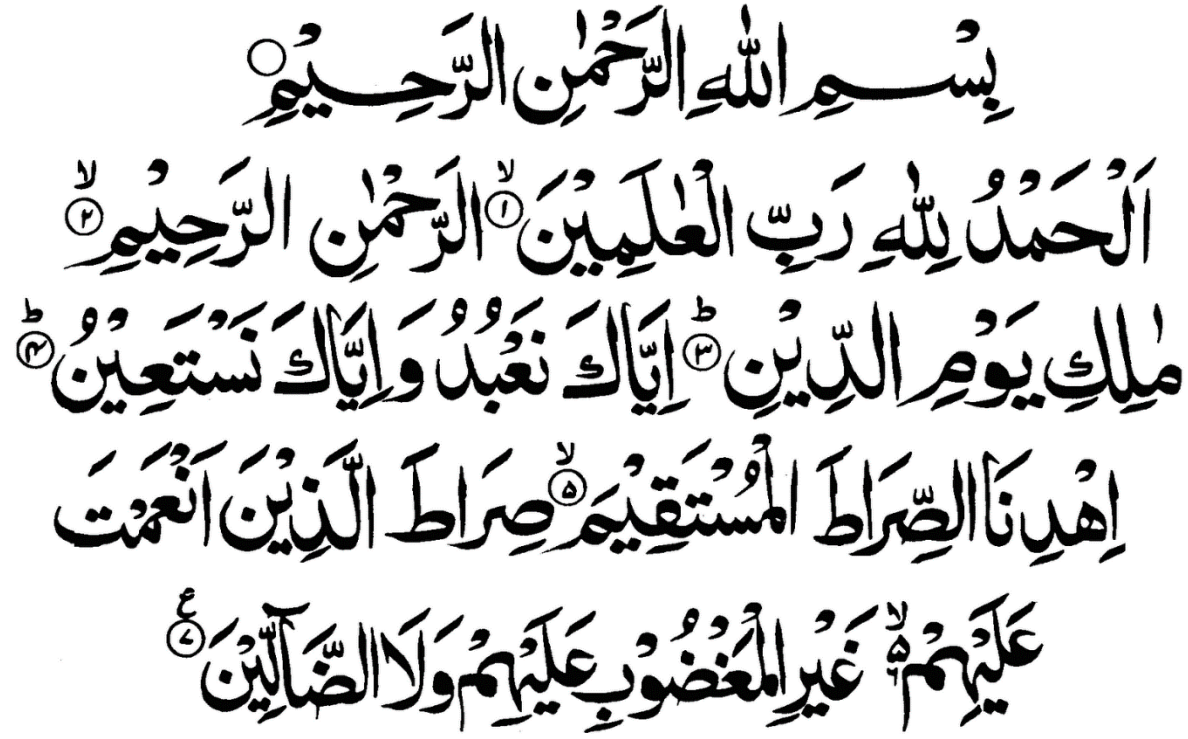

All praise belongs to Allah, Lord of the world, the Most Gracious and the Most Mercifull, Master of the day of the Judgement, You (Alone) we worship and you (Alone) we ask for help (for each and everything), guide us to the Straight Way, The Way of those on whom You have bestowed Your Grace not of those who earned You Anger nor of those who went astray [Al-Fatiha].

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MORPHOLOGICAL, ECOPHYSIOLOGICAL AND BIOCHEMICAL RESPONSES TO SALT STRESS IN EGGPLANT (*SOLANUM MELONGENA* L.)

Sami Hannachi

Thesis submitted in fulfillment of the requirements
for the degree of Doctor (PhD) in Applied Biological Sciences

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Summary

The Mediterranean regions are currently experiencing increasing salt stress problems. Because of its economic importance, the negative impact of salty water on plant productivity has been investigated in many crops. Until now, studies on salt tolerance in eggplant are limited and have been focused on growth parameters and horticultural performances as a selection criterion. However screening salt tolerance of genotypes based on agronomic parameters has many inherent disadvantages such as differential growth and developmental patterns between genotypes if no stress is present, and logistical and time constraints with long-term growth comparisons.

Facing salt stress, the plant uses different morphological and cellular responses to adapt. Thus, the search for traits related to salt tolerance is an important step for the selection of eggplant genotypes to improve their performance under these conditions. In this respect, this work invests the main eco-physiological and biochemical mechanisms concerned in tolerance phenomena to salinity in four eggplant cultivars: water status, osmoregulation, chlorophyll fluorescence, gas exchanges, accumulation of osmolytes as well as the accumulation of malonaldehyde as a result of oxidative damage caused by salt stress. Also accumulation patterns of Na and Cl in leaves and roots are studied.

The effects of salinity were investigated under *in vitro* controlled conditions on germination, seedling growth and biochemical parameters in four eggplant (*Solanum melongena* L.) cultivars. Seeds and subsequent seedling growth were exposed to increasing salt stress (0, 20, 40, 80 and 160 mM NaCl). Germination was strongly reduced at 160 mM for all cultivars. The decline in seed germination parameters, fresh weigh, dry weigh, height and leaf number were more pronounced with the increase of NaCl concentration in the cultivars ‘Adriatica’ and ‘Black Beauty’ than in the cultivars ‘Bonica’ and ‘Galine’. The water content decreased markedly in ‘Adriatica’ and ‘Black Beauty’ and remained quite stable in the other cultivars. Higher levels of MDA and proline were detected in the leaves of ‘Adriatica’ and ‘Black Beauty’. The responses of the germination, seedling growth and biochemical parameters to salt stress indicated two groups with contrasting sensitivity responses. ‘Adriatica’ and ‘Black Beauty’ were more sensitive to the applied salt stresses than ‘Bonica’ and ‘Galine’.

The relative salt tolerance of four eggplant cultivars (*Solanum melongena* L.) was also assessed by chlorophyll fluorescence during the vegetative growth stage under increasing salinity levels. In a pot experiment plants were subjected to saline stress ranging from 0

(control), 20, 40, 80 and 160 mM NaCl for 25 days. The results showed that increasing NaCl concentration hardly affected the maximum quantum yield of PSII (F_v/F_m). The quantum yield of PSII (Φ_{PSII}) decreased significantly in 'Adriatica' and 'Black Beauty' under saline stress. Photochemical quenching (q_P) decreased for 'Black Beauty' and non-photochemical quenching (NPQ) increased for 'Adriatica' under salt stress. For 'Bonica' and 'Galine' chlorophyll fluorescence parameters did not significantly change under salt stress, revealing their photochemical tolerance to salinity. At the end of the experiment plant growth decline under salt stress was more pronounced for 'Adriatica' and 'Black Beauty'. Additionally, a significant correlation between biomass and Φ_{PSII} was observed for 'Adriatica' and 'Black Beauty'. 'Bonica' and 'Galine' tolerated better the applied salt stress and limited effect on primary photochemistry as compared to 'Adriatica' and 'Black Beauty' was observed.

The effect of NaCl stress on plant water status and biochemical parameters was also investigated in this experiment for all eggplant cultivars (*Solanum melongena* L.). Increasing NaCl concentration increased strongly proline, malondialdehyde and carbohydrate leaf contents in the sensitive cultivars 'Adriatica' and 'Black Beauty'. However, the tolerant cultivars 'Bonica' and 'Galine' showed a decrease in carbohydrate accumulation and a significant increase in starch levels under saline stress. The midday leaf water potential (ψ) and leaf osmotic potential (ψ_π) were significantly affected in sensitive cultivars and remained quite stable in tolerant cultivars under salt stress. Leaf Na and Cl^- content were higher in sensitive than in tolerant cultivars. Leaf K, Ca and Mg contents were reduced under salt stress in sensitive cultivars. Increasing salinity did not change Ca and Mg content in tolerant cultivars. The growth responses were integrated in a plant tolerance index which could clearly discriminate sensitive and tolerant cultivars as well for a low salinity level (20 mM) as higher salinity levels.

The application of salt stress also limited photosynthetic efficiency. This has been studied in the third experiment through the assessment of CO_2 assimilation rates, photosynthetic electron flow and photorespiration in two eggplant cultivars, 'Bonica' and 'Black Beauty', differing in their tolerance to salt stress. We used again a pot experiment and four salt stress levels namely 0 (control), 40, 80 and 160 mM of NaCl. A significant decrease in net photosynthesis (A_n) was noticed in both cultivars under increasing salt stress though respiration rates (R_n and R_d) were not affected. The ratio A_n/A_t decreased under increasing salinity while R_d/A_t increased under increasing salt stress in both cultivars. High respiration rates are linked to higher ATP production; therefore both cultivars could maintain sufficient

energy levels under increasing salt stress levels. However, this energy is probably used for different purposes such as osmotic adjustment in ‘Black Beauty’ or for sodium exclusion and tissue tolerance in ‘Bonica’. The ratio J_c/J_t was not affected by increasing salt levels except for ‘Black Beauty’ at 160 mM NaCl. Under 160 mM NaCl level less than 40% of the total electron flow was used for oxygenation of RuBP in ‘Black Beauty’ and ‘Bonica’. Photorespiration (R_l) is an alternative electron sink and this pathway is more pronounced in ‘Bonica’ at 160 mM NaCl.

A concentration of 40 mM NaCl already significantly reduced g_s in ‘Black Beauty’, this for both 13 and 21 DSS. Significant lower g_s was only found for 160 mM NaCl in ‘Bonica’. Transpiration rate (E) reduction induced by salinity was more pronounced in ‘Black Beauty’ than in ‘Bonica’. Biochemical analysis confirmed the results of the other experiments. Proline and MDA accumulation were pronounced in the sensitive cultivar. While ‘Black Beauty’ accumulated considerable amount of sugars (sucrose, glucose and fructose) in the leaves and lower amount of starch under saline conditions, tolerant cultivar showed a decline of sugar content accompanied by a starch accumulation increase. On the other hand leaves of ‘Bonica’ accumulated lower concentration of Na and Cl than ‘Black Beauty’. Moreover at 160 mM NaCl while sodium accumulation was higher in the roots than in the leaves of ‘Bonica’, Black Beauty’ accumulated lower Na in roots than in leaves. Leaf and root K contents reduction were higher in ‘Black Beauty’ than in ‘Bonica’ at 160 mM NaCl. Besides significant differences for the Na/K ratio were only observed in ‘Black Beauty’.

This study showed that different salt tolerance levels are present among commercial cultivars of eggplant. Most parameters could differentiate between the cultivars, sometimes even at low salt levels yet combined physiological and biochemical traits should be considered in screening salt tolerance of eggplant genotypes rather than only one specific trait.

Samenvatting

De Mediterrane regio's zijn gekenmerkt door een verhoogd risico op verzilting van de bodems. De negatieve impact van irrigatie met zout-belast water op de productiviteit werd daarom in verschillende landbouwgewassen onderzocht. Tot heden zijn studies betreffende zouttolerantie bij aubergine beperkt en lag de focus hoofdzakelijk op agronomische aspecten als selectiecriterium. Nochtans heeft een screening op agronomische parameters een aantal inherente nadelen zoals het verschillend groeipatroon van genotypes wanneer geen stress aanwezig is, naast logistieke en tijdsbehoeften bij productiebepalingen in veldproeven.

Onder zoutstress gebruiken planten verschillende morfologische en cellulaire reacties om zich aan deze stress aan te passen. Daarom is onderzoek naar parameters, die zoutstress kunnen vaststellen, een belangrijke stap voor selectie van meer zout tolerante aubergine cultivars. Deze thesis focust op een karakterisering van de belangrijkste fysiologische en biochemische mechanismen, die tussenkomen in de tolerantie voor zoutstress bij vier aubergine cultivars: de plant water status, osmoregulatie, chlorofyl fluorescentie, gas uitwisselingspatronen, en de accumulatie van malonaldehyde als gevolg van oxidatieve schade veroorzaakt door zoutstress. Ook accumulatiepatronen van Na en Cl in blad en wortel zijn bestudeerd.

Zoutstress effecten werden eerst bestudeerd onder gecontroleerde *in vitro* proeven, op de kieming van zaden, de groei van de zaailingen en twee biochemische parameters bij vier aubergine cultivars (*Solanum melongena* L.). Zaden en de hierop volgende jeugdgroei werden onderworpen aan toenemende zoutstress (0, 20, 40, 80 en 160 mM NaCl). Zaadkieming was sterk gereduceerd bij 160 mM NaCl bij alle cultivars. Het negatief effect van toenemende zoutstress op zaadkiemingsparameters, lengte, aantal bladeren, vers- en drooggewicht was sterker bij de cultivars 'Adriatica' en 'Black Beauty' dan bij cultivars 'Bonica' en 'Galine'. De waterinhoud verlaagde sterk bij 'Adriatica' en 'Black Beauty' en bleef ongewijzigd bij de andere cultivars. Hogere concentraties MDA en proline werden vastgesteld in de bladeren van 'Adriatica' en 'Black Beauty'. De reacties op de bestudeerde parameters toonde aan dat twee groepen met contrasterende gevoeligheid warden gevormd. 'Adriatica' en 'Black Beauty' waren eerder gevoelig voor zoutstress, terwijl 'Bonica' en 'Galine' eerder tolerant waren voor de zoutstress.

