Effect of saline irrigation on the carotenoids biosynthesis and fruit development of *Solanum lycopersicum* cv. Micro Tom.

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Content

Abstract

Salinity is an environmental factor that limits plants growth and crop productivity, affecting biochemical and physiological mechanisms, as well as morphology in diverse plants. Decreasing in the photosynthetic capacity triggers important changes in plant development. It has been described that saline stress induce lipids, proteins and nucleic acids oxidative damage. To survive to these negative conditions, plants synthesize antioxidant molecules and detoxifying enzymes, in order to protect plant tissues against propagation of oxidative and cytotoxics species. In this context, Lycopene and ß-carotene have been correlated with antioxidant activities in several fruits. In a plant with moderate tolerance to salinity, such as tomato, high levels of NaCl decrease fruit size, number of leaves and stomatal density, reduce crop yield and negatively affect the carotenoids concentration. Interestingly, tomato plants exposed to low salinity significantly modifies the photosynthetic capacity, enhances carotenoid fruit accumulation and could improve tomato taste by increasing sugar and organic acids content. In this work, the effect of saline irrigation on the carotenoids biosynthesis and fruit development of *Solanum lycopersicum* cv. Micro Tom was studied. The results showed that after 8 weeks of irrigation with a 80mM to 160mM NaCl solutions negatively modified photosynthetic capacity, in terms of net photosynthesis, stomatic conductivity, photochemical efficiency, electron transport rate and non photochemical quenching. Also, after 14 weeks of a 40 to 160 mM salinity treatments, tomato fruits showed an early coloration, increasing the solid soluble content in comparison with the control. However, a reduction in fruit caliber

and fresh weight was observed from 80 mM NaCl onwards. Finally, salinity treatments differentially changed expression of carotenoid biosynthetic genes and increased accumulation of several carotenoids under 120 to 160mM. Altogether, these results suggest that long term salt irrigation correlates with earliness in tomato, a major breeding characteristic in crop plants.

CHAPTER 1

GENERAL INTRODUCTION

Importance of tomato as an agricultural crop and a fruit development model

Tomato (*Solanum lycopersicum*) is a crop originated from the coastal zone adjacent to the Pacific Ocean of South America, extending from Ecuador to Chile (Rick 1978; Harlan 1992). Tomato crop is known as a healthy food, with low calory value and high vitamins and antioxidants content, , being the most consumed horticultural crop around the wrorld (ODEPA, 2013). This species is the most cultivated vegetable in the world, with the greater planted area and production volume. According to ODEPA (2017) countries like China, India, Turkey, USA and Irak have the greatest production, surface planted and consumption, but northern European countries like Netherlands, Belgium, United Kingdom, Finland and Sweden, are leading in terms of yield , because of their effort in production technologies, harvesting more than 350 ton/ha. On the other hand, Chile ranks in n°20 in production with 993.000 tons, n°37 in surface with 15.800 ha and n°39 in yield with 62 ton/ha. In Chile, tomato is cultivated for two main purposes, fresh consumption and agroindustry or processed, being tomato for fresh consumption the third horticultural crop with greater planted surface, after corn and lettuce. About 60% of planted area are concentrated between the regions of Arica y Parinacota, Valparaíso, Metropolitana, O'Higgins and Maule.

In addition to its commercial and nutritive importance, tomato is considered a model vegetable system to understanding fleshy fruit development, because it has a relatively short life cycle and a small genome (950 Mb) (Wein 1997). Moreover, availability of standardized protocols for genetic transformation (Schwartz et al. 2014), making tomato handling relatively easy and fast, allowing both physiological and molecular studies.

Tomato fruit is classified as a climacteric fruit, whose ripening depends directly of the ethylene biosynthesis, perception and signaling (Bapat et al. 2010; Liu et al. 2015). Therefore, the onset of this process is characterized by an increase in ethylene biosynthesis and respiration rate (Giovannoni et al. 2001). In tomato, as in different plant species, fruit development is a complex process starting immediately after fruit set, and it is divided into four stages: fruit set, cell division, cell expansion and ripening (Gillaspy et al. 1993). The first stage (Stage I) begins with fruit set, which occurs after the ovary fertilization and correspond to the conversion from the static flower to the dynamic young fruit condition (Serrani et al. 2007). This earliest phase involves the development of the ovary and the decision to abort or to proceed with further cell division and fruit development (Gillaspy et al. 1993). The second stage (Stage II) is characterized by cell division (which is activated after fertilization and allows the fruit growth), seed formation and the early embryo development (Gillaspy et al. 1993). In the third phase (Stage III) fruit growth continues, mainly by cell expansión given by an increase in the vacuolar volume (Gillaspy et al. 1993), until the fruit reaches its final size, which depends on both, stage I and stage II

(Kumar et al. 2014). Finally, multiple variables participate in ripening, the last step of fruit development. Ripening is initiated after seed maturation has been completed and it is characterized by changes in firmness, accumulation of high levels of carbohydrates in the form of either sugars or starch and organic acids that give the fruit an appealing taste (Ho 1988; Gillaspy et al. 1993). On the other hand, during this last step phenolic compounds are synthesized and the conversion of chloroplasts into carotenoid-accumulating chromoplasts begins, which is concomitant with the first visible color changes (Briggs et al. 1986; Schuch et al. 1989). In tomato, color is an important trait because its used as an indicator of harvest. In Figure 1, different tomato color stages are represented according to Tikizawa et al., 2014.

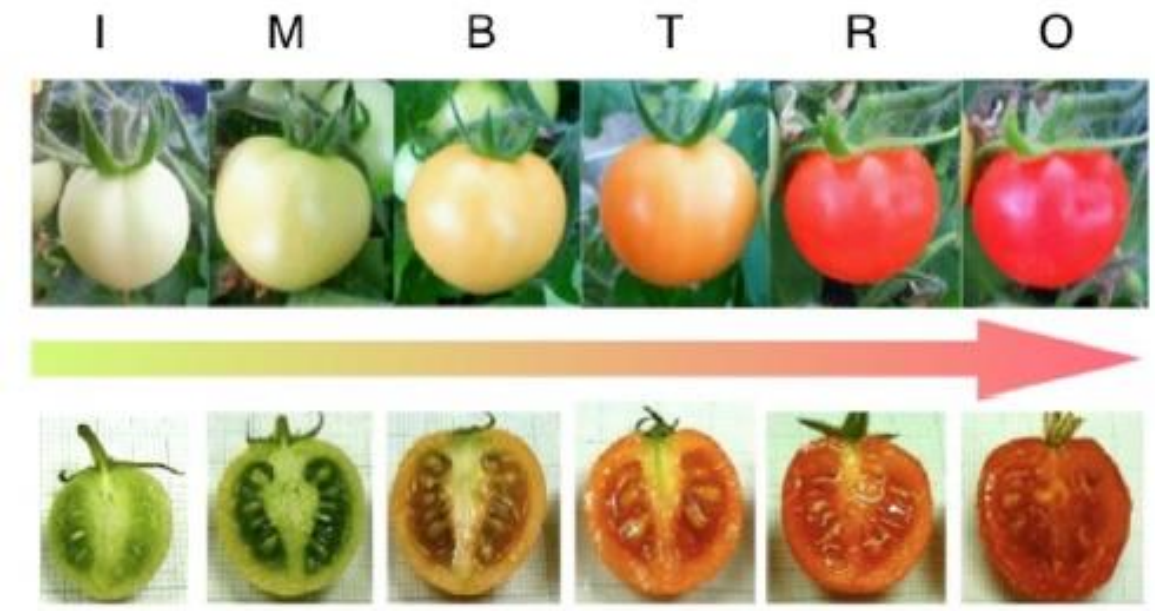

Figure 1. Variation in color tomato stages. Tomato fruit color stages from immature green (I), mature green (M), braker (B), turning (T), red ripe (R) and overripe (O).

Carotenoids compounds and their importance in tomato

Carotenoids are red, orange, and yellow lipid-soluble antioxidant pigments found throughout the plant kingdom. These pigments are embedded in the chloroplasts and chromoplasts membranes and protect photosynthetic organisms against potentially harmful photooxidative processes, being essential structural components of the photosynthetic antenna and reaction center complexes (Bartley et al. 1995; ONG, Augustine and Tee 1992). Carotenoids are generally C_{40} terpenoid compounds formed by the condensation of eight isoprene units (Bartley et al 1995). According to their chemical structure, they can be classified into two types: carotenes and xantophylls (hydroxylates) (Bramley 2002).

The developmental process of tomato fruit involves conversion of chloroplasts into chromoplasts, in which carotenoids, mainly lycopene crystalloids in membrane-shaped structures, are accumulate (Egea et al. 2010), resulting in fruits with appealing color (Bartley et al. 1995). Lycopene concentration increases between 10 to 14 times from green to ripe state (Bramley 2002). Thus, a tomato plant has about 2000-5000 µg of lycopene / 100 g of plant. (Fraser and Bramley 2004; Kandlakunta et al. 2008). For this, tomato fruit is considered as a healthy food (Canene-Adams et al. 2005), an important source of vitamin A and C, and carotenoids with nutritional benefits (Nagao 2011), antioxidant properties (Hadley et al. 2003) and the capacity to protect against prostate cancer (Bramley 2000; La Vecchia. 1999).

Figure 2 shows the structure of the most important molecules synthesized along the carotenoid biosynthetic pathway; while Figure 3 shows the structural genes involved on this route. Several condensation reactions are the first steps of this pathway and result in the synthesis of geranylgeranyl diphosphate (GGPP), a molecule that will be used for the synthesis of phytoene, through the action of Phytoene synthase (PSY). Later, due to a series of desaturation reactions Lycopene is synthesized, and then it is cycled to give rise to α and β carotene. Lycopene epsilon cyclase (LCYε) participates in the synthesis of α carotene, while lycopene beta cyclase (LCYβ) participates in both, α and β carotene biosynthesis. Lutein and Zeaxanthin are synthesized from α and β carotene, respectively. In the first case, by the activity of the ε-hydroxylase CYP97C (cytochrome P450-type monooxygenase); while in the second case, the CYP97A (cytochrome P450-type β-hydroxylase) and/or HYD (ferredoxin-dependent di-iron monooxygenase) is involved (Da Silva Messias et al. 2014; Bartley et al 1995).

Expression patterns studies have allowed to analyse the carotenoid biosynthetic genes at different stages of tomato fruit development, and correlate transcript levels with the accumulation of each metabolite (Ronen et al. 2000).

Different studies have been carried out in order to know if carotenoids biosynthesis pathway is affected by external stimuli. Within these, it has been demonstrated that light (Giuliano et al. 1993; Von Lintig et al. 1997; Xu et al. 2009), salinity (De Pascale et al. 2001), even phytohormones exogenously added (Vishnevestky et al. 1999) can affect carotenoids biosynthesis.

Although different models of carotenoid synthesis regulation have been proposed at

organ level in combination with environmental factors, still most of the molecular mechanisms and their implicancies in carotenoid biosynthesis remain unknown. Figure 4 shows a model proposed by Fanciullino et al., 2014, that accounts for the roles of the different environmental factors like light intensity, temperature and drought, than can trigger plant stress and their effects on carotenoid concentration, exposing photosynthesis as the primary control point affected by these stimuli. Two major integrative factors controlling biosynthetic pathways are considered: the concentration of total soluble sugars, which integrates a range of biological processes and their response to the environment, and the concentration of H_2O_2 which can be considered as representative of the global redox status resulting from exposure to stress conditions. In leaves, stressing conditions negatively affects photosynthesis, which decreases sugar production and leads to putative changes in expression of carotenoid biosynthesis pathway genes and differential accumulation of carotenoids, also caused by a photooxidative stress due to photosynthesis malfunction. Fanciullino also postulated that due to an unknown signal, ROS from the leaf may cause an effect in the fruit, leading to changes in enzymatic activity and differential carotenoid accumulation. In fruits, it is proposed that each stress can differently affect photosynthesis, while temperature may activate it, drought would hae a negative effect on it, and light intensity may not have a clear role on photosynthesis, but the overall environmental effect copes with ontogeny, fruit load, oxidative signaling, changing sugar concentration and carotenoid accumulation, still with several interactions to be proven or unknown.

