on identification of cell-based mechanisms related to transmembrane channels. The three main classes of Na^+ transporters for cellular homeostasis are *HIGH-AFFINITY K$^+$TRANSPORTER1* (*HKT1*), *SALT OVERLY SENSITIVE1* (*SOS1*) and *Na$^+$/H$^+$EXCHANGER* (*NHX*) (YAMAGUCHI; HAMAMOTO; UOZUMI, 2013). The impact of these channels on plant performance is impressive. Introgression of *TmHKT;5-A* into commercial durum wheat varieties (*Triticum turgid* ssp. *durum*) promotes effective Na^+ exclusion of root xylem vessel, leading to enhanced grain yield by 25% on saline soils (JAMES et al., 2012; MUNNS et al., 2012; MUNNS; TESTER, 2008). *SOS* pathway was first described in the *sos1* Arabidopsis mutant. The protein kinase complex, *SOS3/SOS2*, senses changes in Ca^{2+}signal induced by high Na^+ cytosolic concentration, which then stimulates the *SOS1* exchange activity (Na^+/H^+ antiporter) prompting the efflux of excess Na^+ ions (JI et al., 2013; ZHU, 2002). Overexpression of *NHX1* in different plant species, including tomato, promotes Na^+ compartmentalization into the vacuole, minimizing the toxic accumulation of this ion in the cytosol and enhancing salt resistance (APSE et al., 1999; ZHANG; BLUMWALD, 2001; ZHANG et al., 2001).

Complementary to plant membrane manipulations, alterations in plant development and hormonal signalling/synthesis are likely to play a role in the salinity resistance. However, the knowledge about these two aspects in salt response remains limited. In order to determine their roles in the response to salinity, we used tomato genotypes that exhibit alterations on plant development and hormonal status in the same genetic background (*Solanum lycopersicum* L. cultivar Micro-Tom). The following genotypes were used: *Galapagos dwarf* (*Gdw*), *Lanata* (*Ln*), *lutescent* (*l*), *single flower truss* (*sft*), *sft heterozygous* (*sft/+*), *diageotropica* (*dgt*), *entire* (*e*), *Never ripe* (*Nr*), *epinastic* (*epi*), *procera* (*pro*), *notabilis* (*not*), *anti sense Chloroplastic carotenoid cleavage dioxygenase 7* (*35S::asCCD7*) and *Salicylate hydroxylase* (*35S::nahG*). The growth performance, chlorophyll fluorescence, nutritional and hormonal status and productivity of genotypes were evaluated.

The present work was organized in two chapters. The first presents the results of developmental genotypes under salinity and a discussion about the prospect to use such developmental processes in the pursuit of salinity resistance. The second chapter displays the hormonal influence in growth performance and ionic homeostasis under salinity.

References

ALBACETE, A.; GHANEM, M.E.; MARTÍNEZ-ANDÚJAR, C.; ACOSTA M.; SÁNCHEZ-BRAVO, J.; MARTÍNEZ, V.; LUTTS, S.; DODD, I.C.; PÉREZ-ALFOCEA, F. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. **Journal of Experimental Botany**, Oxford, v. 59, n. 15, p. 4119–4131, 2008.

APSE, M.P.; AHARON, G.S.; SNEDDEN, W.A; BLUMWALD, E. Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in Arabidopsis. **Science,** New York, v. 285, n. 5431, p. 1256–1258, 1999.

FAHAD, S.; HUSSAIN, S.; MATLOOB, A.; KHAN, F.A.; KHALIQ, A.; SAUD, S.; HASSAN, S.; SHAN, D.; KHAN, F.; ULLAH, N.; FAIQ, M.; KHAN, M.R.; TAREEN, A. K.; KHAN, A.; ULLAH, A.; ULLAH, N.; HUANG, J. Phytohormones and plant responses to salinity stress: a review. **Plant Growth Regulation**, Dordrecht, v. 75, n. 2, p. 391–404, 2015.

FAO. **Global network on integrated soil management for sustainable use of salt-affected soils**. Rome: FAO, Land and Plant Nutrition Management Service, 2005. Disponível em:<http://www.fao.org/ag/agl/agll/spush>. Acesso em: 30 maio 2016.

GOLDSMITH, E.; HILDYARD, N. **The social and environmental effects of Large Dams**. Cornwall: Worthyvale Manor Camelford, 1984. 404 p.

JACOBSEN, T.; ADAMS, R.M. Salt and silt in ancient Mesopotamian agriculture. **Science**, New York, v. 128, n. 3334, p. 1251–1258, 1958.

JAMES, R.A.; BLAKE, C.; ZWART, A.B.; HARE, R.A.; RATHJEN, A.J.; MUNNS, R. Impact of ancestral wheat sodium exclusion genes Nax1 and Nax2 on grain yield of durum wheat on saline soils. **Functional Plant Biology**, Victoria, v. 39, n. 7, p. 609–618, 2012.

JI, H.; PARDO, J.M.; BATELLI, G.; OOSTEN, M.J. van; BRESSAN, R.A.; LI, X. The Salt Overly Sensitive (SOS) pathway: established and emerging roles. **Molecular Plant**, Oxford, v. 6, n. 2, p. 275–286, 2013.

MOLINERO-ROSALES, N.; LATORRE, A.; JAMILENA, M.; LOZANO, R. *Single flower truss* regulates the transition and maintenance of flowering in tomato. **Planta**, New York, v. 218, p. 427–434, 2004.

MUNNS, R.; JAMES, R.A.; XU, B.; ATHMAN, A.; CONN, S. J.; JORDANS, C.; BYRT, C. S.; HARE, R.A.; TYERMAN, S.D.; TESTER, M.; PLETT, D.; GILLIHAM, M. Wheat grain yield on saline soils is improved by an ancestral Na^+ transporter gene. **Nature Biotechnology**, New York, v. 30, n .4, p. 360–364, 2012.

MUNNS, R.; TESTER, M. Mechanisms of salinity tolerance. **Annual Review of Plant Biology**, Palo Alto, v. 59, n. 1, p. 651–681, 2008.

PÉREZ-ALFOCEA, F.; ALBACETE, A.; GHANEM, M.E.; DODD, I.C. Hormonal regulation of source–sink relations to maintain crop productivity under salinity: a case study of root-to-shoot signalling in tomato. **Functional Plant Biology**, Victoria, v. 37, n. 7, p. 592-603, 2010.

RYU, H.; CHO, Y.-G. Plant hormones in salt stress tolerance. **Journal of Plant Biology**, Seoul, v. 58, n. 3, p. 147–155, 2015.

SHALIT, A.; ROZMAN, A.; GOLDSHMIDT, A.; ALVAREZ, J.P.; BOWMAN, J.L.; ESHED, Y.; LIFSCHITZ, E. The flowering hormone florigen functions as a general systemic regulator of growth and termination. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 106, n. 20, p. 8392–8397, 2009.

SNIR, A.; NADEL, D.; GROMAN-YAROSLAVSKI, I.; MELAMED, Y.; STERNBERG, M.; BAR-YOSEF, O.; WEISS, E. The origin of cultivation and proto-weeds, long before neolithic farming. **PLoS One**, San Francisco, v. 10, n. 7, p. 1–12, 2015. Disponível em: <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0131422>. Acesso em: 13 fev. 2016.

VARGAS, I.; GALLEGOS, H. Sumer: where engineering was born. **Journal of Professional Issues in Engineering**, Utah, v. 116, n. 1, p. 83–92, 1990.

YAMAGUCHI, T.; HAMAMOTO, S.; UOZUMI, N. Sodium transport system in plant cells. **Frontiers in Plant Science**, Lausanne, v. 4, n. 17, p .1-7, 2013.

ZHANG, H.X.; BLUMWALD, E. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. **Nature Biotechnology**, New York, v. 19, n. 8, p. 765–768, 2001.

ZHANG, H.X.; HODSON, J.N.; WILLIAMS, J.P.; BLUMWALD, E. Engineering salt-tolerant *Brassica* plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 98, n. 22, p. 12832–12836, 2001.

ZHU, J.-K. Salt and drought stress signal transduction in plants. **Annual Review of Plant Biology**, Palo Alto, v. 53, n. 1, p. 247–73, 2002.

2 THE ASSESSMENT OF TOMATO (*Solanum lycopersicum* cv. MICRO-TOM) GENOTYPES UNDER SALINITY SUGGESTS NOVEL ACCLIMATORY MECHANISMS IN THE *LUTESCENT* AND *SINGLE FLOWER TRUSS* GENOTYPES

Abstract

Salinity is a challenge for crop productivity since the beginning of agriculture. Salinity is usually a side effect of irrigation and the use of fertilizers, two of the first improvements during agriculture expansion. Despite its importance, our understanding of salt resistance is limited and mostly restricted to cell-based mechanisms. In order to investigate the role of developmental mechanisms in salt response, we employed tomato genotypes affecting different aspects of plant development in the same genetic background (cultivar Micro-Tom). The following genotypes were used: *Galapagos dwarf* (*Gdw*), *Lanata* (*Ln*), *lutescent* (*l*), *single flower truss* (*sft*) and *sft heterozygous* (*sft*/+). Among the genotypes studied, *sft* and *l*, which are involved in flower induction and senescence, respectively, were less affected when exposed to salt stress. Although *l* is considered deleterious due to its precocious senescence, this genotype showed greater shoot biomass and leaf area than the control MT during salinity. The *sft*/+, whose high productivity was recently linked to improved balance marked by vegetative growth extension and high number of inflorescences, changed this balance and lowered its yield more than the control MT did upon salt treatment. This agrees with the known effect of salt stress in flower induction, suggesting that genotypes bred for high productivity based on improved vegetative-to-reproductive balance should be adjusted for the effect of salinity on this developmental response.

Keywords: Flower induction; Heterosis; Plant hormones; Productivity; Senescence; Vegetative-to-reproductive growth

2.1 Introduction

Salt-affected areas have been increased since the invention of irrigation at Sumerian time (ESSINGTON, 2015). According to the latest survey, more than 45 million hectares irrigated are in salinization process worldwide (FAO, 2005). As known, the irrigation not only provides water to plants but also leads to salt deposition in the soil. As evapotranspiration occurs, salt tends to accumulate and concentrate around the roots, reducing water potential.

In plants, salinity induces some deleterious effects such as water absorption capacity reduction, photosynthetic rate decrease, nutritional deprivation, excessive production of reactive oxygen species (ROS), changes in enzymatic activity, membrane damage and cellular homeostasis disruption, and other physiological and biochemical alterations (GUPTA; HUANG, 2014).

The low water potential and hyperosmotic environment cause a stress that may lead to salt stress resistance. According to Levitt (1972), stress resistance of a plant may be defined as a response to specific stress. This terminology includes two basic theoretical subtypes:

avoidance and tolerance. Stress avoidance is achieved by avoiding stress through physical barrier and partial or complete exclusion of stress factor. Stress tolerance is the ability to come to thermodynamic equilibrium with the stress without suffering damage. The plant with stress tolerance is able to prevent, decrease, or repair the stress effects. Stomatal closure, developmental changes and salt exclusion/compartmentalization are considered as stress avoidance responses. In contrast, osmolyte and protein accumulation and reactive oxygen species detoxification are defined as stress tolerance (VERSLUES et al., 2006). Nevertheless, here salt responses were termed as stress resistance in cases where it was not possible or not desirable to refer to a more specific mechanism.

Salinity also causes modifications in plant architecture mainly shoot growth repression and preferential partitioning of photoassimilates to the roots, leading to an increase in root:shoot ratio. Taken together, these responses contribute to ameliorate water imbalance imposed by hyperosmotic environment (ALBACETE et al., 2008). To maintain the growth, meristems activity needs to be coordinated with cell proliferation, since salt stress induces accelerated senescence of mature tissues as reported in *Oryza sativa* (OGAWA et al., 2011), *Zea mays* (URANO; COLANERI; JONES, 2014) and Arabidopsis (WANG et al., 2015).

In tomato plants, the meristematic activity is partially regulated by *SINGLE FLOWER TRUSS* (*SFT*) and *SELF PRUNING* (*SP*) transcription factors. The *SFT* gene (ortholog to Arabidopsis *FLOWERING LOCUS T*, *FT*) promotes flowering acting as a long-sought florigenic signal. On the other hand, SP (related to *TERMINAL FLOWER 1*, *TFL1*, in Arabidopsis) maintains the vegetative stage for antagonizing the SFT floral inductor. The balance between these regulatory genes controls the development and the identity of meristems, and leaves and flowers initiation (MOLINERO-ROSALES et al., 2004; SHALIT et al., 2009).

The manipulation of SFT/SP equilibrium allows altering growth habit and partitioning/allocation of photoassimilates. In tomato plants, the presence of loss-of-function mutation in gene encoding the flowering repressor SP (*sp*) alleles and a single defective copy of allele *sft* (*sft*/+) result on semi-determinate growth habit marked by a vegetative growth extension and greater number of inflorescences compared to determinate and indeterminate genotypes respectively (KRIEGER; LIPPMAN; ZAMIR, 2010). This improved vegetative-to-reproductive balance provides conditions to higher productivity and soluble solids accumulation as well as enhanced water-use efficiency (KRIEGER; LIPPMAN; ZAMIR, 2010; VICENTE et al., 2015). However, the effect of *sft* dosage alteration in salt response remains unknown.

Salinity induces genetic, developmental and physiological responses that not only interfere on development of organs but also impair their maintenance. Among the most obvious salt-effects is leaf senescence that drastically limits plant productivity under salinity (GHANEM et al., 2008). Genetic alterations that decrease senescence are commonly related to improved performance under abiotic stress. The mutation of the Arabidopsis NAC016, a senescence-associated transcription factor, results in plants with higher levels of total chlorophyll and proper balance of photosystem protein under salinity and hyperosmotic conditions (KIM et al., 2013). Tomato genotypes plants harboring genetic modification related to STAY-GREEN (SGR) show altered senescence pattern, since *SGR* genes encode chloroplast-targeted proteins that are induced during senescence (BARRY et al., 2008; PARK et al., 2007). Constitutive expression of *SGR1* gene accelerates chlorophyll degradation in leaves (LUO et al., 2013). In contrast, silencing of *SlSGR1* in tomato by RNA interference lead to reduced chlorophyll degradation in leaves (HU et al., 2011) and higher lycopene and β-carotene synthesis in fruits (LUO et al., 2013).

Nevertheless, the senescence also offers some advantages to plants such as remobilization of leaf nutrients and generation metabolites that serve as precursors of biosynthetic pathways. The resultant molecules are mobilized into developing tissues and organs, maximizing the plant viability (HÖRTENSTEINER; FELLER, 2002). Almeida et al. (2015) reported that the precocious senescence tomato genotype, *lutescent*, is associated to high chlorophyll breakdown that provides recycled phytol for phytol kinase VTE5 and tocopherol biosynthesis in fruits. Tocopherol serves to prevent lipid peroxidation by removal of singlet oxygen and lipid peroxyl radicals (KRIEGER-LISZKAY; FUFEZAN; TREBST, 2008). Moreover, leaf senescence alters carbon and nitrogen distribution that is reflected on source–sink relationship, influencing the development (THOMAS; OUGHAM, 2014).

Other developmental aspects also affect salt response. It is widely known that glandular trichomes are important to salt resistance. These epidermical structures play an important role in salt compartmentalization and excretion in halophytes species (FLOWERS et al., 2010). Recently, a transcriptional profiling of leaf non-glandular trichomes of olive (*Olea europaea*) showed that non-glandular trichome also contributes to abiotic stress tolerance for synthesizing L-arginine aminoacid that serves as nitrogen reserve and precursor of nitric oxide biosynthesis (KOUDOUNAS et al., 2015, TUTEJA; SOPORY, 2008). However, the role of non-glandular trichomes in salt tolerance has scarcely been studied.

Tomato (*Solanum lycopersicum*) plants show a diversity of types of trichomes and mutations that affect from density to ontogeny of trichomes such as *Lanata*, *Wooly*, hair

absent and *hair less* mutations (HUANG; PADDOCK, 1962; REEVES, 1977; SUSSEX, 1992; VENDEMIATTI et al. *in press*). Therefore, tomato plants can be useful as tools to dissect the involvement of trichomes in salt stress tolerance.

Wild relative species of cultivated tomato also comprise a valuable genetic resource of developmental modification. *Solanum* galapagense (LA1401), an endemic specie to Galápagos Islands (SPOONER; PERALTA; KNAPP, 2005), shows highly subdivided leaves and short internodes, and tolerates 350 mM NaCl, equivalent to 70% of sea water salinity (RUSH; EPSTEIN, 1976). The introgression of developmental traits from salt tolerant wild species can provide some advantages of growth under high salt concentrations.

Although, a set of developmental responses affects the tolerance to salinity, in general, the researches related to salt resistance conducted during the last two decades have been focused on identifying cell-based mechanisms related to salt response, especially on transmembrane channels regulation involved in uptake and transport of ions control, and on metabolic alterations responsible for restoring osmotic and ionic homeostasis (HASEGAWA; BRESSAN, 2000; ISCHEBECK et al., 2006; URANO et al., 2010; YAMAGUCHI; HAMAMOTO; UOZUMI, 2013; ZHU, 2002).

Salt tolerance success depends on developmental program readaptation to salinity (CUARTERO et al., 2006). From a reductionist perspective, plant development under salt stress can be understood as an integrator response between molecular effectors and organism plasticity (cell division/expansion/differentiation) which drives morphogenic responses that allow an enhancement of plant survival and reproductive success to salt-stress (POTTERS et al., 2009). Surely, the salt-induced developmental changes are mediated by multicomponent signalling pathways, being plant hormones, transcription factors and repressor proteins the major players in the regulatory system (GOLLDACK; MOHAN; PROBST, 2014).

Nevertheless, the knowledge about the diverse roles of plant development on salinity tolerance remains limited. Hence, in order to investigate the role of developmental mechanisms in salt response, we employed tomato genotypes affecting different aspects of plant development in the same genetic background (cultivar Micro-Tom). The following genotypes were used: *Galapagos dwarf* (*Gdw*), *Lanata* (*Ln*), *lutescent* (*l*), *single flower truss* (*sft*) and *sft heterozygous* (*sft*/+).

We show that among the genotypes studied, *single flower truss* and *lutescent*, which are involved in flower induction and senescence respectively, were less affected when exposed to salt stress. Although *l* is considered deleterious due to its precocious senescence, this genotype presented greater shoot biomass and leaf area during salinity. The *sft*/+, whose

high productivity was recently linked to an improved vegetative-to-reproductive balance, changed this balance and lowered its yield more than the control MT upon salt treatment.

2.2 Material and Methods

2.2.1 Plant material

The tomato (*Solanum lycopersicum* L.) genotypes used are described in Table 1. The genotypes *Galapagos dwarf* (*Gdw*), *Lanata* (*Ln*), *lutescent* (*l*) and *single flower truss* (*sft*) were introgressed into the cv. Micro-Tom (MT), using the same procedures published previously (CARVALHO et al., 2011; PINO et al., 2010). The introgression of respective mutations was previously performed through pollen collection from parents, and its use to fertilization of MT flowers. Subsequently, the resulting F1 hybrids were self-fertilized to obtain recombinant F2 populations, and carry out visual screening for compact size to MT and the phenotype of the respective mutation of interest. Selected plants were backcrossed with MT up to the sixth generation (BC_6), and self-fertilized every second generation to allow the screening for homozygous mutants. After BC_6F_2 the resulting genotypes were considered near-isogenic lines according to Stam and Zeven (1981). All experiments were conducted on BC_6F_3 plants or subsequent generations.

In order to obtain heterozygous *sft* plants (*sft*/+), collected pollen from BC_6F_3 *sft* plants was used to emasculate MT flowers, resulting in F_1 hybrids.

The genotypes *Ln*, *l* and *sft* into the MT background were described before (ALMEIDA et al., 2015; VICENTE et al., 2015; VENDEMIATTI et al., *in press*). The natural allelic variation *Gdw*, from *S. galapagense*, was recently obtained in a PhD thesis (JESUS, 2016).

2.2.2 Preliminary test

A preliminary test was carried out with *Gdw*, *Ln*, *l*, *sft* and *sft*/+ genotypes and the control MT under controlled environmental conditions in a plant growth chamber with temperatures between 25°C (day) and 18°C (night), relative humidity of 50%, 16 h photoperiod and 400 µmol m^{-2} s^{-1} photon flux density at the top of the canopy. The geographic coordinates of the location were: 38°01'23.6"N and 1°10'17.3"W.

Experimental conditions, nutritional solution and salt treatment in preliminary test were quite similar to method reported by Ghanem et al. (2008) except for increase in initial age of plants to transference to hydroponic system and longer period of acclimatation after

transplantation. Previous experiments were conducted to determine the adjustment of these conditions to the dwarf phenotype of MT plants (data not shown).

Table 1 - Phenotypic description of genotypes in the (*Solanum lycopersicum* L.) cv. Micro-Tom (MT) background

Genotype	Effect/Gene function	Origin	Reference
Galapagos dwarf (*Gdw*)	Shortened internodes and increased leaf dissection. Unknown gene function.	LA1401 *S. galapagense*	Jesus (2016)
Lanata (*Ln*)	Excess of trichomes mainly on the stem, leaves and fruits	LA3128 cv. VF36	Szymkowiak and Sussex (1992) Vendemiatti et al. (in press)
lutescent (*l*)	Premature senescence and abscission of old leaves. Yellow fruits do not fully ripe. Unknown gene function.	Heirloom cv. unknown	Barry et al. (2012) Almeida et al. (2015).
single flower truss (*sft*)	Low flowering induction. Indeterminate growth habit. Defective in transcription factor (florigen precursor) of CETS family.	LA2460 cv. MSU 100	Molinero-Rosales et al. (2004); Vicente et al. (2015)
sft heterozygous (*sft*/+)	Altered flowering. Semi-determinate growth habit.	Cross between MT and *sft*	Krieger; Lippman; Zamir (2010)

Seeds of each genotype were germinated in trays with vermiculite and daily watered with half-strength Hoagland's nutrient solution. The maximum field capacity was maintained during the growth of the seedlings.

Twenty five days after sowing, the seedlings had one pair of undeveloped leaves and the average height was 6 cm. At this stage, the seedlings were transferred to a hydroponic system (Water Culture type) containing eight tanks each filled approximately with 20 L half-strength Hoagland's solution continuously aerated. The nutrient solution contained the following chemicals: 5 KNO_3, 1 $NH_4H_2PO_4$, 0.5 $MgSO_4$, 5.5 $Ca(NO_3)_2$ in mM g L^{-1} and 25 KCl, 10 H_3BO_3, 1 $MnSO_4$, 1 $ZnSO_4$, 0.25 $CuSO_4$, 10 Na_2MoO_4 in µM g L^{-1} and 1.87 g·L^{-1} Fe-EDDHA (GHANEM et al., 2008).

After 4-days plant acclimation period, the nutrient solution was supplemented with the respective treatments 0 and 100 mM NaCl. Refilling and replacement of solutions were done each 2 and 10 days, respectively.

In the preliminary experiment, the plants were harvested 21 days after the application of salt treatment (50 DAS and 81 days under salt treatment).

2.2.3 Productivity assay

Since, the plants were not allowed to complete life cycle in preliminary test, it was considered the necessity to conduct a novel experiment to assess the productivity. In this experiment were included only *sft* mutants and the control MT. The selection of genotypes was made based on growth performance of these genotypes under salinity in preliminary test and their salt responses in relation to reproductive-vegetative balance.

The experimental conditions were the same adopted in the preliminary test, expect for duration of experiment that was longer (110 DAS and 81 days under salt treatment). Plants were harvested when at least one of the genotypes reached 70% of fully ripe fruits.

2.2.4 Survival experiment

Some previous experiments were conducted at greenhouse conditions to evaluate effects of NaCl concentration on genotypes. During these experiments were observed that 100 mM NaCl level imposed salt stress conditions without impairing plant survival, while lower salt concentrations did not resulted on unclear responses on growth. When genotypes were exposed to 150 mM NaCl survival was impaired and growth differences less expressive. Upon 200 mM NaCl treatment, most of genotypes had stunted growth and was not able to end the life cycle except for *sft* mutants whose growth performance differed from other genotypes (data not shown). Therefore, an assay was carried out to assess the resistance of *sft* mutants to high salt concentration.

The growth conditions of experiment were as follows: mean temperature of 25°C (32°C maximum and 18°C minimum temperatures during the period), average relative humidity of 40%, 12 h photoperiod, and 250 to 350 μmol photons m^{-2} s^{-1} PAR irradiance, attained by reduction of natural radiation with a reflecting mesh (Aluminet, Polysack Industrial Ltda, Leme, Brazil) in a greenhouse at 22°42'26.7"S 47°37'50.4"W.