De relatief verschillende tolerantiepatronen van de vier aubergine cultivars werd verder onderzocht met chlorofylfluorescentie tijdens de vegetatieve groei. Hiervoor werd een pot

experiment uitgevoerd waarbij planten werden onderworpen aan zoutstres, die toenam van 0 (control), 20, 40, 80 tot 160 mM NaCl, gedurende 25 dagen. Toenemende zoutstress had nauwelijks invloed op de maximum kwantum opbrengst van PSII (F_v/F_m). De kwantum opbrengst van PSII (Φ_{PSII}) nam significant af bij 'Adriatica' en 'Black Beauty' onder zoutstress. De fotochemische quenching (q_P) nam af bij 'Black Beauty' en de niet-fotochemische quenching (NPQ) steeg bij 'Adriatica' onder zoutstress. Chlorofyl-fluorescentie parameters werden niet beïnvloed bij 'Bonica' en 'Galine' onder zoutstress, wat hun hogere tolerantie voor zoutstress aantoonde. Op het einde van het experiment was de vegetatieve groei sterker onderdrukt bij 'Adriatica' en 'Black Beauty'. Bijkomend werd een significante correlatie tussen biomassa en Φ_{PSII} vastgesteld bij 'Adriatica' en 'Black Beauty'.

Het effect van NaCl stress op de plant waterstatus en biochemische parameters werd eveneens in dit experiment onderzocht bij de 4 aubergine cultivars. Toenemende zoutstress verhoogde sterk proline, malondialdehyde en oplosbare koolhydraten in de bladeren van de gevoelige cultivars 'Adriatica' en 'Black Beauty'. Echter bij de tolerante cultivars 'Bonica' and 'Galine' was een afname van de oplosbare koolhydraten merkbaar maar werd een belangrijke toename van zetmeel vastgesteld. De middag plant waterpotentiaal (ψ) en blad osmotische potentiaal (ψ_π) werd significant negatiever bij de gevoelige cultivars maar bleef vrij stabiel bij de tolerante cultivars bij toenemende zoutstress. Blad Na en Cl^- gehalte was hoger bij de gevoelige dan bij de tolerante cultivars. Blad K, Ca en Mg gehaltes namen af bij toenemende zoutstress in de gevoelige cultivars. Toenemende zoutstress beïnvloedde niet de Ca en Mg gehaltes bij tolerante cultivars. De groeirespons werd geïntegreerd in een plant tolerantie index en deze kon duidelijk een onderscheid maken tussen gevoelige en tolerante cultivars, zowel bij lage als hoge zoutstress.

De toepassing van zoutstress reduceerde eveneens de fotosynthetische efficientie. Dit werd bestudeerd in een derde experiment waarbij zowel gasuitwisseling, chlorofylfluorescentie, lineair elektronen transport en fotorespiratie werd bepaald bij 2 cultivars, 'Bonica' en 'Black Beauty', verschillend in hun tolerantie voor zoutstress. Er werd opnieuw een potexperiment opgezet en vier zoutniveaus werden toegepast nl. 0 (controle), 40, 80 en 160 mM NaCl. Zoutstress reduceerde significant de netto-fotosynthese (A_n) bij beide cultivars maar de ademhalingssnelheden (R_n en R_d) werden niet beïnvloed. De verhouding A_n/A_t nam af bij toenemende zoutstress terwijl R_d/A_t toenam bij beide cultivars. Hoge ademhalingsnelheden resulteren in hogere ATP productie, beide cultivars konden hierdoor een voldoende energieniveau behouden onder toenemende zoutstress. Echter, deze energie werd

waarschijnlijk op een verschillende manier aangewend zoals voor osmotische aanpassing in ‘Black Beauty’ en natrium exclusie en weefseltolerantie bij ‘Bonica’. De verhouding J_c/J_t werd niet beïnvloed door toenemende zoutstress met uitzondering voor ‘Black Beauty’ bij 160 mM NaCl. Gemiddeld werd minder dan 40% van de totale elektronen stroom gebruikt voor de oxygenatie van RuBP in ‘Black Beauty’ en ‘Bonica’. Fotorespiratie (R_l) is een alternatieve elektronen sink en deze pathway was sterker uitgesproken bij ‘Bonica’ onder 160 mM NaCl.

De stomatale geleidbaarheid daalde al bij 40 mM NaCl bij ‘Black Beauty’, dit zowel 13 als 21 dagen na de start van het experiment, terwijl bij ‘Bonica’ dit slechts vanaf 160 mM NaCl optrad. De afname van de verdamping (E) was sterker bij ‘Black Beauty’ dan bij ‘Bonica’ onder zoutstress. De biochemische analyses bevestigden de vorige experimenten. Proline en MDA accumuleerden sterk bij de gevoelige cultivar. Eveneens namen de concentraties oplosbare koolhydraten (sucrose, glucose en fructose) sterk toe bij de gevoelige cultivar en werd zetmeel afgebroken bij zoutstress. Bij de tolerante cultivar daalden de concentraties oplosbare koolhydraten en accumuleerde zetmeel in de bladeren. Ook in dit experiment was de zoutaccumulatie in de bladeren lager bij ‘Bonica’ dan bij ‘Black Beauty’. Bijkomend werden bij ‘Bonica’ hogere concentraties zout weerhouden in de wortels dan in de bladeren, terwijl bij ‘Black Beauty’ de sterkste accumulatie voorkwam in de bladeren. Ook de kaliumopname, zoals bleek uit blad en wortelsamenstelling verliep moeilijker bij ‘Black Beauty’ dan bij ‘Bonica’ bij 160 mM NaCl. Dit resulteerde in een significante toename van de Na/K ratio bij ‘Black Beauty’.

Dit onderzoek toonde aan dat verschillende zouttolerantie niveaus aanwezig zijn in commerciële aubergine cultivars. De meeste parameters konden de tolerantie verschillen tussen de cultivars aantonen, dit soms bij vrij lage dosissen zoutstress. Toch is een karakterisering van meerdere fysiologische en biochemische karakteristieken aangewezen voor het screenen van zouttolerantie bij aubergine.

List of abbreviations

Abbreviation	Description	Unit
A_n	Net photosynthetic assimilation rate	[µmol CO_2 m^{-2} s^{-1}]
A_t	Total assimilation rate	[µmol CO_2 m^{-2} s^{-1}]
ATP	Adenosine triphosphate	
BA	Benzylaminopurine	
Ca	Calcium	
CE	Carboxylation efficiency	[mol mol^{-1})
Chl*a*	Chlorophyll a	[µg g^{-1} FW]
Chl*b*	Chlorophyll b	[µg g^{-1} FW]
Chl*a+b*	Total chlorophyll	µg g^{-1} FW
DSS	Day of salt stress	
DW	Dry weight	[g]
E	Transpiration rate	[mmol H_2O m^{-2} s^{-1}]
ETR	Electron transport rate	[-]
F_0	Minimum fluorescence	[-]
F'_0	Minimum fluorescence in light adapted leaf	[-]
F_m	Maximum fluorescence	[-]
F'_m	Maximum fluorescence in light adapted leaf	[-]
F_s	Steady-state fluorescence	[-]
F_v/F_m	Maximum quantum efficiency of PSII photochemistry measured in dark-adapted leaves	[-]
F_v'/F'_m	Efficiency of energy captured by open PSII reaction centre	[-]
FAO	Food and Agriculture Organisation of the United Nations	
FW	Fresh weight	[g]
g_s	Stomatal conductance	[mol H_2O m^{-2} $s^{-}1$]
J_c	Total electron flux used for RuBP carboxylation	[µmol m^{-2} s^{-1}]
J_o	Total electron flux used for RuBP oxygenation	[µmol m^{-2} s^{-1}]
J_{tot}	Total linear electron flow	µmol m^{-2} s^{-1}]
K	Potassium	[%DW]
LED	Light-emitting diode	
LUE	Light use efficiency	[mol mol^{-1}]
Mg	Magnesium	
MDA	Malonaldehyde	[nmol g^{-1} FW]
MDG	Mean daily germination	[%]
MGT	Mean germination time	[day]
MS	Murashige and Skoog	

NAA	Naphthaleneacetic acid	
NADPH	Nicotinamide adenine dinucleotide phosphate-oxidase	
NPQ	Non-photochemical quenching	[-]
N	Nitrogen	[%DW]
P	Phosphorus	[%DW]
PAR	Photosynthetically active radiation	[µmol m^{-2} s^{-1}]
PCA	Principal component analysis	
PFD	Photon flux density	
PSII	Photosystem II	
PSI	Photosystem I	
PTI	Plant tolerance index	
Q_A	The primary electron acceptor	
q_p	Photochemical quenching	[-]
R_d	Mitochondrial respiration rate in the light	[µmol CO_2 m^{-2} s^{-1}]
R_l	Photorespiration	[µmol CO_2 m^{-2} s^{-1}]
R_n	Mitochondrial respiration in the dark	[µmol CO_2 m^{-2} s^{-1}]
RuBP	Ribulose biphosphate	
S	Sulfur	[%DW]
SE	Standard error	
SPSS	Statistical package for the social science	
TDZ	Thidiazuron	
TWC	Tissue water content – fresh weight basis	[%]
WC	Water content – dry weight basis	[%]
WUE	Water use efficiency	[µmol CO_2 (mmol H_2O)$^{-1}$]
Φ_{PSII}	Quantum yield of PSII electron transport	[-]
ψ	Water potential	[MPa]
ψ_π	Osmotic potential	[MPa]

Scope of the thesis

The Tunisian climate is characterized by an irregular inter-regional and inter-annual rainfall. This implies the use of irrigation for agricultural lands but water resources are limited and of low quality as 30% of these resources have a salt content higher than 3g/l (≈ 51.3 mM of NaCl) (Boutiti, 1995). In regions lacking good water quality supplies, the mobilization of all available water resources (underground, surface, treated wastewater and salty water) is found to be highly important for agricultural uses (Chaabouni, 1995). Also, the identification of crops able to valorise this constraining water quality has become very important. Furthermore, the irrigation and inputs of chemical fertilizers usually exceeding the needs of the crop further increases the salinity of the soils after cultivation. For saline soils and irrigation waters, two complementary strategies can be implemented to limit the depressive effects of salt on crop yields. First, we should apply farming techniques to reduce soil salinity and secondly, we could select varieties or species able to minimize the depressive effects of salinity on their performance.

Vegetables have a high cash value and for each vegetable crop there is a wide germplasm available. The vegetable sector has become one of the strategic sectors of the Tunisian economy. The three main solanaceous crops, potato, tomato and pepper are the economically most important vegetables in terms of local consumption and export. The cultivation of eggplant (*Solanum melongena* L.) could be a potentially promising crop for this sector in addition to potatoes, tomato and pepper. This vegetable is also an important greenhouse crop for out of season production, however, secondary salinization due to non-sustainable irrigated horticulture results in a decline in eggplant productivity and as a result growers are reticent to start the cultivation of this crop.

Few comparative studies concerning salt stress have been published on eggplant. These studies focused on growth parameters and horticultural performances as a selection criterion for salinity tolerance (Savvas and Lenz, 2000; Akinci et al., 2004). In other crops research on the effect of salt stress on the growth and yield combined with insight in the various physiological processes that control the productivity led to a strategy to improve yields through increasing productivity. In all this, the obvious question is: how does the understanding of the mechanisms of salt tolerance or sensitivity allows us to use it in improvement programs for the search of genotypes tolerant to salinity in order to preserve our horticultural production with a focus on an economic and ecological sustainability?

Consequently to revive the cultivation of eggplant and operate simultaneously salty water in coastal areas of Tunisia, we investigated the relative salt tolerance of eggplant cultivars. The main aims of this research focus at understanding the physiological adaptation of salt tolerance in eggplant with as final goal the development of selection criteria for salt tolerant genotypes. Both an *in vitro* (started in Tunisia) and *in vivo* approach were undertaken to reach these objectives.

In vitro techniques, with their potential to induce somaclonal variation might indeed be a promising way to improve salt tolerance in the Solanaceae family as it has been successfully used in the selection of salt tolerant tobacco (Nabors et al., 1980) and tomato genotypes (Messai, 2002). Yet, eggplant is a recalcitrant plant species that cannot be easily reproduced through *in vitro* therefore first a protocol for eggplant multiplication is needed.

Salt stress is not only an osmotic stress but also an ionic stress due to accumulation of sodium and chloride at cellular level. Yet, the knowledge of the physiological adaptations of eggplant to salt stress is very limited. This study focuses on the identification of varieties that maintain an adequate growth under irrigation with poor quality water and to investigate the mechanisms involved to counteract the effect of salt stress at a physiological level.

The doctoral thesis is structured into six separate chapters.

The **first chapter** is devoted to a general introduction of the Tunisian horticulture, a description of eggplant and an introduction to factors associated with saline soils and the behaviour of plants under salinity stress.

In the **second chapter** we will present our work concerning *in vitro* regeneration of eggplant seedlings from different somatic tissues such as the cotyledons, fragments of leaves, hypocotyl segments and segments of internodes. Effects of increasing concentrations of the hormone (TDZ) and three types of light quality (white fluorescent light, blue light and red light) were tested to develop a reliable regeneration protocol.

The **third chapter** focuses on the *in vitro* seedling stage. In this chapter we studied the seed germination ability of *S. melongena* (the rate and germination period) in response to salt stress. Seed germination is an important sensitive and critical phase of the plant life cycle. Morphological traits, membrane damage as determined by lipid peroxidation and accumulation of proline are quantified.

The **fourth chapter** focuses on the application of chlorophyll fluorescence for diagnosis of salinity tolerance in four varieties of eggplant. The functioning of the photosynthetic apparatus (chlorophyll fluorescence) can be considered as a specific intrinsic indicator of the first steps of the photosynthesis and its intensity is directly related by an inverse relationship to the photosynthetic efficiency of the plant.

The **fifth chapter** compares and discusses the effects of salt stress on plant water relations, osmotic adaptation and foliar accumulation of sodium and chloride on two relative sensitive and two more tolerant cultivars based on chlorophyll fluorescence screening. This knowledge might be of further advantage to screen eggplant cultivars tolerant to salt stress.