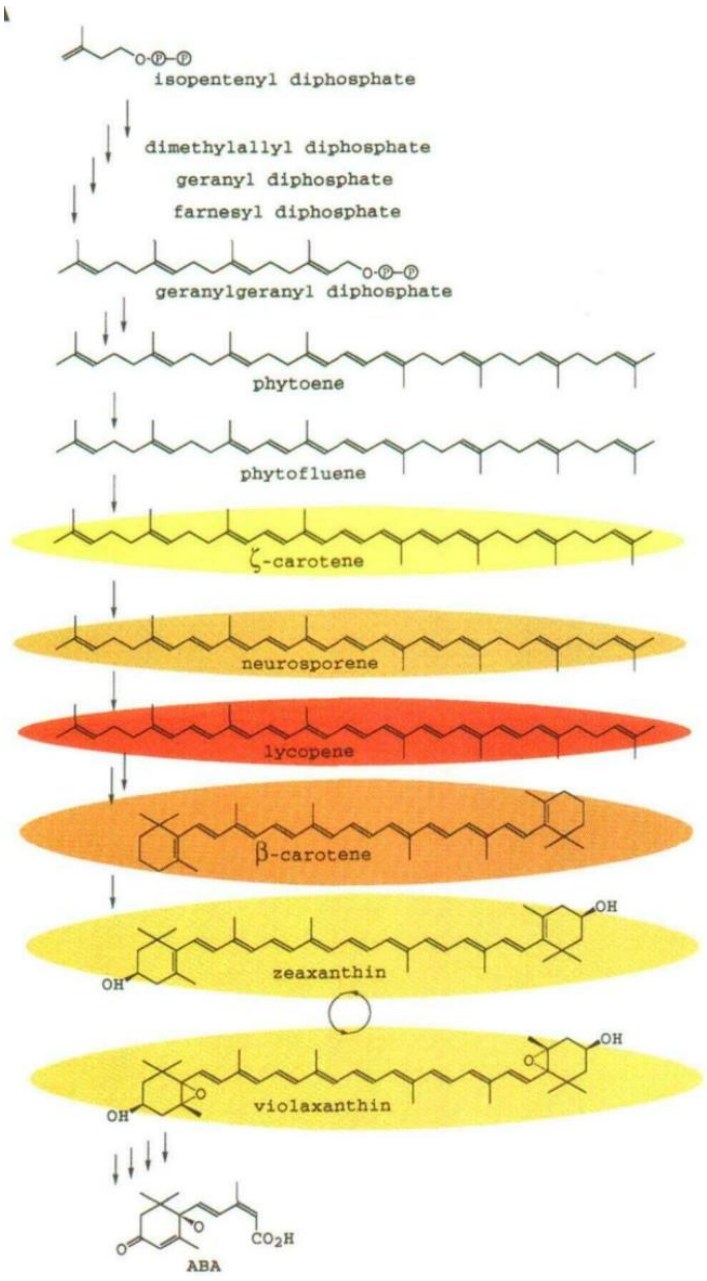

Figure 2. Structure of the most important molecules synthesized along the whole carotenoids route. The prevalent color of each compounds in nature are indicated. Adapted of Bartley G. et al. (1995).

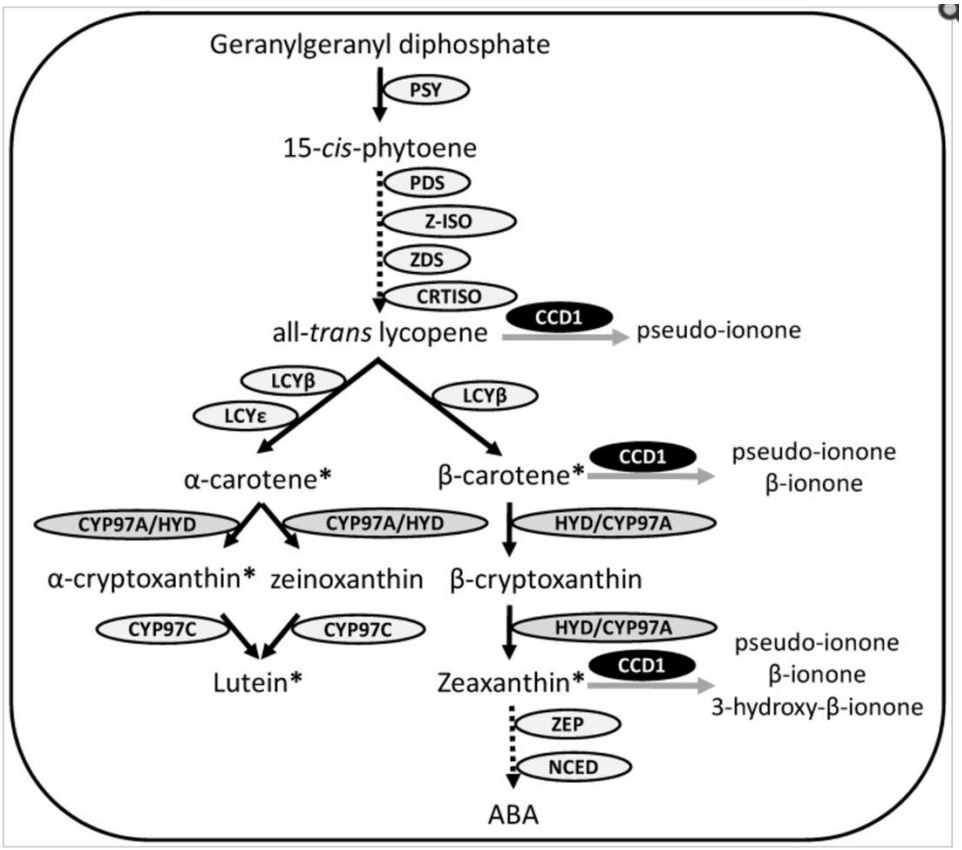

Figure 3. Structural genes involved in carotenoids biosynthetic route. Figure taken from Da Silva M. et al. (2014).

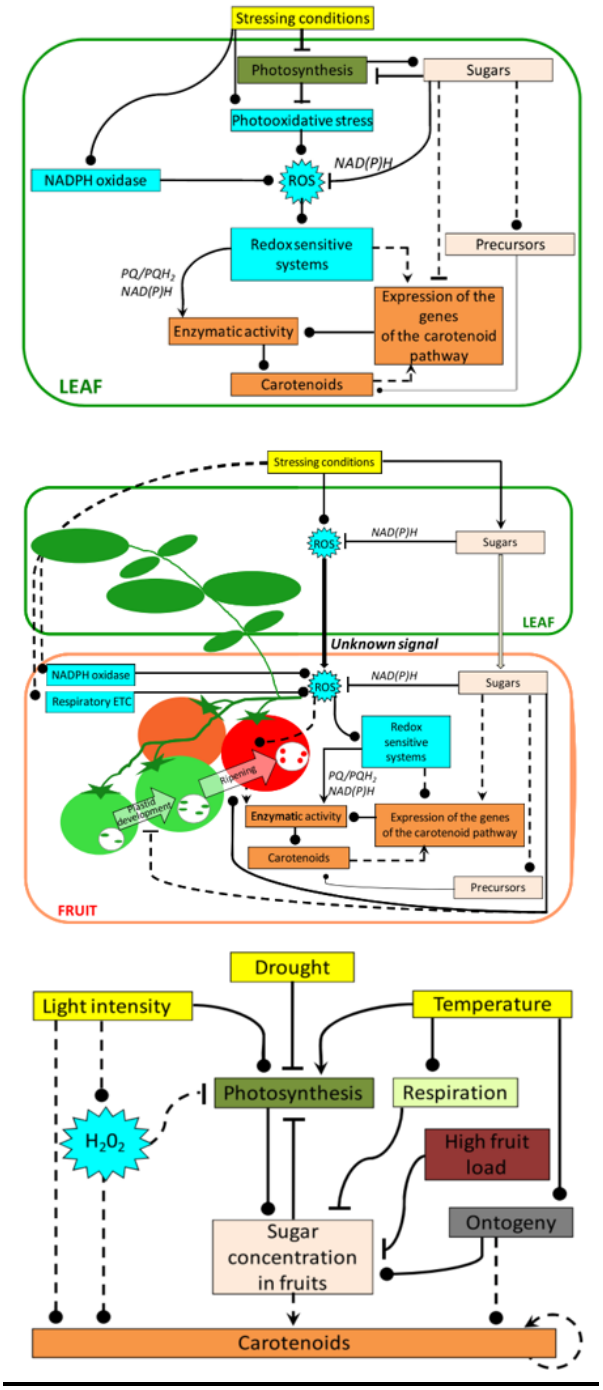

Figure 4. A simplified conceptual model for integrating the major environmental controls over carotenoid build-up in different organs. Figure taken of Fanciullino AL. et al. (2014).

Salinity, an important environmental factor that reduces crop productivity

Salinity is a severe abiotic stress that affect crop productivity, since it limits plant growth through different mechanisms (Orsini et al. 2010; Yao et al. 2011). Currently, 1 billion hectares are affected by salinity around the world (de Vos et al., 2016; Munns 2005). Furthermore, of the 1500 hectares of soil irrigated by rainfall, 32 million have high sodium concentrations (FAO. 2005). Chile is not exempt of this problem, as desertification has led to increase the concentration of NaCl, thus, several tomato crops are established in salinity conditions, especially in the north of the country (Arica and Parinacota) according to Torres 2008. While this area represents 13% of the area of cultivation of tomato for fresh consumption (Eguillor 2010).

It has been described that salt stress can affect plant growth and development in two ways: first, producing an osmotic stress that negatively affects stomatic conductivity, reducing CO_2 uptake and decreasing or inhibiting Calvin's Cycle, in final terms this causes a reduction of the oxygen from the PSI resulting in oxidative stress. The second way is by causing ionic toxicity because of the Na^+ and Cl^- increase, leading to key enzyme malfunction (Munns et al. 2006; Chaves et al. 2009).

Plants have developed several response mechanisms to survive or tolerate salt stress, such as a lower water potential with a restricting root-mediated nutrient uptake that reduces growth rate; also, a reducing leaf growth and inducing leaf senescence, among others (Orsini et al. 2010; Yeo et al. 2007). Other authors described that the sudden reduction in growth rates in vegetative tissue are due to rapid adaptation to NaCl irrigation, since salt affect water relations, hormonal

balance, or carbon supply (Munns R. 2002). These rapid adaptations lead to changes in roots and leaf development decreasing the total photosynthetic capacity of the plant, thus limiting growth (Yeo et al. 2007).

Plants not only control their growth rate, but also synthesize antioxidant molecules and enzymes that protect them against propagation of the oxidative status that salt stress produce (Dhindsa and Matowe. 1981; Foyer 1993). For instance, it has been described that in pepper fruits, Lycopene and ß-carotene are correlated with antioxidant activities, and it has been demonstrated that salinity significantly modified the carotenoid contents of these fruits (Navarro et al. 2006).

Effect of salinity on tomato

In general terms, tomato plant are moderately tolerant to salinity (Bernstein 1964), but plants exposed to high levels of NaCl have a decrease in size, number of leaves and stomatal density (Romero-Aranda et al. 2001), as well as, a decrease in seeds germination and an alteration in the water absorption by roots (Cuartero et al. 1999; Goykovic et al. 2007).

A negative effect was found in photosynthesis in tomato leaves by Zribi et al. (2009), after treating tomato plants with different NaCl concentrations with distinct irrigation regimes. Plants submitted for longer time to high NaCl concentrations exhibited the most severe damage in water potential, stomatic conductivity, non photochemical quenching (NPQ), electron transport rate (ETR) and Photochemical effiency (ΦPSII).

Studies in tomato fruit made by INIA (2010), showed that even from 40 mM of NaCl was possible to detect a mild decrease in fruit fresh weight and caliber in comparison with untreated plants, changes that were more severe with 80 mM irrigation. Other authors found similar results in Micro Tom tomato, where it was observed that after pulses of salt irrigation considering 80, 120 and 160 mM of NaCl, it was possible to observe a significant decrease in fruit fresh weight and an increase in sugar content (Yin et al., 2011).