Seeds were sown in trays containing a 1:1 mixture of commercial potting mix Basaplant® (Base Agro, Artur Nogueira, SP, Brazil) and expanded vermiculite supplemented with 1 $g \cdot L^{-1}$ 10:10:10 NPK and 4 $g \cdot L^{-1}$dolomite limestone ($MgCO_3$ + $CaCO_3$) irrigated daily in order to maintain water potential around field capacity. Upon the appearing of the first true leaf, at 25 days after sowing, the seedlings of each genotype were placed in an expanded

polystyrene plate floating on 80%-strength Hoagland's solution. The composition of the full-strength nutrient solution was the same than that reported in the previous section.

The plants were acclimated for two days on hydroponics (Nutrient Film Technique System) (GHANEM et al., 2008). Salt treatment, 150 mM NaCl, was gradually applied, using three plots of 50 mM NaCl that were added to the nutrient solution at intervals of two days to allow plant adaptation and avoid osmotic shock (VERSLUES et al., 2006). Solutions were refilled daily and changed every week. The harvest was performed at 111 DAS and 80 days of salt treatment.

2.2.5 Vegetative and reproductive growth assessment

The growth assessment were performed 50, 110 and 111 DAS in the preliminary, productivity and survival experiments respectively through the measurement of three, four and 18 plants per treatment.

At each harvest, leaf area was measured using a Li-Cor 3100 area meter (Li-Cor Inc., Lincoln, Nebraska, USA). Subsequently, stem, leaves, inflorescence and fruits were separated and fresh weight determined. The yield was calculated as the sum of the weight of each fruit per plant.

Concentration of total soluble solids (^{o}Brix) was assessed using a handheld refractometer (Atago N1, Tokyo, Japan) from a random sample of 10 fruits per genotype. Then the sugar output per plant was calculated as the product of ^{o}Brix × ripe yield (in kg) (BRY). The level of ripening of fruits used to determine BRY varied from fruits with ripening process advanced (pink color fruit) to completely ripening (red color fruit).

The adjustment of vegetative-to-reproductive growth was calculated as the ratio between reproductive (inflorescence and fruits) and vegetative (steam and leaves) fresh weights. Root biomass was not considered to estimate the balance between reproductive and vegetative growth.

2.2.6 Chlorophyll fluorescence

Prior to the measurement, plants were kept in darkness for 30 min to allow the reversible light-induced quenching to relax. After dark-adaptation, the penultimate leaf before inflorescence from controls and salt-treated plants were detached and placed into a fluorometer IMAGING-PAM M-Series (Heinz Walz GmbH, Effeltrich, Germany).

For determination of chlorophyll fluorescence parameters, the samples were illuminated with actinic light (80 μmol quanta m^{-2} s^{-1} PAR) for several pulses of saturating

light at 2700 μmol quanta m^{-2} s^{-1} PAR for 0.8 s at intervals of 20 s. The quantum yield of photosystem II (PSII), the non-photochemical quenching (NPQ), and nonregulated energy dissipation were analyzed.

2.2.7 Nutritional status

For mineral content determinations, the third leaf of each plant was harvested at the end of the preliminary test. The samples were oven-dried at 60ºC for 72 h and ground into a fine powder. The elemental concentrations were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). Three biological replicates were analyzed.

2.2.8 Hormone extraction and analysis

For the determination of salicylic acid (SA) and jasmonic acid (JA) were collected fourth leaf and middle third part of root system. Since this selected leaf was mature and at non-senescent stage while this root region prevent the collection of new or old roots (ALBACETE et al., 2008). The samples were flash frozen using liquid nitrogen and kept at -80°C. Then the samples were dried in a Christ Alpha 2-4 D freeze-dryer (Martin Christ, Osterode am Harz, Germany) land ground to a fine powder.

The extraction and analysis of hormones were conducted using the same procedures reported by Albacete et al. (2008). Thirty mg of ground material was dropped in 650 μl of cold (4°C) extraction mixture of methanol/water (80/20, v/v). The tubes containing the mixture were mixed three times at intervals of 10 min. Solids were separated by centrifugation (13,000 *g*, 15 min) at 4ºC. The supernatant was taken and maintained at 4ºC.

The solids were mixed with additional 650 μL of the same extraction solution. The steps described above were repeated again once. After the second centrifugation the supernatants were polled and passed through Sep-Pak Plus †C18 cartridge (SepPak Plus, Waters, USA) to remove interfering lipids and part of plant pigments and evaporated at 40ºC under vacuum until organic solvent was removed. The residue was dissolved in 1 ml methanol/water (20/80, v/v) solution using an ultrasonic bath. Afterwards the extracts were centrifuged twice (13,000 g, 15 min, 4ºC). Ten μl of each extract were injected in a U-HPLC-MS system consisting of an Accela Series U-HPLC (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a well plate auto sampler and a capillary pump, coupled to an Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) that uses a heated electrospray ionization (HESI) interface. Mass spectra were obtained using the Xcalibur software version 2.2 (ThermoFisher Scientific, Waltham, MA, USA). For quantification of

the plant hormones, calibration curves were constructed for each analyzed component (1, 10, 50, and 100 µg L^{-1}) and corrected for 10 µg L^{-1}deuterated internal standards. Recovery percentages ranged between 92 and 95%.

2.2.9 Statistical analysis

The data obtained in the experiments were submitted to Levene's test (LEVENE, 1960) and Shapiro-Wilk (SHAPIRO; WILK, 1965) tests to evaluate homoscedasticity and the normal distribution of the residuals, respectively. The parameters shoot FW, reproductive/vegetative and fruit yield of the productivity experiment did not satisfy these assumptions. Hence, they were log transformed to meet the requirements to ANOVA.

The data were analyzed by one-way analysis of variance F-test. When a significant difference was detected, Tukey's test was used to compare the means of genotype inside each salt level. The correlation was tested using Spearman's test. Analyses were performed using the statistical software SAS® 9.2 and PROC GLM program (SAS, 2008).

2.3 Results

2.3.1 Growth and development of genotypes under salinity

The response of a set of tomato genotypes affecting different aspects of plant development to NaCl treatment was assessed. Shoot and root biomass and leaf area varied little among the genotypes under control conditions (Figure 1). The *Gdw* genotype presented the lower shoot and root biomass and the *l* genotype presented the higher root biomass under control conditions although not significant. The experiment duration (50 days after sowing) was determined in order to have the plants in a stage that the genotypes displayed a similar growth.

Salt treatment (100 mM NaCl) considerably reduced shoot growth of all genotypes. However, *l* and *sft/+* were the only genotypes that differed from MT in shoot biomass and photosynthetic area. They showed at least 2.5-fold greater performance in these traits compared to MT.

In general, root growth was less affected than shoot following salinity (Figure 1C). Root fresh weight of all genotypes decreased but not significantly under stress, except for MT, whose drop was about 60% in the salt-stressed plants when compared to control. Root biomass of the *l* genotype presented the higher root biomass among the genotypes although not significantly.

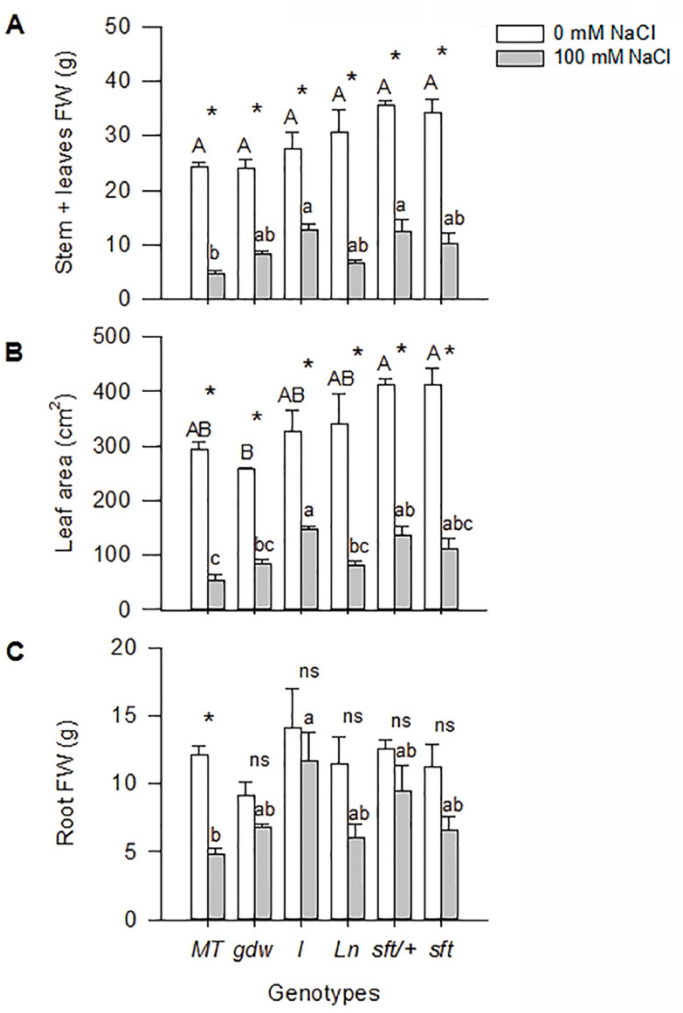

Figure 1 - Effect of salinity on the vegetative development of tomato Micro-Tom (MT) genotypes. Stem and leaf biomass (A), leaf area (B) and root biomass (C) of plants grown in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 21 days. Measurements were performed at 50 days after sowing. Data are means ± SE (n = 3). Asterisks and "ns" indicate, respectively, significant and non significant differences between 0 and 100 mM NaCl for each genotype according to the F test at P<0.05. Bars with different upper and lower case letters represent significant differences between genotypes grown under control and stress conditions, respectively, according to Tukey's test at P <0.05. The absence of upper case letters above the open bars in panels C reflects a lack of significant difference between genotypes according to the F test at P <0.05. A description of the genotypes used is shown in Table 1

2.3.2 Nutritional status

The nutrients and Na leaf concentrations were assessed in the genotypes (Figure 2). The nutritional status exhibited small differences among the genotypes. K, Ca and Mg, and P levels changed according to the genotype in the control treatment. However, the differences were slight between genotypes. When the plants were subjected to 100 mM NaCl, the K, Ca and Mg concentrations decreased and Na, P and Fe concentrations increased, particularly that from Fe. The exception was the *Gdw* genotype, whose Fe concentration did not increase upon NaCl treatment.

Correlation analyses were performed in order to observe how the nutrients were related to each other and to shoot growth under salinity. K level was positively correlated with higher growth (total plant biomass) under control (r= 0.54*) and saline (r= 0.47*) conditions. However, the K values between genotypes were similar when compared by Tukey's test (α = 0.05). The other nutrients were not correlated with total plant biomass in any condition. Ca correlated with Mg levels but only in the salt treatment (r= 0.63**).

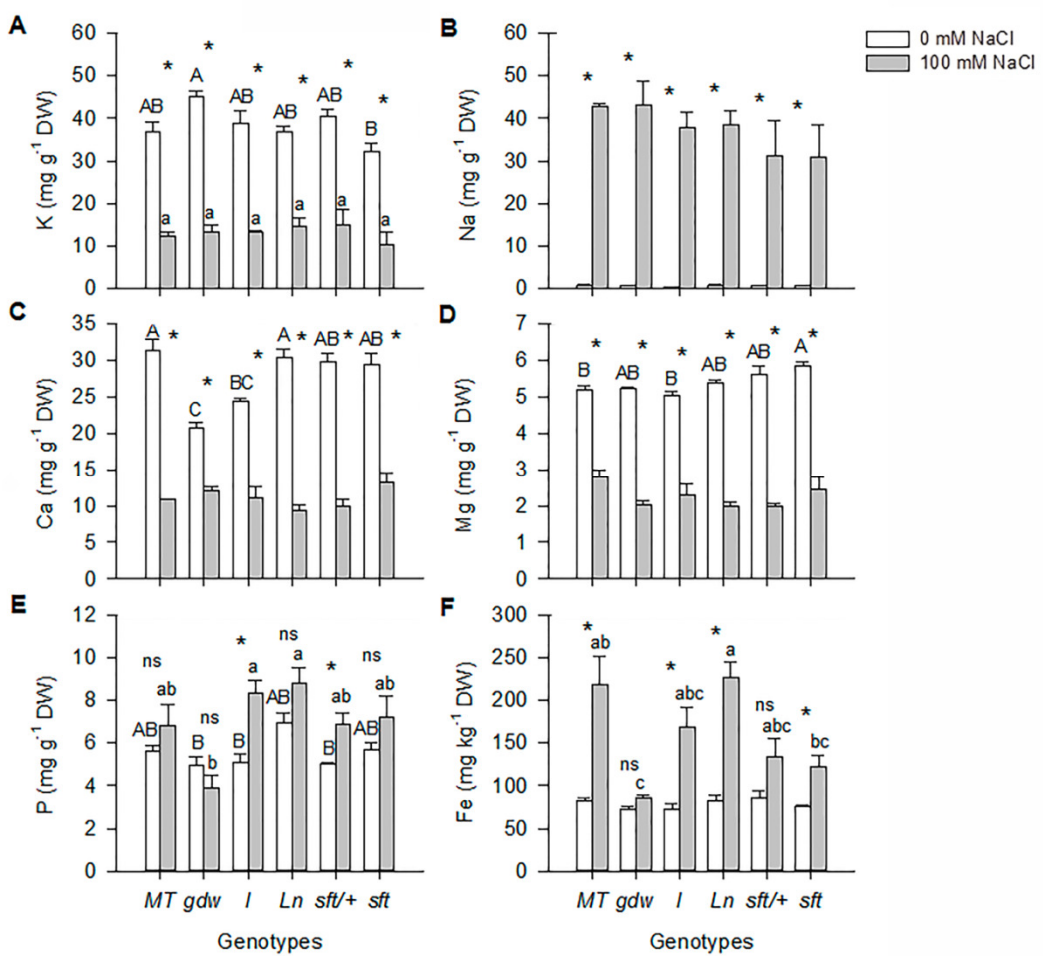

Figure 2 - Effect of salinity on the mineral status in tomato Micro-Tom (MT) genotypes. K (A), Na (B), Ca (C), Mg (D), P (E) and Fe (F) ion concentrations of leaf tissue from plants grown in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 21 days. The leaf samples were taken at 50 days after sowing. Data are means ± SE (n = 3). Asterisks and "ns" indicate, respectively, significant and non significant differences between 0 and 100 mM NaCl for each genotype according to the F test at P<0.05. Bars with different upper and lower case letters represent significant differences between genotypes grown under 0 and 100 mM NaCl, respectively, according to Tukey's test at P<0.05. The absence of letters above the bars in panels B, C, D and F displays a lack of significant difference between genotypes under the respective conditions, according to the F test at P <0.05.A description of the genotypes used is shown in Table 1

As for P concentration, the genotypes with greater growth under salinity, *l* and *sft*/+, were the same ones that displayed a significant higher level of P in the salt treatment, compared to the control. However, the correlation between growth and P level was not

significant. Perhaps, this was due to the absence of this pattern in some genotypes. The P and Fe concentration similarly varied, being strongly associated (r= 0.78****) under saline conditions.

Interestingly, all genotypes presented similarly high Na^+ levels when the plants were salt treated. Therefore, the better performance of *l* and *sft*/+ was not a consequence of Na dilution in tissues of vegetative organs due to the greater growth.

Since plants that harbour *sft* allele have some advantages such as higher water-use efficiency (VICENTE et al., 2015) and low transpiration rate (VICENTE, 2013), it was hypothesized that these features conferred a decrease in Na^+ loading to leaves, which did not occur in this study.

2.3.3 Hormonal profiling

The hormones jasmonic acid (JA) and salicylic acid (SA) are involved in defense response to oxidative stress, which is a consequence of the decline in photosynthesis provoked by the stress. Therefore, their levels in leaves and roots, likewise their relationships with growth were evaluated. Their profiles are show in the Figure 3.

The genotypes exhibited similar contents of JA in leaf tissues and no significant variation in JA root levels under control conditions, except for *l*, which presented a root JA level significantly higher than the control MT.

The NaCl treatment induced changes in JA accumulation. Comparing the genotypes, the leaf JA accumulation was significantly higher in *sft*/+. In general, the differences among the genotypes were more robust in leaves than in roots. Nevertheless, the global mean value of JA level under salinity was higher in roots than in leaves, as expected, since root system is the first organ to enter in contact with salt, and its susceptibility to osmotic and toxic damage induced by high salinity is widely higher than shoot (GARCIA-ABELLAN et al., 2015).

However, the JA level increment between environments (control and 100 mM NaCl) was less noted in roots than shoot, probably because the JA concentration in roots was already higher than leaves under control conditions. Except for *sft* mutant, which exhibited a 6-fold upregulation of JA root content upon salt treatment. However, this JA increase was not associated with a significant root growth reduction (Figure 1C), as observed by Staswick and Tiryaki (2004).

The basal SA concentration varied between genotypes in leaves but not in roots under control conditions. In contrast, the salt treatment led to a differential increase of basal SA level in both organs. A boost in SA accumulation was observed especially in *Gdw* leaves.

These results suggest an increased sensitivity of this genotype to salt stress, since an excessive SA accumulation may means a hypersensitive reaction (CAO et al., 2009; MATEO et al., 2006; RAO; DAVIS, 1999; YUAN; LIN, 2008).

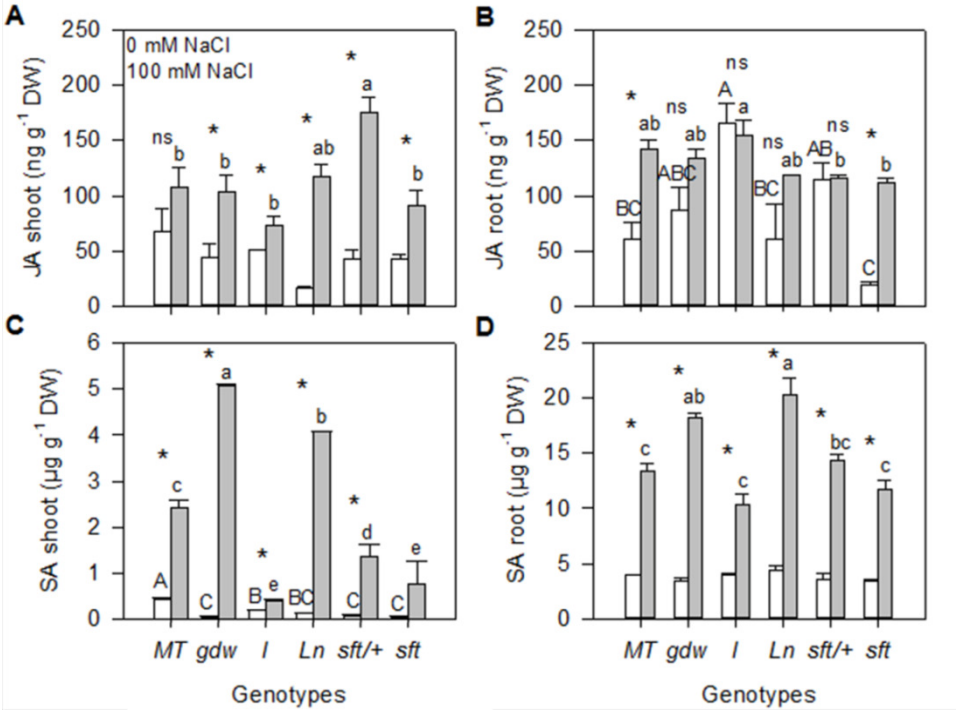

Figure 3 - Effects of NaCl on endogenous hormones related to oxidative stress in tomato Micro-Tom genotypes. Jasmonate concentration on leaves (A) and roots (B) and salicylic acid concentration on leaves (C) and roots (D). The samples were taken from plants growing in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 21 days at 50 days after sowing. Data are means ± SE (n = 3). Asterisks and "ns" indicate, respectively, significant and non significant differences between 0 and 100 mM NaCl for each genotype according to the F test at P <0.05. Bars with different upper and lower case letters represent significant differences between genotypes grown under 0 and 100 mM NaCl, respectively, according to Tukey's test at P <0.05. The absence of letters above the open bars in panels A and D evidences a lack of significant difference between genotypes under control (no salt) condition, according to the F test at P <0.05 . A description of the genotypes used is shown in Table 1

2.3.4 Chlorophyll fluorescence

The effect in photochemical phase was investigated through chlorophyll fluorescence (Figure 4). Different chlorophyll fluorescence parameters were measured to assess modifications in photosynthetic electron transport and energy dissipation caused by salinity. Salt stress decreased quantum yield of photosystem II (PSII) in all genotypes. However, the reduction was less drastic in *l*and *sft*/+ genotypes, especially in the latter. On the other hand, *Gdw*, *Ln* and *sft* were the genotypes more affected. Interestingly, the *Ln* and *sft* dissipated larger part of excessive excitation energy by nonphotochemical quenching (NPQ), i.e. using regulated physiological processes to protect themselves. Moreover, both genotypes showed low quantum yield of nonregulated energy dissipation (NO). In contrast, *Gdw* plants

displayed the highest NO. These results suggest that the photochemical energy conversion and protective regulatory mechanisms could not have been efficient in *Gdw*. This inefficiency may be result of damages in tissues.

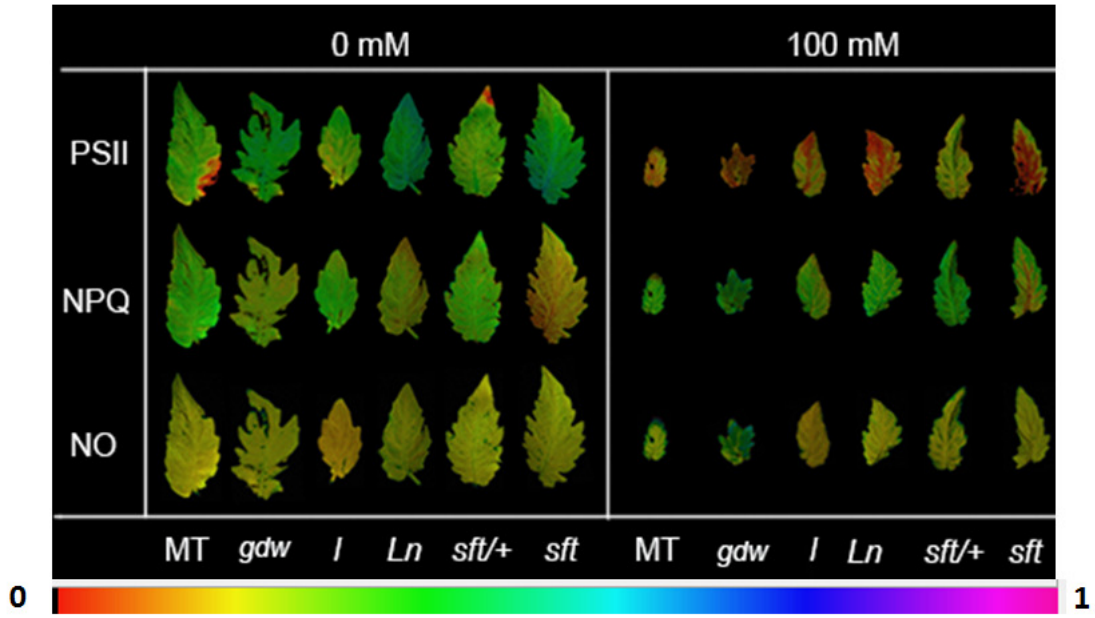

Figure 4 - Effect of salinity on chlorophyll fluorescence of tomato genotypes in the Micro-Tom (MT) genetic background. Quantum yield of photosystem II (PSII), nonphotochemical quenching (NPQ) and nonregulated energy dissipation (NO) of representative leaves from genotypes. The samples were taken from plants growing in half-strength Hoagland's solution in the absence or presence of 100 mM NaCl for 21 days at 50 days after sowing. A description of the genotypes used is shown in Table 1

2.3.5 The impact of salinity in reproductive-vegetative balance and survival

The inhibition of vegetative growth combined with the cost of defense against salt stresses can cause an impairment of reproductive development or even a disturbance of reproductive/vegetative balance. These effects were assessed through the determination of yield-related parameters (Figure 5).

The *l* plants outperformed all the genotypes in the evaluated reproductive parameters under saline conditions, exhibiting the highest yield than other genotypes due to greater fruit fresh weight. Nevertheless, inflorescence biomass of *l* plants did not differ from *Ln* and *sft/+* ones at the pre-established significance level ($\alpha = 0.05$).

The *sft/+* and *sft* mutants presented lower flower and fruit set, even under absence of salt. This could be due to a delay of flowering onset, typical of these genotypes. *sft/+* and *sft* harbour one and two copies of *sft* allele, respectively, a loss-of-function of the *SFT* gene, responsible by the production of florigen in tomato plants (SHALIT et al., 2009). Hence,

these genotypes might have produced higher fruit yield, if the plants have been allowed to grow for more time.