The **sixth chapter** focuses on effects of salt stress on photosynthesis and respiration. Salt-induced photosynthetic dysfunction is investigated by chlorophyll fluorescence, pigment concentration and Na content of the leaves. The variation in salt uptake mechanisms is also studied by analysis of the mineral content of both roots and leaves. Differential responses of a relative sensitive and a more tolerant cultivar (see Chapter 5) are interpreted.

Finally, a general conclusion that summarises all findings and provides perspectives for future research is presented in **Chapter 7**.

Chapter 1

General introduction

Chapter 1 General introduction

1.1 The vegetable sector in Tunisia

In Tunisia the vegetable crops have a considerable economic importance and therefore they are considered as one of the strategic cultures of the country. Vegetable crops in open fields and in greenhouses occupy an average of 140,000 ha. The areas of protected vegetable crops (greenhouses, small tunnels and multi-tunnels greenhouses) represent only 6.2% of this area, so 8,650 hectares divided into:

- 1,250 ha non-heated greenhouses: Peppers are the main crop in unheated greenhouses with 56% of the area, followed by tomato which occupies on average 26% of the area and melon with only 6% of the area. The governorate of Monastir has about 39% of the cultivated vegetable area under non-heated greenhouses (572 ha), followed by the governorates of Sidi Bouzid, Mahdia and Sfax with respectively 14.3%, 13.2% and 12.7% of the total area.
- 7,300 ha under small tunnels: The main crops grown under small tunnels are watermelon and pepper. They occupy 4,178 hectares or 57% of the total area of small tunnels. The governorate of Sfax is the largest producer of vegetables in small tunnels.
- 100 ha in heated greenhouses: Vegetable crops in greenhouses heated by geothermal water are spread over 3 governorates. The governorate of Gabes (37 ha) is specialized in the cultivation of tomato mainly for export. The governorate of Kebili (41 ha) is specialized in the production of cucumber (40% of the area) followed by tomato (28.5%) and melon (22%). The governorate of Tozeur with 22 ha, is dominated by melon production (30% of the area), followed by cucumbers (19%) and okra (18%).

The strategic vegetable crops for the country are potato, tomato and peppers. The production of potato averages about 370,000 tonnes grown on an average area of 25,000 hectares. For the last five years the potato export averages 11,000 tonnes per year. They result mainly of the early season and out of season crop. The tomato cultivation covers an average area of 29,000 ha per year, with an average production of 1.2 million tonnes. This production is

based on field crops (both late season and season crop) and protected cultivation. Exports of fresh tomatoes increased from 2,481 tonnes in 2004/2005 to 13,981 tonnes in the 2013/2014 agricultural year. The pepper cultivation in term of sown area occupies the 3rd place with an area of 20,000 ha and an average production of 346,000 tonnes in the last five years. Pepper exports are increasing during the past decade from 53 tonnes in 2005 to 471 tonnes in 2014. The main importers are Libya, France and the Gulf countries.

The cultivation of eggplant is considered as a secondary vegetable crop with a production area of 67 ha in 2015 (Table 1.1). Although eggplant is mainly a summer vegetable, all-year round production exists by producing also under cold greenhouses and geothermal greenhouses in the southern regions. Ten varieties are listed on the Tunisian official catalogue, the best known are the elongated dark purple or black and ovoid eggplants while yellow and white eggplants are mainly for export. Thus, eggplant contributes to the diversification of vegetables crops and constitutes a new product requested by foreign markets: The average quantity exported in the last five years is around 187 tonnes. France and the Gulf countries are the main destinations of this product. Thus the cultivation of eggplant could constitute a potential niche to be adopted by farmers because it is a promising crop in terms of local demand and attractive fruit prices.

1.2 The importance of eggplant production

An overview of the major eggplant producing countries is given in Table 1.2, China is by far the main producing country followed by India and Iran. The Netherlands with an average production of 46.38 tonnes/ha (average of 2012-2013), have the highest yield of this crop in greenhouse growing conditions, followed by Belgium (38.61 tonnes/ha). For 25 years the area of greenhouse production increased by more than 2,000 ha per year (FAOSTAT, 2015), and this increase was mainly responsible for the dramatic increase in yield (Greer and Driver, 2000). The Mediterranean countries of Europe are one of the largest concentrations of protected crops in the world with about 100,000 hectares dedicated to the production of vegetables grown in greenhouses, and 300,000 ha grown under small tunnels and mulching, which contributed to the increased production of the eggplant. The eggplant with tomato, pepper, cucumber, melon and watermelon are the main protected crops in this region (Cantliffe and Vansickle, 2003).

Table 1.1: Repartition by governorate of area and production of eggplant in the last five years: 2010-2015 (Anonym, 2015).

	2010		2011		2012		2013		2014		2015	
	Area (ha)	Production (tonnes)	Area (ha)	Production (tonnes)	Area (ha)	Production (tonnes)	Area (ha)	Production (tonnes)	Area (ha)	Production (tonnes)	Area (ha)	Production (tonnes)
Nabeul	10	600	20	300	20	400	20	200	25	400	32	480
Sousse	1	0	0	0	4	80	0	0	0	0	0	0
Gafsa	40	1,200	50	800	40	1,000	0	0	4	80	35	500
Gabes	10	100	8.5	127.5	4	60	2	20	0	0	0	0
Total	61	1,900	78.5	1,227.5	68	1,540	22	220	29	480	67	980

Table 1.2: Major eggplant producing countries in the world (FAOSTAT, 2015)

	2012			2013		
Countries	ha	tonnes	tonnes/ha	ha	tonnes	tonnes/ha
China	775,436	27,698,600	35,72	786,977	28,433,500	36,13
India	692,272	12,634,000	18,25	722,019	13,444,000	18,62
Iran	39,501	1,300,000	32,91	42,182	1,345,185	31,89
Egypt	45,256	1,193,854	26,38	41,534	1,194,115	28,75
Turkey	26,001	799,285	30,74	26,598	826,941	31,09
Indonesia	50,567	518,827	10,26	46,433	509,380	10,97
Iraq	21,106	422,336	20,01	23,566	510,918	21,68
Japan	9,861	327,400	33,20	9,700	321,200	33,11
Spain	3,900	245,900	63,05	3,700	206,300	55,75
Netherlands	1,050	47,000	44,76	1,000	48,000	48.00
USA	2,000	65,000	32,50	2,034	67,784	33,32

In Europe, there is a trend towards diversification of eggplant on the market. Consumers show interest in "exotic" varieties with colours, shapes, sizes and flavours from those traditionally marketed (dark purple or lilac oblong berries). For now, however, the most common varieties on the European market are high-yielding varieties with oblong shaped dark purple fruits.

Eggplant production is of considerable economic importance in Europe (Table 1.2, Table 1.3), this includes breeders and seed companies, growers and phytochemists, all concerned with a better use of genetic resources of eggplants. The consumption of eggplant in the European Union is increasing but the trade balance remains positive (Table 1.3).

The production of eggplant in the EU provides thus a source of income for the producers of the region. At the same time, it ensures that the consumer receives a fresh product with good quality, which is locally produced, and therefore prices are relatively fair. The Netherlands produce eggplants in greenhouses on substrates. Spain and Italy obtain most of their eggplant production through the protected-culture system (tunnels, mulching). Greenhouses and protected cultivation generally ensure a better quality and stability of the harvest (Bougoul et al., 2005).

Table 1.3: Trade of eggplant within the European Union (FAOSTAT, 2015)

Year	2010	2011	2012	2013
Import (1,000 tonnes)	175,749	178,686	193,685	196,560
Export (1,000 tonnes)	190,505	205,663	216,276	221,681

1.3 Description of eggplant

1.3.1 Origin

Solanum melongena, the common or brinjal eggplant, occurs in wild or semi-wild form in India. Various data indicate that the species that evolved in Africa, *S. incanum,* gave rise to a distinct species which spread to South-East Asia as the wild ancestor of *S. melongena* (Lester, 1998). India or Indochina is recognized as the centre of the eggplant diversity. Primitive eggplant characteristics are tall plants with large, piny leaves, flowering in clusters with andromonoecy. Their fruits are small, green and bitter in taste, with a thick skin and hard flesh.

Eggplant was described in India in 3rd century B.C, production started in 4th century in China and in 9th century in Africa. Although cultivated from prehistorical times, eggplant appears to have been unknown to the Western World for many centuries. *Melongena* was an Arabic name for one eggplant cultivar and Avicenna mentioned it as a medicinal and vegetable plant.

Domestication, mutation, natural intercrossing, human selection and hybridization brought extensive genetic diversity of eggplant cultivars, now grown all over the world. Cultivar differences concern mainly the colour, shape and height of fruits, but chemical composition of the fruits, earliness of fruiting, yield, environmental requirements, etc. are also taken into consideration. Fruit colour varies from light to dark purple, almost black, green, or white. Fruit length is between 4-45 cm, and thickness 2-35 cm, and weight ranges between 15-1500 g. The fruits are set as single or in clusters, up to 5 fruits. Physiologically ripe fruits become brown, red or yellow (Swarup, 1995).

African eggplants – *S. aethiopicum* and *S. macrocarpon*, are the most popular native, traditional vegetables in West and Central Africa, but the productivity of these crops is still relatively low and the growing area and yields have not been recorded. The centre of diversity of these eggplants is Western Africa. African eggplants are grown mainly in gardens and small fields near villages. *S. aethiopicum* is a fruit and leaf vegetable. It is a herbaceous

shrub with hairy or glabrous leaves and hermaphroditic flowers, self or cross-pollinated, single or in clusters. The fruits are consumed raw or cooked. They are light to dark green, white or blackish in colour, with a bitter taste that varies depending on its saponin content. The fruit shape is round, elongate-round or oval with smooth or grooved surface and taste varies from sweet to bitter, particularly in the case of oval-fruit cultivars. At full maturity, the fruits turn red or reddish-orange due to high carotene content. Fruit surfaces vary from smooth to grooved or ribbed. The leaves are often consumed in the same way as spinach (Seek, 1997; Macha, 2005).

S. macrocarpon is grown for its large, glabrous leaves (50 × 30 cm), used as a green vegetable. Fruits have a large, often clasping calyx. They are sub-spherical and large (3-10 cm in diameter, 2-6 cm long), cream white, green-white or green. Fruits are sweeter in taste compared to *S. aethiopicum*. At full maturity fruits turn yellow, orange or brown with cracked surface (Bukenya, 1994; Macha, 2005).

1.3.2 Classification

The classification of eggplant is as follows (Lawande and Chavan, 1998; Mace et al., 1999; Collonier et al., 2001):

Class	*Magnoliopsida*		
Family	*Solanaceae*	Genus	*Solanum*
Subfamily	*Solaoideae*	subgenus	*Leptostemonum*
Tribe	*Solaneae*	Species	*melongena*

Solanum melongena was originally described by Linnaeus (1753) in his "*Species Plantarum*", where he described the two species which are the corner stones of the eggplant complex, i.e. *S. incanum* and *S. melongena*. Other classifications followed in order to explain the complex pattern of wild, domesticated and semi-domesticated plants that form the "*S. incanum*-*S. melongena* complex" (Table 1.4-1.5, Mace et al., 1999).

The domestication of *Solanum* vegetables in Africa depended on the development of agricultural systems and the availability of suitable wild or introduced species (Lester and Daunay, 2003). Studies by Daunay et al. (2001) recognize three African vegetable *Solanum* species (Figure 1.1), and *Solanum melongena* L., which was domesticated rather in South East Asia than in Africa, but whose closely related wild species are indigenous in Tropical Africa and the crop is extensively grown in both northern and southern Africa.

Table 1.4: Ancient taxonomical classification of *Solanum* (Mace et al., 1999)

Authors	Achievement	Remark
Linnaeus "Species Plantarum")	Described S. *incanum* and S. melongena.	Led to confusion because of the morphological plasticity of these species
Dunal (1852)	Intended to give the exact number of African *Solanum* species.	-
Dammer (1915)	Increased the number of African *Solanum* sp. to 200.	Did not clarify the delimitations of these species.
Bitter (1923)	Begun to unravel the confusion surrounding African *Solanum* species by using the species-aggregate concept.	Indicates close relationship between groups of species but does not force premature nomenclatural decisions for that group.

Table 1.5: Taxonomical classification of *Solanum* according to their centre of origin (Lester and Hasan, 1991; Mace et al., 1999)

Wild taxa of *S. incanum sensu lato*, from Africa			Weedy and cultivated taxa of *S. melongena* from Asia		
Group A	*S. campylacanthum*	East and South Africa	Group E	*S. melongena* (*S.* insanum)	India
Group B	*S. panduriforme*	South Africa	Group F	*S. melongena* (*S. cumingii*)	S.E Asia
Group C	*S. incanum*	North Africa, Arabia	Group G	*S. melongena* (S. *ovigerum*)	S.E Asia
Group D	*S. lichtensteinii*	South Africa	Group H	*S. melongena* (*S. melongena*)	world-wide

The botanical classification of eggplant recognizes three major botanical varieties under the species *melongena* (Lawande and Chavan, 1998):

- S. *melongina* var. *serpentium* (Snake Aubergine)
- *S. melongena* var. *depressum* (Dwarf Aubergine)
- *S. melongena* var. *esculentum* (Common Aubergine).