Borghesi et al., 2011, discovered that depending on the cultivar, intensity of color and carotenoid content in fruit puree may vary in response to single application of salt treatments. Interestingly, other authors have reported that high salt concentrations negatively affect tomato carotenoid byosynthesis (Juan et al. 2005; Coesel et al. 2008), while low concentrations increase carotenoid accumulation, sugar and organic acids content (Adams 1989; 1991; De Pascale 2001). Apparently, different responses to salt irrigation depends on the amount and intensity of the stress, and on the type of cultivar. Usually, the most salt-resistant tomato cultivars are characterized by have a reduced uptake of Na^+ and Cl^-, and the capacity of increase the sucrose and carotenoids synthesis, as well as, a reduced lipid peroxidation and oxidative damage (Juan et al. 2005). It is important to note that the carotenoids levels in a cultivar may determine relative stress tolerance (Knox and Dodge 1985; Sairam 2002). Lycopene is one of the carotenoids that shows the greatest variation under salt stress in tomato fruits because it is the main carotenoid in this organ (Porretta 1991).

It is noteworthy to mention the concept of earliness, which is referred to the time it takes the plant from sowing to harvestable product. It is assumed that variation in earliness can be due to an earlier switch from vegetative to reproductive growth or due to faster ripening of the fruit (Gur et al, 2010). Earliness can influence the harvest index, being both traits with important agronomic value in processing tomatoes.

CHAPTER 2

HYPOTHESIS AND OBJECTIVES

2.1 HYPOTHESIS

Considering that:

1) Salinity is a severe abiotic stress that affects tomato crop productivity in agriculture, since it limits plant growth by negatively affecting photosynthesis, a process that has been proposed to be a key factor in terms of connecting how environmental stressors modify carotenoid and sugar content from leaf to fruits.
2) Salt irrigation also reduces fruit quality indicators, such as fruit caliber and fresh weight, and increases total soluble solids.
3) Fruit coloration and carotenoids content have been reported to be differentially affected depending on the type of salt irrigation and tomato cultivar, but little is known about how their biosynthetic pathway respond under prolonged salt condition and its implicances to fruit overall development.

We propose the HYPOTHESIS:

Prolonged salt stress promotes fruit earliness and increases carotenoid synthesis of Solanum lycopersicum cv. Micro Tom.

2.2 OBJECTIVES

General objective:

To characterize the effect of prolongated saline irrigation on the carotenoids biosynthesis and fruit development parameters of *Solanum lycopersicum* cv. Micro Tom.

Specific objectives:

1) To determine the effect of saline irrigation on the photosynthetic capacity of tomato plants cv. Micro Tom

2) To analyze the effect of saline irrigation in fruit development of tomate plants cv. Micro Tom.

3) To characterize the carotenoids accumulation on tomato fruits cv. Micro Tom in response to saline irrigation.

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CHAPTER 3

Salinity modifies photosynthetic capacity, fruit development and carotenoids accumulation in tomato (*Solanum lycopersicum* L. cv. 'Micro-Tom') plants

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Abstract

In tomato, it has been described that high salt stress significantly dampens photosynthetic capacity and plant growth and, depending of the salt concentration as well as tomato variety, carotenoids biosynthesis could be either positively or negatively affected in fruits. However, evidences that connect such processes are poorly understood. In order to demosntrated a relationship between these parameters, tomato plants (*Solanum lycopersicum* L. cv. 'Micro-Tom') were exposed to saline irrigation with different NaCl concentrations. Interestingly, our results showed that constant saline irrigation (in the range of 40mM up to 160 mM) affected negatively photosynthetic components more severely after 8 weeks. After 14 weeks of salinity treatmentsa significantly reduction in the numbers of clusters and an early fruit development were observed, mainly due to accumulation of higher amounts of lycopene, total soluble sugars together with a reduction in fruit caliber and fresh weight (in moderate saline treatments or above). Relative expression of carotenoid pathway genes showed that in fruits under 11 weeks of salinity treatment with higher salt concentrations, lycopene biosynthesis related genes were induced, also β-Carotene and Lutein biosynthesis genes with moderate saline concentrations, showing an early response of the carotenoid pathway in comparison with the control. These results suggest that prolonogated stress correlates with an early display of ripening parameters.

Abbreviations: Fm, maximal fluorescence; Fv, variable fluorescence; Fv/Fm, the maximal photochemical efficiency of PSII; HPLC, high performance liquid

chromatography; PSII, photosystem II; NPQ, non-photochemical quenching; ROS, reactive oxygen species; WST, weeks of salt treatments.

Introduction

Salt stress is an environmental constraint that reduce photosynthetic efficiency and negatively affects plant growth and development (Yao et al. 2011).In plants like wheat, salinity inhibits the photosynthetic capacity (Munns et al. 2006; Chaves et al. 2009; El-Hendawy et al. 2017) due to alterations in the photosynthetic metabolism (Lawlor et al 2002) and a decrease in the CO_2 availability (Flexas et al., 2004).

Tomato (*Solanum lycopersicum*) is negatively affected by this condition, depending of the salt concentration and plant variety (Yin et al. 2010; Adams et al 1989; Adams 1991). This species is moderately tolerant to salinity (Bernstein 1964). However, tomato plants exposed to high levels of NaCl shown a decrease in size, number of leaves and stomatal density (Romero-Aranda et al. 2001), as well as, an alteration in water absorption by the roots (Cuartero et al. 1999). Interestingly, although salinity negatively affects the normal development of vegetative tissue, it has been shown that low salinity concentrations may improve the taste by increasing sugar and organic acids content, and depending on the cultivar, also, allowing an increase in carotenoid concentration in commercial tomato fruit (Adams 1989; 1991; De Pascale 2001). On the other hand, the most salt-resistant non-commercial tomato varieties are characterized by greater synthesis of carotenoids in high concentrations of salt (Juan et al. 2005; Coesel et al. 2008).

In tomato, carotenoids are synthesized in leaves, flowers and fruits. Lutein is the main carotenoid present in leaves; the xanthophylls, violaxanthin and neoxanthin in flowers; and lycopene in fruits. Each one of those conferring the characteristic yellow

and red colors to each tissue (Slimestad et al. 2008; Galpaz et al. 2006). Although lycopene is the main carotenoid in the fruit, others intermediates from this biosynthetic pathway may be found in lower concentrations, such as phytoene and phytofluene (colourless intermediates), β-carotene (responsible for orange color), and even γ-carotene, δ-carotene, lutein, neurosporene and α-carotene (Fray et al. 1993; Galpaz et al. 2006). In this way, the type of carotenoids present in each cultivar is highly conditioned by the genotype and environmental conditions to which the plant is exposed (Tiwari et al. 2013). Regarding carotenoids biosynthesis, the first committed step is catalyzed by the enzyme phytoene synthase (PSY), allowing the condensation of two geranylgeranyl pyrophosphate (GGPP) molecules to form phytoene. Then, through four dehydrogenation reactions, this colorless molecule is converted to lycopene, the carotenoid responsible for the red color of ripe tomato fruit (Fraser et al. 1999), steps involving the enzymes phytoene desaturase (PDS) and z-carotene desaturase (ZDS) (Lois et al. 2000). Finally, lycopene epsilon cyclase (LCYε) participates in the synthesis of α-carotene, while lycopene beta cyclase (LCYβ) participates in both, α and β-carotene biosynthesis (Da Silva Messias et al. 2014; Bartley et al 1995).

In order to detect an anticipated fruit development under constant saline irrigation, we characterized the effect on carotenoids biosynthesis pathway and metabolite accumulation in two developmental stages in *Solanum lycopersicum* cv. Micro Tom fruits, since tomato coloration is used as harvest criteria. We irrigated plants twice a week with different NaCl solutions (40, 80, 120 and 160 mM), and then analyzed carotenoids accumulation and genes associated with their biosynthesis. Also, in

order to determine a relation between plant photosynthetic capacity and carotenoids biosynthesis in response to salinity, different photosynthetic parameters were analyzed.

The results showed that constant saline irrigation negatively affects photosynthetic components after 8 week of sanility treatment (8 WST) more severily, reducing net photosynthesis in 22%, from the lower NaCl concentration (40 mM), up to a 50% reduction at 80 mM or much concentrated solutions, in comparison with the control condition, severely damaging the photoquemical apparatus by reducing photochemical efficiency and electron transport rate in 50%, and increasing in 50% the non photochemical quenching. In terms of plant phenotype, plants from 8 and 10 WST with 160 mM reduced in 25% the amount of total inflorescences per plant, leading to significant reduction of the number of fruit clusters per plant at 14 WST with 120 and 160 mM of NaCl solutions, exhibiting a 25 and 62% decrease, respectively in comparison with the control. Fruit quality was also measured by two methologies, the first named “chronological approach” measured fruits at 14 WST in all treatments, harvested when the control fruits were in red ripe stage and independently of the intensity of red coloration of each saline treatment, showing a significant reduction of 33% in fruit fresh weight with 80 mM, ecuatorial caliber presented a 40% decrease from 40 to 160 mM and total soluble content increased by 20% with 40mM to 27% with 160 mM, and also showing that red color intensity augmented signifantly in 25% with 80 mM and even more with the higher concentration of NaCl irrigation, comparing with the control, evidencing that fruits with the same time of exposure to saline irrigation had more red coloration, barely

appreaciable by sight, but quantified using a colorimeter and also presenting a significant increase in total carotenoid content from 40 to 160 mM of NaCl. The second strategy, named “Phenological strategy”, fixed the color criteria for harvest, selecting only red riped fruits of the different traetments regardless of their time under salinity treatment, showing that color did not present significant differences, neither total carotenoid content, evidencing a correct fruit selection, but showing similar results than the chronological approach in terms of fruit fresh weight, caliber and total soluble content, indicating that fruits with the same colour, had different overall quality due to salinity treatments. At molecular level, fruits from the chronological approach, were analized for relative expression of *PSY1*, *PDS* and *ZDS* showing upregulation under 160 mM in fruits at 11 WST, further evidencing a significant decrease in fruits at 14 WST at 120 and 160 mM. On the other hand, *LyCB* was upregulated from 80mM to 160 mM in 11 WST fruits decreasing at 14 WST ones. In terms of carotenoid levels, Lycopene and Violaxanthyn showed a significant increase in 11 WST fruits under 120 and 160 mM, lutein and β-carotene augmented their concentration at 11 WST from 40 to 160 mM. At 14 WST, lycopene showed a significant increase in all saline treatment conditions in comparison to the control, lutein showed an increase from 40 to 120 mM, meanwhile β-carotene and violaxanthin did not show significant differences. The overall results show that tomato fruit showed a significant early development under saline irrigation.

Materials and Methods

Plant Materials, growth conditions and salt treatment

Solanum lycopersicum cv. Micro-Tom seeds were imbibed at 4 ºC in darkness for 48 h, and then sown in plates with Murashige & Skoog culture medium 0,5X, without vitamins. Plates were placed on benches in a cultivation chamber under controlled experimental condition. The 7-day-old seedlings were transferred into pots with a mix of peat and vermiculite (2:1) and maintain under controlled environmental conditions, with relative humidity of 60–80%, temperature 23-25ºC, and 16 h/8 h photoperiod (day/night) with 120 mol $m^{-2}s^{-1}$. Plants were irrigated twice a week with deionized water and fertilized with 0.5X Hoagland solution (Sigma-Aldrich) for 120 days.

For salt treatments, plants with 5 to 6 leaves approximately, were irrigated twice a week with 0, 40, 80, 120 and 160 mM NaCl solutions (keeping the Hoagland fertilization), for 14 weeks until harvest. The experimental design was randomized into a complete block with three biological replicates (plants) for each treatment.