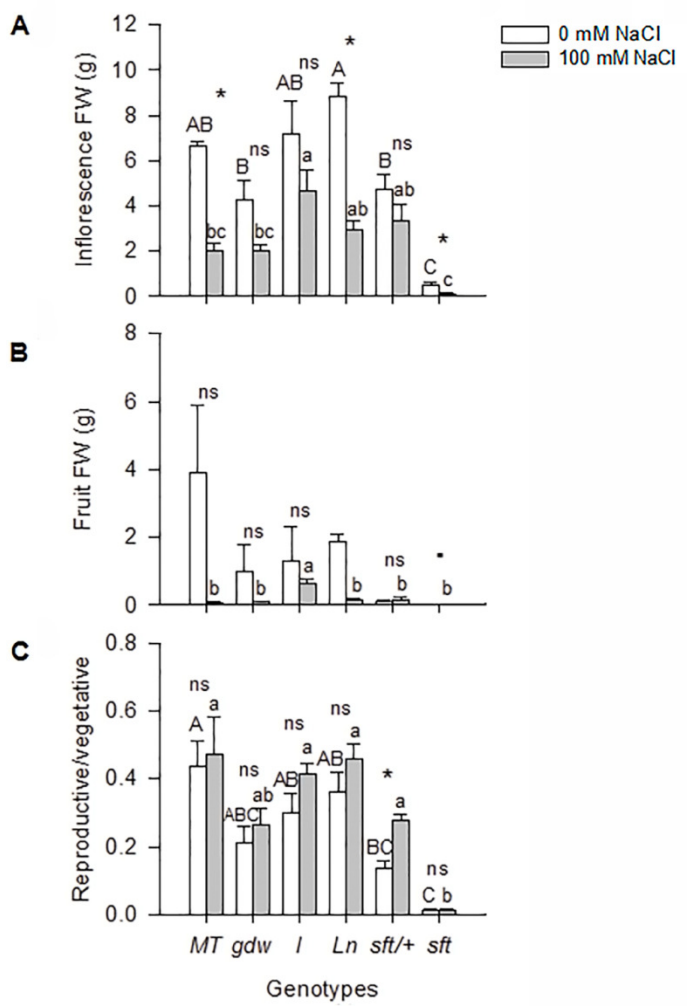

Figure 5 - Effect of salinity on the reproductive development of tomato Micro-Tom (MT) genotypes. Inflorescence (A) and fruit (B) biomass and reproductive/vegetative fresh weight (FW) ratio (C) of plants grown in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 21 days. Measurements were performed at 50 days after sowing. Data are means ± SE (n = 3). Asterisks and "ns" indicate, respectively, significant and non significant differences between 0 and 100 mM NaCl for each genotype according to the F test at P <0.05. Bars with different upper and lower case letters represent significant differences between genotypes grown under 0 and 100 mM NaCl, respectively, according to Tukey's test at P <0.05. The absence of upper case letters above the open bars in panels B denotes a lack of significant difference between genotypes according to the F test at P <0.05. A description of the genotypes used is shown in Table 1

An interesting result is the reproductive/vegetative balance which was strongly altered in saline conditions in *sft/+* plants (Figure 5C). They showed an increase of 98.5% reproductive/vegetative ratio when compared to control environment. The salinization also led *sft/+* reach higher inflorescence fresh weight values than MT (Figure 5A), although statistically not significant.

Since in the preliminary test the plants were not allowed to complete the life cycle, it was considered the necessity to conduct a novel experiment to assess the yield chosen genotypes under stress.

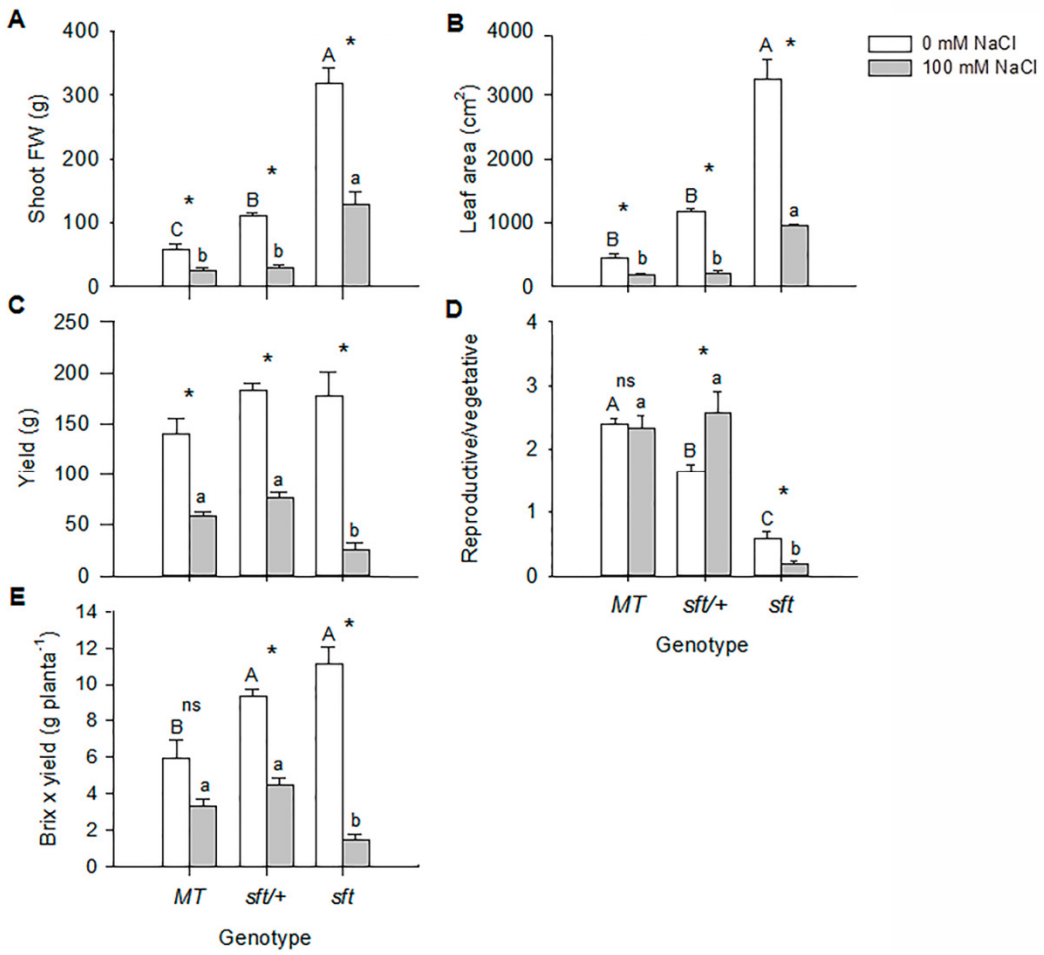

Figure 6 - Effect of salinity on productivity parameters of different tomato genotypes in the Micro-Tom (MT) genetic background. Shoot biomass (A), leaf area (B), fruit biomass (C), reproductive/vegetative growth ratio (D) and °Brix x yield were measured in the MT and two near isogenic lines harbouring the *single flower truss* mutation in homozygous (*sft*) and heterozygous (*sft*/+) form growing in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 85 days. Measurements were performed at110 days after sowing. Data are means ± SE (n = 4). Asterisks and "ns" indicate, respectively, significant and non significant differences between 0 and 100 mM NaCl for each genotype according to the F test at P <0.05. Bars with different upper and lower case letters represent significant differences between genotypes grown under 0 and 100 mM NaCl, respectively, according to Tukey's test at P <0.05. The absence of letters above the open bars in panels C and D evidences a lack of significant difference between genotypes under control (no salt) condition, according to the F test at P <0.05

The *sft*/+ growth was featured by a robust vegetative development under absence of salt stress. In addition, *sft*/+ showed yield and °Brix x yield (BRY) higher than MT, although the differences were not statistically significant for yield (Figures 6C and E, Figure 7).

Moreover, the observed results agreed with Vicente et al. (2015) that reported a vegetative-to-reproductive balance of MT-*sft*/+ as 20% more vegetative than MT even so the MT-*sft*/+ yield is higher about 30%.

Interestingly, the reproductive-to-vegetative balance was drastically altered under salt stress in heterozygous *sft*/+, i.e. the plants being more reproductive. Taken together, these results suggest a tendency for the photosynthate partitioning to reproductive development in detriment of vegetative growth under salinity. On the other hand, a reduction of vegetative growth provoked by salinization must be also considered. Although the observed results have been probably a consequence of a combination of a set of responses. Moreover, the *sft*/+ and sft showed a more vigorous growth than all other genotypes.

In view of the more vigorous growth of *sft*/+ and its identification with the "heterosis" effect (KRIEGER; LIPPMAN; ZAMIR, 2010), an experiment was carried out to assess the survival of the mutants at 150 mM NaCl from the seedling stage until fruit ripening (Figure 8 A).

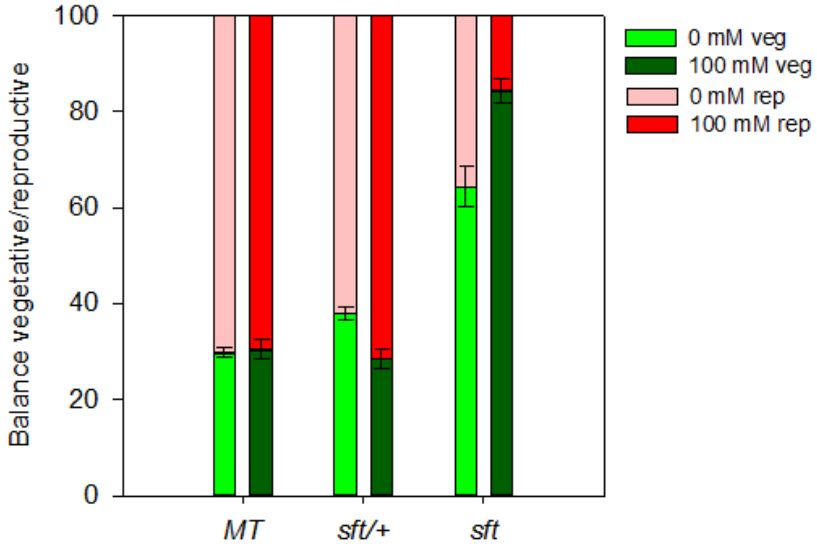

Figure 7 - Effect of salinity on vegetative-to-reproductive balance of *sft* mutants in the Micro-Tom (MT) genetic background calculated as the relative contribution of vegetative and reproductive components. Fresh matter partition between vegetative part (green bars) and fruits (pink/red bars) from plants grown in half-strength Hoagland's solution in the absence (light green and pink colors) or presence of 100 mM NaCl (deep green and red colors) for 85 days. Measurements were performed at 110 days after sowing. Data are means percent (n = 4).

Remarkably, *sft*/+ reached a survival rate of about 80%, 4- and 1.3-fold increases, when compared to MT and *sft*, respectively (Figure 8 B).

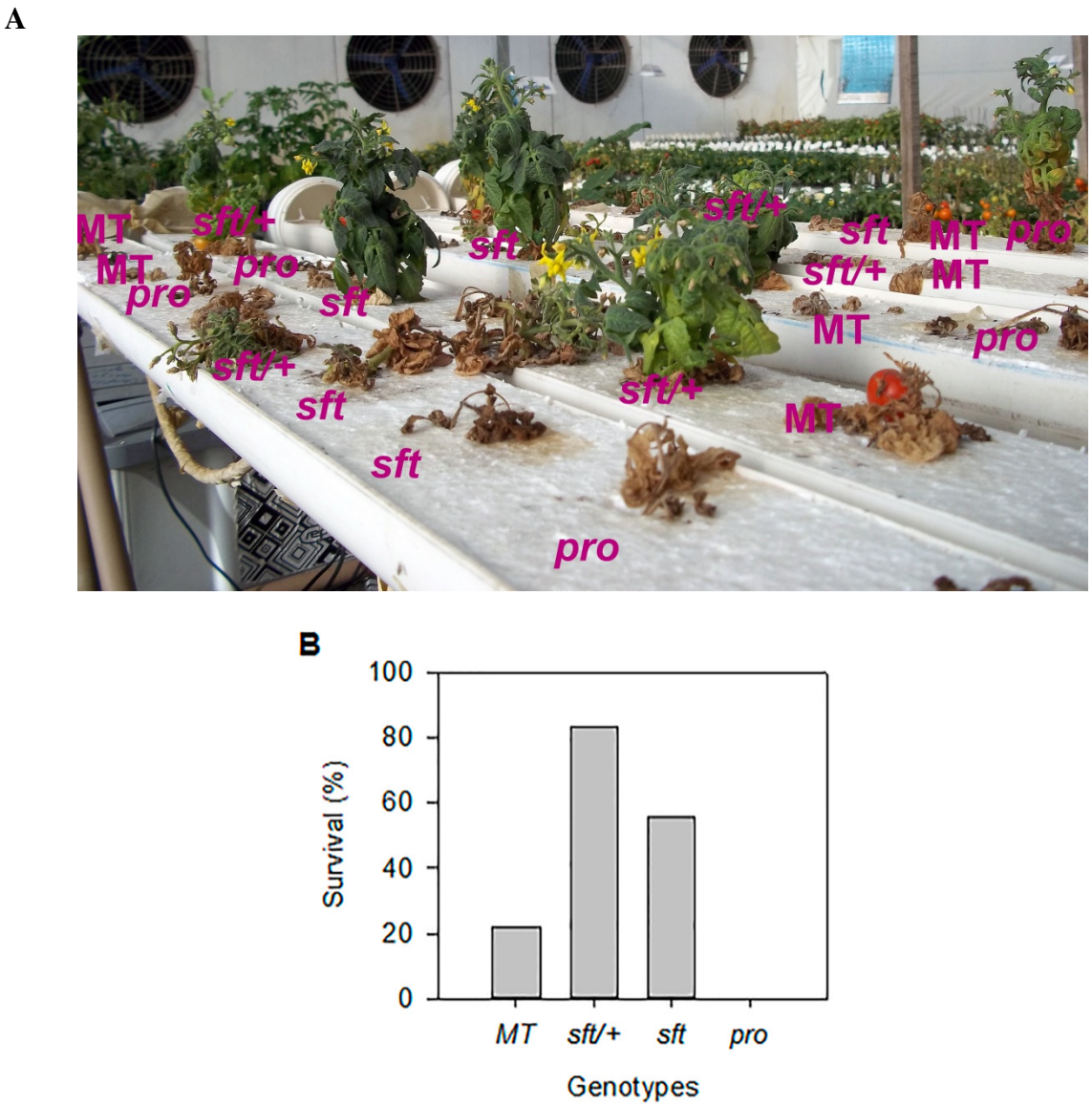

Figure 8 - Effect of salinity on the survival rate of different tomato genotypes in the Micro-Tom (MT) genetic background. (A) Representative plants of MT and two near isogenic lines harbouring the single flower truss mutation, in homozygous (*sft*) and heterozygous (*sft*/+) form, and the *procera* (*pro*) mutation, after 80 days growing in greenhouse on 70%-strength Hoagland's solution plus 150 mM NaCl. (B) Survival rate of the genotypes. The image was taken after 104 days after sowing and the survival rate (n = 18) was evaluated after 111 days after sowing

2.4 Discussion

Although plant resistance to high NaCl concentrations is considered to be complex, due to its polygenic nature, many studies have demonstrated that the transference or alterations in the expression of one or few genes can cause remarkable changes in the plant survival capacity under salinity (revised by Flowers, 2004). Furthermore, previous reports have helped identify and understand the mechanisms and effectors that regulate growth under salinity. Here, it was shown that tomato genotypes into a same genetic background (cv. Micro-Tom) are useful tools to achieve these aims.

Salt treatment (100 mM NaCl) impaired shoot and root growth of all genotypes. The observed shoot development and reduced leaf area relative to root growth in all genotypes upon NaCl treatment could be a consequence of a fall of the water potential in the first phase of salt stress, which impairs substantially the expansion of young leaves, while root tips were not unduly affected (Figure 1). This favors the maintenance of nutrient supply (FRANCO-NAVARRO et al., 2016), even considering the smaller uptake under hyperosmotic conditions (Figure 2). Furthermore, this differential adjustment in shoot and root growth allows water saving, due to the decrease in the transpiration area, contributing to lower Na loading to shoot and a longer conservation of soil moisture (MUNNS; TESTER, 2008). According to Albacete et al. (2008), the novel balance between shoot and root development upon salt exposition is partly regulated by cytokinins and auxins, whose concentrations increase in roots and decline in leaf.

Although affected by salinity, *l* and *sft/+* presented greater shoot biomass and leaf area when compared to MT (Figure 1). The NaCl treatment induced a decrease of quantum yield of photosystem II (PSII) in all genotypes, but *l* and *sft/+* genotypes were less affected (Figure 4).

Regarding the *l* genotype, Almeida et al. (2015) reported an improved tocopherol biosynthesis, at least in fruits, for the same MT-*l* line used here. This was attributed to the premature senescence phenotype of *l* (BARRY et al., 2012), and associated with high chlorophyll degradation that provides recycled phytol for the phytol kinase VTE5 (ISCHEBECK et al., 2006; VALENTIN et al., 2006) and tocopherol biosynthesis (ALMEIDA et al., 2015; DORP et al., 2015; QUADRANA et al., 2013).

Therefore, the typical elevated chlorophyll degradation rate of *l* plants, which occurs in the whole plant, probably improved the tocopherol synthesis not only in fruits (ALMEIDA et al., 2015), but also in leaves. Thus, when the plants were subjected to NaCl treatment, the antioxidative response might have occurred more readily and effectively against unfavourable changes caused by salinity (RAIOLA et al., 2015), ameliorating salinity effects during *l* plants growth. As reported by Skłodowska et al. (2009), severe salt stress by itself leads to a substantial increase of tocopherol (vitamin E) accumulation in tomato chloroplasts.

El-Bassiouny and Sadak (2015) demonstrated that α-tocopherol leaf application reduced lipid peroxidation and activities of polyphenol oxidase, conferring tolerance to salinity in flax cultivars. Moreover, the sucrose mobilization might have been favored by tocopherol content due to the fact that this molecule is needed for sugar distribution, as suggested by Asensi-Fabado et al. (2015). These authors observed an 80% sucrose exudation

rate decrease in leaves of tocopherol-deficient potato plants compared to wild-type ones. Further experiments monitoring the tocopherol content during the exposition of MT-*l* plants to salinity should shed more light on this subject.

Although the *lutescent* genotype used here was not identified at the molecular level yet, its mutation probably underlies a gene functionally related to the tomato *lutescent2* mutant, which is orthologous to the *ETHYLENE-DEPENDENT GRAVITROPISM DEFICIENT AND YELLOW-GREEN1* (*EGY1*) gene of *Arabidopsis thaliana* (BARRY et al., 2012). The two tomato genotypes and the one from Arabidopsis show identical phenotypes: reduced chlorophyll content, abnormal chloroplasts and retarded growth (TANKSLEY et al., 1992; BARRY et al., 2012; CHEN; BI; LI, 2005). It is likely that they also present similar responses to stress such as lower H_2O_2 production (LI et al., 2012) and faster proteolysis of photosystem proteins, which is necessary to the rapid turnover and repair of stress-damaged proteins (ADAM, 2013; SUN et al., 2010).

A common response to salt stress is the increase in SA concentration, which potentiates the generation of ROS in photosynthetic tissues (BORSANI; VALPUESTA; BOTELLA, 2001), contributing to widespread cell damage through lipid peroxidation (MONTEIRO et al., 2011). The *l* plants showed lower shoot values of SA, when compared to the other genotypes (Figure 3C). This may indicate that these plants were less stressed than the other genotypes, as seen by it is less affected quantum yield of photosystem II (Figure 4). Although an increase in SA content is likely to contribute to the activation of cellular protective enzymes, this phenomenon has not been always associated with the induction of stress tolerance (CAO et al., 2009; LUO et al., 2009; SHI et al., 2006; WAHID et al., 2007). Nevertheless, JA and SA concentrations were not directly correlated with growth parameters under salinity (rho > 0.05).

Regarding the *sft*/+ mutant, it showed higher yield and ^{o}Brix x yield (BRY) and higher survival than MT under salinity, although the differences were not statistically significant for BRY (Figures 6 and 7). The *sft*/+showed an increased vegetative-to-reproductive balance under non stressed conditions, but this was inverted upon salt treatment (Figure 6D and 7). As reported previously, the plants that have one or two copies of *sft* allele have a phenotype marked by more vegetative growth in detriment of reproductive development (SHALIT et al., 2009; VICENTE et al., 2015). Here, it was shown that this balance can be changed dramatically by salt treatment. Despite of majority of plant species generally favouring the reproductive development under stress conditions (MWANAMWENGE et al., 1999; WADA; TAKENO, 2010), a sharp response like the one observed here in *sft*/+is interesting, since its

implies that salt stress can be used to manipulate the typical *sft/+* partitioning, which was previously associated to heterosis for yield (JIANG et al., 2013; KRIEGER; LIPPMAN; ZAMIR, 2010).

In general, plants use their resources not only to withstanding the stress but also to achieve reproductive success. The gibberellin (GA) pathway is believed to be a key point in the control of vegetative and reproductive development and GA metabolism is responsiveness to abiotic stress (YANG; MA; XU et al., 2014). The exposure to salt stress is known to elicit a higher expression of GA_2 oxidase (GA_2ox), which inactivates active GAs (SHAN et al., 2014). As a consequence, the reduction of active GAs results in more DELLA repressors, inhibiting plant growth (ACHARD et al., 2006, 2008; MAGOME et al., 2008). Since GAs are also necessary for flower induction (REBERS et al., 1999), salt treatments extends the duration of the vegetative phase by means of a DELLA-dependent reduction of the transcript levels of flower inductor *LEAFY* (*LFY*), but not of *FLOWERING LOCUS T* (*FT*) (ACHARD et al., 2006; LI et al., 2007), which is ortholog to tomato *SINGLE FLOWER TRUSS* (*SFT*). However, the reduction of GA upon salt stress favours the photoassimilates redistribution from vegetative to reproductive growth when the plant resumes flowering (CORBESIER; LEJEUNE; BERNIER et al., 1998; SHAN et al., 2014).

Furthermore, it is important to have in mind that the genotypes used here are in the MT background, which harbors the homozygous *sp* allele (CARVALHO et al., 2011), resulting in determinate and compact growth habit (STEVENS; RICK, 1986). The presence of the *sp* allele means that MT plants do not have the *SP* anti-terminator antagonizing the *SFT* floral inductor (LIFSCHITZ et al., 2014; LIFSCHITZ; AYRE; ESHED, 2006; VICENTE et al., 2015).Therefore, upon salt treatment, the shift from a more vegetative to a more reproductive balance in *sft/+* and the absence of this change in the *sft* (Figure 6D and 7) were probably due to the action of the wild type *SFT* allele present in the heterozygous plant and absent in the homozygous one.

On the other hand, it was reported for Arabidopsis, maize and soybean, that under abiotic stress there is a decrease in the expression of the central repressor of the floral transition, *FLOWERING LOCUS C* (*FLC*), mediated by *miR169* upregulation. This allows the accumulation of the florigenic signal *FT* and *LFY*, resulting on the promotion of early and robust flowering (XU et al., 2014). Although, the *FLC* pathway has not been described in *S. lycopersicum*, it is conceivable that over-expressing of this same microRNA in tomato causes an enhancement of drought tolerance (ZHANG et al., 2011).

It is likely that late-flowering was critical point for the growth advantage of *sft*/+ under salinity. Transgenic plants of Arabidopsis overexpressing the tomato transcriptional factor *SlCDF3* (DNA binding with One Finger), which also affects flowering time, showed similar responses to *sft*/+ mutants. *SlCDF3* and *sft*/+ presented a delay of flowering time but also a better performance under drought and salt stress when compared to wild type (CORRALES et al., 2014; VICENTE et al., 2015). Probably, during this extra time before flowering onset, physiological mechanisms to ameliorate the stress is occurring in these genotypes, such as the nutrient recycling and synthesis of metabolic defense compounds including the JA- and SA-inducible (CORRALES et al., 2014).

The highest value of shoot JA concentration was observed in *sft*/+ (Figure 4). This hormone mediates salinity response, as reported Ding et al. (2016), which observed that *lox3* Arabidopsis mutant that did not produce *LOX3* enzyme that is involved in JA synthesis showed on hypersensitivity to salt stress. JA induces senescence processes and chlorophyll-breakdown (SELTMANN; HUSSELS; BERGER, 2010) whose metabolites generated from chlorophyll degradation can be used in the synthesis of molecules with antioxidant action as discussed before. Interestingly, the *l* mutation elicits the same mechanisms but in JA-independent manner. However, the JA accumulation impairs the synthesis of small subunit of Rubisco and chlorophyll a/b binding proteins (SELTMANN; HUSSELS; BERGER, 2010). It has been shown that Rubisco-activase is down-regulated by JA in a COI1-dependent manner (SHAN et al., 2011), affecting negatively photosynthesis.