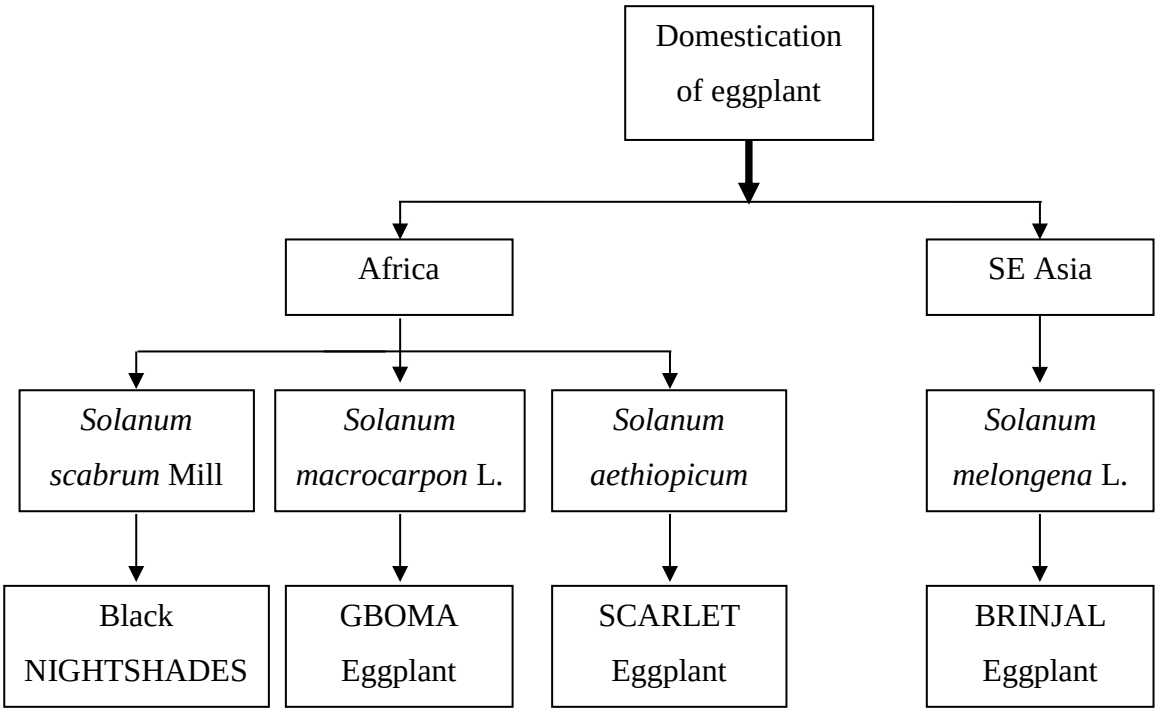

Figure 1.1: The domestication of *Solanum* sp. (Lester and Daunay, 2003)

S. melongena, *S. aethiopicum* and *S. macrocaepon* are diploid plants (2n=2x=24) (Daunay, 1997). *S. melongena* and its varieties are very diverse in fruit form and colour (Prinz, 1989; Lawande and Chavan, 1998). The variation is continuous, which means that the existing sub-classifications into botanical varieties and subspecies have no horticultural value. For certain, this biodiversity of eggplant could be used to select new types for greenhouse cultivation all over the world. Controlled hybridization within *S. melongena* lead to the development of many hybrid F1 cultivars (heterozygous but homogeneous phenotype).

1.3.3 Botanical description of eggplant

It is a tropical perennial that does not support frost. The stem develops monopodial for the first 6 to 10 leaves which coincide with the vegetative phase. Once flower initiation starts the stem develops sympodial with dichotomous development generally each two leaves. The plant thus has a bushy habit that can reach a height of 0.5 m to 2.5 m. The stems and the upper surface of the eggplant leaves are covered with stellate hairs which make them rough to touch. The stem is thick and has a strong woody ring at its base; the bark is thin, green or reddish due to anthocyanin. It can be with or without thorns. The large leaves alternate, are angular or lobed, usually with strong thorny ribs. They are greyish green with purple discoloration on the ribs.

Eggplants are autogamous. Flowers appear in the axils, sometimes solitary, but often grouped in cymes of two, three or even five flowers. The flowers are large, 3 to 5 cm of diameter having a coloured corolla violet or purple and whose lower face is fluffy. The chalice, coloured green or purplish colour, covers the upper part of the fruit; it is very indented and jagged, smooth or spiny. The flowers are usually long-styled hermaphrodites, but in the distal part of the cymes, they are often short-styled or males (Daunay et al., 1997).

Flowers can stay open for 8 to 10 days but will close every evening; they are more receptive in the morning between 6:00 and 11:00 in summer (Rao, 1980). Pollen remains viable for 3 days while the receptivity of the stigma is good till the 2nd day and declines thereafter (Rao, 1980).

The first flower appears between 55 and 110 days after sowing but for most varieties, this takes about 70 to 80 days. Fruit development ranges between 20-40 days between flowering and fruit harvest at the commercial stage though it takes 40 to 80 days to reach full physiological maturity (Daunay, 2002).

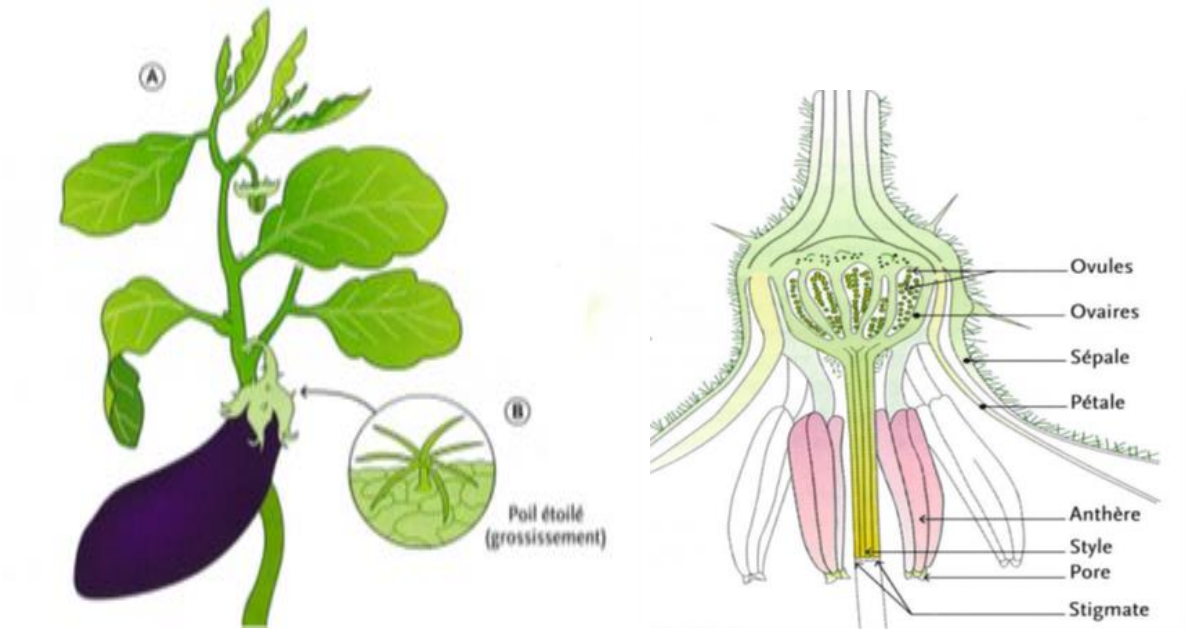

Figure 1.2: Eggplant, flower and fruit morphology (Daunay, 2002)

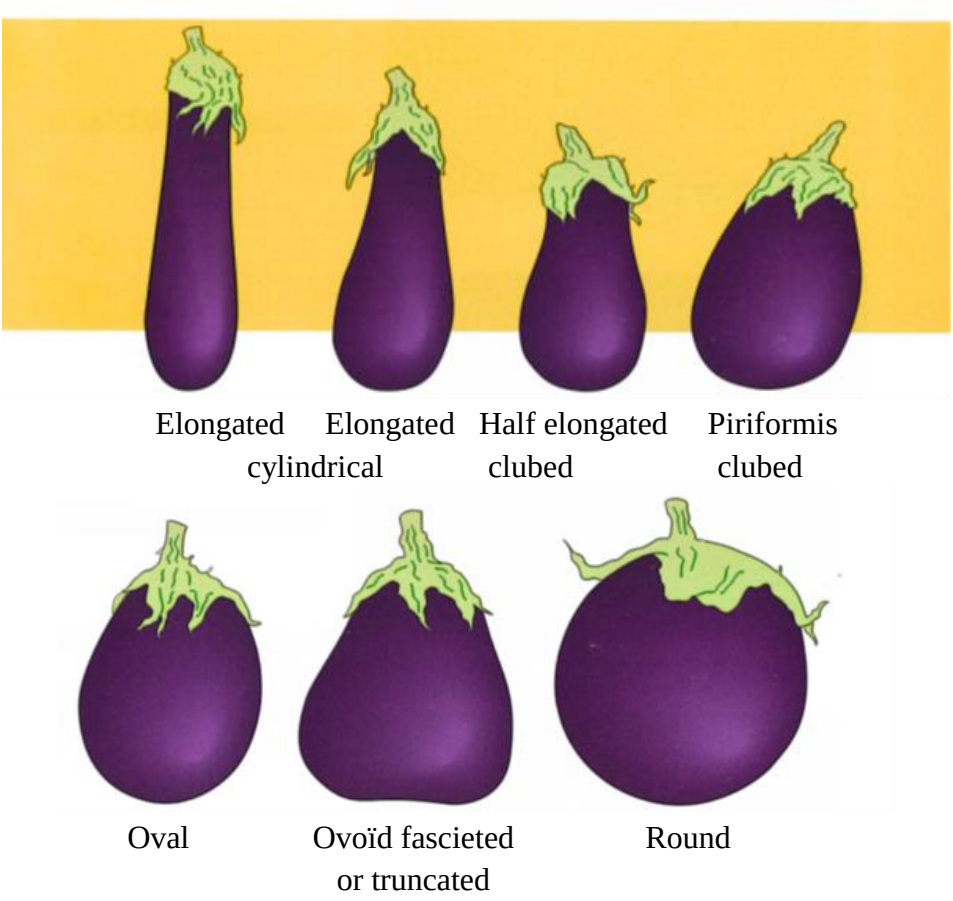

Figure 1.3: Main forms of fruits found in Europe (Adinolfi and Bianchi, 1983)

Botanically, the fruits are full bays, with the seeds arranged in two or more carpels. Their shape varies depending on the cultivar from round to pear-shaped, oblong or elongated and lengths varying from 4 to 5 cm to over 30 cm (Figure 1.3). The bitter and pungent flavour of many varieties of eggplant is due to the presence of solasonine, a glycoalkaloid in the placental area of the fruit and saponins, localized especially in the seeds (Aubert et al., 1989). When the fruits are cut or injured, their flesh takes a dark brown coloration due to the presence of phenolic compounds that rapidly oxidize in air (Rubatzky and Yamaguchi, 1997).

Seeds are small, yellowish brown, smooth and hairless and kidney-shaped. There are 200-250 seeds per gram. Germination is sometimes irregular following harvest but this seed dormancy is easily lifted by a cold treatment. They support desiccation and can retain their germination capacity for several decades if stored in dry and cool conditions (15% RH and 6°C).

The root system is characterised by a strong taproot. In addition, a large number of more superficial, horizontally spreading roots develop. The entire root system is relatively shallow (50 cm) but powerful enough to explore a large volume of earth.

1.3.4 Physiology of flowering, fruit set and fruit ripening

Growth and flowering are continuous throughout the life of the plant and, in view of the competition between vegetative growth and fruiting; eggplant is prone to waves of production. Low light intensities, with high temperatures and/or excess nitrogen fertilization, are favourable for vegetative development at the expense of flowering and fruit set. Sometimes the flowers have sepals overly developed which take the form of leaves or the main stem. This is a result of low temperatures below 10-12°C (Daunay, 2002).

S. melongena is a self-pollinating species, with a tendency to highly variable outcrossing that can reach over 70% depending on climatic conditions and the presence of pollinating insects such as bumblebees and honeybees, wild or domestic. As tomato, eggplant is a buzz-pollinated species. Pollinators vibrate the flower and anthers, dislodging pollen (Vaissière, 2002). In cold and wet weather, fruit set can be significantly improved by the use of insect pollinators. It is also possible to use auxin based hormones which cause the formation of parthenocarpic fruits. *S. melongena* cultivars have a natural parthenocarpic behaviour; this character was introduced by breeders in modern varieties. The fruit is harvested still immature. At this point, its skin is smooth and shiny. The overall colour of the fruit in the commercial stage is a result of that of the exocarp (skin) and mesocarp and endocarp (flesh) (Table 1.6).

Anthocyanin is responsible for the wide variety of colours of eggplant fruit and its content varies considerably among different cultivars. The white fruit cultivars lack this pigment (Sidhu et al., 1982). The anthocyanin coloration of eggplant may be sensitive or insensitive to light. If the biosynthesis is light-sensitive, parts of the fruit with low light exposure are brighter (reduced anthocyanin formation). If light does not interfere with the anthocyanin biosynthesis the fruit has a homogeneous colour regardless of the exposure of the skin (Daunay, 2002). Finally physiological maturity also affects the colour. A fruit that has exceeded its harvest stage becomes dull yellow (on white or light green cultivar) or more or less dark brown ('Black' and purple cultivars).

The fruit firmness changes also with physiological age: the more a fruit is aged, the higher the firmness. There is also a link between fruit diameter and firmness: the thicker the fruit the more it is firm (Hennart, 1996).