Plant sampling and phenotypic analysis of *Solanum lycopersicum* cv. Micro-Tom

Vegetative and reproductive tissues of *Solanum lycopersicum* Plants cv. Micro-Tom, exposed to continuous NaCl irrigation, were analyzed for 14 weeks. In the case of

vegetative tissue, samples were taken at 2 and 8 weeks of salt treatments (WST) for analysis of the photosynthetic parameters. To evaluate fruit development, the first two clusters for each plant were sampled, following two aproches. The first aproach considered a chronological sampling according to the fruits in the control treatment and the second one was done independent of the control condition. For the chronological approach, samples at 11 and 14 WST were taken from all treatments for the analysis of fruit phenotypic and physiological parameters, using control as a guide at 11 WST (Control mature green) and 14 WST (Control red ripe). Additionaly, the carotenoids content and gene expression were measured. For the second approach, fruits of the different treatments were harvested at mature green and red ripe, independent of control's developmental stage. Leading to harvest fruits from different treatments in distinct days, approximately for mature green and red ripe fruits 160 mM fruits were harvested 5,5 days, 120 and 80 mM at 3,5 days and 40 mM at 1,5 days before the control condition. For phenotypic and physiological analysis of fruits, the number of fruits per plant was registered and fresh weight and caliber was determined. Total soluble solids were measured in each fruit with a refractometer (R0020002, Veto y Cia. Ltda.). Color intensity was measured using a Konica Minolta CR-400, using the CieLAB color scale. Determinating L*, a* and b* values. A color is defined by an achromatic component L* (relative darkness or lightness) and chromatic descriptors (a* and b* values), indicating the red and yellow color intensity, respectively.

Photosynthetic Parameters

The net photosynthesis rate and stomatal conductance were measured in the first and second leaves of three randomly selected plants exposed to 2 and 8 weeks of saline irrigation, with a portable CO_2 infrared gas analyzer (Licor LR6400, Lincoln, NE, USA). This equipment controls light (300 µmol $m^{-2}s^{-1}$), temperature (20 ºC), humidity and CO_2 concentration.

Chlorophyll fluorescence parameters

Leaf chlorophyll fluorescence parameters were measured with a portable pulse amplitude modulated fluorimeter (FMS 2; Hansatech Instruments, Norfolk, UK), in the same leaves used for measuring photosynthetic parameters, previously adapted to darkness for 20 min. Maximum quantum yield (Fv/Fm), effective quantum yield (Φ PSII), and electron transport rate (ETR) were estimated according to Genty et al. (1989), whereas nonphotochemical quenching (NPQ) was estimated as described by Maxwell and Johnson (2000).

HPLC analysis

Carotenoids were extracted from five grams of tomato fruit, including skin, pulp and seed. Samples were processed with of 1 g quartz sand and 0.5 g ascorbic acid, in a mortar. The initial extraction procedure was carried out according Biacs and Daood (1994), followed by the extraction of carotenoids with 1.2-dichloroethan in a liquid –

liquid partitioning. Phases were separated according to a previously procedure described by Daood (2014), as well as carotenoids separation; quantification was done by a HPLC–DAD.

Gene expression analysis

Fruits from the first and second clusters of each plant were frozen with liquid nitrogen and total RNA extraction was carried out using the CTAB-Spermidine method, modified by Poupin et al. (2007). RNA was treated with RQ1 DNAse (Promega) and quantified by spectrophotometry using a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific™). The RNA purity was assessed using the A260/280 and A260/230 ratios given by the NanoDrop® instrument. For cDNA synthesis, RNA was reverse transcribed using RevertAid Reverse Transcriptase (Thermo Fisher), following manufacter´s instructions.

RT-qPCR analysis

Quantitative real-time PCR was carried out in an Applied Biosystem StepOne qPCR, using the corresponding Applied Biosystem 2.3 software and the SensiMixTM Plus SYBR commercial kit (Quantace). Primers suitable for amplification of housekeeping, carotenoid pathway and salt stress markers genes, were designed with Primer-BLAST tool available on NCBI webpage (http://www.ncbi.nlm.nih.gov/tools/primer- blast/). The list of the primer pairs is shown in Table 1. Dissociation curves were generated for each reaction. Threshold values (Ct) were employed to quantify relative gene expression according to Livak

and Schmittgen (2001).

Statistical analysis

All variables were analyzed using an ANOVA analysis followed by Tukey´s Multiple Comparison test were performed using GraphPad Prism, GraphPad Software (San Diego, CA). p-value < 0.05 were considered as significant differences.

Results

Effect of saline irrigation over photosynthetic parameters of *Solanum lycopersicum* plants cv. "Micro Tom".

To determine the impact of long-term salt stress in plant growth and development, 3week-old *Solanum lycopersicum* cv Micro Tom plants were exposed to saline irrigation twice a week with 0, 40, 80, 120 and 160 mM NaCl solutions (see Material and Method). Foliar photosynthetic parameters were evaluated at 2 and 8 weeks of salinity treatment (WST). Net Photosynthesis (NP) rate given by CO_2 assimilation was decreasing with the increasing of salt concentration (Fig 1A and 1B). Since NP reduction is partly due to a reduced stomatal conductance (SC) in several plant species (Munns et al. 2006, Chaves et al. 2009), SC was also analyzed at 2 and 8 weeks. At 2 WST, SC was reduced by 18 (non-significant) and 43% by 120 and 160 mM, respectively (Fig 1C). At 8 WST plants treated with 120 and 160, showed a

significant reduction in about 49 and 63%, respectively (Fig 1D), compared to the control condition.

To further evaluate the effects of salt stress-induced damage to photosynthetic apparatus, effective quantum yield (ΦPSII), electron transport rate (ETR) and non-photochemical quenching (NPQ) were measured. At 2 weeks of treatments ΦPSII and ETR were similar to normal for the samples treated with all salt concentrations (Fig 2A and 2C). However, NPQ analysis showed that 80, 120 and 160 mM NaCl could lead to significantly increase, compared with control samples (Fig 2F). At 8 weeks, differences were observed from 80 mM NaCl onwards, for these three measured parameters (Fig 2B, D and G), indicating that there was a cumulative negative effect on different phtosyntetic parameters, thus the time of exposure to salt irrigation is important, even within the least severe treatment.

Effects of salt stress on development of tomato plants

Plant phenotype

Total number of clusters per plant were quantified during two stages: flowering and ripening. In the first stage (at 9 weeks of saline irrigation, approximately) plants treated with 160 mM NaCl showed a reduction of 25% respect to the control plants, presenting an average number of 6 clusters with flowers per plant. In the case of plants treated with 40, 80 y 120 mM NaCl did not show any differences on this variable (Fig 3A). Likewise, counting the amount of clusters at 14 WST, plants treated with 120 and 160 mM NaCl showed a reduction of 27 and 50% respectively

in the number of viable clusters with fruits that did not abort during saline irrigation, showing an average number of 5 and 3 surviving clusters within the plant (Fig. 3B). For the next phenotype variables, results were standarized considering only the first two clusters of all treatments because they were similar in terms of development within each experimental condition, and also because 160 mM treated plants showed only 3 average remaining clusters at 14 WST, in contrast with other traetments showing significantly higher amounts of clusters per plant, limiting cluster comparisson. At 7 WST total number of flowers were counted and individually marked with colored strings, showing a similar initial number per cluster within all saline treatments (Fig 4A). At 9 WST the percentage of fruit set was estimated according to the initial number of flowers per cluster, showing a significant reduction in plants treated with 160 mM of NaCl presenting a decrease of approximately 30% in comparison with the control (Fig 4B). The percentage of fruit retained in relation to the initial number of flowers, 160 mM trated plants showed a 30% significant reduction, in comparison with control condition (Fig 4 C). Finally, fruit load per plant was measured by counting the total amount of fruits per plant at 14 WST, showing a 16, 33 and 50% significant decrease in 80, 120 and 160 mM salt treatments, respectively, in contrast with control condition (Fig 4D).

Fruit development parameters

Considering the chronogical approach, fruits from all treatments were harvested according to the control developmental stage. At 11 WST (mature green in control plants) fruits from treatments 0, 40, 80 and 120 mM presented green coloration,

indicating a similar stage of fruit development, while fruits from plants irrigated with 160 mM showed a yellowish coloration in comparison with the control condition. These results suggest that 160 mM salt treatment can induce the apparition of ripening parameters faster than lower salinity regimes. (Fig. 5A). At 14 WST, fruits from all treatments did not show visually differences from control (red ripe). Then, fruits were evaluated using a colorimeter that measure CieLAB color scale. Regarding a* color parameters (Fig. 5C), tomato fruits from salt-stressed plants irrigated with 80, 120 and 160 mM NaCl, presented higher values compared with control treatment. No significant differences with non-stressed or control plants were observed when a moderate salt concentration was used (40 mM NaCl, Fig. 5C). The salinity effect on b* index was similar to those observed in a* values (Fig. 5D). On the contrary, L* values were only affected in tomato fruits from plants under 80 mM NaCl treatment, in which a slight decrease in fruit brightness was observed (Fig. 5E). In contrast, no significant differences were found following the phenological approach, harvesting fruits from different treatments at mature green and red ripe, independently from the control condition developmental stage (Fig. 5F, G and H), indicating that fruits presented similar coloration within treatments using this approach.

Variables of fruit quality were also measured using the two different approaches (see Materials and Methods). Chronogically (14 WST fruits), when the control condition reached the red ripe stage, all treatments showed a significantly reduction in fruit fresh weight (FW) together with the increase in salinity, reaching approximately 50% reduction in those irrigated with 120 and 160 mM NaCl, with respect to the non-

treated controls (Fig. 6A). Interestingly, there were no significant changes in FW in tomato fruits when plants were grown under a moderate stress (40 mM NaCl). Also, fruit caliber decreased in all salt treatment with respect to the control (Fig. 6B). When comparing salt treatment, no significant differences were observed. Contrary to the pattern of variation of FW and fruit diameter, total soluble solid content (Brixº) increased in plants under salt conditions, especially in those fruits from plants treated with high salt concentration. A concentration-dependent increment of Brixº was observed in fruits from salt-treated plants, with an increase of about 28 and 47% under 40 mM and 160 mM NaCl treatment, respectively (Fig. 6C). Similar results were observed for fresh weight and brix degrees by Yin Y-G in 2010. Interestingly, fruits harvested using the phenological approach, at red ripe, presented likely the same results, with mild effect on each variable. It was found that fruit fresh weight had the same behavior as the chronological approach (Fig. 6E), fruit caliber was significantly reduced in all salt treatments with the same intensity (Fig. 6D) and that the total soluble solid accumulation had a sigfinicant accumulation in salt treatments (Fig. 6F).

Carotenoids accumulation and expression of genes related to their synthesis

To determine the changes in the carotenoids concentration in fruits obtained from plants exposed to different salinity treatments, total carotenoids accumulation was measured using samples from the chronogical (11 and 14 WST) and phenological (mature green and red ripe) approaches (Fig. 7A, B, C and D, respectively). Significantly higher carotenpids concentration were found in 11 WST with all saline

treatments (Fig. 7A and B), while no significant differences were found with the phenological strategy neither in mature green nor red ripe color stage (Fig 7. C and D). Analysis for individual carotenoid accumulation was made only in fruits with the chronological strategy for both 11 and 14 WST.

Individual carotenoid content was quantified from fruits of 11 and 14 WST by HPLC-DAD (Fig. 8 A, B, C and D). At 11 WST, lycopene content increased more than 50% in plants treated with 120 and 160 mM NaCl, while in plants at 14 WST, an increase on this carotenoid was observed from 40 mM onwards (Fig 8A). For lutein, the results were very similar, however at 14 WST plants irrigated with 160 mM NaCl showed similar lutein concentration than the control ones (Fig 8B). The β carotene content showed differences between treatments only at 11 WST, while in the case of the violaxantina content, the results were more variable, and diffrences were observed in both sampling times (11 and 14 WST), but with different concentration (Fig. 8 C). Interestingly violaxantin, a member of the xanthofile carotenoid group, was significantly accumulated in c 120 and 160 mM of NaCl at 11 WST, but no differences were found at 14 WST (Fig 8 D).

It is important to note that for all carotenoids measured, 120 and 160 mM NaCl treatments have an important effect on their accumulation, at 11 WST. This is in agreement with the results obtained for the total carotenoid content quantification (Fig 7A), since a higher content was observed in fruits of plants treated with 120 and 160 mM NaCl.