Regarding the robustness and the increased survival of *sft*/+ mutants under salinity (Figure 8), they can also be associated with heterosis effect. The heterozygosity of the single gene for loss-of-function alleles of *SFT* led to an improvement of vigour under control conditions (KRIEGER; LIPPMAN; ZAMIR, 2010) and drought (VICENTE et al., 2015). Probably, the vigour of *sft*/+ plants was determinant to growth advantage under salinity. Moreover, as demonstrated by Ryan et al. (2015), there is a link between vigour, biomass accumulation and enhanced tolerance to abiotic stress.

Currently, the majority of work on salinity resistance focuses on the tolerance, i.e. the level of growth impairment through the comparison of plant development between optimal and stressing environment (FLOWERS, 2004). However, the most important parameter could be the plant performance in the agricultural environment. Therefore, even genotypes that displayed high growth reduction when cultivated in saline areas, but that have growth and yield superior to other lines, may be advantageous. In these terms, the *sft*/+ genotype can be a potential breeding source for the development of hybrids lines to be used in saline soils.

Nevertheless, additional studies focusing on sugar metabolism and stress defense under salinity would be useful to achieve a better understanding about the performances of *sft/+* and *l* genotypes.

2.5 Conclusions

The salt treatment tends to influence flower induction, leading to an alteration of reproductive-to-vegetative balance. As reported by Vicente et al. (2015), the heterosis of *sft/+* (KRIEGER; LIPPMAN; ZAMIR, 2010) influences this same growth balance. In this work, we observed that the vegetative and reproductive growth pattern of *sft/+* was broken under salinity. Therefore, the use of this pseudoheterosis in order to achieve higher yield must be used with care in areas subjected to salinity as demonstrated in this work. On the other hand, the precocious senescence of *lutescent*, although considered deleterious at a first glimpse, may be advantageous as a novel acclimatory mechanism under salinity.

References

ACHARD, P.; CHENG, H.; GRAUWE, L.; DECAT, J.; SCHOUTTETEN, H.; MORITZ, T.; STRAETEN, D. van der.; PENG, J.; HARBERD, N.P.; Integration of plant responses to environmentally activated phytohormonal signals. **Science**, New York, v. 311, n. 5757, p. 91–94, 2006.

ACHARD, P.; GONG, F.; CHEMINANT, S.; ALIOUA, M.; HEDDEN, P.; GENSCHIK, P. The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. **The Plant Cell**, Rockville, v. 20, n. 8, p. 2117–2129, 2008.

ADAM, Z. Emerging roles for diverse intramembrane proteases in plant biology. **Biochimica et Biophysica Acta (BBA) Biomembranes**, Amsterdam, v.1828, n. 12, p. 2933–2936, 2013.

ALBACETE, A.; GHANEM, M.E.; MARTÍNEZ-ANDÚJAR, C.; ACOSTA M.; SÁNCHEZ-BRAVO, J.; MARTÍNEZ, V.; LUTTS, S.; DODD, I.C.; PÉREZ-ALFOCEA, F. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. **Journal of Experimental Botany**, Oxford, v. 59, n. 15, p. 4119–4131, 2008.

ALMEIDA, J.; ASÍS, R.; MOLINERI, V.N.; SESTARI, I.; LIRA, B.S.; CARRARI, F.; PERES, L.E.P.; ROSSI, M. Fruits from ripening impaired, chlorophyll degraded and jasmonate insensitive tomato mutants have altered tocopherol content and composition. **Phytochemistry**, New York, v. 111, n. 1, p. 72–83, 2015.

ASENSI-FABADO, M.A.; AMMON, A.; SONNEWALD, U.; MUNNÉ-BOSCH, S.; VOLL, L.M. Tocopherol deficiency reduces sucrose export from salt-stressed potato leaves independently of oxidative stress and symplastic obstruction by callose. **Journal of Experimental Botany**, Oxford, v. 66, n. 3, p. 957–971, 2015.

BARRY, C.S.; MCQUINN, R.P.; CHUNG, M.Y.; BESUDEN, A.; GIOVANNONI, J.J. Amino acid substitutions in homologs of the STAY-GREEN protein are responsible for the green-flesh and chlorophyll retainer mutations of tomato and pepper. **Plant Physiology**, Lancaster, v. 147, n. 1, p. 179–187, 2008.

BARRY, C.S.; ALDRIDGE, G.M.; HERZOG, G.; MA, Q.; MCQUINN, R.P.; HIRSCHBERG, J.; GIOVANNONI, J.J. Altered chloroplast development and delayed fruit ripening caused by mutations in a zinc metalloprotease at the *lutescent* locus of tomato. **Plant Physiology**, Lancaster, v. 159, n. 3, p. 1086-1098, 2012.

BORSANI, O.; VALPUESTA, V.; BOTELLA, M.A. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. **Plant Physiology**, Lancaster, v. 126, n. 3, p. 1024-1030, 2001.

CAO, Y.; ZHANG, Z.-W.; XUE, L.-W.; DU, J.-B.; SHANG, J.; XU, F.; YUAN, S.; LIN, H.-H. Lack of salicylic acid in Arabidopsis protects plants against moderate salt stress. **Journal of Biosciences: Zeitschrift für Naturforschung**, Tubingen, v. 64, n. 3/4, p. 231–238, 2009.

CARVALHO, R.F.; CAMPOS, M.L.; PINO, L.E.; CRESTANA, S.L.; ZSÖGÖN, A.; LIMA, J.E.; BENEDITO, V.A; PERES, L.E.P. Convergence of developmental mutants into a single tomato model system: "Micro-Tom" as an effective toolkit for plant development research. **Plant Methods**, London, v. 7, n. 1, p. 1-14, 2011.

CHEN, G.; BI, Y.R.; LI, N. *EGY1* encodes a membrane-associated and ATP-independent metalloprotease that is required for chloroplast development. **The Plant Journal**, Oxford, v. 41, n. 3, p. 364–375, 2005.

CORBESIER, L.; LEJEUNE, P.; BERNIER, G. The role of carbohydrates in the induction of flowering in *Arabidopsis thaliana*: comparison between the wild type and a starchless mutant. **Planta**, New York, v. 206, n. 1, p. 131–137, 1998.

CORRALES, A.-R.; NEBAUER, S.G.; CARRILLO, L.; FERNÁNDEZ-NOHALES, P.; MARQUÉS, J.; RENAU-MORATA, B.; GRANELL, A.; POLLMANN, S.; VICENTE-CARBAJOSA, J.; MOLINA, R.-V.; MEDINA, J. Characterization of tomato Cycling Dof Factors reveals conserved and new functions in the control of flowering time and abiotic stress responses. **Journal of Experimental Botany**, Oxford, v. 65, n. 4, p. 995–1012, 2014.

CUARTERO, J. Increasing salt tolerance in the tomato. **Journal of Experimental Botany,** Oxford, v. 57, n. 5, p. 1045–1058, 2006.

DING, H.; LAI, J.; WU, Q.; ZHANG, S.; CHEN, L.; DAI, Y.S.; WANG, C.; DU, J.; XIAO, S.; YANG, C. Jasmonate complements the function of Arabidopsis lipoxygenase3 in salinity stress response. **Plant Science**, Cambridge, v. 244, n. 1, p. 1–7, 2016.

DORP, K. von.; HÖLZL, G.; PLOHMANN, C.; EISENHUT, M.; ABRAHAM, M.; WEBER, A.P.M.; HANSON, A.D.; DÖRMANN, P. Remobilization of phytol from chlorophyll degradation is essential for tocopherol synthesis and growth of Arabidopsis. **The Plant Cell**, Rockville, v. 27, n. 10, p. 2846–2859, 2015.

EL-BASSIOUNY, H.M.S.; SADAK, M.S. Impact of foliar application of ascorbic acid and α-tocopherol on antioxidant activity and some biochemical aspects of flax cultivars under salinity stress. **Acta Biológica Colombiana**, Bogotá, v. 20, n. 2, p. 209–222, 2015.

ESSINGTON, M.E. **Soil and water chemistry:** an integrative approach. 2.ed. Boca Raton: CRC Press, 2015. 656 p

FAO. **Global network on integrated soil management for sustainable use of salt-affected soils**. Rome: FAO, Land and Plant Nutrition Management Service, 2005. Disponível em:<http://www.fao.org/ag/agl/agll/spush>. Acesso em: 30 maio 2016.

FLOWERS T. J. Improving crop salt tolerance. **Journal of Experimental Botany**. Oxford, v. 55, n. 396, p. 307–319, 2004.

FLOWERS, T.J.; GALAL, H.K.; BROMHAM, L. Evolution of halophytes: Multiple origins of salt tolerance in land plants. **Functional Plant Biology**, Victoria, v. 37, n. 7, p. 604–612, 2010.

FRANCO-NAVARRO, J.D.; BRUMÓS, J.; ROSALES, M.A.; CUBERO-FONT, P.; TALÓN, M.; COLMENERO-FLORES, J.M. Chloride regulates leaf cell size and water relations in tobacco plants. **Journal of Experimental Botany**, Oxford, v. 67, p. 873–891, 2016.

GARCIA-ABELLAN, J.O.; FERNANDEZ-GARCIA, N.; LOPEZ-BERENGUER, C.; EGEA, I.; FLORES, F.B.; ANGOSTO, T.; CAPEL, J.; LOZANO, R.; PINEDA, B.; MORENO, V.; OLMOS, E.; BOLARIN, M.C. The tomato res mutant which accumulates JA in roots in non-stressed conditions restores cell structure alterations under salinity. **Physiologia Plantarum**, Copenhagen, v. 155, n. 3, p. 296–314, 2015.

GHANEM, M.E.; ALBACETE, A.; MARTÍNEZ-ANDÚJAR, C.; ACOSTA, M.; ROMERO-ARANDA, R.; DODD, I. C.; LUTTS, S.; PÉREZ-ALFOCEA, F. Hormonal changes during salinity-induced leaf senescence in tomato (*Solanum lycopersicum* L.). **Journal of Experimental Botany**, Oxford, v. 59, n. 11, p. 3039–3050, 2008.

GOLLDACK, D.; LI, C.; MOHAN, H.; PROBST, N. Tolerance to drought and salt stress in plants: Unraveling the signaling networks. **Frontiers in Plant Science**, Lausanne, v. 5, n. April, p. 1-10, 2014.

GUPTA, B.; HUANG, B. Mechanism of Salinity Tolerance in Plants: Physiological, Biochemical, and Molecular Characterization. **International Journal of Genomics**, New York, v. 2014, n. 2014, p. 1–18, 2014.

HASEGAWA, P.M.; BRESSAN, RAY A; BOHNERT, J.-K. Z.H.J. Plant Cellular Andmolecular Responses To High Salinity. **Annual Review of Plant Physiology and Plant Molecular Biology**, Palo Alto, v. 51, p. 463–499, 2000.

HORTENSTEINER, S.; FELLER, U. Nitrogen metabolism and remobilization during senescence. **Journal of Experimental Botany**, Oxford, v. 53, n. 370, p. 927–937, 2002.

HU, Z.L.; DENG, L.; YAN, B.; PAN, Y.; LUO, M.; CHEN, X.Q.; HU, T.Z.; CHEN, G.P. Silencing of the *LeSGR1* gene in tomato inhibits chlorophyll degradation and exhibits a stay-green phenotype. **Biologia Plantarum**, Praha, v. 55, n. 1, p. 27–34, 2011.

HUANG, P.C.; PADDOCK, E.F. The Time and Site of the Semidominant Lethal Action of "*Wo*" in *Lycopersicon esculentum*. **American Journal of Botany**, Baltimore, v. 49, n. 4, p. 388–393, 1962.

ISCHEBECK, T.; ZBIERZAK, A.M.; KANWISCHER, M.; DÖRMANN, P. A salvage pathway for phytol metabolism in *Arabidopsis*. **The Journal of Biological Chemistry**, Baltimore, v. 281, n. 5, p. 2470–2477, 2006.

JESUS, F.A. de. **Caracterização de uma variação genética natural de *Solanum galapagense* controlando o comprimento do entrenó e arquitetura foliar em tomateiro**. 2015. 70 p. Tese (Doutorado em Fisiologia e Bioquímica de Plantas) – Escola Superior de agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2015. Disponível em: <http://www.teses.usp.br/teses/disponiveis/11/11144/tde-28032016-135123/en.php>. Acesso em: 30 maio 2016.

JIANG, K.; LIBERATORE, K.L.; PARK, S.J.; ALVAREZ, J.P.; LIPPMAN, Z.B. Tomato yield heterosis is triggered by a dosage sensitivity of the florigen pathway that fine-tunes shoot architecture. **PLoS Genetics**, San Francisco, v. 9, n. 12, p. 1-13, 2013. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/24385931>. Acesso em: 15 jan. 2016.

KHAN, M.I.R.; SYEED, S.; NAZAR, R.; ANJUM, N.A. An Insight into the Role of Salicylic Acid and Jasmonic Acid in Salt Stress Tolerance. In: KHAN, A.N.; NAZAR, R.; IQBAL, N.; ANJUM, A.N. (Ed.). **Phytohormones and Abiotic Stress Tolerance in Plants**. Berlin: Springer Berlin Heidelberg, 2012. chap. 12, p. 277–300.

KIM, Y.S.; SAKURABA, Y.; HAN, S.H.; YOO, S.C.; PAEK, N.C. Mutation of the arabidopsis NAC016 transcription factor delays leaf senescence. **Plant and Cell Physiology**, Tokyo, v. 54, n. 10, p. 1660–1672, 2013.

KOUDOUNAS, K.; MANIOUDAKI, M.E.; KOURTI, A.; BANILAS, G.; HATZOPOULOS, P. Transcriptional profiling unravels potential metabolic activities of the olive leaf non-glandular trichome. **Frontiers in Plant Science**, Lausanne, v. 6, n. 8, p. 1–10, 2015.

KRIEGER U.; LIPPMAN Z. B.; ZAMIR D. The flowering gene *SINGLE FLOWER TRUSS* drives heterosis for yield in tomato. **Nature Genetics,** New York, v. 42, n. 5, p. 459–463, 2010.

KRIEGER-LISZKAY, A.; FUFEZAN, C.; TREBST, A. Singlet oxygen production in photosystem II and related protection mechanism. **Photosynthesis Research**, Dordrecht, v. 98, n. 1–3, p. 551–564, 9 out. 2008.

LEVENE H. Robust tests for equality of variances. In: OLKIN I. **Contributions to probability and statistics**: essays in honor of Harold Hotelling. Palo Alto: Stanford University Press, 1960. p. 278–292.

LEVITT, J. **Responses of Plants to Environmental Stresses**. New York: Academic Press, 1972. 698 p.

LI, B.; LI, Q.; XIONG, L.; KRONZUCKER, H.J.; KRÄMER, U.; SHI, W. Arabidopsis plastid *AMOS1/EGY1* integrates abscisic acid signaling to regulate global gene expression response to ammonium stress. **Plant Physiology**, Lancaster, v.160, n. 4, p. 2040–2051, 2012.

LI, K.; WANG, Y.; HAN, C.; ZHANG, W.; JIA, H.; LI, X. GA signaling and CO/FT regulatory module mediate salt-induced late flowering in *Arabidopsis thaliana*. **Plant Growth Regulation**, Dordrecht, v. 56, n. 3, p. 195–206, 2007.

LIFSCHITZ, E.; AYRE, B.G.; ESHED, Y. Florigen and anti-florigen: a systemic mechanism for coordinating growth and termination in flowering plants. **Frontiers in Plant Science**, Lausanne, v. 5, n. 165, p. 465, 2014.

LIFSCHITZ, E.; EVIATAR, T.; ROZMAN, A.; SHALIT, A.; GOLDSHMIDT, A.; AMSELLEM, Z.; ALVAREZ, J.P.; ESHED, Y. The tomato *FT* ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 103, n. 16, p. 6398–6403, 2006.

LUO, M.H.; YUAN, S.; CHEN, Y.E.; LIU, W.J.; DU, J.B.; LEI, T.; WANG, M.B.; LIN, H.H. Effects of salicylic acid on the photosystem 2 of barley seedlings under osmotic stress. **Biologia Plantarum**, Praha, v. 53, n. 4, p. 663–669, 2009.

LUO, Z.; ZHANG, J.; LI, J.; YANG, C.; WANG, T.; OUYANG, B.; LI, H.; GIOVANNONI, J.; YE, Z. A STAY-GREEN protein SlSGR1 regulates lycopene and β-carotene accumulation by interacting directly with *SlPSY1* during ripening processes in tomato. **New Phytologist**, Oxford, v. 198, n. 2, p. 442–452, 2013.

MAGOME, H.; YAMAGUCHI, S.; HANADA, A.; KAMIYA, Y.; ODA, K. The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, *GA2ox7*, under high-salinity stress in Arabidopsis. **The Plant Journal**, Oxford, v. 56, n. 4, p. 613–626, 2008.

MATEO, A.; FUNCK, D.; MÜHLENBOCK, P.; KULAR, B.; MULLINEAUX, P.M.; KARPINSKI, S. Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. **Journal of Experimental Botany**, Oxford, v. 57, n. 8, p. 1795–1807, 2006.

MOLINERO-ROSALES, N.; LATORRE, A.; JAMILENA, M.; LOZANO, R. *Single flower truss* regulates the transition and maintenance of flowering in tomato. **Planta**, New York, v. 218, n.3, p. 427–434, 2004.

MONTEIRO, C.C.; CARVALHO, R.F.; GRATÃO, P.L.; CARVALHO, G.; TEZOTTO, T.; MEDICI, L.O.; PERES, L.E.P.; AZEVEDO, R.A. Biochemical responses of the ethylene-insensitive never ripe tomato mutant subjected to cadmium and sodium stresses. **Environmental and Experimental Botany**, Oxford, v. 71, n. 2, p. 306–320, 2011.

MUNNS, R.; TESTER, M. Mechanisms of salinity tolerance. **Annual Review of Plant Biology**, Palo Alto, v. 59, n. 1, p. 651–681, 2008.

MWANAMWENGE, J.; LOSS, S.; SIDDIQUE, K.H.; COCKS, P. Effect of water stress during floral initiation, flowering and podding on the growth and yield of Faba bean (*Vicia faba* L.). **European Journal of Agronomy**, Amsterdam, v. 11, n. 1, p. 1–11, 1999.

OGAWA, D.; ABE, K.; MIYAO, A.; KOJIMA, M.; SAKAKIBARA, H.; MIZUTANI, M.; MORITA, H.; TODA, Y.; HOBO, T.; SATO, Y.; HATTORI, T.; HIROCHIKA, H.; TAKEDA, S. *RSS1* regulates the cell cycle and maintains meristematic activity under stress conditions in rice. **Nature Communications**, London, v. 2, p. 278, 2011.

PARK, S.-Y.; YU, J.-W.; PARK, J.-S.; LI, J.; YOO, S.-C.; LEE, N.-Y.; LEE, S.-K.; JEONG, S.-W.; SEO, H. S.; KOH, H.-J.; JEON, J.-S.; PARK, Y.-I.; PAEK, N.-C. The Senescence-Induced Staygreen Protein Regulates Chlorophyll Degradation. **The Plant Cell,** Rockville, v. 19, n. 5, p. 1649–1664, 2007.

PINO, L.E.; LOMBARDI-CRESTANA, S.; AZEVEDO, M.S.; SCOTTON, D.C.; BORGO, L.; QUECINI, V.; FIGUEIRA, A.; PERES, L.E.P. The *Rg1* allele as a valuable tool for genetic transformation of the tomato 'Micro-Tom' model system. **Plant Methods**, London, v. 6, n. 23, p.1-23, 2010.

POTTERS, G.; PASTERNAK, T.P.; GUISEZ, Y.; JANSEN, M. A. K. Different stresses, similar morphogenic responses: Integrating a plethora of pathways. **Plant, Cell and Environment**, New York, v. 32, n. 2, p. 158–169, 2009.

QUADRANA, L.; ALMEIDA, J.; OTAIZA, S.N.; DUFFY, T.; SILVA, J.V.C.; GODOY, F.; ASÍS, R.; BERMUDEZ, L.; FERNIE, A.F.; CARRARI, F.; ROSSI, M. Transcriptional regulation of tocopherol biosynthesis in tomato. **Plant Molecular Biology**, Dordrecht, v. 81, n. 3, p. 309–325, 2013.

RAIOLA, R.; TENORE, G.C.; BARONE, A.; FRUSCIANTE, L.; RIGANO, M.M. Vitamin E content and composition in tomato fruits: beneficial roles and bio-fortification. **International Journal of Molecular Sciences,** Basel, v. 16, n. 2, p. 29250–29264, 2015.

RAO, M.V.; DAVIS, K.R. Ozone-induced cell death occurs via two distinct mechanisms in Arabidopsis: the role of salicylic acid. **The Plant Journal**, Oxford, v. 17, n. 6, p. 603–614, 1999.

REBERS, M.; KANETA, T.; KAWAIDE, H.; YAMAGUCHI, S.; YANG, Y.Y.; IMAI, R.; SEKIMOTO, H.; KAMIYA, Y. Regulation of gibberellin biosynthesis genes during flower and early fruit development of tomato. **The Plant Journal**, Oxford, v. 17, n. 3, p. 241–250, 1999.

REEVES, A.F. Tomato Trichomes and Mutations Affecting Their Development. **American Journal of Botany**, Baltimore, v. 64, n. 2, p. 186–189, 1977.

RUSH, D.W.; EPSTEIN, E. Genotypic Responses to Salinity: Differences between Salt-sensitive and Salt-tolerant Genotypes of the Tomato. **Plant Physiology**, Lancaster, v. 57, n. 2, p. 162–6, 1976.

RYAN, P.R.; LIAO, M.; DELHAIZE, E.; REBETZKE, G.J.; WELIGAMA, C.; SPIELMEYER, W.; JAMES, R.A. Early vigour improves phosphate uptake in wheat. **Journal of Experimental Botany**, Oxford, v. 66, n. 22, p. 7089–7100, 2015.

SAS INSTITUTE. **SAS/STAT® 9.2:**user's guide. Cary, 2008.

SELTMANN, M. A; HUSSELS, W.; BERGER, S. Jasmonates during senescence. **Plant Signaling & Behavior**, Georgetown, v. 5, n. 11, p. 1493–1496, 2010.

SHALIT, A.; ROZMAN, A.; GOLDSHMIDT, A.; ALVAREZ, J.P.; BOWMAN, J.L.; ESHED, Y.; LIFSCHITZ, E. The flowering hormone florigen functions as a general systemic regulator of growth and termination. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 106, n. 20, p. 8392–8397, 2009.

SHAN, C.; MEI, Z.; DUAN, J.; CHEN, H.; FENG, H.; CAI, W. *OsGA2ox5*, a gibberellin metabolism enzyme, is involved in plant growth, the root gravity response and salt stress. **PloS One**, San Francisco, v. 9, n. 1, p. 1-10, 2014. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/24475234>. Acesso em: 22 maio 2016.

SHAN, X.; WANG, J.; CHUA, L.; JIANG, D.; PENG, W.; XIE, D. The role of Arabidopsis Rubisco activase in jasmonate-induced leaf senescence. **Plant Physiology**, Lancaster, v. 155, n. 2, p. 751–764, 2011.

SHAPIRO, S.S.; WILK, M.B. An analysis of variance test for normality (complete samples). **Biometrika**, London. v. 52, n. 3/4, p. 591–611, 1965.

SHI, Q.; BAO, Z.; ZHU, Z.; YING, Q.; QIAN, Q. Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. **Plant Growth Regulation**, Dordrecht, v. 48, n. 2, p.127–135, 2006.

SKŁODOWSKA, M.; GAPIŃSKA, M.; GAJEWSKA, E.; GABARA, B. Tocopherol content and enzymatic antioxidant activities in chloroplasts from NaCl-stressed tomato plants. **Acta Physiologiae Plantarum**, Warszawa, v. 31, n. 2, p. 393–400, 2009.

SPOONER, D.M.; PERALTA, I.E.; KNAPP, S. Comparison of AFLPs with Other Markers for Phylogenetic Inference in Wild Tomatoes [*Solanum* L. Section *Lycopersicon* (Mill.) Wettst.]. **Taxon**, Utrecht, v. 54, n. 1, p. 43–61, 2005.

STAM, P.; ZEVEN, A.C. The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. **Euphytica**, Dordrecht, v. 30, n. 2, p. 227–238, 1981.