Table 1.6: Fruit colour at harvest stage according to the flesh and the skin colour (Messiaen, 1998; Daunay, 2002)

Exocarp	Mesocarp	Endocarp
Colourless	Light green	White
Purple	Purple	Pink purple
Streaked with purple	Purple and green striped	Purple and white striped
Uniform purple	"Black", very dark purple	purple

Eggplant fruits have a reasonable nutritional value which can be compared with the nutritional value of tomato (Sutarno et al., 1993). The chemical composition (Table 1.7) and texture of the eggplant fruit makes them attractive for human consumption worldwide. In addition, certain species of *Solanum* are rich sources of various types of steroidal alkaloids and saponins, which are of great interest for pharmaceutical research. Eggplant is known to have medicinal characteristics (Lawande and Chavan, 1998); it is widely used in traditional medicine against haemorrhoids, ulcers, diabetes, asthma, cholera, bronchitis, dysuria, high blood cholesterol levels, ear infections and toothaches (Sutarno et al., 1993).

Table 1.7: Chemical composition of eggplant (per 100 g edible portion from different eggplant cultivars) (Lawande and Chavan, 1998).

Constituent	Content	Constituent	Content
Oxalic Acid	18 mg	Sodium	3.0 mg
Calcium	18 mg	Copper	0.17 mg
Magnesium	16 mg	Potassium	2.0 mg
Phosphorus	47 mg	Sulphur	44 mg
Iron	0.9 mg	Chlorine	52 mg
Moisture content	92.7 %	Vitamin A	124 UI
Carbohydrates	5.8 g	Thiamine	0.4 mg
Protein	0.98 g	Riboflavin	0.11 mg
Fat	0.18 g	B-Carotene	0.74 µg
Fibre	3 g	Vitamin C	12 mg
Energy	24 kcal		
Steroidal saponin	5-10mg		

Despite the similarity in the chemical composition of eggplant cultivars (Lopes-Andreu et al., 1992), there are differences that are typical for the different cultivars groups. The physical characteristics of fruit, e.g. shape, colour, presence of spines on the calyx or foliar colour, influence the chemical composition (Dighe, 1995). Other factors such as cultural techniques, the availability of water in soil, irrigation and fertilization can also affect the mineral content of eggplant (Russo, 1996). Bajaj et al. (1979) found that the long-fruited cultivars contain, on average, a large amount of dry matter, amino protein, water soluble sugars, free reducing sugars, anthocyanins, phenols and glycoalkaloids as solanine. The percentages of nitrogen were similar for purple, green and white eggplant cultivars (Dighe, 1995). However, the white fruit cultivars contain twice as much crude fibre as the purple and green cultivars (Dighe, 1995); while the amino acid levels were higher in cultivars with purple fruit and lower in white fruit cultivars (Flick et al., 1978).

The presence of glycoalkaloids, which often occur between members of *Solanacea* family, are responsible for bitterness in eggplant fruit and its high levels (20 mg/100 g fresh weight) produces a bitter taste and off-flavour. Potassium, chlorine, magnesium and calcium are present at high concentrations but are highest in the green and lowest in the purple cultivars (Bajaj et al., 1990).

1.4 Salt stress: Causes and responses of plants

1.4.1 Introduction

From an agricultural point of view, salinity is the accumulation of dissolved salts in the soil water to an extent that inhibits plant growth (Gorham, 1992). There are mainly two forms of soil salinity: primary and secondary salinity. Primary salinity results from the accumulation of salts in the soil or groundwater through natural processes over a long period of time. Two natural processes cause primary salinity. The first is the weathering of parent materials containing soluble salts. The second is the deposition of oceanic salt carried through wind and rain. Secondary salinization results from human activities that change the hydrologic balance of the soil between water applied (irrigation or rainfall) and water used by crops and transpiration. The most common causes of secondary salinization are (i) land clearing and the replacement of perennial vegetation with annual crops, and (ii) irrigation schemes using salt rich irrigation water or having insufficient drainage water.

Salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. Estimates vary, but approximately 7% of the world's total land area is affected by salinity (Flowers et al., 1997). Most importantly, the percentage of cultivated land affected by salt is even greater. Furthermore, there is also a dangerous trend of a 10 % per year increase in the saline area throughout the world (Ponnamieruma, 1984). In addition, salinity is a problem for agriculture because also only few crop species and genotypes are adapted to saline conditions. Although irrigation covers only about 15% of the cultivated land of the world, irrigated land has at least twice the productivity of rain-fed land, and may therefore produce one-third of the world's food. The reduced productivity of irrigated lands due to secondary salinity is, therefore, a serious issue. With the projected increase in populations of 4.3 billion people (World meters, 2016) coupled with increased urbanization in developing countries, the world's agriculture is faced with an enormous challenge to maintain, let alone increase, our present level of food production (Owen, 2001). Reducing the spread of salinization and increasing the salt tolerance of crops and improving species or genotypes to salt tolerance, particularly the high yielding ones are, therefore, issues of global importance.

In Tunisia, 30% of available water contains 3 g/l or ±51.3 mM of salt (= threshold value for salty water, Ennabli (1995)) and this proportion increases from north to south (10% in north and 50% in south, Chaabouni 1995). In irrigated agriculture, water with 2 to 3.5g/l (≈ 34.2 to 59.85 mM) of salt are the most used and those grading from 3.5 to 4.5g/l(≈ 59.85 to 76.95 mM) come second (Braudeau and Hachicha, 1998). The use of saline water varies according to region and the importance of water resources (Table 1.8).

Table 1.8: Importance of saline groundwater exploited in Tunisian agriculture (Boutiti, 1995)

Governorate	Salinity (g/l)	Salinity (mM)	Occurence of saline groundwater (100%)
Ariana	4-5	68.4-85.5	75
Béja	4-6	68.4-102.6	40
Ben Arous	1-6	17.1-102.6	100
Bizerte	4	68.4	33
Gabes	5-12	85.5-205.2	100
Gafsa	3-10	51.3-171	100
Jendouba	4-6	68.4-102.6	16
Kairouan	3-4	51.3-68.4	30
Kasserine	3-6	51.3-102.6	54
Kébilli	5-6	85.5-102.6	100
Kef	4-5	68.4-85.5	57
Monastir	5	85.5	75
Nabeul	4-6	68.4-102.6	66
Sfax	5-15	85.5-256.5	100
Sidi Bouzid	3.5-10	59.85-171	40
Siliana	7-16	119.7-273.6	55
Tozeur	4-6	68.4-102.6	66
Zaghouane	5-6	85.5-102.6	80

1.4.2 Causes and types of salinity

1.4.2.1 Quality of irrigation water

The suitability for the use of water in irrigation should be based on the chemical composition of the residual alkalinity and the electrical conductivity. Braudeau and Hachicha, (1998) divided the waters into five classes according to their electrical conductivity (Table 1.9).

The sodium adsorption ratio (SAR) is used as an index of risk of alkaline water. SAR describes the proportion of sodium to calcium and magnesium in the solution and is given by the following expression:

$$SAR = Na / \sqrt{(Ca + Mg) / 2}$$

Where Na, Ca and Mg are the concentrations of these ions in meq/l.

SAR of irrigation water is thus connected to the exchangeable sodium percentage (ESP) by the soil. It is also used to measure the sodicity of soils.

Table 1.9: Water classification according to the electrical conductivity (EC) (Braudeau and Hachicha, 1998)

Class	EC	Characteristics
C1	EC<0.25dS/m	Low salinity water.
C2	0.25<EC<0.75dS/m	Medium salinity water.
C3	0.75<EC<2.25dS/m	Water with high salinity.
C4	2.25<EC<5dS/m	Water with very high salinity.
C5	5<EC<20dS/m	Exceptional saline water

Table 1.10: Water classification according to the sodium adsorption ratio (SAR) (Lacharme, 2001).

Class	SAR	Characteristics
S1	SAR < 10	low sodic water used for irrigation of almost all soils with little danger.
S2	10<SAR<18	moderately sodic water having a danger of appreciable alkalization in the ground fine texture.
S3	18<SAR<26	strongly sodic water can cause the appearance of the contents of exchangeable Na, dangerous in most soils.
S4	26<SAR<100	strongly sodic waters and generally unusable for irrigation unless salinity is low or average

1.4.2.2 Saline soils

Salinization is the set of mechanisms according to which the soil is enriched with soluble salts and acquires a more or less strong, salty character (Braudeau and Hachicha, 1998). In irrigated lands, the water applied to the soil is consumed by the crop or evaporates directly from the moist soil. The excess salt remains and accumulates in the soil causing salinization. Irrigation with salty water hastens this process (Chaabouni, 1995).

Considering the average requirement of 6,000 m³/ha for irrigated areas of North and Centre Tunisia, 11,000 m³/ha for the oasis of Gabes and 20,000 m³/ha for the rest of the southern oasis, areas threatened by salinization are estimated to be 68,000 ha for irrigated plots from groundwater and 2,000 ha for irrigated plots from deep aquifers (Boutiti, 1995).

Two main types of salt affected soils have been described namely alkaline and sodic soils.

1.4.2.2.1 Sodic soils

The term sodic soil is used to refer to situations where the soil physical behaviour is affected by the presence of exchangable sodium irrespective of the Na amount present. The sodium affects the behaviour of the diffuse double layer of the clay particles in relation to swelling, clay dispersion and physical degradation (Sumner, 1993).

Sodisation is measured as the percentage of the cation exchange complex occupied by sodium (ESP).

$$ESP = \frac{Exchangeable\ Na\ (meq\ per\ 100\ g\ soil) x\ 100}{Cation\ Exchange\ Capacity\ (meq\ per\ 100\ g\ soil)}$$

When a threshold sodium level is reached, generally around 10% or more (ESP ≥ 10%), clays tend to deflocculate (disperse). A soil is considered sodic if the ESP is higher than 15%.

Sodic soils consist of fine-textured soils, with high contents of smectite clay, with low permeability and poor drainage (Rafiq, 1990). In such soils, swelling and dispersion of soil aggregates causes the size and number of water-conducting pores (macropores) to decrease, resulting in a slow leaching. The salty water held in micropores remains largely immobile under steady-state flow conditions, since the micropores do not take much part in the water flow (Russo, 1989).

1.4.2.2.2 Alkaline soils

These sodic soils contain Na^+ salts capable of alkaline hydrolysis ($Na_2\ CO_3$); this is an increase in pH (pH > 9) of the soil under the effect of the accumulation of bases (Lacharme, 2001).

The presence of CO_3^{2-} ions, causes $CaCO_3$ (which is only slightly soluble) to precipitate as solid calcium carbonate. Hence, the calcium ions Ca^{2+} are immobilized and the Na^+ ions left in solution can bind to the colloidal complex (Braudeau and Hachicha, 1998).

$$2\ Na^+ + CO_3^{2-} + Ca^{2+} \rightarrow 2\ Na^+ + CaCO_3\ (solid)$$

According to Sumner (1993) alkaline soils are also defined as soils in which the ESP is >15 and the EC of the saturation extract is <4 dS with pH between 8.5 and 10.

1.4.3 Plant responses to salt stress

1.4.3.1 Introduction

Plants can be categorized according to their biomass production under salt stress. Four main responses can be distinguished (Prasad, 1997). Eu-halophytes (*Salicornia*) have growth stimulation at moderate salt stress while facultative halophytes have enhanced growth at low salt levels (*Plantago maritima*). Glycophytes can be subdivided in plants with low salt tolerance (e.g. *Hordeum*) and very low salt tolerance (*Glycine max*). Most crop plants are glycophytes.

The general effect of salinity is a reduced growth rate resulting in smaller leaves, shorter internodes and sometimes fewer leaves. The initial and primary effects of salinity, especially at low to moderate concentrations, are due to their osmotic effects (Munns and Termat, 1986; Jacoby, 1999). Roots are also reduced in length and mass but depending on the genotype may become thinner or thicker (Munns and Tester, 2008). Plants with their root system in a medium with heterogeneous salt concentration, such as occurs in the soil, develop more roots and absorb more water in the less saline part of the medium. Over days, reduction in cell elongation and also cell division leads to slower root appearance and smaller final size. Cell dimensions change, with more reduction in area or/and in depth, so roots are smaller and thicker or longer and thinner.

Maturity rate may be delayed or advanced depending on the species. The degree to which growth is reduced by salinity differs greatly with species and to a lesser extent with varieties within a species. The severity of the salinity response is also mediated by environmental interactions such as relative humidity, temperature, radiation and air pollution (Shannon et al., 1994).

Depending upon the composition of the saline solution, ion toxicities or nutritional deficiencies may arise because of a predominance of a specific ion or competition effects among cations or anions (Khan, 2001; Parida et al., 2005; Cheng et al., 2015). The osmotic effects of salinity contribute to reduced growth rate, changes in leaf colour, and developmental characteristics such as root/shoot ratio and maturity rate. Ionic effects are manifested more generally in leaf and meristem damage or as symptoms typical for nutritional disorders. Thus, high concentration of Na or Cl may accumulate in leaves or portions thereof and result in ‘scorching’ or ‘firing’ of leaves; whereas, nutritional deficiency symptoms are generally similar to those that also occur in the absence of salinity.

Calcium deficiency symptoms are common when Na/Ca ratio is high in the soil water. Calcium is known to play a crucial role in maintaining the structural and functional integrity of plant membranes in addition to its considerable role in cell wall stabilization, regulation of ion transport and selectivity and activation of cell wall enzymes. The low Ca/Na ratio of a saline medium plays a significant role in growth inhibition in addition to causing significant changes in morphology and anatomy of plants. It is possible to speculate that Ca controls Na influx by gating channels in the plasmalemma that are permeable to Na (Kaya et al., 2002).