In order to elucidate the response to salinity of several carotenoid biosynthetic genes and compare it with the carotenoid content shown in Fig. 8, samples were taken at 11 and 14 WST and *PSY1, PDS, ZDS* and *LyCB* transcript accumulation was quantified. At 11 WST, *PSY1*, *PDS* and *ZDS* showed a significant transcript accumulation in fruits from plants exposed to 160 mM NaCl compared to the control, but no significant differences were found between plants treated with lower salt concentrations and control plants (Fig 9 A, B and C). On the other hand, at 14 WST, *PSY1*, *PDS* and *ZDS* transcripts accumulation decreased in plants exposed to high concentrations of salt (120 and 160 mM, Fig. 9 A, B and C right), which is similar to the decrease in the lutein concentration (Fig 8 B). Interestingly, at 11 WST *LyCB* transcripts accumulation increases from 80 mM NaCL onwards, while only an increase with 80 mM of salt treatment was observed at 14 weeks (Fig. 8). These results suggest that probably accumulation of B-carotenoid or lutein occurs with lesser salt content than lycopene.

Discussion

Salinity is one of the most severe abiotic stress affecting crop productivity, since its limits plant growth through different mechanisms (Orsini et al. 2010; Yao et al. 2011). In tomato, salinity has a significant economic impact, as it not only affects vegetative growth, but also affects fruit development. Moreover, depending on the cultivar, salt stress culminates with a negative or positive effect, due to the stress tolerance of each cultivar (Juan et al. 2005, Coesel et al. 2008). In tomato as well as other specie,

one of the main features that can be affected by salinity is the photosynthetic capacity, which in turn, can be related to the decrease of plant growth and productivity (Munns et al. 2006, Chaves et al. 2009).

Several parameters allow to quantify plant photosynthetic capacity. In this work the net photosynthesis rate, the stomatal conductance, as well as, several leaf chlorophyll fluorescence parameters, were measured (Fig. 1 and 2). The reduction in the PSII quantum yield (Φ PSII) and in the electron transport rate induced by salinity, in addition to the increment in the non-photochemical quenching (NPQ), showed that salt stress could negatively affect the ability of plants to use light, and the capacity to produce photoassimilates. Besides, it could weaken the quality of the thylakoid membranes, which may have direct negative effects on plant development. Similar results were observed in wheat by El-Hendawy et al. (2017), as well as, in tomato by Zhang et al. (2012).

It should be noted that the results showed in fig. 1 and 2 allowed to demonstrate that the effects of salt are concentration-dependent, since the results obtained at low concentrations are, mostly, very different to the results obtained at high concentration. In the case of the reproductive tissue, previous studies have shown that salinity negatively affects the number of tomato fruits, but at the same time improves their quality (Adams 1989; 1991; De Pascale et al. 2001). However, these contradictory effects absolutely depend on the cultivar and on the ability of plants to tolerate this stress (Juan et al. 2005, Coesel et al. 2008). The results of this work allowed to determine that high salt concentrations affect the total number of fruits, reducing both, the number of clusters per plant and the number of fruits per cluster.

However, although fruits presented a higher content of total soluble solids, which positively affects their quality, at the same time, showed a decrease in fresh weight and fruit caliber, which in turn are consider as negative quality traits (Fig. 6). One of the most interesting results of this work was that the effect over the photosynthetic capacity and fruit quality begins with a moderate salinity. De Pascale et al. (2001) previously observed that an increasing concentration of salt in irrigation water negatively affected fruit fresh weight and increased total soluble content. Thus, it is possible that the decrease in the photosynthetic capacity could have important effects on fruits development, affecting the parameters before mentioned.

It is important to note that damages in the photosintethic apparatus can allow that the energy cannot be appropriately dissipated, as was reported by Demmig-Adams and Adams (1992), and consequently reactive oxygen species (ROS) can be synthesized as a consecuence of the stresso. Plants must reward the damages through the synthesis of antioxidant molecules such as carotenoids, among which, violaxanthin, through the synthesis of zeaxanthin has important protective functions (Havaux and Niyogi 1999). In this work we observed increasing accumulation of violaxanthin at 11WST and other carotenoids, specially lycopene at 14 WST, with different salt concentrations, suggesting that stress signals like ROS production can upregulate carotenoids biosynthesis in fruits. Even though sme tomato landraces where shown to be more tolerant to salt stress than cultivars, and also accumulated more carotenoids in fruits (Massaretto et al., 2018), further experiments need to be done in order to validate this hypothesis. It is important to note that changes observed in the carotenoids content in response to salinity may be cultivar-

dependent. For example, De Pascale et al., (2001) used a hybrid called HC01, which showed a significant increase in lycopene and total carotenoids content under low to moderate salt irrigation, together with an important decrease at higher concentrations, in opposite to the results of this study. Also, Massaretto et al., (2018), showed that tomato landraces like Negro Yeste presented higher basal amount of lycopene, higher salt tolerance and an increased accumulation of lycopene in response to high salt irrigation than cultivar Moneymaker. However, in all cases this is possibly related to the cultivar´s ability to cope with stress and to defend against the oxidative damage that it could generate. It could be interesting to further investigate if tomato cultivars with higher carotenoids content in fruits, could tolerate a more severe salt stress rather than low carotenoids cultivars and try to find out a way to increase carotenoids levels to better compensate salt stress.

Likewise, carotenoids biosynthetic genes analyzed in this study (Fig. 8) showed a different profile at the transcript levels between 11WST and 14 WST in response to salinity, possibly because the fruit stress response is modified with the stage of development, showing an earliness response, as a biological strategy to quickly produce offspring. At 11 WST, an increase in total carotenoids levels was observed from 40 mM onwards; particularly lycopene and violaxanthin increased from 120 to 160 mM and lutein and β-carotene from 40 mM onwards. However, *PSY1, PDS and ZDS* transcript levels showed an increment only with 160 mM, probably due to timming. RNA accumulation is not linearly related with metabolite accumulation at the exact same time, since there are several types of regulations taking place, from transcription to post transduction, that were not analyzed on this study. On the other

hand, transcripts levels of carotenoids genes pathway were overall diminished but significant amount of lycopene was detected in 14 WST fruits, within all salinity treatments. Variations in the carotenoids content in response to salinity, may not only occurred due to changes in the expression of the biosynthetic pathway genes, but be consecuence of a post-transcriptional regulation or a modification in the storage capacity of these metabolites in the plant.

Fig. 10 shows a summary of the effect observed in fruits from plants exposed to saline irrigation and how it negatively affects photosynthesis, promoting significant differences in transcript levels of genes related to carotenoids biosynthesis, as well as, in the concentration of the differents carotenoids measured and fruit development variables, such as earliness.

This work allows to correlate the effect of salinity on the vegetative tissue of tomato plants cultivar "Micro Tom" and the effect produced over the fruits. The results observed in the different fruit stages analyzed, showed that there is an important relationship between the photosynthetic response and plant productivity.

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Figures and Tables

Table 1. List of primers used for gene expression analysis

GENE	PRIMERS	SEQUENCE (5´→ 3´)
UBIQUITIN	Forward	GGACGGACGTACTCTAGCTGAT
	Reverse	AGCTTTCGACCTCAAGGGTA
ACTIN	Forward	GAAATAGCATAAGATGGCAGACG
	Reverse	ATACCCACCATCACACCAGTAT
PSY1	Forward	ATTTGCTGGAAGGGTGACCGAT
	Reverse	GCTCAATTCTGTCACGCCTTTC
PSY2	Forward	GGACGAGATTGAAGCAAACGAC
	Reverse	TGCATAAGCAATGGGCAACGTC
PDS	Forward	CTTCAATGGAAGGCGCTGTCT
	Reverse	ACGCTTGCTTCCGACAACTTCT
ZDS	Forward	TTGGAGCGTTCGAGGCAATTGA
	Reverse	GCCAATGCAAGATCTGCAAAGC
CRTL-B	Forward	TCAGAGAGTCGTTGGAATCGGT
	Reverse	AACAGGAGCCGCAGCTAGT
CRTL-E	Forward	TGGTCTTACATACCGGTTGGTG
	Reverse	TGTGGCTGGATGAACCATGCTA

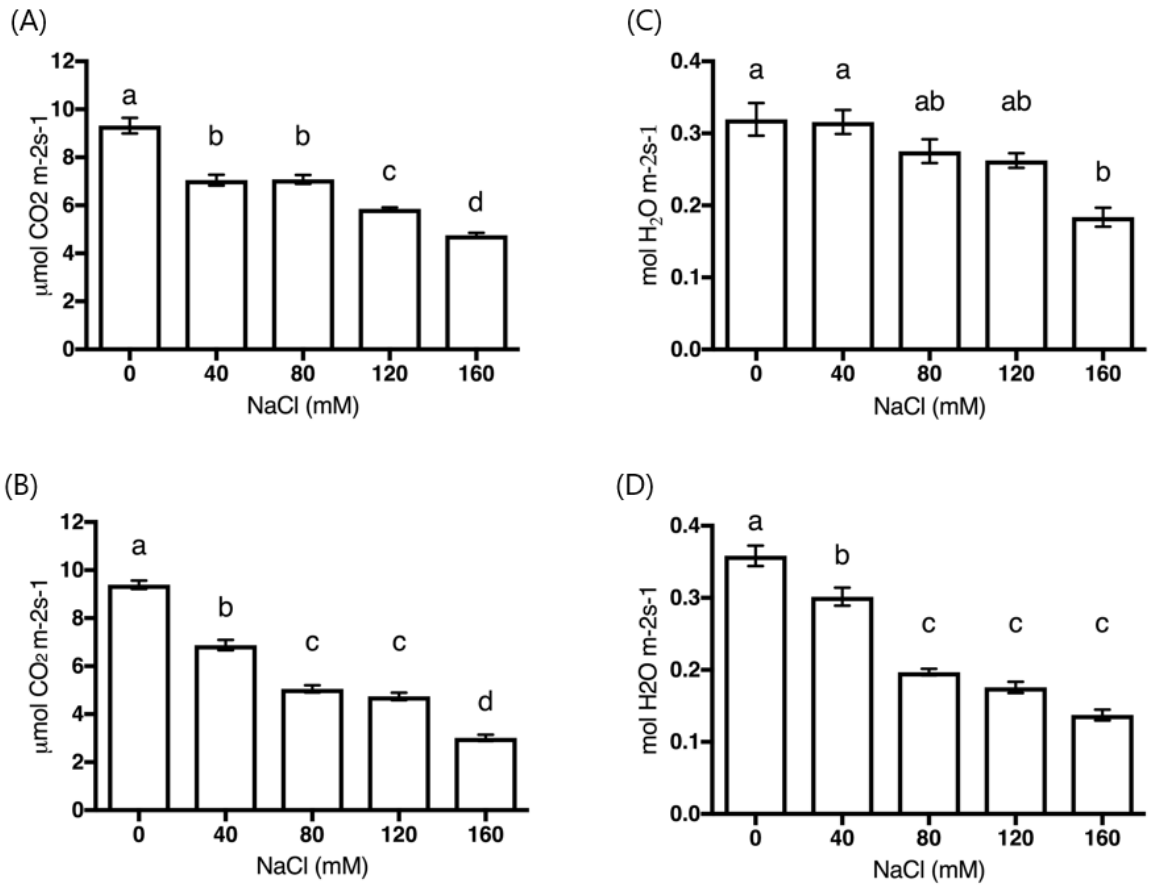

Fig. 1: Net photosynthesis rate and stomatal conductance in plant exposed to saline irrigation. **A**. Net photosynthesis at 2 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **B**. Net photosynthesis at 8 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **C**. Stomatal conductance at 2 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **D**. Stomatal conductance at 8 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. Values are means ±SD (n=3). Different letters indicate statistical significance of means estimated using Anova and Tukey test ($p < 0.05$).