STASWICK, P.E.; TIRYAKI, I. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. **The Plant Cell**, Rockville, v. 16, n. 8, p. 2117–2127, 2004

STEVENS, M.A.; RICK, C.M. Genetics and breeding. In: ATHERTON, J.; RUDICH, J. (Ed.). **The tomato crop:** a scientific basis for improvement. London: Chapman & Hall, 1986. p. 35–100.

SUN, X.; FU, T.; CHEN, N.; GUO, J.; MA, J.; ZOU, M.; LU, C.; ZHANG, L. The stromal chloroplast Deg7 protease participates in the repair of photosystem II after photoinhibition in Arabidopsis. **Plant Physiology**, Lancaster, v. 152, n. 3, p. 1263–1273, 2010.

SZYMKOWIAK, E.J.; SUSSEX, I.M. The internal meristem layer (L3) determines floral meristem size and carpel number in tomato periclinal chimeras. **The Plant Cell**, Rockville, v. 4, n. 9, p. 1089–1100, 1992.

TANKSLEY, S.D.; GANAL, M.W.; PRINCE, J.P.; VICENTE, M.C.; BONIERBALE, M.W.; BROUN, P.; FULTON, T.M.; GIOVANNONI, J.J.; GRANDILLO, S.; MARTIN, G.B. High density molecular linkage maps of the tomato and potato genomes. **Genetics**, Bethesda, v. 132, n, 4, p. 1141–1160. 1992.

THOMAS, H.; OUGHAM, H. The stay-green trait. **Journal of Experimental Botany**, Oxford, v. 65, n. 14, p. 3889–3900, 2014.

TUTEJA, N.; SOPORY, S.K. Chemical signaling under abiotic stress environment in plants. **Plant Signaling & Behavior**, Georgetown, v. 3, n. 8, p. 525–536, 2008.

URANO, D.; COLANERI, A.; JONES, A.M. Gα modulates salt-induced cellular senescence and cell division in rice and maize. **Journal of Experimental Botany**, Oxford,v. 65, n. 22, p. 6553–6561, 2014.

URANO, K.; KURIHARA, Y.; SEKI, M.; SHINOZAKI, K. “Omics” analyses of regulatory networks in plant abiotic stress responses. **Current Opinion in Plant Biology**, London, v. 13, n. 2, p. 132–138, 2010.

VALENTIN, H.E.; LINCOLN, K.; MOSHIRI, F.; JENSEN, P.K.; QI,Q.; VENKATESH,T. V.; KARUNANANDAA, B.; BASZIS, S.; NORRIS, S.; SAVIDGE, B.; GRUYS, K.J.; LAST, R. The Arabidopsis vitamin E pathway *gene 5-1* mutant reveals a critical role for phytol kinase in seed tocopherol biosynthesis. **The Plant Cell**, Rockville, v. 18, n. 1, p. 212–224, 2006.

VENDEMIATTI, E.; ZSÖGÖN, A.; SILVA, G.F.F.; JESUS, F.A.; CUTRI, L.; TANAKA, F.; NOGUEIRA, F.T.S.; PERES, L.E.P. Loss of type-IV glandular trichomes in tomato (*Solanum lycopersicum*) is a heterochronic trait reversible by the promotion of juvenile phase of vegetative development. **New Phytologist**, London, 2016. In press.

VERSLUES, P.E.; AGARWAL, M.; KATIYAR-AGARWAL, S.; ZHU, J.; ZHU, J.-K. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. **The Plant Journal**, Oxford, v. 45, n. 4, p. 523–539, 2006.

VICENTE, M.H.; ZSÖGÖN, A.; SÁ, A.F.L.; RIBEIRO, R.; PERES, L.E.P. Semi-determinate growth habit adjusts the vegetative-to-reproductive balance and increases productivity and water-use efficiency in tomato (*Solanum lycopersicum*). **Journal of Plant Physiology**, Stuttgart, v. 177, n. 1, p. 11–19, 2015.

VICENTE. M.H. **Regulação do balanço vegetativo-reprodutivo pelo crescimento semi-determinado em tomateiro (*Solanum lycopersicum* L. cv. Micro-Tom) e seu impacto na produtividade e eficiência no uso da água.** 2013. 87 p. Dissertação (Mestrado em Fisiologia e Bioquímica de Plantas) – Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2013. Disponível em: <http://www.teses.usp.br/teses/disponiveis/11/11144/tde-10092013-161029/publico/Mateus_Henrique_Vicente.pdf>. Acesso em: 20 maio 2016.

WADA, K.C.; TAKENO, K. Stress-induced flowering. **Plant Signaling & Behavior**, Georgetown, v. 5, n. 8, p. 944–947, 2010.

WAHID, A.; PERVEEN, M.; GELANI, S.; BASRA, S.M.A. Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. **Journal of Plant Physiology**, Stuttgart, v. 164, n. 3, p. 283–294, 2007.

WANG, Y.; SHEN, W.; CHAN, Z.; WU, Y. Endogenous Cytokinin Overproduction Modulates ROS Homeostasis and Decreases Salt Stress Resistance in *Arabidopsis Thaliana*. **Frontiers in Plant Science**, Lausanne, v. 6, n. November, p. 1004, 2015.

WOLTERS, H.; JÜRGENS, G. Survival of the flexible: hormonal growth control and adaptation in plant development. **Nature Reviews Genetics**, London, v. 10, n. 5, p. 305–317, 2009.

XU, M.Y.; ZHANG, L.; LI, W.W.; HU, X.L.; WANG, M.B.; FAN, Y.L.; ZHANG, C.Y.; WANG, L. Stress-induced early flowering is mediated by *miR169* in *Arabidopsis thaliana*. **Journal of Experimental Botany**, Oxford, v. 65, p. 89–101, 2013.

YAMAGUCHI, T.; HAMAMOTO, S.; UOZUMI, N. Sodium transport system in plant cells. **Frontiers in Plant Science**, Lausanne, v. 4, n. 17, p .1-7, 2013.

YANG, Y.; MA, C.; XU, Y.; WEI, Q.; IMTIAZ, M.; LAN, H.; GAO, S.; CHENG, L.; WANG, M.; FEI, Z.; HONG, B.; GAO, J. A zinc finger protein regulates flowering time and abiotic stress tolerance in chrysanthemum by modulating gibberellin biosynthesis. **The Plant Cell**, Rockville, v. 26, n. 5, p. 2038–2054, 2014.

YUAN, S.; LIN, H.-H. Role of salicylic acid in plant abiotic stress. **Zeitschrift fur Naturforschung. Teil C: Biochemie, Biophysik, Biologie, Virologie**, Tubingen, v. 63, n. 5/6, p. 313–320, 2008.

ZHANG, X.; ZOU, Z.; GONG, P.; ZHANG, J.; ZIAF, K.; LI, H.; XIAO, F.; YE, Z. Over-expression of *microRNA169* confers enhanced drought tolerance to tomato. **Biotechnology Letters,** Dordrecht, v. 33, n. 2, p. 403–409, 2011.

ZHU, J.-K. Salt and drought stress signal transduction in plants. **Annual Review of Plant Biology**, Palo Alto, v. 53, n. 1, p. 247–273, 2002.

3 HORMONAL CONTROL OF SALINITY RESPONSES: ASSESSMENT OF TOMATO (*Solanum lycopersicum* L.) GENOTYPES AFFECTING SIX HORMONAL CLASSES IN A SAME GENETIC BACKGROUND (cv. MICRO-TOM).

Abstract

Plants exposed to saline environments display reduced growth due to adverse effects of specific ions on metabolism and water relations. The plant hormones perform essential functions in response to salinity, regulating plant development and consequently salt-resistance or sensitivity. In order to investigate the role of different hormone classes in salt response, we employed mutants and transgenic tomato plants affecting different aspects of hormonal status/signaling in the same genetic background (cultivar Micro-Tom). The following genotypes were used: *anti sense Chloroplastic carotenoid cleavage dioxygenase 7 - 35S::asCCD7* (*CCD7*), *Salicylate hydroxylase - 35S::nahG* (*nahG*), *diageotropica* (*dgt*), *entire* (*e*), *epinastic* (*epi*), *procera* (*pro*), *notabilis* (*not*) and *Never ripe* (*Nr)*. In general, all genotypes presented predictable salinity responses such as reduced growth, enhanced root:shoot ratio, increased endogenous levels of abscisic acid (ABA) and ethylene, decreased auxin and cytokinins contents and augmented Na:K ratio. The ABA deficient mutant, *not,* changed some of these responses, such as its the no significant reduction in root:shoot ratio. In the multivariate analysis of genotypes four kinds of salt response among the genotypes were observed: *i*) High shoot growth in spite of high Na:K ratio presented by the strigolactone deficient and high branching *CCD7* transgene; *ii*) High shoot growth and reduced accumulation of Na in tissues (probably due to dilution) presented by the auxin constitutive response *e* mutant; *iii*) The opposite response observed in "*ii*" presented by the low auxin sensitivity *dgt* mutant and *iv*) growth inhibition combined with reduced levels of Na and higher accumulation of K presented by the *not* mutant. Such specific behaviors point for novel levels of salt response regulation, suggesting a role for auxin in Na dilution in tissues and specific mechanisms of ABA and strigolactones in the control of growth under salinity. On the other hand, the lack of the DELLA repressor in the gibberellin constitutive *pro* mutant and ethylene insensitivity in the *Nr* mutant had less effect in the growth upon salt treatment.

Keywords: Abscisic acid; Auxin; Ethylene; Gibberellins; Mutant; Plant hormones; Salicylic acid; Strigolactone; Salt sensitivity

3.1 Introduction

Plants are thought to have evolved not from sea algae but from a class of fresh water green algae (charophytes) (McCOURT et al., 2004). Therefore, it is conceivable that sodium (Na), the most abundant element in the marine environment, did not play any essential and exclusive role in plants. Besides, Na causes adverse effects on metabolism and water balance in many terrestrial plant species, including crop plants. As a consequence, plant growth, productivity or even survival are impaired. Agriculture activities such as irrigation and fertilization have aggravated this situation by contributing to soil salinization.

In view of this, efforts have been made to understand the mechanisms involved in salt response. There is considerable evidence that the differential regulation of growth is crucial during salt response (ACHARD et al., 2006; ZHU, 2001). Plant hormones are considered the major players in this process, since they not only orchestrate plant development but also regulate salt-induced effectors such as the activity of ion transporters and osmoprotectors (TANG et al., 2015; ZHANG et al., 2006).

During salt stress, the metabolic performance is impaired as a result of Na^+ competition with K^+ for enzyme activation and reduction of water potential (SHABALA; CUIN, 2008). Plant hormones regulate ionic flux contributing to ionic and osmotic homeostasis (HAN; YIN; HUANG, 2015). Hence, ethylene hormone decreases K^+ loss, restricts Na^+ influx and regulates xylem Na^+ loading (JIANG et al., 2013). Ethylene strongly inhibits cell expansion when in high concentration (TAO et al., 2015), but fine tunes cell death, restraining this event under salinity (PAN et al., 2016). Abscisic acid (ABA) accumulation is stimulated during various environmental stresses (HICHRI et al., 2016; MAGGIO et al., 2007). This hormone contributes to the increase of xylem water potential and water uptake (FRICKE et al., 2004). ABA accumulation leads to retention of both K^+ and Ca^+, and promotes the biosynthesis of osmolytes, which counteract with the toxic Na^+ (GURMANI et al., 2011). In addition, ABA prevents excess ethylene production under abiotic stress (SHARP et al., 2000; SPOLLEN et al., 2000). The hormone auxin (IAA) increases the number and the activity K channels (PHILIPPAR et al., 1999, 2004). IAA concentration is adjusted differentially under salt stress, increases in the roots but substantially reduces in the shoot causing reduction of leaf expansion and the number of leaves (ALBACETE et al., 2008).

Furthermore, salinity perturbs considerably the status of other hormones. The levels of active gibberellins (GA) decrease on salt, leading to the DELLA growth repressor accumulation (ACHARD et al., 2006; 2008; KING; MORITZ; HARBERD, 2001), inhibiting leaf expansion and stem elongation under the coordination of IAA, ethylene and ABA (WEISS; ORI et al., 2007). The expression of the tomato strigolactone (SL) biosynthesis gene *SlCCD7* is reduced under abiotic stress, impairing SL production (RUIZ-LOZANO et al. 2015). This hormone is reported as a positive regulator of stress signaling networks, upregulating the expression of stress-related and/or ABA-responsive genes and downregulating genes required for CK degradation (HA et al., 2007; 2014). In salt presence, salycilic acid (SA) levels are incremented, stimulating to compatible osmolyte metabolism and helping in salt resistance (MINOUNI et al., 2016).

Taken together, the understanding of the coordinated hormonal status modifications during salt stress would be useful to engineer and manage plant salt tolerance in the future (HAN; YIN; HUANG, 2015). However, the knowledge of involved mechanisms still remains limited.

In diverse studies, the hormonal effects on plant development are explored through applications of plant hormones or growth regulators under salinity conditions. These approaches have contributed to broaden the knowledge in this area along the years (FLEISHON et al.; 2011; MAGGIO et al., 2010; revised by SHAHBAZ et al., 2012).

However, one of the most successful approaches for the study of hormone physiology has been the use of contrasting genotypes, whether mutant, transgenic or derived from natural genetic variation (JESUS et al., 2014). In the present work, we used tomato genotypes affecting hormonal status in the same genetic background (*Solanum lycopersicum* L. cultivar Micro-Tom) to assess their roles in the salinity response. The used genotypes harbored genetic modifications such as low sensitive and increased response to auxin, overproduction and sensitivity to ethylene, constitutive response to gibberellins, abscisic acid deficiency, and low levels of strigolactones and salicylic acid. The hormonal influence in growth performance and ionic homeostasis were discussed.

3.2 Material and Methods

3.2.1 Plant material

The tomato (*Solanum lycopersicum* L.) genotypes used here are described in Table 1. The genotypes *anti sense Chloroplastic carotenoid cleavage dioxygenase 7 - 35S::asCCD7* (*CCD7*), *Salicylate hydroxylase - 35S::nahG* (*nahG*), *diageotropica* (*dgt*), *entire* (*e*), *epinastic* (*epi*), *procera* (*pro*), *notabilis* (*not*) and *Never ripe* (*Nr*) were introgressed into the cv. Micro-Tom (MT), using the same procedures published previously (CARVALHO et al., 2011; PINO et al., 2010). The introgression of respective genetic alterations was previously performed through pollen collection from parents, and its use to fertilization of MT flowers. Subsequently, the resulting F_1 hybrids were self-fertilized to obtain recombinant F_2 populations, and conducted a visual screening for compact size to MT and the phenotype of the respective mutation of interest to *dgt*, *e*, *epi*, *pro*, *not* and *Nr* genotypes. To conduct the introgression of transgenic events, *CCD7* and *nahG*, the same procedures described before were performed but the selection was done using kanamycin application (400 mg L^{-1}), since the transgenic parents of *CCD7* and *nahG* are kanamycin-resistant plants. Selected plants were backcrossed with MT up to the sixth generation (BC_6), and self-fertilized every second

generation to allow the screening for homozygous mutants. After BC_6F_2 the resulting genotypes was considered near-isogenic lines (STAM; ZEVEN, 1981). All experiments were conducted on BC_6F_3 plants or subsequent generations.

The mutations *dgt*, *epi*, *pro*, *not* and *Nr* into the MT background were described in previous publications (ALMEIDA et al., 2015; CARRERA et al., 2012; CARVALHO et al., 2011; FRACETTO et al., 2013; GRATÃO et al., 2012; LIU et al., 2016; LOMBARDI-CRESTANA et al., 2012). The transgenic lines *35S::nahG* (BRADING et al., 2000) and *35S::asCCD7* (VOGEL et al., 2010) were obtained previously in different backgrounds (see Table 1) and then introgressed into MT in the present work.

3.2.2 Growth conditions and treatment experiments

The vegetative and reproductive growth assays were conducted under controlled environmental conditions in a plant growth chamber with relative humidity of 50%, temperatures between 25°C (day) and 18°C (night), with a 16/8 h light/dark regime and 400 µmol m^{-2} s^{-1} photon flux density at the top of the canopy The geographic coordinates of the location were: 38°01'23.3"N and 1°10'18.0"W.

Experimental conditions, nutrient solution and salt treatment in preliminary test were quite similar to method reported by Ghanem et al. (2008) except for increase in initial age of plants to transference to hydroponic system and longer period of acclimatation after transplantation. Previous experiments were conducted to determine the adjustment of these conditions to the dwarf phenotype of MT plants (data not shown).

Seeds of each genotype were germinated in trays with expanded vermiculite and watered daily with half-strength Hoagland's nutrient solution. Transplantation of seedlings to hydroponic system occurred at 15 days after sowing (DAS) in the first experiment. On the following experiments, the plants were transferred to hydroponics upon the appearance of the first pair of leaves at 25 DAS. The hydroponic system consisted of ten tanks each containing approximately 20 L half-strength Hoagland's solution continuously aerated. The composition of stock nutrient solution was in g L^{-1}: 5 KNO_3, 1 $NH_4H_2PO_4$, 0.5 $MgSO_4$, 5.5 $Ca(NO_3)_2$ mM g L^{-1} and 25 KCl, 10 H_3BO_3, 1 $MnSO_4$, 1 $ZnSO_4$, 0.25 $CuSO_4$, 10 Na_2MoO_4 in µM g L^{-1} and 1.87 g·L^{-1} Fe-EDDHA.

After 4-days acclimation period, plants grown under absence of NaCl (control) or 100 mM of NaCl that was added to the nutrient solution. The solutions were refilled and replaced every 2 and 10 days, respectively.

The duration of salt treatment was 21 days in vegetative experiments and 81 days in the reproductive experiments respectively. When these respective periods were achieved, the plants were harvested.

3.2.3 Vegetative growth assessment

The growth assessments were performed 40 and 50 DAS in the first and second vegetative experiments respectively and 100 DAS in the productivity experiment through the measurement of three plants per treatment. The total leaf area was measured using a Li-Cor 3100 area meter (Li-Cor Inc., Lincoln, Nebraska, USA). Subsequently, stem, leaves and fruits were separated and fresh weight determined.

3.2.4 Nutritional status

In order to quantify the K^+ and Na^+ accumulation in leaves, the third leaf of each plant was harvested at the end of vegetative experiments. The samples were oven-dried at 60^{o}C for 72 h and ground into a fine powder. The elemental concentrations were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). All measurements were performed in triplicates.

3.2.5 Hormone extraction and analysis

For the determination of indole-3-acetic acid, zeatin, ethylene precursor 1-aminocyclopropane-1-carboxylic acid and abscisic acid contents, the fourth leaf of plants were flash frozen using liquid nitrogen and kept at -80°C. Then the samples were dried in a Christ Alpha 2-4 D freeze-dryer (Martin Christ, Osterode am Harz, Germany) and ground to a fine powder.

The method adopted to obtain the hormone amounts and aminocyclopropane-1-carboxylic acid was described by Albacete et al. (2008). Firstly, 30 mg of ground material were dropped in 650 µL of cold (4°C) extraction mixture of methanol/water (80/20, v/v) into tubes that were mixed three times at intervals of 10 min. Solids were separated by centrifugation (13,000 g, 15 min) at 4ºC. The supernatant was taken and maintained at 4ºC. The solids were mixed with additional 650 µL of the same extraction solution. The steps described above were repeated again once. After the second centrifugation the supernatants were polled and passed through Sep-Pak Plus †C18 cartridge (SepPak Plus, Waters, USA) to remove interfering lipids and part of plant pigments and evaporated at 40ºC under vacuum until organic solvent was removed.

Table 1- Phenotypic description of genotypes in *Solanum lycopersicum* L. cv. Micro-Tom (MT) background

Hormones	Genotypes	Effects	Origin	References
Abscisic acid (ABA)	*notabilis (not)*	ABA deficiency. Defective for 9-cis-epoxycarotenoid dioxygenase (NCED), a carotenoid cleavage enzyme.	LA0617 cv. Lukulus	Burbidge et al. (1999), Neill and Horgan et al. (1985)
Salicylic acid (SA)	*35S::nahG*	Reduced SA levels due to its degradation by the overexpressed salicylic hidroxylase.	Transgenic line in cv. Money Maker	Thara et al. (1999)
Auxin	*diageotropica (dgt)*	Low sensitivity. Defect in a cyclophilin biosynthesis gene (a putative signal transduction component).	LA1529 cv. unknown	Kelly and Bradford (1986) Oh et al. (2006)
	entire (e)	Increased response. Loss of function of the AUX/IAA9, a transcriptional repressor of auxin.	LA2922 cv. Ailsa Craig	Dengler (1984) Wang et al. (2005)
Strigolactone (SL)	*35S::asCCD7*	Low endogenous levels. Transgenic plants silenced for carotenoid cleavage dioxygenase gene, an enzyme necessary for SL biosynthesis.	Transgenic line in cv. M82	Vogel et al. (2010)
Ethylene	*Never ripe (Nr)*	Low sensitivity. Defective for ethylene receptor (LeETR3).	LA0162 cv. Pearson	Hamilton et al. (1990) Yen et al. (1995)
	epinastic (epi)	Ethylene overproduction. Unknown gene function.	LA2089 cv.VFN8	Barry et al. (2001) Fujino et al.(1988)
Gibberellin	*procera (pro)*	Constitutive response. Contains a point mutation in a gene that converts VHVD putative DNA-binding domain of the tomato DELLA gene into VHEID.	LA0565 cv. Condine Red	Bassel et al. (2008) Jones (1987)

The residue was dissolved in 1 ml methanol/water (20/80, v/v) solution using an ultrasonic bath. Afterwards, the extracts were centrifuged twice (13,000 g, 15 min, 4ºC). Ten µl of each extract samples were injected into a U-HPLC-MS system consisting of an Accela Series U-HPLC (ThermoFisher Scientific, Waltham, MA, USA) equipped with a well plate auto sampler and a capillary pump, coupled to an Exactive mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA) that uses a heated electrospray ionization (HESI) interface. Mass spectra were obtained using the Xcalibur software version 2.2 (ThermoFisher Scientific, Waltham, MA, USA). For quantification of the plant hormones, calibration curves were constructed for each analyzed component (1, 10, 50, and 100 µg L^{-1}) and corrected for 10 µg L^{-1} deuterated internal standards. Recovery percentages ranged between 92 and 95%.

3.2.6 Statistical analysis

The data obtained in the experiments were submitted to Levene's (LEVENE, 1960) and Shapiro-Wilk (SHAPIRO; WILK, 1965) tests to evaluate homoscedasticity and the normal distribution of the residuals, respectively.

The data were analyzed by one-way analysis of variance F-test. When a significant difference was detected, Duncan's test was used to compare the means of genotype inside each salt level. ANOVA, means test and correlation analyses were performed using the statistical software SAS® 9.2 and PROC GLM program (SAS, 2008). The principal components analysis was carried out using R software.

3.3 Results

3.3.1 Growth and development of genotypes: *pro*, *epi* and *not*

Firstly, we evaluated genotypes *pro*, *epi* and *not*, which enhance gibberellin perception, increment ethylene production and produce less abscisic acid, respectively (Table 1).

Shoot and root biomass varied among the genotypes even under control conditions (Figure 1), expressing the effect of the hormonal mutation itself on plant growth. The genotypes MT, *pro* and *not* showed similar growth. In contrast, *epi* displayed shoot and root growth increased 45 and 100 %, respectively when compared to the control MT. The ratio between root and shoot was higher in the mutants when compared to MT in non-salt treatment. The lower root:shoot ratio of MT was mostly a result of its low root biomass.

Salt treatment (100 mM NaCl) provoked extensive decrease in shoot and root biomass in all genotypes. In some degree, this growth reduction was proportional to the growth

performance of the respective mutant under control conditions, except for *not*. Thus, in general, genotypes that grew more under control conditions, showed sharper growth inhibition when they were subjected to salt treatment. The comparison of biomass values among the genotypes under saline conditions did not reveal great differences.