All salinity effects are not automatically negative; salinity may have some favourable effects on yield, quality, and disease resistance. At low salinity, relative growth rate and relative leaf growth rate of pea plants did not decrease significantly (Najafi et al., 2007). In grafted water melon low salinity improved fruit quality, total fruit yield, dry matter, glucose, fructose and total soluble solids (Colla et al., 2006). In potato, low salinity increased leaf area and dry matter compared to control treatment (Van Hoorn et al, 1993). Tomato apparently favours the growth of foliage at the expense of fruit formation under saline conditions (Katerji et al., 1998). Pardossi et al. (1999) working on celery noticed that increasing salinity had little or no influence on plant growth, water relations, and the tissue concentration of macronutrients, but it enhanced the uptake of Na and Cl, which accumulated markedly in the mature leaves and to a much lesser extent in the actively growing leaves. Moreover salinization also improved the yield quality by reducing the accumulation of nitrate–nitrogen and the incidence of `blackheart' in young leaves

Generally, salinity can inhibit plant growth by three major ways (Greenway and Munns, 1980):

- Water deficit arising from the more negative water potential (elevated osmotic pressure) of the soil solution;
- Specific ion toxicity usually associated with either excessive chloride or sodium uptake; and
- Nutrient ion imbalances when the excess of Na^+ or Cl^- leads to a diminished uptake of K^+, Ca^{2+}, NO_3^- or P, or to impaired internal distribution of one or another of these ions.

1.4.3.2 Effects of salinity on plant phenology and biomass

One immediate response of plants to elevated salinity is a decrease in the rate of leaf expansion. Consequently, the total leaf area of the plant is reduced. The common decrease in

leaf expansion is associated with a loss in cell turgor pressure rather than a salt-specific effect. This is supported by Na^+ and Cl^- levels which are below toxic concentrations in the expanding cells themselves. For example, Hu and Schmidhalter (1998) showed that wheat growing in 120 mM NaCl reacted with a 25% reduction in growth rate, Na^+ in the cells of expanding leaves was maximal only 20 mM, and Cl^- only 60 mM. However, a review by Ball (1988) on mangrove found that the common decrease in leaf expansion is not related to a loss in turgor pressure and is most likely a result of a change in hormonal signalling from roots to leaves.

In the salt-sensitive genotypes, in which salt is not effectively excluded from the transpiration stream, salt will build up to toxic levels in the leaves, resulting in death of old leaves and new leaves becoming injured and succulent (Munns and James, 2003). Consequently, the number of green and healthy leaves will ultimately decline. There is then a race against time to initiate flowers and produce seeds while there are still an adequate number of green leaves left to supply the necessary photosynthesis (Mass and Poss, 1989; Munns, 1993). Consequently, seed number and seed size are reduced.

Although salinity can induce a rapid reduction in root growth (Neumann, 1995), shoot growth decreases proportionally more than root growth, causing an increase in the root/shoot ratio. In addition, salinity significantly decreased tiller number and their appearance in wheat (Mass and Poss, 1989). Salinity significantly reduces the total dry matter yield, and the degree of reduction in total dry matter depending on genotypes and salt concentrations (Pessarakli and Huber, 1991). Salinity causes stunting of shoots.

The phenological responses to salt stress are complex and change with the developmental stages of the plant (Neumann, 1995). For example, many crops show a reduced tolerance to salinity during seed germination, but greater tolerance during later growth stages and vice versa in other crops. Results of salt tolerance for some crops have shown that wheat, sorghum and cowpea (Mass and Poss, 1989) were most sensitive during the vegetative and early reproductive stages, less sensitive during flowering, and least sensitive during the grain filling stage. In contrast, sugar beet and sunflower are relatively more sensitive during germination and most tolerate at late growth stage (Mass and Poss, 1989), while the tolerance of soybeans may increase or decrease during different growth periods depending on the variety. Levy (1992) working on potato showed that salinity delays the emergence of the plants, reduces the growth of stems and tubers and hastens maturity. Therefore, information on the growth stage response to salinity is important in adopting suitable genetic and management strategies for

saline soils. For example, if a crop is more sensitive during one stage than another, it may be possible to irrigate with saline water during the more tolerant stages of growth and use low-salinity water only during the sensitive stages of growth.

In glycophytes, growth rate is generally reduced by salinity even at low concentrations (Greenway and Munns, 1980). NaCl reduced the total above ground biomass and delayed flowering and maturity in rice (Castillo et al., 2007). Increasing salinity decreased significantly plant growth in tomato (Zribi et al., 2009). Pasternack et al. (1984) stated that the sensitivity of onions to salt stress during the early stages of growth could be due to a small and shallow rooting system. Wannamaker and Pike (1987) showed that the salinity seriously affects germination in onion (50% reduction for an EC of 130 $dS.m^{-1}$). François (1994) working on garlic concluded that salinity negatively affect all components of the yield (weight and diameter of the bulb, the number of plants per unit area). The yield in carrot roots decreased by 14% for each unit of increase in the salt stress (Malcolm Smith, 1971).. Graifenberg et al. (1996) classed fennel as a sensitive crop to salinity. Indeed fennel bulbs accumulated more Na^+ and Cl^- than leaves and roots. In addition the Na^+ generates the deficiency of K^+ in the bulbs which may contribute to the reduction in growth. Salinity increases Na^+ and Cl^- in tissues basal to the apical meristem in lettuce and contributes to the reduction of Ca^{2+}, K^+ and PO_4^{2-} (Lazof and Läuchli, 1991). This disruption of the ionic composition generates a nutritional imbalance in the apical meristem which might signal growth reduction in expanding leaves.

1.4.3.3 Effect of salinity on physiological aspects

Salinity stress involves changes in various physiological processes. One approach toward understanding of physiological responses to salinity is to follow the series of events after salinity initiates. Such time studies do not prove causal relations, but they can eliminate some possibilities. For example, if leaf expansion slows before photosynthesis does, then the decrease in photosynthesis cannot cause the decrease in leaf expansion (Munns, 1993; Yeo, 1998).

The initial effects of increasing soil salinity are very similar to those observed when plants are exposed to drought. Reductions in leaf water potential will reduce stomatal conductance and stomata will close. This simultaneously restricts the entry of CO_2 into the leaf, reducing photosynthesis (Baker and Rosenqvist, 2004). At higher concentrations, NaCl may also directly inhibit photosynthesis due to oxidative stress (Stepien and Johnson, 2009). Salt stress contributes to the accumulation of toxic compounds (free reactive oxygene) which induce an

oxidative damage. The inhibition of assimilation in salt-stressed plants is accompanied by a decrease in electron transport through PSII, indicated by the decline in ΦPSII and the photochemical quenching, and cumulative damage to PSII, indicated by the progressive drop in F_v/F_m (François, 1994; Zribi et al., 2009; Stepien and Johnson, 2009).

The uptake of NaCl competes with that of other nutrient ions, especially K^+ leading to potassium deficiency (Ball et Farquhar, 1984). Although leaves of the halophyte grey mangrove have been reported to accumulate high NaCl concentration, changes in photosynthesis were associated with change in leaf K^+ concentration (Ball et al., 1987). More than 50 enzymes require K^+ as a cofactor, and these are particularly susceptible to high Na^+ and high Na^+/K^+ ratios (Munns et al., 2005). A substantial decrease in the photosynthetic capacity of spinach leaves has been attributed to the reduction in K^+ supply under high-salinity conditions which lead to a reduction in the quantum yield, due to the malfunctioning of photosystem II (Chow et al., 1990).

A strong positive correlation has been found between the photosynthetic capacity of leaves and their nitrogen content, most of which is used for the synthesis of components of the photosynthetic apparatus (Evans et Terashima, 1987; Sugiharto et al., 1990). A high chloride level reduces the uptake of nitrate. Furthermore, a specific negative ion effect of chloride on photosynthesis has been found in tomato plants (Heuer and Feigin, 1993), and Cl^- has also been found to be closely associated with the inhibition of photosynthesis in bell pepper plants (Bethke and Drew, 1992). A direct effect of NaCl on the photosynthesis process has also been found in pea plant (Fedina et al., 1994). In chickpea, photosynthetic rates were reduced more by chloride than by sulphate salinity (Datta and Charma, 1990). In salt stressed barley plant, reduced Mn concentrations have been correlated with a reduced CO_2 assimilation rate (Cramer and Nowak, 1992). Photosynthetic activity in rice could be significantly increased by potassium applications (Bohra and Doerfling, 1993) and the net photosynthetic rate of barley was remarkably increased by nitrogen nutrition (Shen et al., 1994).

Salinity also caused chloroplasts to aggregate which leads to ultrastructural changes of the assimilating organs (Glagoleva et al., 1992). These include dilatation of thylakoid membranes and enlarged mesophyll cells (Brugnoli and Bjorkman, 1992; Mitsuya et al., 2000). Salt stress significantly reduced chlorophyll content in many plants (Hernandez et al., 1995; Zhu et al., 2002).

1.4.3.4 Effect of salinity on biochemical aspects

Salt stress leads to oxidative stress as observed by the accumulation of toxic compounds such as reactive oxygen species (ROS) in plants, which include peroxides, superoxides and hydroxyl radicals (Burdon et al., 1996; Shen et al., 1997; Tsugane et al., 1999). These toxic molecules can then damage cellular membranes, membrane-bound structures, enzymes and DNA especially in mitochondria and chloroplasts, and can therefore severely impair plant growth and survival (Allen, 1995). Increasing salinity is associated with a decrease in auxin, gibberellin and cytokinin levels in plant tissues, and an increase in abscisic acid (Moorby and Besford, 1983). Such changes in hormone levels are thought to be a primary process regulating the reduction in growth associated with salinity. There is little evidence that salinity directly affects the hormone balance within the plant, and the greatest change in hormone levels caused by saline conditions results from water deficit (Blume, 1988).

Most research on the effect of salinity on the enzyme activity and metabolism of proteins was performed *in vitro* (Noble and Rogers, 1992). Generally, enzymes are inhibited *in vitro* by salt irrespective if they are extracted from glycophytes or halophytes (Greenway and Munns, 1980). Tolerance to salinity is always correlated with efficient antioxidant systems (Gosset et al., 1994; Sreenivasulu et al., 2000; Bowers et al., 2000; Ashraf and Harris, 2004; Demiral and Turkan, 2004).

1.5 Mechanisms of salinity tolerance of plants

1.5.1 Osmotic adjustment

Osmotic adjustment by means of solute accumulation in plant cells is a process by which the water potential of a cell can be decreased without an associated decrease in cell turgor. It is a net increase in solute content per cell that is independent of the volume changes that result from loss of water (Taiz and Zeiger, 2002). Osmotic adjustment in plants subjected to salt stress can occur by the accumulation of high concentrations of either inorganic ions or organic solutes (or both). Their relative contribution varies among species, among cultivars and even between different compartments within the same plant (Ashraf, 1994a; Ashraf and Bashir, 2003).

Ion accumulations in the cytosol (mainly K^+) and in the vacuole (Na^+, especially in salt tolerant cultivars/species) are found to be important for the osmotic adjustment of plant cells (Gorham et al., 1985).

The compatible organic osmolytes generally found in higher plants are low molecular weight sugars, organic acids, polyols, and nitrogen containing compounds such as amino acids, amides, imino acids, soluble low molecular weight proteins such as LEA (late embryogenesis- abundant) proteins and dehydrins. The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity or drought, often accompanied by a significant decrease in net CO_2 assimilation rate (Popp and Smirnoff, 1995; Murakeozy et al., 2003). Amino acids have been reported to accumulate in higher plants under salinity stress (Ashraf, 1994b; Mansour, 2000). The important amino acids in this respect include alanine, arginine, glycine, serine, leucine and valine, together with the imino acid, proline, and the non-protein amino acids, citrulline and ornithine (Rabe, 1990; Mansour, 2000). Proline accumulates in larger amounts than other amino acids in salt stressed plants (Ashraf, 1994a; Ali et al., 1999; Abraham et al., 2003). Of the different quaternary compounds, glycine betaine is known to play an important role in osmotic adjustment in salt stressed plants (Mohanty et al., 2002; Yang et al., 2003) although not all plants can biosynthesize this compound. While assessing the role of some amino acids and glycine-betaine in osmoregulation of spinach plants subjected to salt stress, Martino et al. (2003) found that osmoregulation due to accumulation of some free amino acids and glycine betaine, was one of the predominant strategies used by spinach plants to tolerate saline stress. Osmotic adjustment has undoubtedly gained considerable recognition as a significant and effective mechanism of salinity resistance in crop plants. Compatible solutes seem to function as a chaperone protecting enzymes and membrane structures, and as a scavenger reducing radical oxygen species under salt stress conditions (Horie et al., 2012).