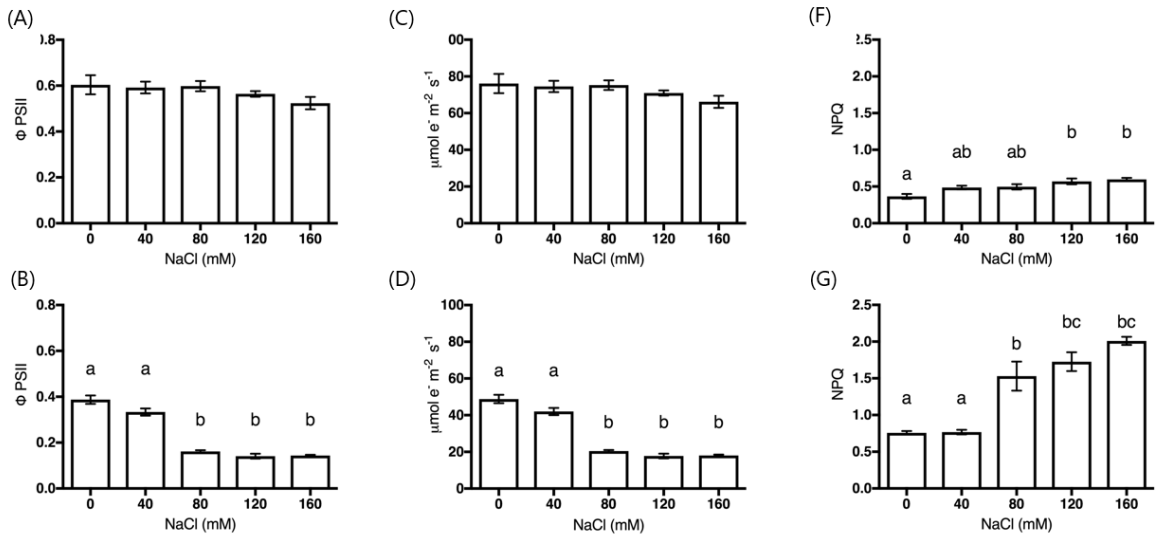

Fig. 2: Leaf chlorophyll fluorescence parameters of plant exposed to saline irrigation. **A.** PSII quantum yield (PSII) at 2 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **B.** PSII quantum yield (PSII) at 8 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **C**. Electron transport rate (ETR) at 2 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **D**. Electron transport rate (ETR) at 8 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. F. Non-photochemical quenching (NPQ) at 2 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **G**. Non-photochemical quenching (NPQ) at 8 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. Values are means ±SD (n=3). Different letters indicate statistical significance of means estimated using Anova and Tukey test ($p < 0.05$).

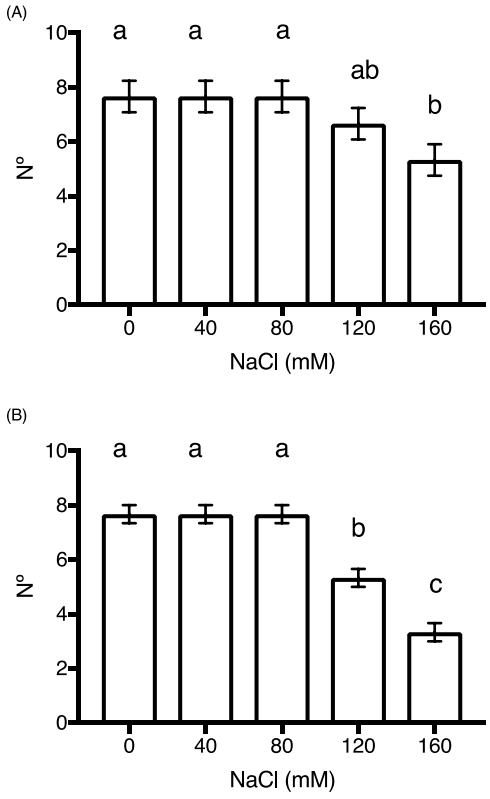

Fig. 3: Total number of clusters and clusters with ripe fruits per plant in response to saline irrigation. **A.** Total number of clusters per plant in those exposed to 14 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **B.** Clusters with ripe fruits per plant in those exposed to 14 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. Values are means ±SD (using three biological replicates, each one containing a pool of data from six plants per treatment). Different letters indicate statistical significance of means estimated using Anova and Tukey test ($p < 0.05$).

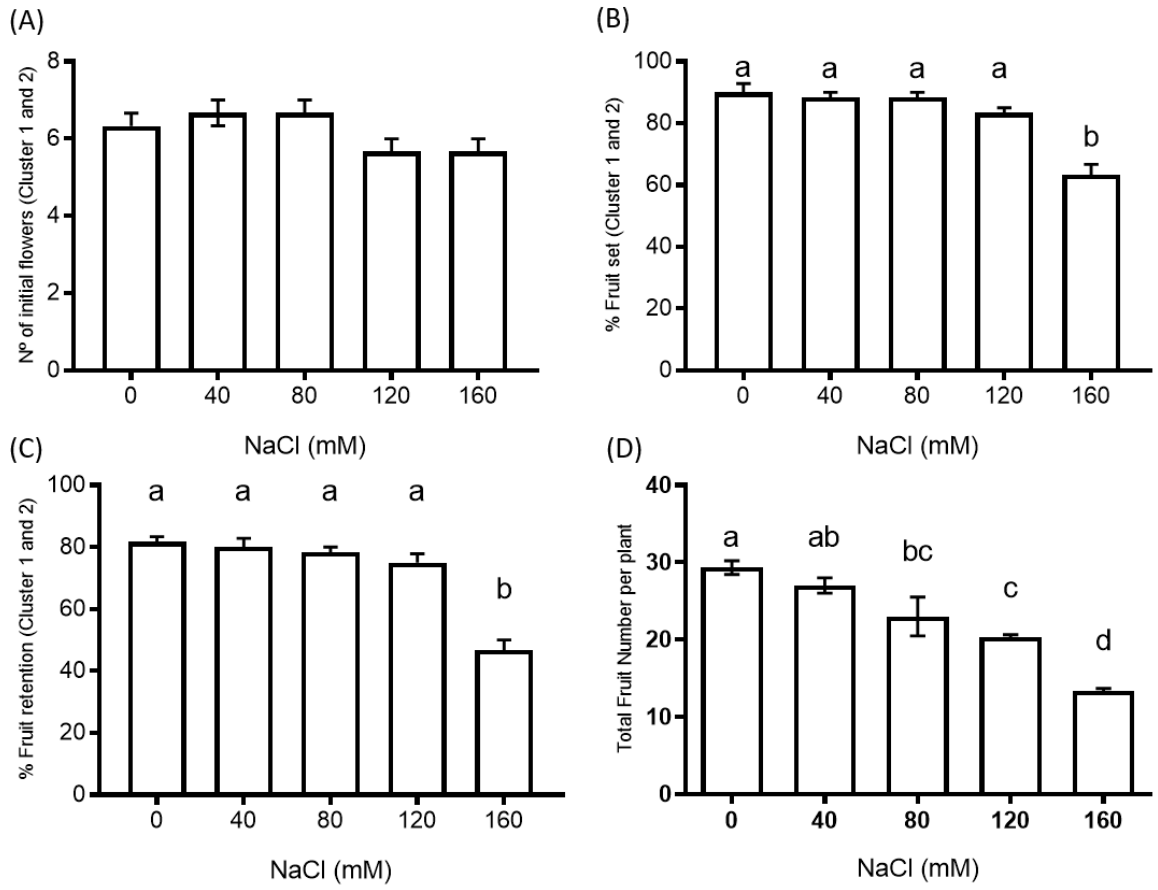

Fig. 4: Number of Initial flowers, percentage of fruit set, percentage of fruit retention and total number of fruits per plant. **A.** Initial flowers at 7 WST in clusters 1 and 2 of. **B.** Percentage of fruit set at 9 WST. **C.** Percentage of fruit retention at 14 WST. **D.** Total number of fruits per plant at 14 WST. Values are means ±SD (using three biological replicates, each one containing a pool of data from six plants per treatment). Different letters indicate statistical significance of means estimated using Anova and Tukey test ($p < 0.05$).

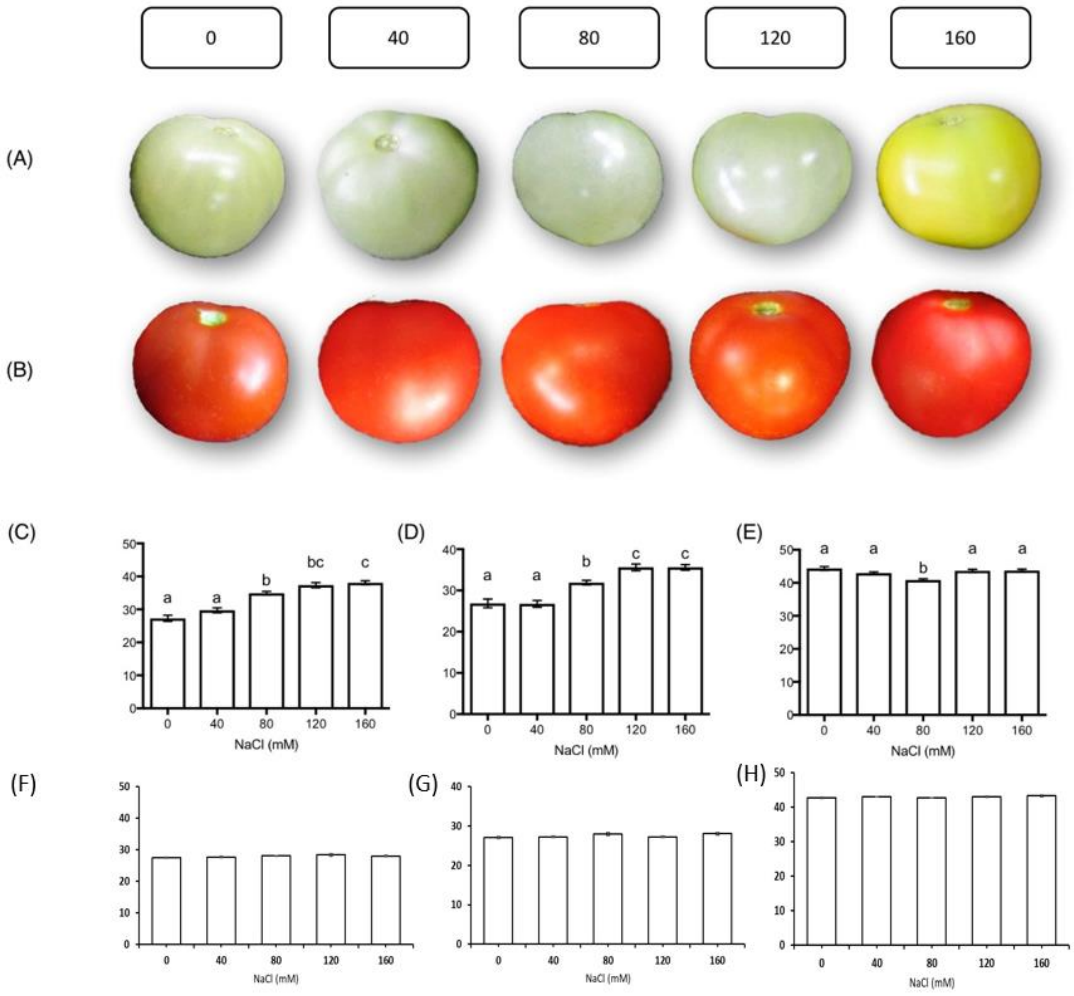

Fig. 5: Color differences of fruits from plants exposed to saline irrigation. **A.** Photography of fruits from plants exposed to 11 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **B.** Photography of fruits from plants exposed to 14 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **C, D and E.** Indexes A, B and L, respectively, of fruits from plants exposed to 14 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **F, G and H.** Indexes A, B and L, respectively, of red ripened fruits from plants exposed to irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a

week. Values are means ±SD (using three biological replicates, each one containing a pool of data from six plants per treatment). In C, D and E different letters indicate statistical significance of means estimated using Anova and Tukey test ($p < 0.05$).