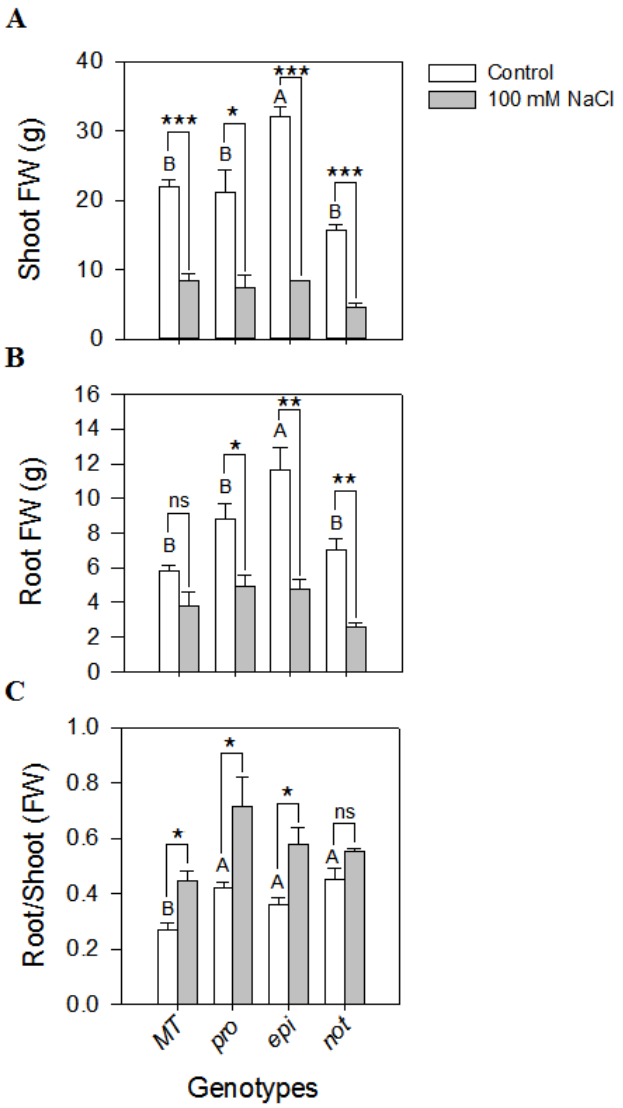

Figure 1 - The vegetative growth affected by salinity of tomato genotypes *procera* (*pro*), e*pinastic* (*epi)* and *notabilis* (*not*) in the MT genetic background. Stem and leaf (A) and root fresh weight (B) and root: shoot ratio (C) of plants grown in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 21 days. Measurements were performed at 40 days after sowing. Data are means ± SE (n = 3). Asterisks and "ns" indicate, respectively, significant at *P <0.05, **P <0.01, ***P <0.001 and non significant differences between 0 and 100 mM NaCl for each genotype according to the F test. Bars with different upper and lower case letters denote significant differences between genotypes grown under control and stress conditions, respectively, according to Duncan's test at P <0.05. The absence of upper case letters above the open bars in the panels reflects a lack of significant difference between genotypes according to the F test at P <0.05. A description of hormone alterations involved in each genotype can be found on Table 1

The ethylene overproducer *epi* genotype contributed to higher shoot biomass accumulation and more vigorous plants in control conditions. However, this genotype

lowered its shoot growth more than other genotypes upon salt stress. *epi* plants showed 3.8-fold and 2.4-fold decreases in shoot and root fresh weights, respectively, when exposed to salt treatment.

Among the morphological impacts of salinity on plant development were the presence of wrinkled leaves, inhibited leaf area and smaller number of these organs (Figure 2).

In general, salinity affected less root growth than shoot one. However, root fresh weight was reduced by 64% in ABA deficient *not* mutant under salinity. As a consequence, root system of *not* was more affected by salinity than other genotypes. Nevertheless, this mutant exhibited similar root:shoot ratio under control conditions and salinity due to low range between shoot and root fresh weights values under salinity.

The *pro* mutant exhibited lesser lateral root extension and roothairs, altering remarkably root architecture on salt (Figure 2). Even so, *pro* plants displayed less root growth inhibition, which propitiated higher root:shoot ratio.

In order to verify if a better performance under salinity was related to lesser Na^+ contents and higher K^+ levels in the leaves, the concentration of these elements were determined (Figure 3). The genotypes accumulated very similar levels of these minerals under control conditions. The Na^+ concentrations were almost nulls and K^+ levels varied little around 38 mg g^{-1} under control conditions. However, major differences among genotypes could be noted upon salt treatment, especially in MT that exhibited the highest Na content under salinity. Salt treatment promoted similar rising in Na concentrations in *pro*, *epi* and *not* plants.

Remarkably, *not* plants were less affected in relation to K^+ uptake than *epi* and *pro,* despite the fact that the three genotypes showed very close leaf Na contents under saline conditions. Moreover, K^+ concentrations in *not* did not differ under control and saline conditions, according to the F test (α= 0.05). In terms of Na:K ratio, *epi, not* and *pro* genotypes showed lower ratios than MT plants whose ratio was the highest upon salt treatment.

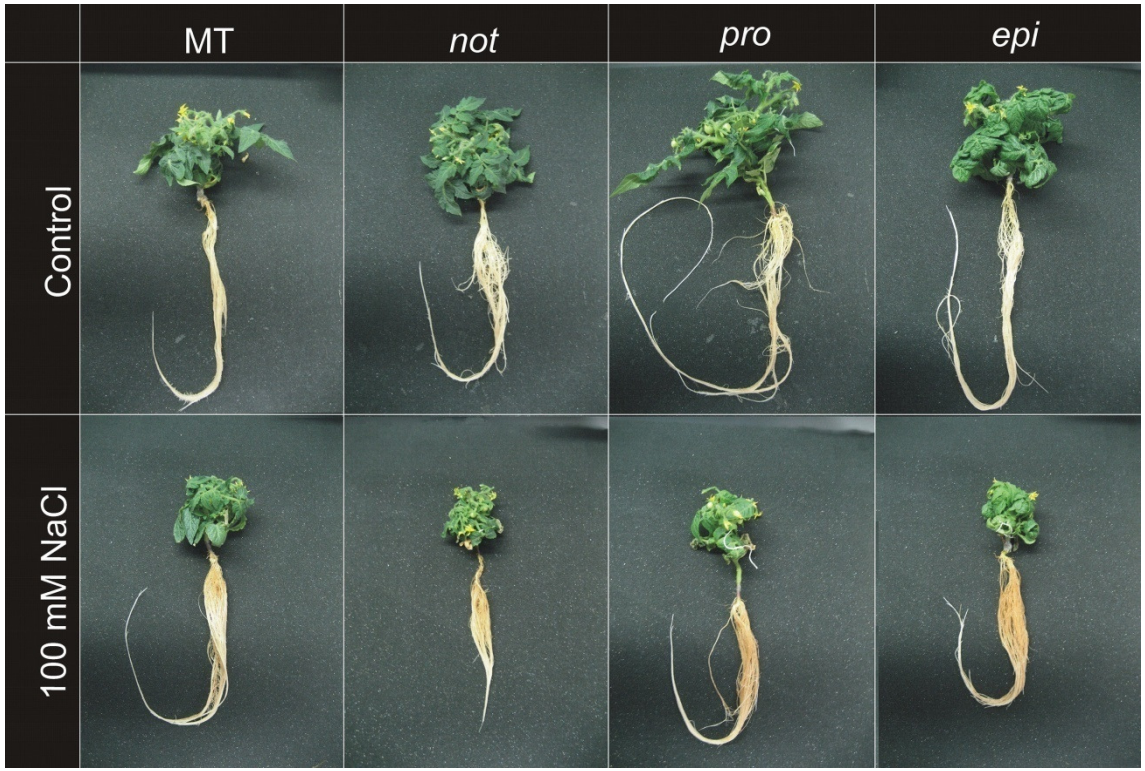

Figure 2 -Representative plants of hormonal genotypes *notabilis* (*not*), *procera* (*pro*) and *epinastic* (*epi)* and and the control Micro-Tom (MT) after 21 days growing in greenhouse on 50%-strength Hoagland's solutionin absence or presence of 100 mM NaCl. The image was taken after 40 days after sowing

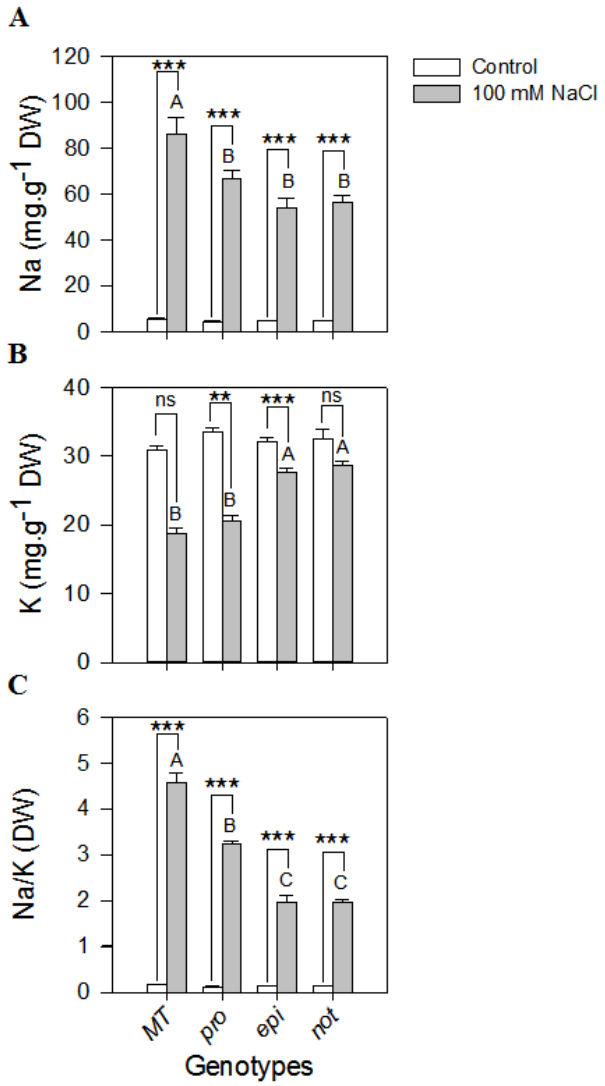

Figure 3 - The ionic accumulation in leaf of tomato Micro-Tom (MT) genotypes exposed to salinity. K (A), Na (B) and Na:K ratio (C) ion concentrations of leaf tissue from plants grown in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 21 days. The leaf samples were taken at 5040days after sowing. Data are means ± SE (n = 3). Asterisks and "ns" indicate, respectively, significant at *P <0.05, **P <0.01, ***P <0.001 and non significant differences between 0 and 100 mM NaCl for each genotype according to the F test. Bars with different upper and lower case letters represent significant differences between genotypes grown under 0 and 100 mM NaCl, respectively, according to Duncan's test at P<0.05. The absence of letters above the bars in the panels displays a lack of significant difference between genotypes under the respective conditions, according to the F test at P<0.05.A description of the genotypes used is shown in Table 1

The endogenous levels of hormones: abscisic acid (ABA), zeatin (Z) and indole-3-acetic acid (IAA) and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) were determined in order to evaluate salt-induced variations. The concentration of hormones varied among the genotypes under both conditions (Figure 4). Mutant *not* produced less ABA under control conditions and salt treatment, as expected due to its defective biosynthesis of this hormone. Still, under control conditions, *not* also showed higher Z levels. *epi* plants

accumulated more ACC according to its mutation that causes increment in ethylene production, beyond higher IAA level together to *not* plants.

Salinity increased ABA and ACC concentrations, and reduced Z and IAA leves in all genotypes. The hormones IAA and Z levels were lesser affected in MT. The ACC increments were more pronounced in *pro* plants. Genotype *epi* showed elevated ABA and ACC contents but also high concentrations of Z and IAA under salinity.

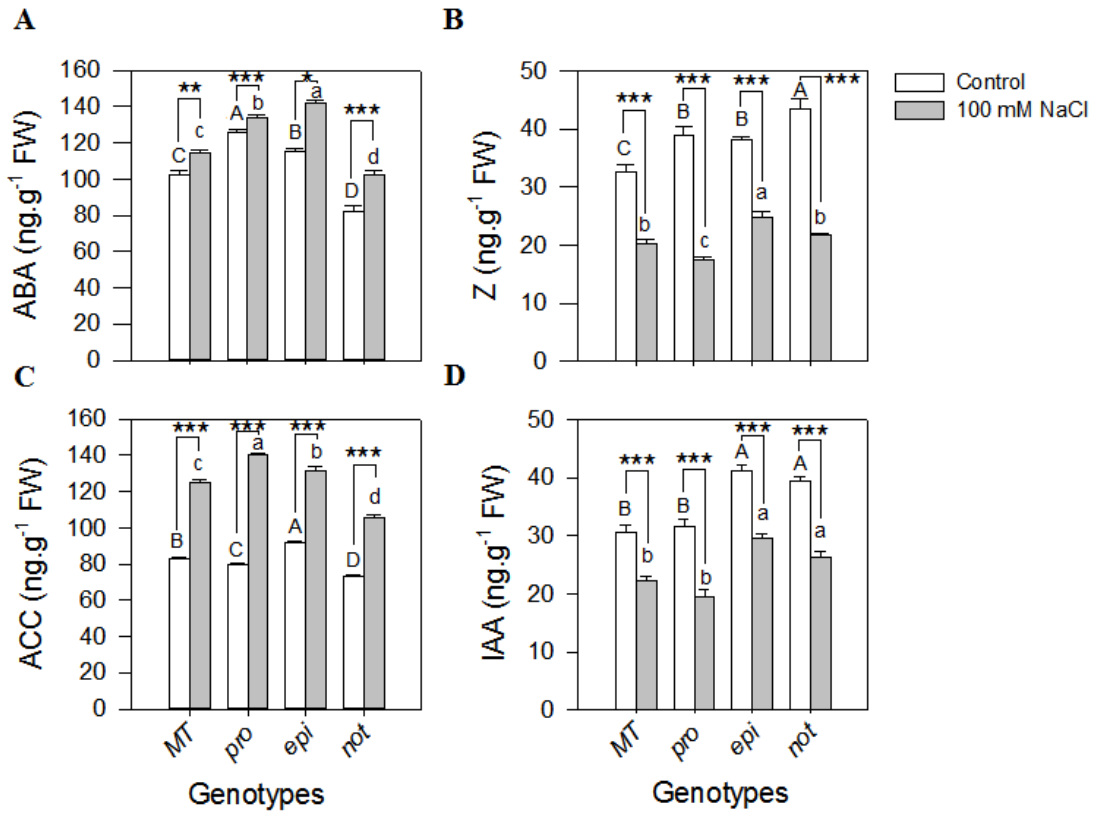

Figure 4 - Effects of NaCl on endogenous hormones and ethylene precursor on leaves of tomato Micro-Tom genotypes. Abscisic acid (ABA) (A), Zeatin (Z) (B), Ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) (C) and Indole-3-acetic acid (IAA) (D). The samples were obtained from plants growing in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 21 days at 40 days after sowing. Data are means ± SE (n = 3). Asterisks and "ns" indicate, respectively, significant at *P<0.05, **P<0.01, ***P<0.001 and non significant differences between 0 and 100 mM NaCl for each genotype according to the F test. Bars with different upper and lower case letters represent significant differences between genotypes grown under 0 and 100 mM NaCl, respectively, according to Duncan's test at P<0.05.A description of the genotypes used is shown in Table 1

3.3.2 Growth and development of *CCD7*, *nahG*, *dgt*, *e* and *Nr*

The investigation of plant hormones roles in salt stress tolerance were extended adopting more genotypes that affected also other hormonal classes or influenced in another way the hormones that were evaluated in the previous experiment (Table 1).

Shoot and root biomass and leaf area varied considerably according to the genotype under control conditions (Figure 5). In general, *e* and *CCD7* plants accumulated higher shoot

and root fresh weights. Nevertheless, *e*, which has a constitutive response to auxin, exhibited higher growth than *CCD7*, even though this difference was not significant. Whereas, *dgt* plants growth was deeply limited by its low sensitivity to IAA.

The elevated biomass presented by *CCD7* is likely effect of its reduced endogenous strigolactone levels, which impair branching control (SHINOHARA; TAYLOR; LEYSER, 2013). Moreover, *CCD7* achieved total leaf area higher than other genotypes as a consequence of its excessive branching (Figure 6).

The balance between root and shoot was relatively similar among genotypes under control conditions, except for *dgt* plants whose plant development was more restricted (Figure 5). However, *dgt* did not differ significantly from other genotypes regarding this trait.

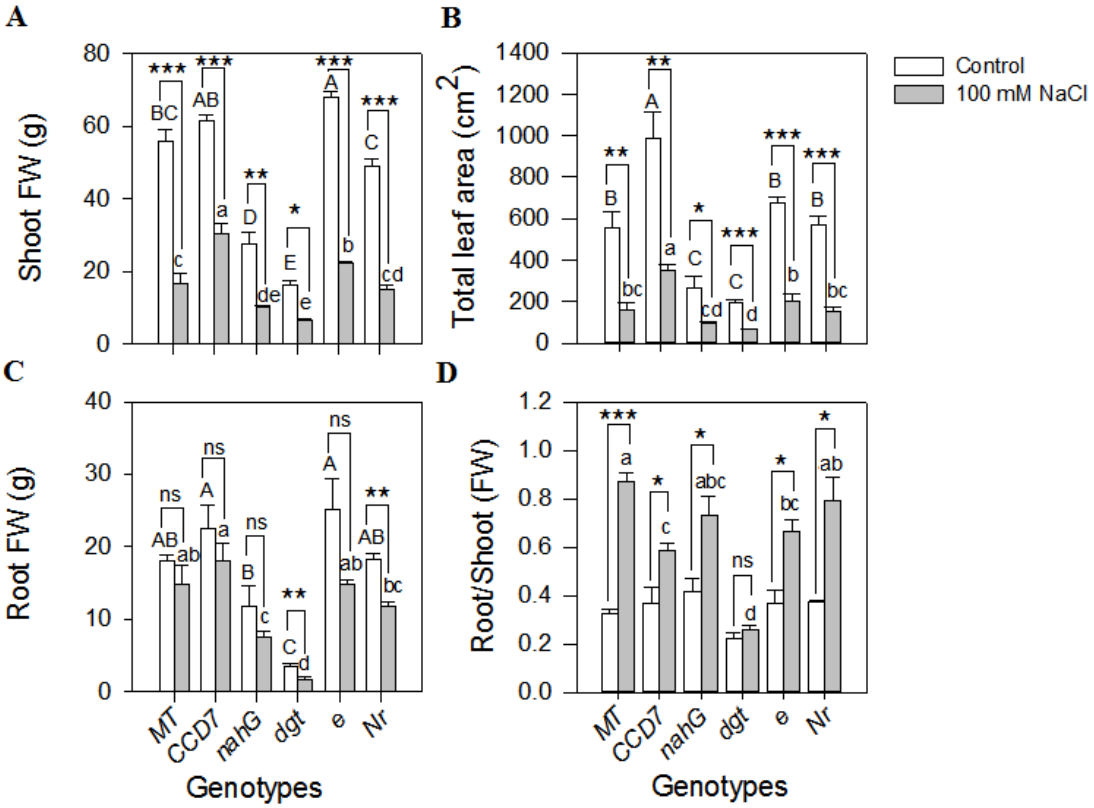

Figure 5 - The vegetative development affected by salinity in Micro-Tom (MT) plants and *Chloroplastic carotenoid cleavage dioxygenase 7* (*35S::asCCD7*), *Salicylate hydroxylase* (*35S::nahG*), *diageotropica* (dgt), *and entire* (*e*) and *Never ripe* (*Nr*) genotypes in MT genetic background. Shoot biomass (A), total leaf area (B), root biomass (C) and root: shoot ratio (D) of plants grown in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 21 days. Measurements were performed at 50 days after sowing. Data are means ± SE (n = 3). Asterisks and "ns" indicate, respectively, significant at *P<0.05, **P<0.01, ***P<0.001 and non significant differences between 0 and 100 mM NaCl for each genotype according to the F test. Bars with different upper and lower case letters denote significant differences between genotypes grown under control and stress conditions, respectively, according to Duncan's test at P <0.05. The absence of upper case letters above the open bars in panel C reflects a lack of significant difference between genotypes according to the F test at P<0.05. A description of hormone alterations involved in each genotype can be found on Table 1

When the plants were subjected to 100 mM NaCl, the genotypic effect was potentiated, *i.e.* sharper differences were noticed (Figures 5 and 6). Plants marked by lower ethylene sensitivity, as the result of the introgressed mutation *Never* ripe, were more affected than other genotypes. This mutant suffered about 3.5-fold reduction of shoot biomass, when its growth was compared under control and saline conditions.

MT, *e* and *Nr* plants showed quite similar shoot growth reduction, whereas, *CCD7* showed 15% lesser decrease in shoot biomass mean value than *Nr*. A substantial growth restriction on shoot was also caused by salinity in *dgt* and *nahG* plants. However, F-test results indicated smaller variations in shoot biomass of *dgt* and *nahG* plants among the NaCl treatments when compared to other genotypes. Probably, these subtler statistical differences resulted from growth differences within these respective genotypes. During this experiment, few foliar abscission was observed under salinity. However, the photosynthetic area dropped in all genotypes as a consequence of smaller expansion and lower leaf formation on salt. *Nr* plants presented leaf area lower than other genotypes, although no significant difference was observed between *Nr*, *dgt* and *e* about this trait (Figure 5 B).

In general, salinity impaired root development lesser than shoot growth. Hence, the root elongation was maintained in the majority of genotypes. Only *Nr* and *dgt* showed root growth inhibition to distinguish significantly salt treatments (0 and 100 mM NaCl). However, a novel adjustment in root:shoot ratio under salinity could be clearly seen in all genotypes as a result of severe shoot growth impairment, except for *dgt*. The control MT, which displayed the highest root:shoot ratio under salinity, showed root biomass relatively similar under control and saline environments but expressive shoot growth inhibition on salt.

To investigate the relationship between leaf accumulation of Na^+ and K^+ and hormonal responses under salinity, these elements were determined. The genotypes presented Na^+ concentrations practically equivalents under control conditions (Figure 7). However, Na^+ levels considerably varied among the genotypes upon salt treatment (Figure 7).

Remarkably, *e* plants showed lesser accumulation of Na^+ than other genotypes under salinity, which can be attributed to Na dilution in tissues of vegetative organs due to greater *e* growth. The *CCD7* and *nahG* did not differ from *e* by Duncan's test (α= 0.05), even though Na^+ levels were little higher in *CCD7* and *nahG* plants than in *e*. However, shoot biomass accumulation of *CCD7* and *e* plants were significantly similar but higher than *nahG* plants (Figures 5A and 7A). Mutants *dgt*, *Nr* and MT showed high Na^+ accumulation, especially the latter.