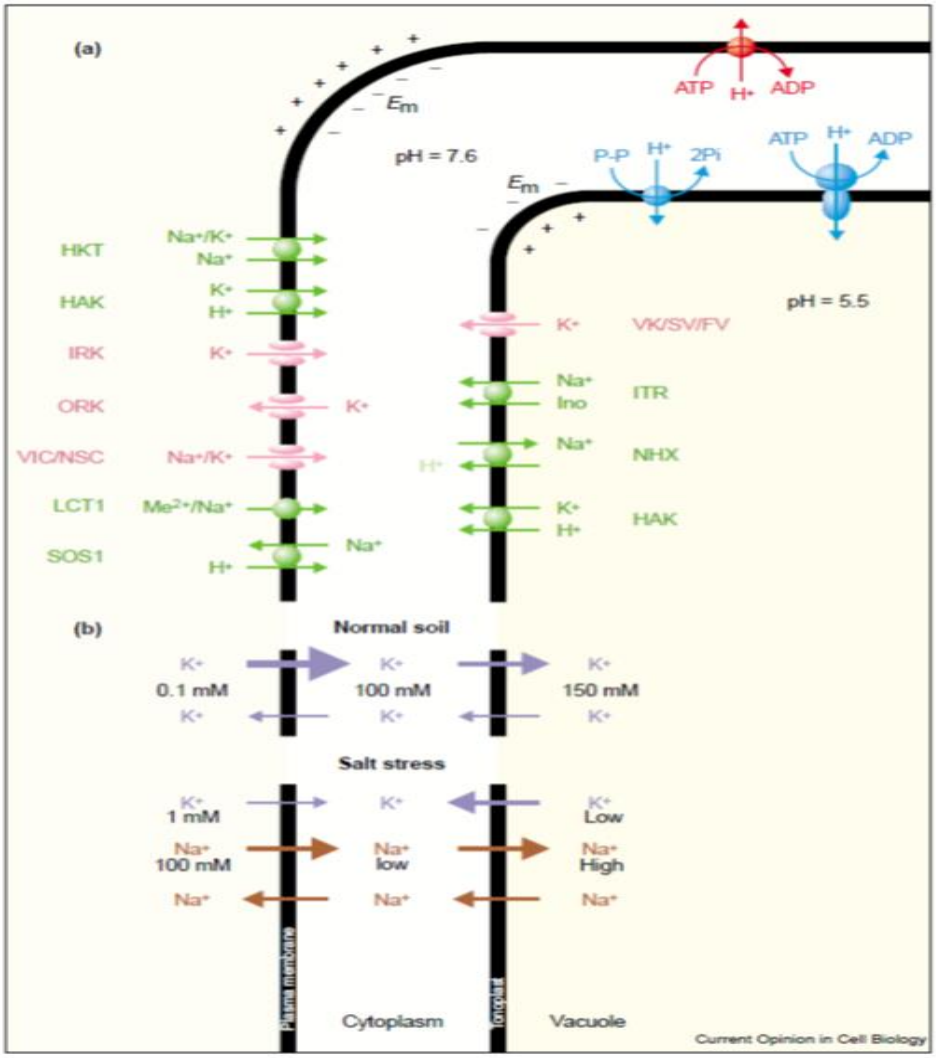

Figure 1.1: Transporters and fluxes of K+ and Na+ in the plant cell (Serrano and Rodriguez-Navarro, 2001)

Legend:(a) The P-type H^+-pumping ATPase shown in red at the upper part of the scheme energizes the plasma membrane, developing a membrane potential which may vary between –100 and –200 mV and a pH gradient of 2–3 units. Under the effect of this electrical potential, and occasionally the effect of the pH gradient, several transporters (shown in green — HKT is a K^+ Na^+ symporter or Na^+ uniporter; HAK is probably a K^+H^+ symporter; SOS1 is a Na^+/H^+ antiporter; and LCT1 transports divalent cations or Na^+) and channels (shown in pink — IRK, inward rectifying K^+ channels; ORK, outward rectifying K^+ channels; VIC, voltage insensitive channels; NSC, non-selective channels) mediate K^+ and Na^+ movements across the plasma membrane. A V-type H^+- pumping ATPase and a H^+-pumping pyrophosphatase (shown in blue) energize the tonoplast developing a membrane potential, which may vary between –80 and –20 mV, and a pH gradient of 1–2 pH units. Under the effect of this electrical potential and the pH gradient, several transporters (shown in green — NHX is a Na^+/H^+ antiporter; ITR is a *myo*inositol- Na^+ symporter; a HAK type K^+- H^+ symporter has not been identified, but can be predicted in certain circumstances from the K^+ distribution across the tonoplast) and channels (shown in pink — SV, slow-activated vacuolar channels; VK, vacuolar K^+ channels; FV, fast-activated vacuolar channel) mediate K^+ and Na^+ fluxes across the tonoplast.

(b) In non-salt-stressed cells, K^+ is taken up from the external medium and accumulated in the cytoplasm and vacuole; a part of this K^+ may be returned to the external medium either as a normal balancing efflux or during osmotic adjustments; these fluxes are mediated by channels, but the existence of K^+/H^+ antiporters is also probable. The presence of 100 mM Na^+ in the external medium inhibits the K^+ influx mediated by HKT, HAK and IRK, and triggers a Na^+ influx mediated by HKT, VIC/NSC, and LCT1; Na^+ may return to the external medium crossing the tonoplast, by mediation of ITR, and the plasma membrane, by mediation of SOS1.

1.5.2 Ion exclusion or inclusion

Plants respond to salinity stress either by accumulating inorganic ions as osmotica for maintenance of water balance, the halophytic response, or by partial exclusion of ions and the synthesis of organic osmotica for osmotic adjustment, the glycophytic response.

Effective strategies for glycophytes to cope with salinity stress are to keep cytosolic Na^+ levels low at the cellular level and to keep shoot Na^+ concentrations low at the whole plant level. Glycophytes thus accumulate a certain level of Na^+ in their roots and exclude it from their shoots, especially from the meristems and from leaves that are actively expanding and photosynthesizing. Such plants are referred to as Na^+ excluders (Wyn Jones, 1981; Ashraf, 1994a). Regulation of Na^+ uptake by cells and long distance Na^+ transport seems to be a crucial adaptation of plants to salt stress (Munns et al., 2000).

In contrast, certain species efficiently accumulate high amounts of Na^+ in the shoots and are thus known as Na^+ includers. For example, most dicotyledonous halophytes are Na^+ includers, and some salt tolerant glycophytes such as barley fall into this category (Collander, 1941).

The underlying mechanisms of Na^+ entries into plant roots via both symplastic and apoplastic pathways are largely unknown. Although there is strong evidence that Na^+ moves passively through a general cation channel from the saline growth medium into the cytoplasm of plant cells (Blumwald, 2000; Mansour et al., 2003), active transport of Na^+ through Na^+/H^+ antiports is also known (Niu et al., 1993; Shi et al., 2003). During intrusive Na^+ entries into the root, plants can exert "selectivity" at three independent biological membranes: the plasma membrane of epidermal/cortical cells, the tonoplast of cells in roots and shoots, and the plasma membrane of the xylem parenchyma cell (Horie et al., 2012) (Figure 1.1). Salt tolerance in most plants is associated with low uptake and accumulation of Na^+, which is mediated through the control of influx and/or by active efflux from the cytoplasm to the vacuoles and also back to the growth medium (Grattan and Grieve, 1999; Blumwald, 2000) (Figure 1.1). This control mechanism is dependent on the regulation of proton pumps and antiporters operating at both plasma membrane and tonoplast. For example, overexpression of the vacuolar Na^+/H^+ antiporter that sequesters Na^+ in vacuoles (NHX1) improved the salinity tolerance in *Arabidopsis*, tomato, and brassicas (Aharon et al., 2003) (Figure 1.1). Tavakkoli et al. (2011) demonstrated that Na^+ and Cl^- exclusion among barley genotypes are

independent mechanisms and different genotypes expressed different combinations of the two mechanisms.

The variation in mechanism of ion uptake could be due to some multiple adaptations to toxic ions operating concurrently within a specific plant. These mechanisms can occur in all cells within the plant, or can occur in specific cell types, showing adaptations at cellular or whole plant level (Tester and Davenport, 2003; Carden et al., 2003). It is evident from these reports that glycophytes can use both ion exclusion or inclusion mechanisms in response to saline substrates. These two mechanisms depend on the pattern of ion distribution between leaves and on ion compartmentation within the cell (Cheeseman, 1988; Ashraf, 1994a; Munns, 2002). As a further complication, time-courses of ion accumulation can be different in an organ-specific way (Ashraf and Bashir, 2003).

1.5.3 K^+/Na^+ and Ca^{2+}/Na^+ ratios discrimination

Under saline conditions, due to excessive amounts of exchangeable Na^+, high Na^+/K^+ and Na^+/Ca^{2+} ratios occur in the soil. Plants subjected to such environments, take up high amounts of Na^+, whereas the uptake of K^+ and Ca^{2+} is considerably reduced. Reasonable amounts of both K^+ and Ca^{2+} are required to maintain the integrity and functioning of cell membranes (Marschner, 1995; Davenport et al., 1997; Wenxue et al., 2003).

It is now generally accepted that K^+/Na^+ homeostasis is a key feature of plant salinity tolerance (Ashraf, 2004). The underlying mechanism for maintenance of adequate K^+ in plant tissue under salt stress seems to be dependent upon selective K^+ uptake and selective cellular K^+ and Na^+ compartmentation and distribution in the shoots (Poljakoff-Mayber and Lerner, 1999; Munns et al., 2000; Carden et al., 2003). Plants use low- and high-affinity transporters for uptake of K^+ from the growth medium (Blumwald, 2000). There are three classes of low affinity K^+ channels [Inward rectifying channels (KIRC), K^+ outward rectifying channels (KORCs), and voltage-independent cation channels (VIC)], which play important roles in maintaining cellular K^+/Na^+ ratios (Amtmann and Sanders, 1998) (Figure 1. 1). In addition, two families of high-affinity transporters have also been reported to play a role in K^+ transport (Quintero and Blatt, 1997), and they also determine the K^+/Na^+ ratio in plant cells. For example, the KUP-HAK, high-affinity K^+ transporters have been found in *Arabidopsis* (Quintero and Blatt, 1997; Kim et al., 1998; Fu and Luan, 1998) and barley (Santa- Maria et al., 1997) (Figure 1. 1). These transporters couple K^+ transport to the H^+ gradient and are very selective for K^+. However, Na^+ blocks them in even small concentrations (Kim et al., 1998;

Fu and Luan, 1998). High K^+/Na^+ selectivity in plants under saline conditions has been suggested as an important selection criterion for salt tolerance (Gorham et al., 1997; Ashraf, 2002; Wenxue et al., 2003). Cheng et al. (2015) suggest that higher-affinity K^+ uptake might play a key role in higher salt tolerance and it might be a reliable indicator for breeding new species of salt-tolerant wheat.

Kafkafi (1984) concluded that roots of the salt tolerant *Beta vulgaris* had a greater affinity for K^+ relative to Na^+ than did the salt sensitive *Phaseolus vulgaris*. Recently, however, Munns and James (2003) have found that although Na^+ exclusion had a positive relationship with salinity tolerance of different tetraploid wheats, K^+/Na^+ ratio showed little relationship.

Calcium plays an important role in protecting the structure and the functioning of plant membranes besides the control of regulation of ion transport and enzymes activities. (Rengel, 1992; Marschner, 1995). Wu et al. (2012) suggest that Ca^{2+} could regulate K^+/Na^+ homeostasis in rice at low salinity by enhancing the selectivity for K^+ over Na^+, reducing the Na^+ influx and efflux, and lowering the futile cycling of Na^+.

The maintenance of calcium acquisition and transport under salt stress is an important determinant of salinity tolerance (Soussi et al., 2001; Unno et al., 2002). In most cases salt tolerance of a crop cultivar can be increased by an increase in the Ca^{2+} concentration in the saline growth medium. For example, supplemental calcium alleviated the adverse effect of salt stress on the germination and vegetative growth of bean (Awada et al., 1995) and pigeon pea (Subbarao et al., 1990). In contrast, no significant effect on the uptake of Na^+ by rice of varying Na^+/Ca^{2+} ratios was found by Yeo and Flowers (1985). The relationship between salt tolerance and Ca^{2+} retention among different plant species was investigated by Unno et al. (2002) using salt tolerant maize (*Zea mays*) and squash (*Cucurbita maxima*), and salt sensitive reed canary grass (*Phalaris arundinacea*) and cucumber (*Cucumis sativus*). Ca^{2+} was released extensively from root sections and intact roots of the salt sensitive plants when exposed to saline medium. The distribution of Ca^{2+} in shoot decreased greatly in the salt sensitive plants under salt stress. These results suggest that the ability of plants to retain Ca^{2+} is associated with their salt tolerance. It can be concluded that K^+/Na^+ and Ca^{2+}/Na^+ ratio and selectivity are effective in discriminating between salt tolerant and salt sensitive plants of many crops

Chapter 2

In vitro regeneration of eggplant

Chapter 2 In vitro regeneration of eggplant

2.1 Introduction

In vitro culture, with its potential to induce somaclonal variation (chromosomal rearrangements) proved to be a promising method to select for salt tolerant genotypes in a number of crops (Karan and Subudhi, 2012). Also molecular marker techniques were used successfully to transfer alleles of interest from wild relatives into commercial cultivars (Tanksley and McCouch, 1997). Marker-assisted selection (MAS) and QTL mapping to select genotypes with desirable salt tolerant traits offers also a great promise for plant breeding for traits as abiotic stress tolerance (Karan and Subudhi , 2012).

The *in vitro* culture approach reduces the time required for the release of new variety compared to mutation breeding and has been useful in breeding programs (Zhu et al., 2000). According to Rai et al. (2011) *in vitro* selection is based on the induction of genetic variation among cells, tissues and/or organs in cultured and regenerated plants. The selection of somaclonal variation appearing in the regenerated plants may be genetically stable and useful in crop improvement. A general approach to select for salt-tolerant crops is given in Figure 2.1. After selection of suitable explants callus production is initiated. These undifferentiated cells will then be cultivated on media with increasing osmolarity. Calli which survive are then used to regenerate plants with a higher salt tolerance. Queiro et al. (2007) established from potato callus cultures (by direct selection or gradual selection) cell lines able to grow on media containing (50, 100, 150 or 200 mM NaCl). The same authors reported that the NaCl-tolerant calli showed a decrease in relative growth rate and water content, with higher reduction in the 150 mM tolerant callus. Hassan and Willkins (1988) obtained a salt tolerant callus line of *Lycopersicon peruvianum* by exposing the cells in suspension cultures and then regenerate callus on increasing concentrations of salt (50-350 mM). Moreover, this selected line grew better in media containing salt than in those without it. It retained its salt tolerance after subculture for 3 passages (3 months) on salt-free medium.