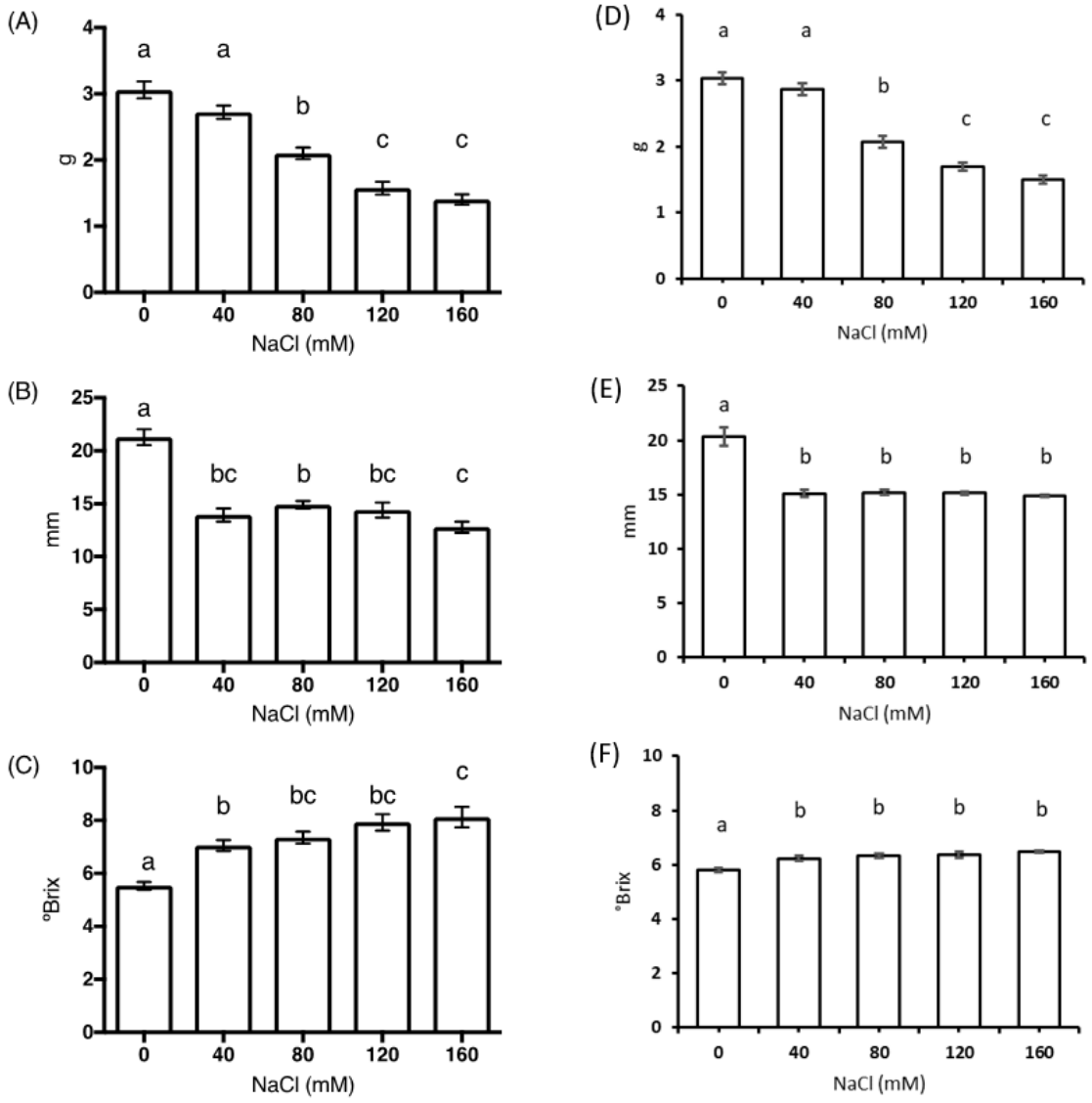

Fig. 6: Tomato fruits development of plant exposed to saline irrigation. **A.** Fresh weight of fruits of exposed to 14 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **B.** Caliber of fruit of plants exposed to 14 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **C.** Brix degrees (%) of fruit of plants exposed to 14 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **D.** Fresh weight of red ripe fruit of plants exposed to irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week, during complete plant development. **E.** Fruit caliber of red ripe fruit of plants exposed to irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week, during complete plant development. **F.** Total soluble solid content of red ripe fruit of plants exposed to irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week, during complete plant development.Values are means ±SD (using three biological replicates, each one containing a pool of data from six plants per treatment). Different letters indicate statistical significance of means estimated using Anova and Tukey test ($p < 0.05$).

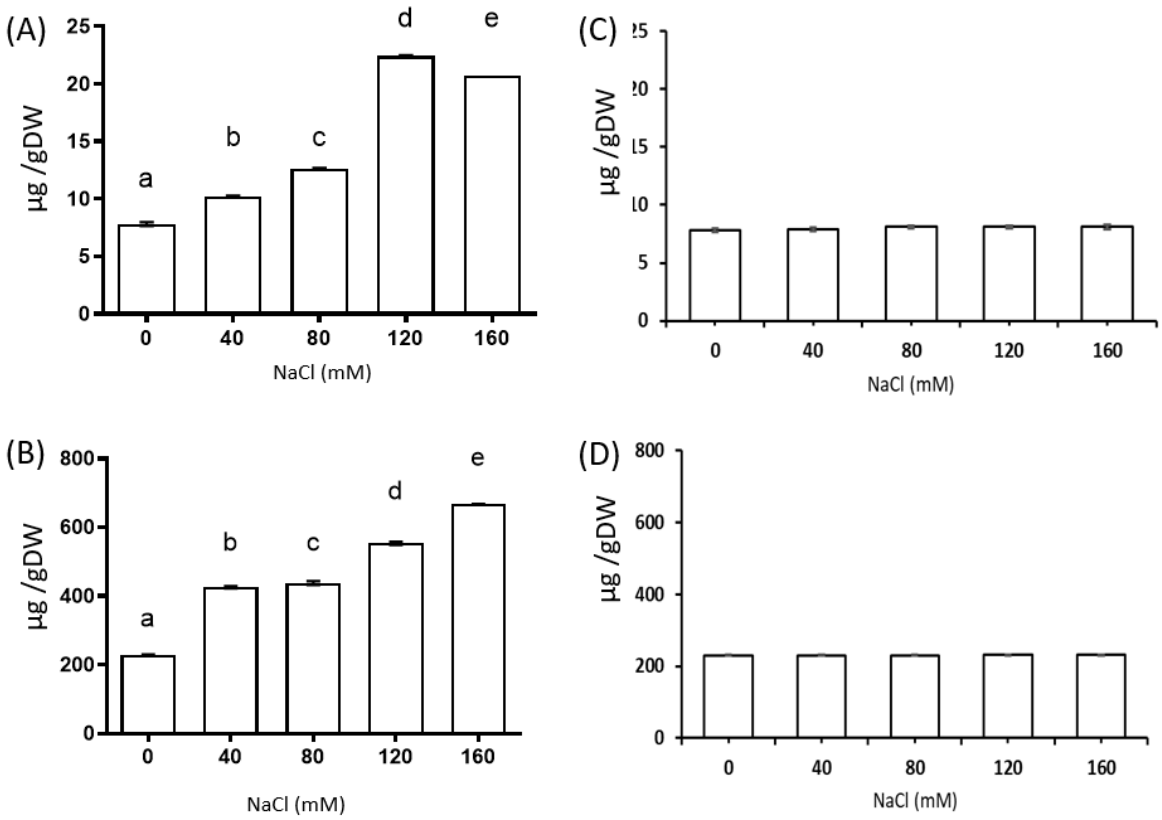

Fig. 7: Total carotenoids accumulation in fruits from plants exposed to saline irrigation. **A.** Total carotenoid content in fruits from plants exposed to 11 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **B.** Total carotenoid content in fruits from plants exposed to 14 weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **C.** Total carotenoid content in mature green fruits from plants exposed to of irrigation with 0, 40, 80, 120 and 160

mM NaCl solutions, twice a week, during the complete plant development. **D.** Total carotenoid content in red ripe fruits from plants exposed to of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week, during the complete plant development. Values are means ±SD (using three biological replicates, each one containing a pool of data from six plants per treatment). Different letters indicate statistical significance of means estimated using Anova and Tukey test ($p < 0.05$).

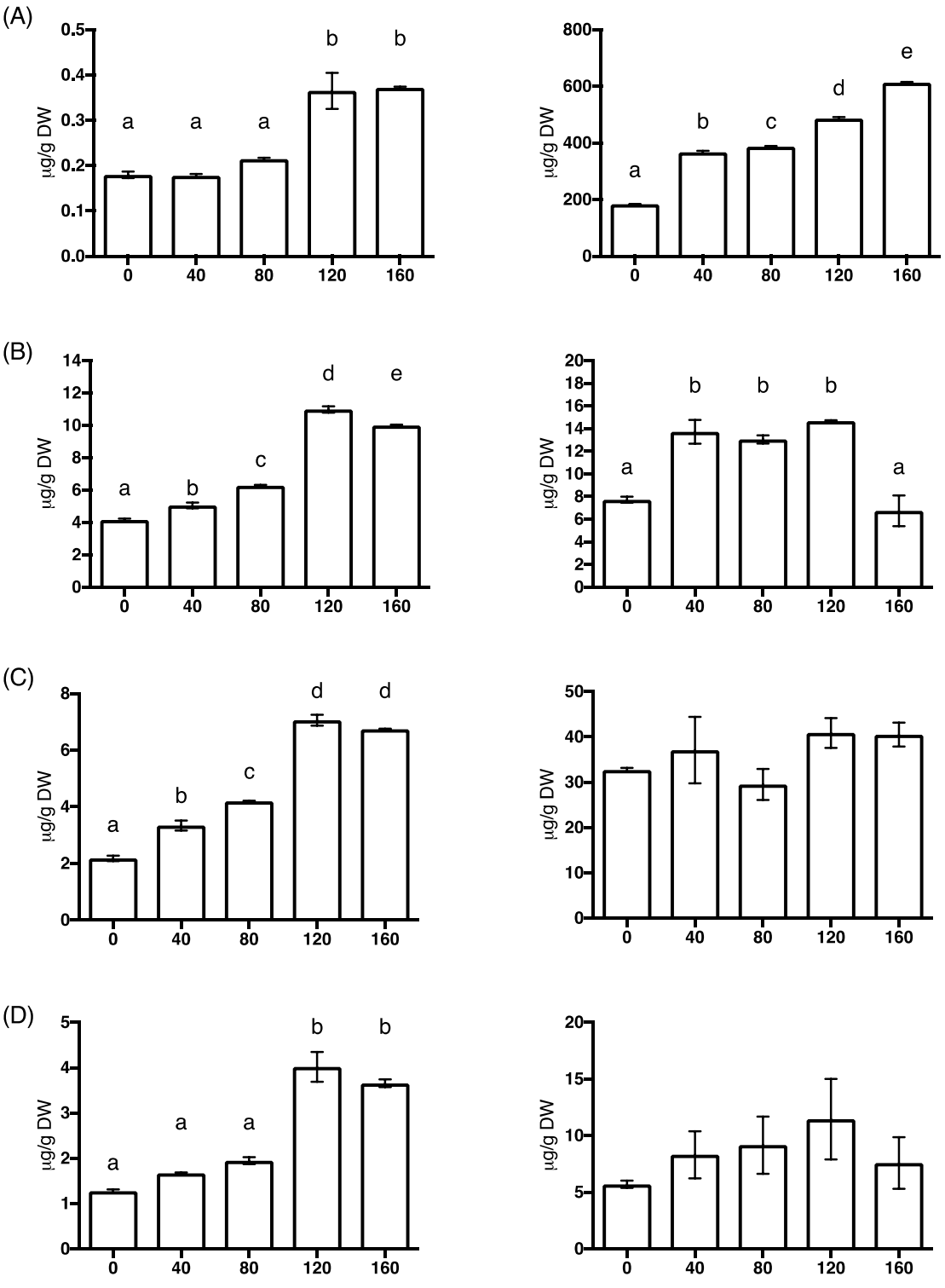
(A)
µg/g DW
0.5
0.4
0.3
0.2
0.1
0.0
a a a b b
0 40 80 120 160
800
600
400
200
0
a b c d e
0 40 80 120 160
(B)
µg/g DW
14
12
10
8
6
4
2
0
a b c d e
0 40 80 120 160
20
18
16
14
12
10
8
6
4
2
0
a b b b a
0 40 80 120 160
(C)
µg/g DW
8
6
4
2
0
a b c d d
0 40 80 120 160
50
40
30
20
10
0
0 40 80 120 160
(D)
µg/g DW
5
4
3
2
1
0
a a a b b
0 40 80 120 160
20
15
10
5
0
0 40 80 120 160

Fig. 8: Carotenoids accumulation in fruits from plants exposed to saline irrigation. **A.** Lycopene content in fruits from plants exposed to 11 (left) and 14 (right) weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **B.** Lutein content in fruits from plants exposed to 11 (left) and 14 (right) weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **C.** β-carotene content in fruits from plants exposed to 11 (left) and 14 (right) weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **D.** Violaxantin content in fruits from plants exposed to 11 (left) and 14 (right) weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. Values are means ±SD (using three biological replicates, each one containing a pool of data from six plants per treatment). Different letters indicate statistical significance of means estimated using Anova and Tukey test ($p < 0.05$).