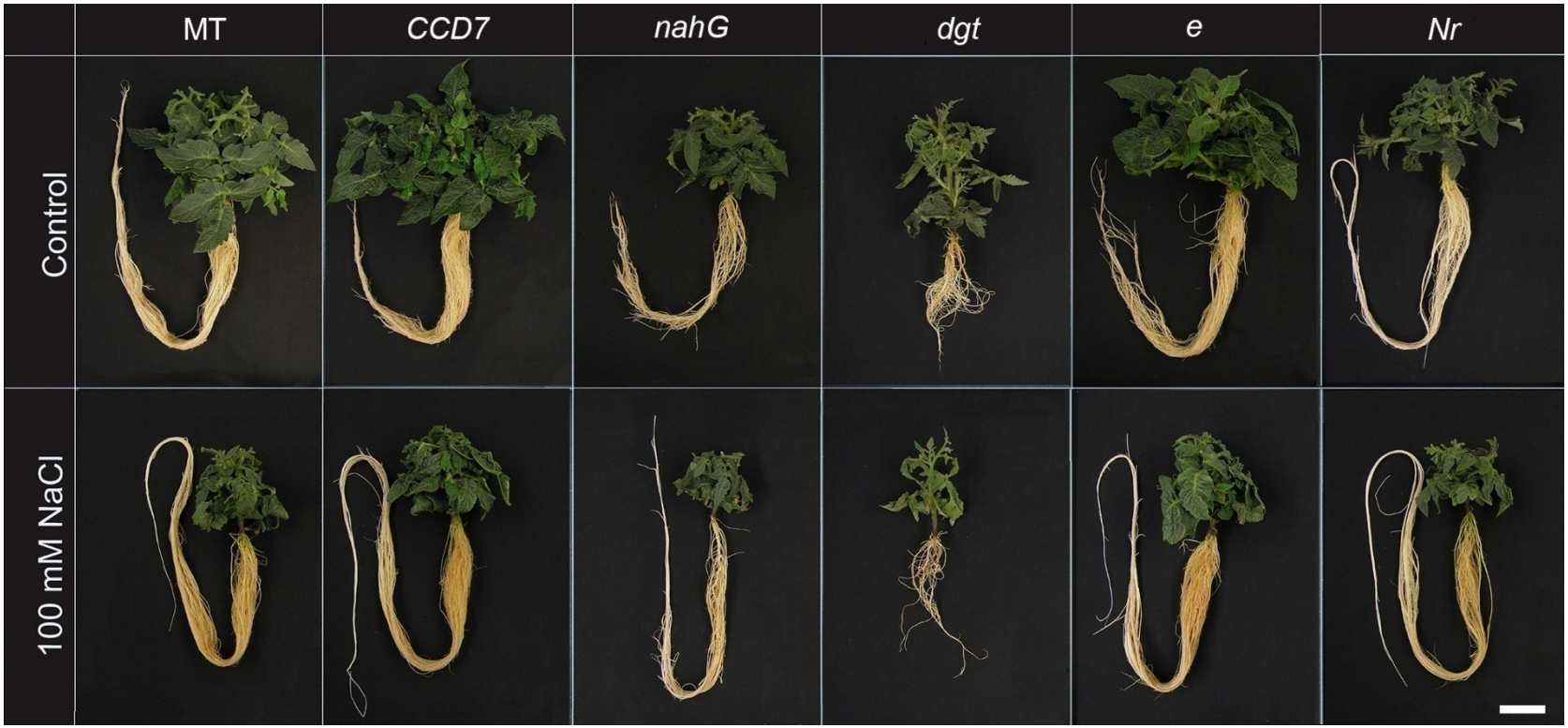

Figure 6 - Effect of salinity on vegetative growth in the Micro-Tom (MT) genetic background. Representative plants of genotypes *anti sense Chloroplastic carotenoid cleavage dioxygenase 7* (*35S::asCCD7*), *Salicylate hydroxylase* (*35S::nahG*), *diageotropica* (*dgt*), *entire* (*e*) and *Never ripe* (*Nr*) and the control Micro-Tom (MT) after 21 days growing in greenhouse on 50%-strength Hoagland's solution in absence or presence of 100 mM NaCl. The image was taken after 50 days after sowing. Scale bar = 5 cm

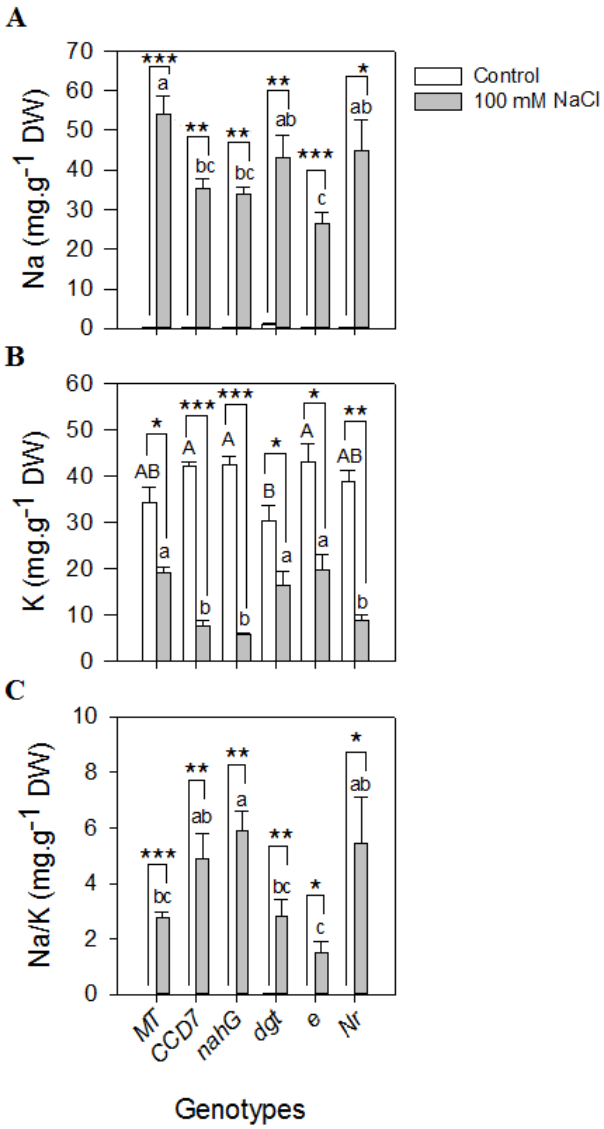

Figure 7 - Effect of salinity on the mineral status in tomato Micro-Tom (MT) genotypes. Na (A), K (B) and Na:K ratio (C) ion concentrations of leaf tissue from plants grown in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 21 days. The leaf samples were acquired at 50 days after sowing. Data are means ± SE (n = 3). Asterisks and "ns" indicate, respectively, significant at *P<0.05, **P<0.01, ***P<0.001 and non significant differences between 0 and 100 mM NaCl for each genotype according to the F test. Bars with different upper and lower case letters represent significant differences between genotypes grown under 0 and 100 mM NaCl, respectively, according to Duncan's test at P<0.05. The absence of letters above the bars in the panels denotes a lack of significant difference between genotypes under the respective conditions, according to the F test at P<0.05.A description of the genotypes used is shown in Table 1

The genotypes, *CCD7*, *nahG* and *e* exhibited higher K^+ levels but that did not differ from MT and *Nr* in non-salt treatment. Salinity impaired accumulation of K^+ in all genotypes. However, K^+ concentration was less affected in MT, *dgt* and *e* plants under salinity and consequently these genotypes exhibited lower Na:K ratio.

3.3.3 The impact of hormones on reproductive development under salinity

In order to assess how hormones influenced reproductive performance under salinity, an independent experiment was carried out using this same set of genotypes. The plants were subjected to salinity until fruit formation. The results exhibited some differences in relation to shoot biomass growth between vegetative and reproductive experiments (Figures 5 and 8).

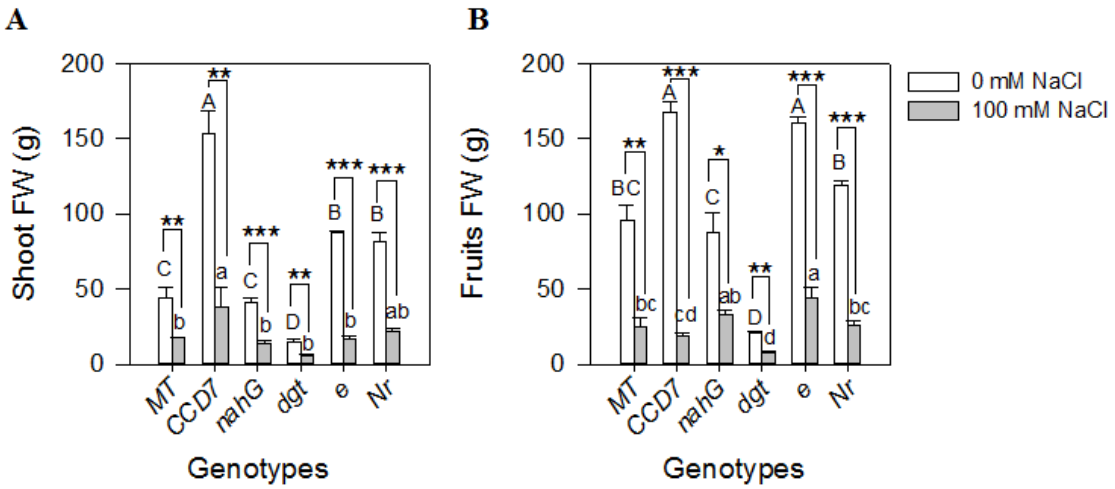

Figure 8 - The vegetative growth and productivity affected by salinity in Micro-Tom (MT) genotypes. Shoot biomass (A) and fruit biomass (B) of plants grown in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 85 days. Measurements were performed at 100 days after sowing. Data are means ± SE (n = 3). Asterisks and "ns" indicate, respectively, significant at *P<0.05, **P<0.01, ***P<0.001 and non significant differences between 0 and 100 mM NaCl for each genotype according to the F test. Bars with different upper and lower case letters represent significant differences between genotypes grown under 0 and 100 mM NaCl, respectively, according to Duncan's test at P<0.05

The *CCD7* plants continued to show higher shoot biomass under control and saline conditions even when the experiment duration was extended (Figure 8). However, *CCD7* and *e* growths were no longer similar on salt. Shoot and root growth of *e* was approximately 1.7-fold and 2.3-fold lower than *CCD7* under control conditions and salinity respectively. Nevertheless, these two genotypes presented the same fruit fresh weight under salt absence but when the plants were subjected to salt stress, the *e* mutant was about 58% more productive than *CCD7* (Figure 8).

Apparently, the improper branching regulation under stress conditions led to a partition of assimilates to shoot growth in detriment of fruits formation. Moreover, *CCD7* plants displayed expressive Na Shoot and root growth sensitivity since they suffered more substantial reductions in reproductive growth than plants of other genotypes when compared the biomass under control and saline conditions (Figure 8).

3.3.4 Hormones influences Na/K balance in vegetative growth

The effects of hormonal classes were evaluated through different experiments. However, growth varied between experiments due to photon flux density change. The growth environment was enhanced with 150 µmol m^{-2} s^{-1} on the second experiment. Nevertheless, this PAR difference did not debilitate the study, since in all experiments the control MT was present. In order to solve this constraint, the performances of genotypes were standardized from mean values of control MT in the same experiment to each trait under saline conditions. The approach allowed performing comparison of all genotypes in relation to MT and analyzing data through multivariate technique. The adopted statistical procedure was principal component (PC) analysis (PCA).

The PC1 represents the patterns of Na^+ and K^+ accumulations and PC2 shoot growth of salt-stressed plants, both in comparison to the control MT performance under salinity (Figure 10). The Na^+ and K^+ levels were more important to explain the data variance of hormonal genotypes (PC1:51.52%) than shoot growth (PC2: 35.68%). The genotypes plotted near to zero in any PC indicated certain similarity to MT about the traits represented in each PC.

Interestingly, the analysis indicated a weak correlation between lower leaf Na:K ratio concentrations and higher shoot biomass under salinity, although, clearly Na^+ accumulation has impaired the growth.

The genotypes *e*, *epi* and *not* exhibited more K and lesser Na on the leaves than MT. Besides, the lack DELLA repressor in the gibberellin constitutive *pro* showed little difference in terms of K concentration compared to MT.

High Na contents were observed in *CCD7*, *nahG*, *Nr* and *dgt* plants. Even so *CCD7* showed impressive shoot growth whereas *dgt* mutant presented low shoot biomass. The *Nr* plants showed similar growth to MT.

The low SL endogenous level and IAA increased response in *CCD7* and *e* plants respectively led to higher shoot growth. Moreover, *CCD7* accumulated large Na^+ amounts but median K level. In contrast, *e* plants showed higher K^+ leaf concentration than Na^+ one.

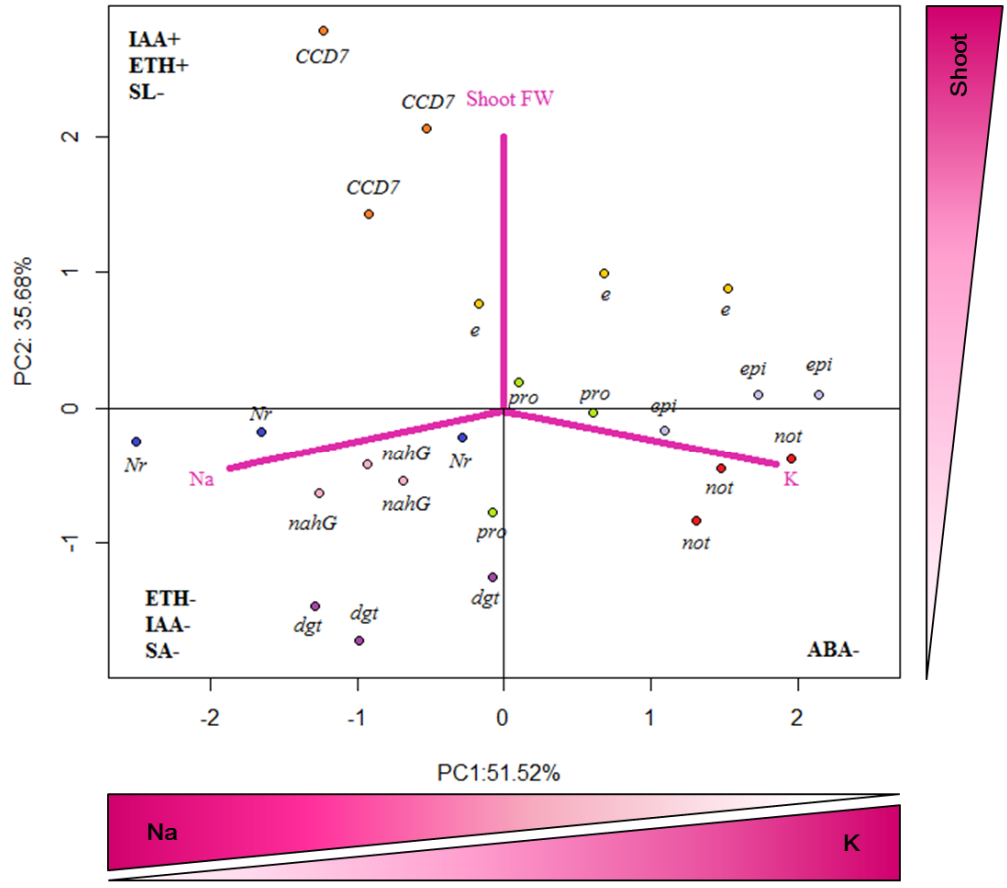

Figure 10 - Principal component analysis (PCA) biplot for K:Na homeostasis (PC1) and shoot growth (PC2) under salinity. Data show the effect of hormone alteration responses in these traits compared to control Micro-Tom. Indole-3-acetic acid (IAA), ethylene (ETH), strigolactone (SL), salycilic acid (SA) and abscisic acid (ABA).

3.4 Discussion

The use of genotypes harboring hormonal alterations in a single genetic background (*Solanum lycopersicum* cv. Micro-Tom) allowed the comparative study of the hormonal effects on growth under control and saline conditions. The screening of eight genotypes with hormonal alterations: *not*, *epi*, *Nr*, *pro*, *dgt*, *e*, *nahG* and *CCD7* (Table 1) propitiated the observation of the effect of six hormonal classes: ABA, ethylene, GA, IAA, SA and SL in salt response.

The results indicated that salt induced restriction on vegetative and reproductive growth of all genotypes tested (Figures 1, 5 and 8). The comparison of growth among the genotypes revealed that hormonal alterations responses conferred by mutations and transgenes led to different levels of salt sensitivity (Figure 10).

Since *pro* contains a point mutation causing a loss of function in the single tomato *DELLA* gene, the growth repression activity performed via DELLA proteins as impaired

(BASSEL et al., 2008). This probably contributed to the higher root:shoot ratio on this genotype when compared to control MT under salinity (Figure 1). However, it was reported that *pro* plants retain a residual DELLA activity (TUINEN et al., 1999), beyond DELLA-independent GA-response pathway (LIVNE et al., 2015; MAYMON et al., 2009). This may explain the fact that *pro* displayed shoot growth similar to MT, and not higher under salinity, in disagreement to previous finding in Arabidopsis DELLA-deficient genotypes reported by Achard et al. (2006). Arabidopsis quadruple-DELLA mutant shows higher root biomass accumulation and greater shoot growth than wild-type when it is subjected to 100 mM NaCl (ACHARD et al., 2006; CHENG et al., 2004).

The *epi* plants presented better performance than *pro* under salinity, which was expressed by higher biomass and lower Na:K ratio. Studies conducted with Arabidopsis ethylene overproducer *eto1* mutant indicated that ethylene enhances salinity tolerance for reducing K^+ loss and restricting Na^+ influx (JIANG et al., 2013). Furthermore, there are evidences that ethylene may also regulate K^+ transporter (DEMIDCHIK et al., 2010; PENG et al., 2014; SHABALA; CUIN, 2008; YANG et al., 2015).

Interestingly, the ethylene overproducer genotype *epi* and mutant *not,* that produces less ABA under stress showed similar Na:K ratio (Figures 1 and 3). ABA is reported as negative regulator of ethylene production under abiotic stress (SHARP et al., 2000; SPOLLEN et al., 2000). Hence the ABA deficiency in *not* might have led to a fail in avoiding the excessive ethylene accumulation, contributing to similar Na:K ratios among these two genotypes in spite of their growth differences. However, the ethylene precursor ACC concentration was not enhanced in *not* mutant (Figure 4). Consistent with ethylene role in Na:K homeostasis, the mutant *Nr*, which blocks ethylene responses, showed higher Na^+ accumulation and lower K^+ level than *epi* and the control MT (Figures 7 and 10).

Although *not* plants presented low Na:K ratio, they exhibited shoot biomass accumulation on salt almost equivalent to one third of that observed under control conditions (Figure 1C). Conceivably, the ABA deficiency impaired various stress responses, since ABA biosynthesis is the most important stress signal transduction pathway among all the plant responses to stresses (ZHANG et al., 2006). As a consequence, the perception of incoming stresses and regulation of the physiological mechanisms to salt tolerance were neglected in *not* plants, resulting in impressive inhibition to growth.

A crosstalk between ABA and SL was recently described, the latter upregulating ABA-responsive genes. SL-deficient Arabidopsis *max* mutants exhibited increased leaf stomatal density and lower ABA-induced stomatal closure, compared to wild type (HA et al.,

2014). In agreement, the tomato SL deficient plants displayed higher stomatal conductance and transpiration rate (Figure S1).

Moreover, *CCD7* exhibited an impressive decrease under salinity compared to its growth under control conditions (Figures 4A and 8A) and impaired salt-induced adjustment of shoot/root growth (Figure 4C). Hence, this hormone is need to suppress shoot branching for inhibiting the outgrowth of axillary buds (GOMEZ-ROLDAN et al., 2008; UMEHARA et al., 2008).

Furthermore, SL was described as a regulator of root growth and formation, inducing primary root formation but suppressing adventitious root growth (DE CUYPER et al., 2015; KAPULNIK et al., 2011; KOREN et al., 2013; RASMUSSEN et al., 2012; RUYTER-SPIRA et al., 2011). However, SL deficiency in *CCD7* did not affect root biomass accumulation (Figure 5C). The auxins also modulate root beyond also shoot growth and development through various mechanisms mediated by local accumulation (RYU; CHO, 2005).

Root development was impaired under salinity in *dgt*, which has a low IAA sensitivity, but was not enhanced in *e* that presents an increased IAA response (DENGLER, 1984; OH et al., 2006; WANG et al., 2005). The shoot fresh weight was increased in *e* plants and decreased in the *dgt* mutant on salt (Figure 5). As already known, the modification in IAA signaling and perception influences the initiation and growth of plants organs and consequently the biomass (RYU; CHO, 2005; FAHAD et al., 2015; WANG et al., 2001). In addition, it was observed by Albacete *et al*. (2008) that IAA concentration was reduced on the leaves and increased in roots under salinity, which may explain the favored biomass allocation to roots, in detriment to shoot development observed here (Figure 5). Nevertheless, *e* plants showed expressive growth decrease upon salt treatment compared to control conditions, which is in line with the observation that enhanced auxin response impaired the signaling crosstalk between auxin and salt stress (JUNG; PARK, 2011).

The *dgt* and *e* mutants presented similar K^+ concentration under salinity. However, *e* salt-stressed plants exhibited lower leaf Na^+ level than *dgt*, probably due to the Na^+ dilution propitiated by its higher growth.

The low SA endogenous levels in plants expressing constitutively the bacterial *nahG* gene, encoding Salicylate hydroxylase (BRANDING et al., 2000; THARA et al., 1999) led to a severe inhibition of shoot and root growth beside impaired leaf expansion of under salinity (Figures 5 and 6). In addition, these transgenic plants showed K^+ level decrease similar to *Nr* and *dgt* plants, but Na^+ concentration similar to MT (Figures 7 and 10). These results were expected since SA takes part of the maintenance of a suitable membrane potential that affects

various metabolic mechanisms including K^+ channel (JAYAKANNAN et al., 2001). Nevertheless, Wasti et al. (2007) reported that exogenous SA application led to the maintenance of optimum K:Na ratio and more accumulation of photosynthetic pigments and osmoprotectants in tomato plants under salinity.

3.5 Conclusions

Based on the results presented here and the PCA, four kinds of salt response among the genotypes were observed: *i*) High shoot growth in spite of high Na:K ratio presented by the strigolactone deficient and high branching *CCD7* transgene; *ii*) High shoot growth and reduced accumulation of Na in tissues (probably due to dilution) presented by the auxin constitutive response *e* mutant; *iii*) The opposite response observed in "*ii*" presented by the low auxin sensitivity *dgt* mutant and *iv*) growth inhibition combined with reduced levels of Na and higher accumulation of K presented by the *not* mutant, which produces less ABA. Such specific behaviors point to novel levels of salt response regulation, suggesting a role for auxin in Na dilution in tissues and specific mechanisms of ABA and strigolactones in the control of growth under salinity.

References

ACHARD, P.; GONG, F.; CHEMINANT, S.; ALIOUA, M.; HEDDEN, P.; GENSCHIK, P. The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. **The Plant Cell**, Rockville, v. 20, n. 8, p. 2117–2129, 2008.

ACHARD, P.; CHENG, H.; DE GRAUWE, L.; DECAT, J.; SCHOUTTETEN, H.; MORITZ, T.; STRAETEN, D. van der; PENG, J.; HARBERD, N.P. Integration of plant responses to environmentally activated phytohormonal signals. **Science**, New York, v. 311, n. 5757, p. 91–94, 2006.

ALBACETE, A.; GHANEM, M.E.; MARTÍNEZ-ANDÚJAR, C.; ACOSTA M.; SÁNCHEZ-BRAVO, J.; MARTÍNEZ, V.; LUTTS, S.; DODD, I.C.; PÉREZ-ALFOCEA, F. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. **Journal of Experimental Botany**, Oxford, v. 59, n. 15, p. 4119–4131, 2008.

ALMEIDA, J.; ASÍS, R.; MOLINERI, V.N.; SESTARI, I.; LIRA, B.S.; CARRARI, F.; PERES, L.E.P.; ROSSI, M. Fruits from ripening impaired, chlorophyll degraded and jasmonate insensitive tomato mutants have altered tocopherol content and composition. **Phytochemistry**, New York, v. 111, p. 72–83, 2015.

BARRY, C.S.; FOX, E.A.; YEN, H.; LEE, S.; YING, T.; GRIERSON, D.; GIOVANNONI, J.J. Analysis of the ethylene response in the *epinastic* mutant of tomato. **Plant Physiology**, Lancaster, v. 127, n. 1, p. 58–66, 2001.

BASSEL, G.W.; MULLEN, R.T.; BEWLEY, J.D. *Procera* is a putative DELLA mutant in tomato (*Solanum lycopersicum*): effects on the seed and vegetative plant. **Journal of Experimental Botany**, Oxford, v. 59, n. 3, p. 585–593, 2008.

BRADFORD, K.J.; YANG, S.F. Stress-induced ethylene production in the ethylene-requiring tomato mutant *diageotropica*. **Plant Physiology**, Lancaster, v. 65, n. 2, p. 327–330, 1980.

BRADING, P.A.; HAMMOND-KOSACK, K.E.; PARR, A.; JONES, J.D.G. Salicylic acid is not required for Cf-2- and Cf-9-dependent resistance of tomato to *Cladosporium fulvum*. **The Plant Journal**, Malden, v. 23, n. 3, p. 305–318, 2000.

BURBIDGE, A.; GRIEVE, T.M.; JACKSON, A.; THOMPSON, A.; MCCARTY, D.R.; TAYLOR, I.B. Characterization of the ABA-deficient tomato mutant *notabilis* and its relationship with maize Vp14. **The Plant Journal**, Malden, v. 17, n. 4, p. 427–431, 1999.

CARRERA, E.; RUIZ-RIVERO, O.; PERES, L.E.P.; ATARES, A.; GARCIA-MARTINEZ, J.L. Characterization of the *procera* tomato mutant shows novel functions of the *Sl*DELLA protein in the control of flower morphology, cell division and expansion, and the auxin-signaling pathway during fruit-set and development. **Plant Physiology**, Lancaster, v. 160, n. 3, p. 1581–1596, 2012.

CARVALHO, R.F.; CAMPOS, M.L.; PINO, L.E.; CRESTANA, S.L.; ZSÖGÖN, A.; LIMA, J.E.; BENEDITO, V.A.; PERES, L.E.P. Convergence of developmental mutants into a single tomato model system: "Micro-Tom" as an effective toolkit for plant development research. **Plant Methods**, London, v. 7, n. 1, p. 1-18, 2011.

CHENG, H.; QIN, L.; LEE, S.; FU, X.; RICHARDS, D.E.; CAO, D.; LUO, D.; HARBERD, N.P.; PENG, J. Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. **Development**, Cambridge, v. 131, n. 5, p. 1055–1064, 2004.

CUYPER, C.; FROMENTIN, J.; YOCGO, R.E.; KEYSER, A.; GUILLOTIN, B.; KUNERT, K.; BOYER, F.D.; GOORMACHTIG, S. From lateral root density to nodule number, the strigolactone analogue GR24 shapes the root architecture of *Medicago truncatula*. **Journal of Experimental Botany**, Oxford, v. 66, n. 1, p. 137–146, 2015.

DEMIDCHIK, V.; CUIN, T.A.; SVISTUNENKO, D.; SMITH, S.J.; MILLER, A.J.; SHABALA, S.; SOKOLIK, A.; YURIN, V. Arabidopsis root K+-efflux conductance activated by hydroxyl radicals: single-channel properties, genetic basis and involvement in stress-induced cell death. **Journal of Cell Science**, London, v. 123, n. 9, p. 1468–1479, 2010.

DENGLER, N.G. Comparison of leaf development in normal (+/+), *Entire* (*e*/*e*), and *Lanceolate* (*La*/+) plants of tomato, *Lycopersicon esculentum* "Ailsa Craig". **Botanical Gazette**, Chicago, v. 145, n. 1, p. 66–77, 1984.

FAHAD, S.; HUSSAIN, S.; MATLOOB, A.; KHAN, F.A.; KHALIQ, A.; SAUD, S.; HASSAN, S.; SHAN, D.; KHAN, F.; ULLAH, N.; FAIQ, M.; KHAN, M.R.; TAREEN, A. K.; KHAN, A.; ULLAH, A.; ULLAH, N.; HUANG, J. Phytohormones and plant responses to salinity stress: a review. **Plant Growth Regulation**, Dordrecht, v. 75, n. 2, p. 391–404, 2015.

FLEISHON, S.; SHANI, E.; ORI, N.; WEISS, D. Negative reciprocal interactions between gibberellin and cytokinin in tomato. **The New Phytologist**, London, v. 190, n. 3, p. 609–617, 2011.

FRACETTO, G.G.M.; PERES, L.E.P.; MEHDY, M.C.; LAMBAIS, M.R. Tomato ethylene mutants exhibit differences in arbuscular mycorrhiza development and levels of plant defense-related transcripts. **Symbiosis**, Philadelphia, v. 60, n. 3, p. 155–167, 2013.

FRICKE, W.; AKHIYAROVA, G.; VESELOV, D.; KUDOYAROVA, G. Rapid and tissue-specific changes in ABA and in growth rate in response to salinity in barley leaves. **Journal of Experimental Botany**, Oxford, v. 55, n. 399, p. 1115–1123, 2004.

FUJINO, D.W.; BURGER, D.W.; YANG, S.F.; BRADFORD, K.J. Characterization of an ethylene overproducing mutant of tomato (*Lycopersicon esculentum* Mill. Cultivar VFN8). **Plant Physiology**, Lancaster, v. 88, n. 3, p. 774–779, 1988.

GHANEM, M.E.; ALBACETE, A.; MARTÍNEZ-ANDÚJAR, C.; ACOSTA, M.; ROMERO-ARANDA, R.; DODD, I. C.; LUTTS, S.; PÉREZ-ALFOCEA, F. Hormonal changes during salinity-induced leaf senescence in tomato (*Solanum lycopersicum* L.). **Journal of Experimental Botany**, Oxford, v. 59, n. 11, p. 3039–3050, 2008.

GOMEZ-ROLDAN, V.; FERMAS, S.; BREWER, P.B.; PUECH-PAGÈS, V.; DUN, E.A.; PILLOT, J.-P.; LETISSE, F.; MATUSOVA, R.; DANOUN, S.; PORTAIS, J.-C.; BOUWMEESTER, H.; BÉCARD, G.; BEVERIDGE, C.A.; RAMEAU, C.; ROCHANGE, S.F. Strigolactone inhibition of shoot branching. **Nature**, London, v. 455, n. 7210, p. 189–194, 2008.

GRATÃO, P.L.; MONTEIRO, C.C.; CARVALHO, R.F.; TEZOTTO, T.; PIOTTO, F.A.; PERES, L.E.P.; AZEVEDO, R. A. Biochemical dissection of *diageotropica* and *Never ripe* tomato mutants to Cd-stressful conditions. **Plant Physiology and Biochemistry**, Paris, v. 56, p. 79–96, 2012.

GURMANI, A.R.; BANO, A.; ULLAH, N.; KHAN, H.; JAHANGIR, M.; FLOWERS, T.J. Exogenous abscisic acid(ABA) and silicon (Si) promote salinity tolerance by reducing sodium (Na^+) transport and bypass flow in rice (*Oryza sativa*). **Australian Journal of Crop Science**, Lismore, v. 7, n. 9, p. 1219–1226, 2013.

HA, C. Van; LEYVA-GONZÁLEZ, M.A.; OSAKABE, Y.; TRAN, U.T.; NISHIYAMA, R.; WATANABE, Y.; TANAKA, M.; SEKI, M.; YAMAGUCHI, S.; DONG, N. Van; YAMAGUCHI-SHINOZAKI, K.; SHINOZAKI, K.; HERRERA-ESTRELLA, L.; TRAN, L.-S.P. Positive regulatory role of strigolactone in plant responses to drought and salt stress. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 111, n. 2, p. 851–856, 2014.

HAMILTON, A.J.; LYCETT, G.W.; GRIERSON, D. Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. **Nature**, London, v. 346, n. 6281, p. 284–287, 1990.

HAN, Y.; YIN, S.; HUANG, L. Towards plant salinity tolerance-implications from ion transporters and biochemical regulation. **Plant Growth Regulation**, Dordrecht, v. 76, n. 1, p. 13–23, 2015.

HICHRI, I.; MUHOVSKI, Y.; CLIPPE, A.; ŽIŽKOVÁ, E.; DOBREV, P.I.; MOTYKA, V.; LUTTS, S. *SlDREB2*, a tomato dehydration-responsive element-binding 2 transcription factor, mediates salt stress tolerance in tomato and Arabidopsis. **Plant, Cell & Environment**, New York, v. 39, n. 1, p. 62–79, 2016.

JAYAKANNAN, M.; BOSE, J.; BABOURINA, O.; RENGEL, Z.; SHABALA, S. Salicylic acid improves salinity tolerance in Arabidopsis by restoring membrane potential and preventing salt-induced K+ loss via a GORK channel. **Journal of Experimental Botany**, Oxford, v. 64, n. 8, p. 2255–2268, 2013.

JESUS, F.A. de, ZSÖGÖN, A.; PERES, L.E.P. Physionomics. In: BORÉM, A.; FRITSCHE-NETO, R. (Ed.). **Omics in Plant Breeding**. New Jersey: John Wiley, 2014. chap. 6, p. 104-126.

JIANG, C.; BELFIELD, E.J.; CAO, Y.; SMITH, J.A. C.; HARBERD, N.P. An Arabidopsis soil-salinity-tolerance mutation confers ethylene-mediated enhancement of sodium/potassium homeostasis. **The Plant Cell**, Rockville, v. 25, n. 9, p. 3535–3552, 2013.

JONES, M.G. Gibberellins and the *procera* mutant of tomato. **Planta**, New York, v. 172, n. 2, p. 280–284, 1987.

JUNG, J.-H.; PARK, C.-M. Auxin modulation of salt stress signaling in Arabidopsis seed germination. **Plant Signaling & Behavior**, Georgetown, v. 6, n. 8, p. 1198–1200, 2011.

KAPULNIK, Y.; RESNICK, N.; MAYZLISH-GATI, E.; KAPLAN, Y.; WININGER, S.; HERSHENHORN, J.; KOLTAI, H. Strigolactones interact with ethylene and auxin in regulating root-hair elongation in Arabidopsis. **Journal of Experimental Botany**, Oxford, v. 62, n. 8, p. 2915–2924, 2011.

KELLY, M.O.; BRADFORD, K.J. Insensitivity of the *diageotropica* tomato mutant to auxin. **Plant Physiology**, Lancaster, v. 82, n. 3, p. 713–717, 1986.

KING, K.E.; MORITZ, T.; HARBERD, N.P. Gibberellins are not required for normal stem growth in *Arabidopsis thaliana* in the absence of GAI and RGA. **Genetics**, Bethesda, v. 159, n. 2, p. 767–76, 2001.

KOREN, D.; RESNICK, N.; MAYZLISH GATI, E.; BELAUSOV, E.; WEININGER, S.; KAPULNIK, Y.; KOLTAI, H. Strigolactone signaling in the endodermis is sufficient to restore root responses and involves SHORT HYPOCOTYL 2 (SHY2) activity. **The New Phytologist**, London, v. 198, n. 3, p. 866–874, 2013.

LEVENE, H. Robust tests for equality of variances. In: OLKIN, I. **Contributions to probability and statistics:** essays in honor of Harold Hotelling. Palo Alto: Stanford University Press, 1960. p. 278–292.

LIU, M.; GOMES, B.L.; MILA, I.; PURGATTO, E.; PERES, L.E.P.; FRASSE, P.; MAZA, E.; ZOUINE, M.; ROUSTAN, J.-P.; BOUZAYEN, M.; PIRRELLO, J. Comprehensive profiling of ethylene response factor expression identifies ripening-associated *ERF* genes and their link to key regulators of fruit ripening in tomato. **Plant Physiology**, Lancaster, v. 170, n. 3, p. 1732–1744, 2016.

LIVNE, S.; LOR, V.S.; NIR, I.; ELIAZ, N.; AHARONI, A.; OLSZEWSKI, N.E.; ESHED, Y.; WEISS, D. Uncovering DELLA-independent gibberellin responses by characterizing new tomato *procera* mutants. **The Plant Cell**, Rockville, v. 27, n. 6, p. 1579–1594, 2015.

LOMBARDI-CRESTANA, S.; AZEVEDO, M.S.; E SILVA, G.F.F.; PINO, L.E.; APPEZZATO-DA-GLORIA, B.; FIGUEIRA, A.; NOGUEIRA, F.T.S.; PERES, L.E.P. The tomato (*Solanum lycopersicum* cv. Micro-Tom) natural genetic variation *Rg1* and the DELLA Mutant *procera* control the competence necessary to form adventitious roots and shoots. **Journal of Experimental Botany**, Oxford, v. 63, n. 15, p. 5689–5703, 2012.

MAGGIO, A.; BARBIERI, G.; RAIMONDI, G.; PASCALE, S. de. Contrasting effects of GA_3 treatments on tomato plants exposed to increasing salinity. **Journal of Plant Growth Regulation**, New York v. 29, n. 1, p. 63–72, 2010.

MAGGIO, A.; RAIMONDI, G.; MARTINO, A.; PASCALE, S. de. Salt stress response in tomato beyond the salinity tolerance threshold. **Environmental and Experimental Botany**, Oxford, v. 59, n. 3, p. 276–282, 2007.

MAYMON, I.; GREENBOIM-WAINBERG, Y.; SAGIV, S.; KIEBER, J.J.; MOSHELION, M.; OLSZEWSKI, N.; WEISS, D. Cytosolic activity of SPINDLY implies the existence of a DELLA-independent gibberellin-response pathway. **Plant Journal**, Malden, v. 58, n. 6, p. 979–988, 2009.

McCOURT, R.M.; DELWICHE, C.F.; KAROL, K.G. Charophyte algae and land plant origins. **Trends Ecology & Evolution**, Barking, v. 19, n. 12, p. 661-666, 2004.

MIMOUNI, H.; WASTI, S.; MANAA, A.; GHARBI, E.; CHALH, A.; VANDOORNE, B.; LUTTS, S.; AHMED, H. B. Does Salicylic Acid (SA) improve tolerance to salt stress in plants? A study of SA effects on tomato plant growth, water dynamics, photosynthesis, and biochemical parameters .**OMICS: A Journal of Integrative Biology**, Larchmont, v. 20, n. 3, p. 180–190, 2016.

NEILL, S.J.; HORGAN, R. Abscisic acid production and water relations in wilty tomato mutants subjected to water deficiency. **Journal of Experimental Botany**, Oxford, v. 36, n. 8, p. 1222–1231, 1985.

OH, K.; IVANCHENKO, M.G.; WHITE, T.J.; LOMAX, T.L. The *diageotropica* gene of tomato encodes a cyclophilin: A novel player in auxin signaling. **Planta**, New York, v. 224, n. 1, p. 133–144, 2006.

PAN, Y.-J.; LIU, L.; LIN, Y.-C.; ZU, Y.-G.; LI, L.-P.; TANG, Z.-H. Ethylene antagonizes salt-induced growth retardation and cell death process via transcriptional controlling of ethylene-, BAG- and senescence-associated genes in *Arabidopsis*. **Frontiers in Plant Science**, Lauseanne, v. 7, p. 1–10, 2016.

PENG, J.; LI, Z.; WEN, X.; LI, W.; SHI, H.; YANG, L.; ZHU, H.; GUO, H. Salt-induced stabilization of EIN3/EIL1 confers salinity tolerance by deterring ROS accumulation in *Arabidopsis*. **PLoS Genetics**, San Francisco, v. 10, n. 10, p. e1004664, Oct. 2014. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/25330213>. Acesso em: 05 maio 2016.

PHILIPPAR, K.; IVASHIKINA, N.; ACHE, P.; CHRISTIAN, M.; LÜTHEN, H.; PALME, K.; HEDRICH, R. Auxin activates *KAT1* and *KAT2*, two K^+-channel genes expressed in seedlings of *Arabidopsis thaliana*. **The Plant Journal**, Malden, v. 37, n. 6, p. 815–827, 2004.

PHILIPPAR, K.; FUCHS, I.; LUTHEN, H.; HOTH, S.; BAUER, C.S.; HAGA, K.; THIEL, G.; LJUNG, K.; SANDBERG, G.; BOTTGER, M.; BECKER, D.; HEDRICH, R. Auxin-induced K^+ channel expression represents an essential step in coleoptile growth and gravitropism. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 96, n. 21, p. 12186–12191, 1999.

PINO, L.E.; LOMBARDI-CRESTANA, S.; AZEVEDO, M.S.; SCOTTON, D.C.; BORGO, L.; QUECINI, V.; FIGUEIRA, A.; PERES, L.E.P. The *Rg1* allele as a valuable tool for genetic transformation of the tomato 'Micro-Tom' model system. **Plant Methods**, London, v. 6, n. 23, p. 1-23, 2010.

RASMUSSEN, A.; MASON, M.G.; DE CUYPER, C.; BREWER, P.B.; HEROLD, S.; AGUSTI, J.; GEELEN, D.; GREB, T.; GOORMACHTIG, S.; BEECKMAN, T.; BEVERIDGE, C. A. Strigolactones suppress adventitious rooting in Arabidopsis and pea. **Plant Physiology**, Lancaster, v. 158, n. 4, p. 1976–1987, 2012.

RUIZ-LOZANO, J.M.; AROCA, R.; ZAMARREÑO, Á.M.; MOLINA, S.; ANDREO-JIMÉNEZ, B.; PORCEL, R.; GARCÍA-MINA, J.M.; RUYTER-SPIRA, C.; LÓPEZ-RÁEZ, J.A. Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. **Plant, Cell and Environment**, New York, v. 39, n. 2, p. 441–452, 2016.

RUYTER-SPIRA, C.; KOHLEN, W.; CHARNIKHOVA, T.; ZEIJL, A. van; BEZOUWEN, L. van; DE RUIJTER, N.; CARDOSO, C.; LOPEZ-RAEZ, J.A.; MATUSOVA, R.; BOURS, R.; VERSTAPPEN, F.; BOUWMEESTER, H. Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in Arabidopsis: another belowground role for strigolactones? **Plant Physiology**, Lancaster, v. 155, n. 2, p. 721–734, 2011.

RYU, H.; CHO, Y.-G. Plant hormones in salt stress tolerance. **Journal of Plant Biology**, Seoul, v. 58, n. 3, p. 147–155, 2015.

SAS INSTITUTE. **SAS/STAT® 9.2:**user's guide. Cary, 2008.

SHABALA, S.; CUIN, T.A. Potassium transport and plant salt tolerance. **Physiologia Plantarum**, Copenhagen, v. 133, n. 4, p. 651–669, 2008.

SHAPIRO, S.S.; WILK, M.B. An analysis of variance test for normality (complete samples). **Biometrika**, London. v. 52, n. 3/4, p. 591–611, 1965.

SHAHBAZ, M.; ASHRAF, M.; AL-QURAINY, F.; HARRIS, P.J.C. Salt tolerance in selected vegetable crops. **Critical Reviews in Plant Sciences**, Boca Raton, v. 31, n. 4, p. 303–320, 2012.

SHARP, R.E.; LENOBLE, M.E.; ELSE, M.A.; THORNE, E.T.; GHERARDI, F. Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: evidence for an interaction with ethylene. **Journal of Experimental Botany**, Oxford, v. 51, n. 350, p. 1575–1584, 2000.

SHINOHARA, N.; TAYLOR, C.; LEYSER, O. Strigolactone can promote or inhibit shoot branching by triggering rapid depletion of the auxin efflux protein pin1 from the plasma membrane. **PLoS Biology**, San Francisco, v. 11, n. 1, p. e1001474, 2013. Disponível em: <http://dx.plos.org/10.1371/journal.pbio.1001474>. Acesso em: 30 maio 2016.

SPOLLEN, W.G.; LENOBLE, M.E.; SAMUELS, T.D.; BERNSTEIN, N.; SHARP, R.E. Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. **Plant Physiology**, Lancaster, v. 122, n. 3, p. 967–976, 2000.

TANG, X.; MU, X.; SHAO, H.; WANG, H.; BRESTIC, M. Global plant-responding mechanisms to salt stress: physiological and molecular levels and implications in biotechnology. **Critical Reviews in Biotechnology**, Boca Raton, v. 35, n. 4, p. 425–437, 2015.

TAO, J.-J.; CHEN, H.-W.; MA, B.; ZHANG, W.-K.; CHEN, S.-Y.; ZHANG, J.-S. The role of ethylene in plants under salinity stress. **Frontiers in Plant Science**, Lauseanne, v. 6, p. 1059, 2015.

THARA, V.K.; TANG, X.; GU, Y. Q.; MARTIN, G.B.; ZHOU, J.M. *Pseudomonas syringae* pv tomato induces the expression of tomato *EREBP*-like genes *Pti4* and *Pti5* independent of ethylene, salicylate and jasmonate. **The Plant Journal**, Malden, v. 20, n. 4, p. 475–483, 1999.

TUINEN, A. van; PETERS, A.H.L.J.; KENDRICK, R.E.; ZEEVAART, J.A.D.; KOORNNEEF, M. Characterisation of the *procera* mutant of tomato and the interaction of gibberellins with end-of-day far-red light treatments. **Physiologia Plantarum**, Copenhagen, v. 106, n. 1, p. 121–128, 1999.

UMEHARA, M.; HANADA, A.; YOSHIDA, S.; AKIYAMA, K.; ARITE, T.; TAKEDA-KAMIYA, N.; MAGOME, H.; KAMIYA, Y.; SHIRASU, K.; YONEYAMA, K.; KYOZUKA, J.; YAMAGUCHI, S. Inhibition of shoot branching by new terpenoid plant hormones. **Nature**, London, v. 455, n. 7210, p. 195–200, 2008.

VOGEL, J.T.; WALTER, M.H.; GIAVALISCO, P.; LYTOVCHENKO, A.; KOHLEN, W.; CHARNIKHOVA, T.; SIMKIN, A.J.; GOULET, C.; STRACK, D.; BOUWMEESTER, H.J.; FERNIE, A.R.; KLEE, H.J. *SlCCD7* controls strigolactone biosynthesis, shoot branching and

mycorrhiza-induced apocarotenoid formation in tomato. **The Plant Journal**, Malden, v. 61, n. 2, p. 300–311, 2010.

WANG, H.; JONES, B.; LI, Z.; FRASSE, P.; DELALANDE, C.; REGAD, F.; CHAABOUNI, S.; LATCHÉ, A.; PECH, J.-C.; BOUZAYEN, M. The tomato *Aux/IAA* transcription factor *IAA9* is involved in fruit development and leaf morphogenesis. **The Plant Cell**, Rockville, v. 17, n. 10, p. 2676–2692, 2005.

WANG, Y.; MOPPER, S.; HASENSTEIN, K.H. Effects of salinity on endogenous ABA, IAA, JA, and SA in *Iris hexagona*. **Journal of Chemical Ecology**, New York, v. 27, n. 2, p. 327–342, 2001.

WASTI, S.; MIMOUNI, H.; SMITI, S.; ZID, E.; AHMED, H.B. Enhanced salt tolerance of tomatoes by exogenous salicylic acid applied through rooting medium. **OMICS: A Journal of Integrative Biology**, Larchmonte, v. 16, n. 4, p. 200–207, 2012.

WEES, S.C.M. van; GLAZEBROOK, J. Loss of non-host resistance of *Arabidopsis* Nah*G* to *Pseudomonas syringae* pv. *phaseolicola* is due to degradation products of salicylic acid. **The Plant Journal**, Malden, v. 33, n. 4, p. 733–742, 2003.

WEISS, D.; ORI, N. Mechanisms of cross talk between gibberellin and other hormones. **Plant Physiology**, Lancaster, v. 144, n. 3, p. 1240–1246, 2007.

YANG, C.; MA, B.; HE, S.-J.; XIONG, Q.; DUAN, K.-X.; YIN, C.-C.; CHEN, H.; LU, X.; CHEN, S.-Y.; ZHANG, J.-S. *MAOHUZI6/ethylene insensitive3-like1* and *ethylene insensitive3-like2* regulate ethylene response of roots and coleoptiles and negatively affect salt tolerance in rice. **Plant Physiology**, Lancaster, v. 169, n. 1, p. 148–165, 2015.

YANG, Q.; CHEN, Z.-Z.; ZHOU, X.-F.; YIN, H.-B.; LI, X.; XIN, X.-F.; HONG, X.-H.; ZHU, J.-K.; GONG, Z. Overexpression of *SOS (Salt Overly Sensitive)* genes increases salt tolerance in transgenic *Arabidopsis*. **Molecular Plant**, v. 2, n. 1, p. 22–31, 2009.

YEN, H.C.; LEE, S.; TANKSLEY, S.D.; LANAHAN, M.B.; KLEE, H.J.; GIOVANNONI, J.J. The tomato *Never-ripe* locus regulates ethylene-inducible gene expression and is linked to a homolog of the *Arabidopsis ETR1* gene. **Plant Physiology**, Lancaster, v. 107, n. 4, p. 1343–1353, 1995.

ZHANG, J.; JIA, W.; YANG, J.; ISMAIL, A.M. Role of ABA in integrating plant responses to drought and salt stresses. **Field Crops Research**, Amsterdam, v. 97, n. 1, p. 111–119, 2006

ZHU, J.K. Plant salt tolerance. **Trends in Plant Science**, Kidlington, v. 6, n. 2, p. 66–71, 2001.

SUPPLEMENT

Supplement A

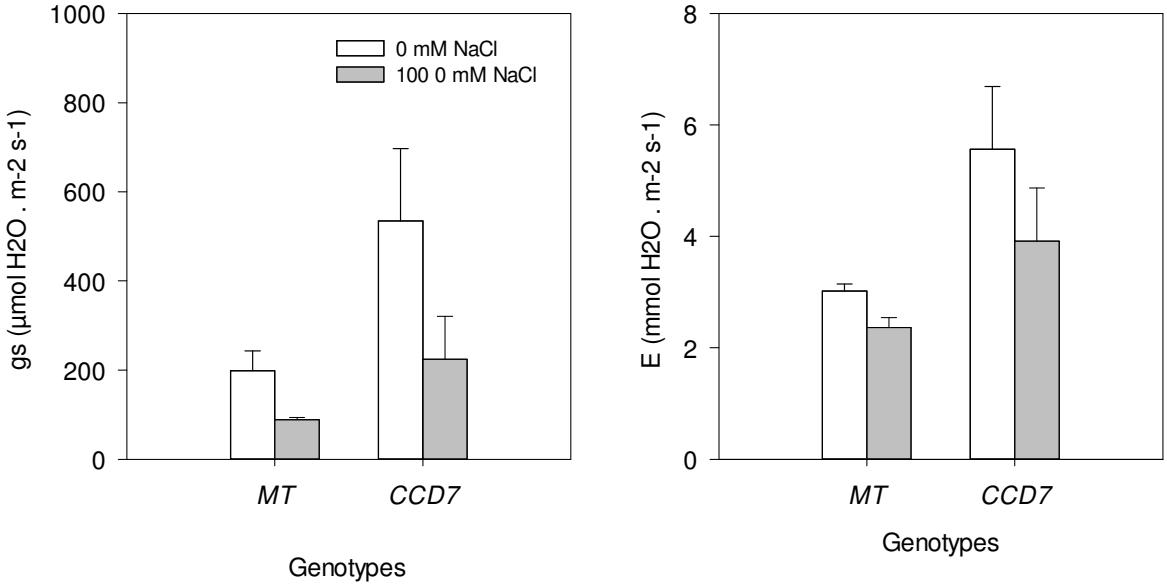

Figure S1 - Effect of strigolactone deficiency on gas exchange in tomato Micro-Tom (MT) genotypes. Stomatal conductance (A) and transpiration rate (B) of leaves from plants grown in half-strength Hoagland's solution in the absence (open bars) or presence of 100 mM NaCl (closed bars) for 21 days. The leaf samples were acquired at 50 days after sowing. Data are means ± SE (n = 3)