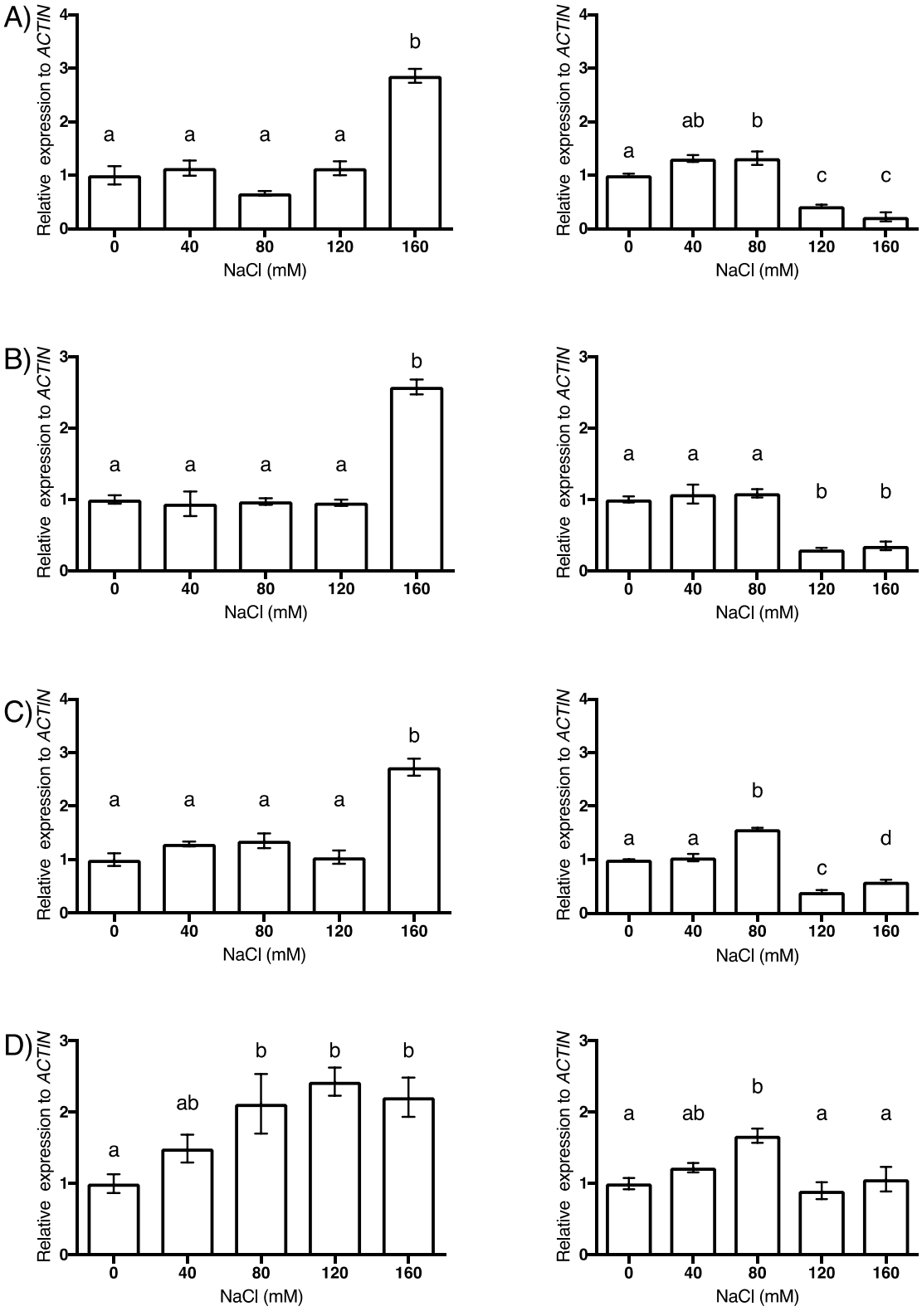
A)
B)
C)
D)
Relative expression to ACTIN
NaCl (mM)
0
40
80
120
160
a
ab
b
c
d

Fig. 9: Quantification of transcript levels of genes related with carotenoids biosynthesis, by quantitative PCR, in fruits from plants exposed to saline irrigation. **A.** *PSY1* transcript levels quantification in fruits from plants exposed to 11 (left) and 14 (right) weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **B.** *PDS* transcript levels quantification in fruits from plants exposed to 11 (left) and 14 (right) weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **C.** *ZDS* transcript levels quantification in fruits from plants exposed to 11 (left) and 14 (right) weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. **D.** *LyCB* transcript levels quantification in fruits from plants exposed to 11 (left) and 14 (right) weeks of irrigation with 0, 40, 80, 120 and 160 mM NaCl solutions, twice a week. Values are means ±SD (using three biological replicates, each one containing a pool of data from six plants per treatment). Different letters indicate statistical significance of means estimated using Anova and Tukey test ($p < 0.05$).

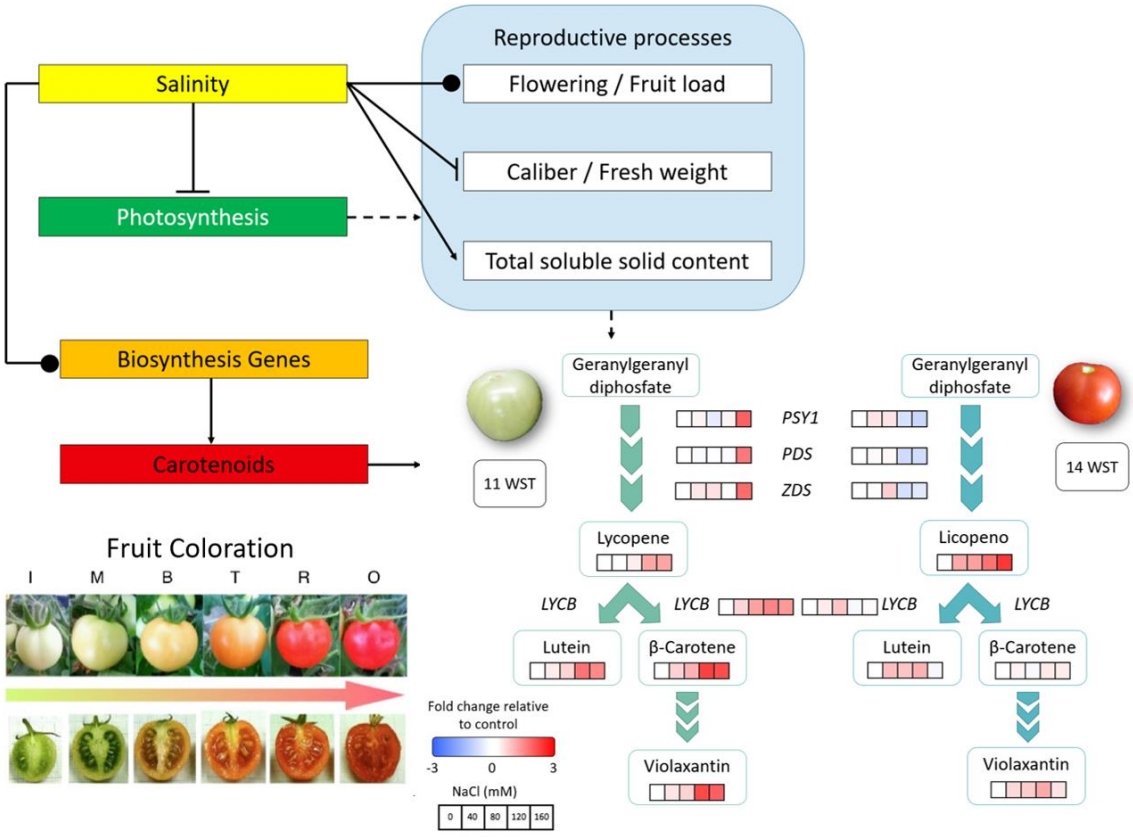

Fig. 10: Model proposed to summarize the results obtained from this study. The negative effect of salinity in photosynthesis and the processes of fruit development that were affected are shown. Lines indicate negative interactions, positive ones are shown with arrows, dual effects with circles and stripped lines denote unknown processes.

CHAPTER 4

CONFERENCE PRESENTATION

Leiva-Ampuero, A; Vega, A. Characterization of *SlNAP1* and *Slnap2*, NAC-like transcription factors encoding genes in *Solanum lycopersicum*. V Plant biology Meeting. Olmué, Chile 1-3 December. 2010.

Leiva-Ampuero, A; Stange Klein, C; Inostroza, C; Reyes-Díaz, M; Vega, A. Effect of long-term salinity stress on carotenoid biosynthesis in *Solanum lycopersicum* fruits. X Plant biology Meeting. Valdivia, Chile 1-4 December. 2015.

CHAPTER 5

CONCLUDING REMARKS

Tomato (*Solanum lycopersicum*) is one of the most important crops in the world, as is the most cultivated vegetable and with a largest planted area. In addition to its commercial importance, tomato is a model vegetable system to understand fleshy fruit development, which makes it a crop of great agricultural, scientific and technological importance.

Plants have generated important adaptive responses to biotic and abiotic stress, leading to a diverse set of morphological and biochemical characteristics. In this thesis, we analyzed the effect of salinity, one of the most severe abiotic stresses affecting crop productivity (Orsini F. et al, 2010; Yao et al. 2011), on the development of tomato plants and their fruits.

The production of tomato may show significant economic losses when is affected by salinity, since, it can negatively affect plant growth and the normal fruit development. Although tomato is moderately tolerant to salinity (Bernstein, L. 1964), plants exposed to high levels of NaCl show a decrease in size, number of leaves, stomatal density (Romero-Aranda R. et al. 2001) and seeds germination, among others (Cuartero, J. et al. 1999). While in the case of fruits, the effect can be positive or negative, depending on the cultivar, since low salinity concentrations may improve the taste by increasing sugar and organic acids content, and induce an increment of the carotenoids concentration (Adams, P. 1989; 1991; De Pascale, S. 2001).

The results obtained in this work showed that saline irrigation, from low to high salt concentrations, caused a decrease on plants photosynthetic capacity and several phenotypic and physiological changes triggered by salt irrigation factor. Moreover, we observed an earliness response, characterized by changes in color of fruit from high salinity treated plants. We assessed the effect of salinity over some genes related to the carotenoids biosynthesis pathway (*PSY1, PDS and ZDS*), revealing important differences in transcript abundance at high salt concentrations after 11 weeks of salinity treatment. Additionally, the carotenoids quantification also revealed important differences between the above analyzed parameters, which is not related in accuracy with the response of the analyzed genes at 11 and 14 weeks of treatments. These findings suggest that in tomato, the basal salinity response machinery is supplemented with secondary metabolic routes that are activated by a different developmental signal. Likewise, the results observed in the different fruit stages analyzed, showed that there is an important relationship between the photosynthetic response and plant productivity.

In the future, it could be interesting to develop field trials in order to seek for industrial applications for these finding and accelerate carotenoid accumulation, for exemple in processed tomato industry, were more colored fruits are needed or to fasten the harvest date. It could be worthy to develop functional genomics in order to have a global knowledge and to understand how the different metabolic routes are affected at different times in response to saline stress, aiming to promote carotenoids accumulation in fruits.