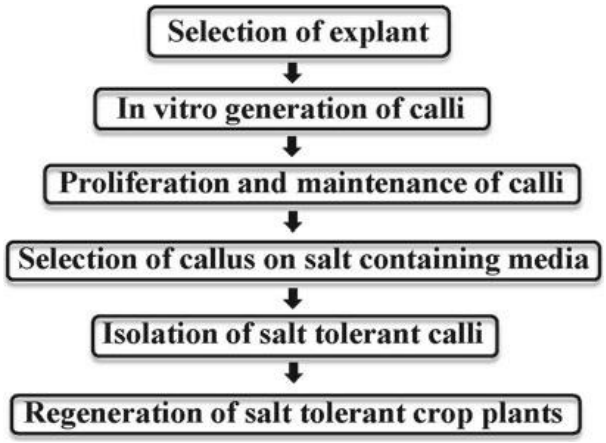

Figure 2.1: An in vitro procedure for regeneration of salinity tolerant crop plants (Karan and Subudhi , 2012)

A bottleneck in this approach is the necessity for a high regeneration of plants from calli. In eggplant in vitro regeneration of different explant types has been reported both via embryogenesis (Rao and Singh, 1991; Saito and Nishimura, 1994; Sharma and Rajam, 1995) and organogenesis (Allichio et al., 1982; Gleddie et al., 1983; Mukherjee et al., 1991; Sharma and Rajam, 1995). Magioli et al. (1998) found that eggplant cotyledons and leaves were the most responsive explant types. The regeneration capacity of plant tissue is however, highly dependent on the cultivar. For example, Sharma and Rajam (1995) studying four Indian eggplant cultivars showed that the variety Pusa Kranti produced more shoots from cotyledon fragments than from any other explant type. In other Solanaceae, such as potato, cultivation of leaf discs of 14 potato varieties showed a wide variation in newly formed shoots, one genotype produced up to 45 units per explant (Wheeler et al., 1985).

The balance between two plant hormones, auxin and cytokinin, determines the state of differentiation and dedifferentiation. In potato, NAA is the most often used auxin for efficient formation of callus (Al Wareh et al., 1989). It is a very strong auxin which exerts its action over a wide concentration ranging from 0.03 to 5 mg/l (Pijnacker and Ferwerda, 1990). Also thidiazuron (TDZ) has been shown to induce callus formation in a variety of species and sometimes higher proliferation rates were obtained compared to other growth regulators (Murthy and Saxena, 1998).

Organ regeneration in plants can be broadly categorized as either direct or indirect. The presence of auxins and cytokinins in the medium is required for inducing shoots. TDZ is also known for its potential to induce adventitious buds (Murthy and Saxena, 1998). Indeed, TDZ application results in a high endogenous cytokinin activity in *in vitro* conditions (Wang et

al.,1986; Fiola et al.,1990; Saxena et al.,1992) as it inhibits degradation of cytokinins through inhibition of cytokinin-oxidase (catabolic pathway) (Hare and Van Staden, 1994). For example, low concentrations of TDZ induced shoots from both cotyledons and leaf explants of eggplant (Magioli et al., 1998). Concentration plays a significant role in the regeneration of shoots and also the interaction with other cytokinins such as BA influenced shoot induction (Magioli et al., 1998).

Plants growing *in vitro* are also affected by environmental factors such as light and temperature (Kozai and Smith, 1995). Light (the quality of the spectrum, the photon flux and photoperiod) is a very important factor and affects growth and development of plants *in vitro*. Light quality plays also an important role in morphogenesis and photosynthesis (Hoenecke et al., 1992; Saebo et al., 1995). Callus induction and shoot regeneration are closely related to light quality. Piao (2002) showed that fluorescent light was better than blue and red for the in vitro multiplication of potato. Tennessen et al. (1994) have suggested that red light inhibits the growth of shoots. Werbrouck et al. (2012) showed that both the number of shoots and callus growth was enhanced by blue light in *Ficus benjamina*. However, an interaction with light intensity is also described as *Prunus* has the highest proliferation rate at low intensity of red light but under higher intensity, red light does not differ from white or blue light in its proliferation rate (Baraldi et al., 1988). Inconsistent responses may thus be the result of genotypic variation and/or variation in the experimental conditions.

Screening *in vitro* callus under salt stress could allow selecting eggplant callus lines or tolerant seedlings to salinity. Also, tissue culture systems have been used as a useful tool to elucidate the cellular mechanisms involved in salt tolerance by using selected NaCl-tolerant cell lines (Davenport et al., 2003). We hypothesised that a reliable *in vitro* regeneration system could be established exploiting the responsiveness of different explant types in the eggplant cultivar Bonica.Therefore we investigated effects of different TDZ concentration as well as the effect of light quality on both callogenesis and shoot proliferation.

2.2 Materials and methods

2.2.1 Plant Material

Seeds of eggplant (‘Bonica’) were obtained from Vilmorin, France and were treated with thiram (diamide tetramethyl thio-peroxydicarbonique). Seeds were surface-sterilized with 70% alcohol and rinsed with distilled water. Then, seeds were soaked in a solution of 0.02%

Dreft (5-15% non-ionic surfactants, 15-30% anionic surfactants) and 5% HazTab (1,3,5 Dichloro-Triazine-Trionedihydrate-Dichlorosodium) for 20 min followed by a second soaking in a solution of mercuric chloride (0.5%) for 10 min. After three rinses with sterile distilled water the seeds were germinated on agar-solidified (0.8 %) MS medium with 3% (w/v) sucrose in 0.7 L glass vessels. The pH was adjusted to 5.8 with 1 N NaOH before and then adding solidified agar (Sigma).

Plant material was maintained in a growth chamber at 28±2°C and a 16-h photoperiod regime provided by cool-white fluorescent lamps with a photon flux density of 36 µmol m^{-2} s^{-1}.

2.2.2 Effect of TDZ on callogenesis and organogenesis

Hypocotyl, epicotyl, (1 mm long) as well as leaf and cotyledon segments (50 mm^2) were excised from 25- to 30-day-old seedlings. Explants were cultured for 30 days on MS medium with 3 % sucrose and 0.8 % agar supplemented with 0, 0.1, 0.2 or 0.4 µM TDZ. Four explants per glass vessel were used, this in 4 replications. The efficiency of the regeneration was measured after 30 days for callogenesis (percentage of regenerated callus, the percentage of callus with buds, the number of shoots/callus and the fresh weight of callus) and for organogenesis (percentage of explants that developed buds, number of shoots per explant and the fresh weight of formed shoots).

2.2.3 Effect of light quality on callogenesis and organogenesis

Best calli (in terms of fresh weight) formed in the presence of 0.2 µM of TDZ were fragmented and transferred onto MS medium supplemented with 0.4 µM TDZ and then transferred under two LED light radiations: blue light and red light (Green Power LED string, Philips, Eindhoven) and cool white fluorescent light (Figure 2.2). Four calli per glass vessel were used, this in 4 replications and calli were cultured for 25 days.

2.2.4 Statistical Analysis

Data were tested by analysis of variance using SPSS Version 19 (SPSS Inc., Chicago) followed by a Student-Newman-Keuls test (P=0.05). Data are presented as an average of four replications ± standard error (SE).

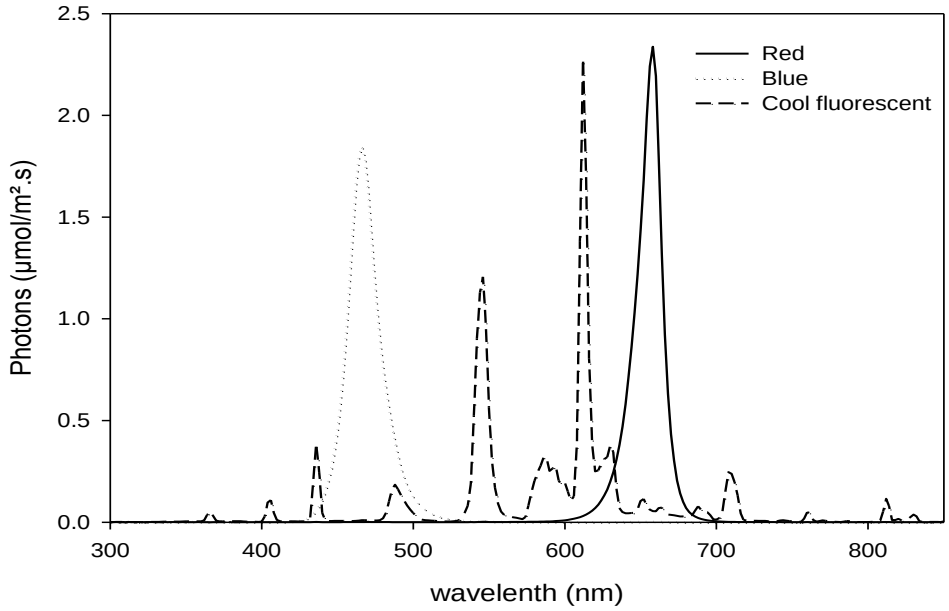

Figure 2.2: Spectrum of the LED strings (Red, blue, fluorescent cool white light)

2.3 Results

2.3.1 Effect of TDZ on callogenesis

Effects of increasing concentrations TDZ on callogenesis are given in Table 2.1. A high variation within treatments was found (Photo 2.1).

For the epicotyl and hypocotyl explants, callogenesis is present both in the absence and presence of TDZ. However, a concentration of 0.4 µM TDZ yielded 100% callus formation. In the absence of TDZ there is no callogenesis for the cotyledon and leaf explants. However in the presence of TDZ callogenesis takes place. A concentration of 0.4 µM TDZ is found to be much better in terms of percentage of callogenesis (Table 2.1).

We also distinguished between the type of callogenesis (non-differentiated and differentiated bud forming calli). For the epicotyl explants, the concentration 0.4 µM TDZ yields 100% differentiated callus with visible buds, although all treatments form a statistical unique class. For the hypocotyl explants, 0.1 µM of TDZ is slightly better (40%) than the other TDZ concentrations. For the cotyledon explants 0.4 µM of TDZ is found to be slightly better compared to the other TDZ concentration in term of callus with buds (30%). And finally for

the leaf explant, the highest percentage of calli with buds was achieved in the presence of 0.4 µM TDZ (Table 2.1).

Shoots were already initiated on certain calli. For the different types of explants the optimal shoot induction rates were achieved in the presence of 0.4 µM of TDZ though for the hypocotyl explants no TDZ also yielded a higher number of shoots (Table 2.1).

The effectiveness of callus induction was also expressed in the callus fresh weight. For the epicotyl explant, the callogenesis weight is significantly higher if 0.2 µM of TDZ is used (Table 2.1). For the hypocotyl explant, the treatments 0.1µM, 0.2 µM and 0.4 M TDZ, form a higher callus weights compared to the control. The callus weights formed on the cotyledon and foliar explants were not influenced by the TDZ concentration.

Table 2.1: TDZ-induced changes in callus percentage, percentage of callus with buds, the number of shoots per callus, callogenesis weight from different types of explants.

	TDZ	Epicotyl	Hypocotyl	Cotyledon	Leaf
Callogenesis (%)	0µM	55.0±18.9^{a}	55.0±26.3^{ab}	0±0^{c}	0±0^{c}
	0 .1µM	80.0±13.5^{a}	46.7±11.2^{ab}	70.0±17.3^{ab}	84.2±6.6^{b}
	0.2µM	52.5±11.1^{a}	30.0±12.2^{b}	56.2±12.1b	48.7±16.4^{a}
	0.4µM	100.0±0^{a}	100.0±0^{a}	100.0±0^{a}	93.7±4.7^{a}
Callus with buds (%)	0µM	50.0±17.3^{a}	20.0±10.8^{a}	0±0^{a}	0±0^{b}
	0.1µM	52.0±11.1^{a}	40.0±20.4^{a}	25.0±11.9^{a}	5.0±2.04^{b}
	0.2µM	66.6±19.1^{a}	5.0±5.0^{a}	25.0±8.7^{a}	20.0±9.1^{ab}
	0.4µM	100.0± 0^{a}	30.0±4.6^{a}	30.0±7.4^{a}	30.0±7.5^{a}
Number of shoots per callus	0µM	4.5±1.70^{a}	4.0±1.47^{a}	-	-
	0 .1µM	3.5±0.64^{a}	0.5±0.28^{b}	3.7±1.49^{a}	1.0±0.70^{b}
	0.2µM	2.7±0.47^{a}	0.7±0.47^{b}	2.2±0.75^{a}	0.7±0.47^{b}
	0.4µM	6.5±1.19^{a}	3.5±0.28^{ab}	5.5±1.04^{a}	5.5±1.32^{a}
Callogenesis weight (g)	0µM	2.5±0.55^{b}	1.7±1.51^{b}	-	-
	0.1µM	3.3±0.81^{b}	5.7±1.20^{a}	4.3±1.88^{a}	6.6±0.75^{a}
	0.2µM	11.8±3.51^{a}	5.5±0.98^{a}	5.6±0.88^{a}	3.9±0.88^{a}
	0.4µM	8.4± 1.27^{ab}	6.1±0.91^{a}	6.4±0.38^{a}	5.6±1.04^{a}

Means followed by the same lowercase within each column are not significantly different at P= 0.05 according to the Student-Newman-Keuls test (n=4)

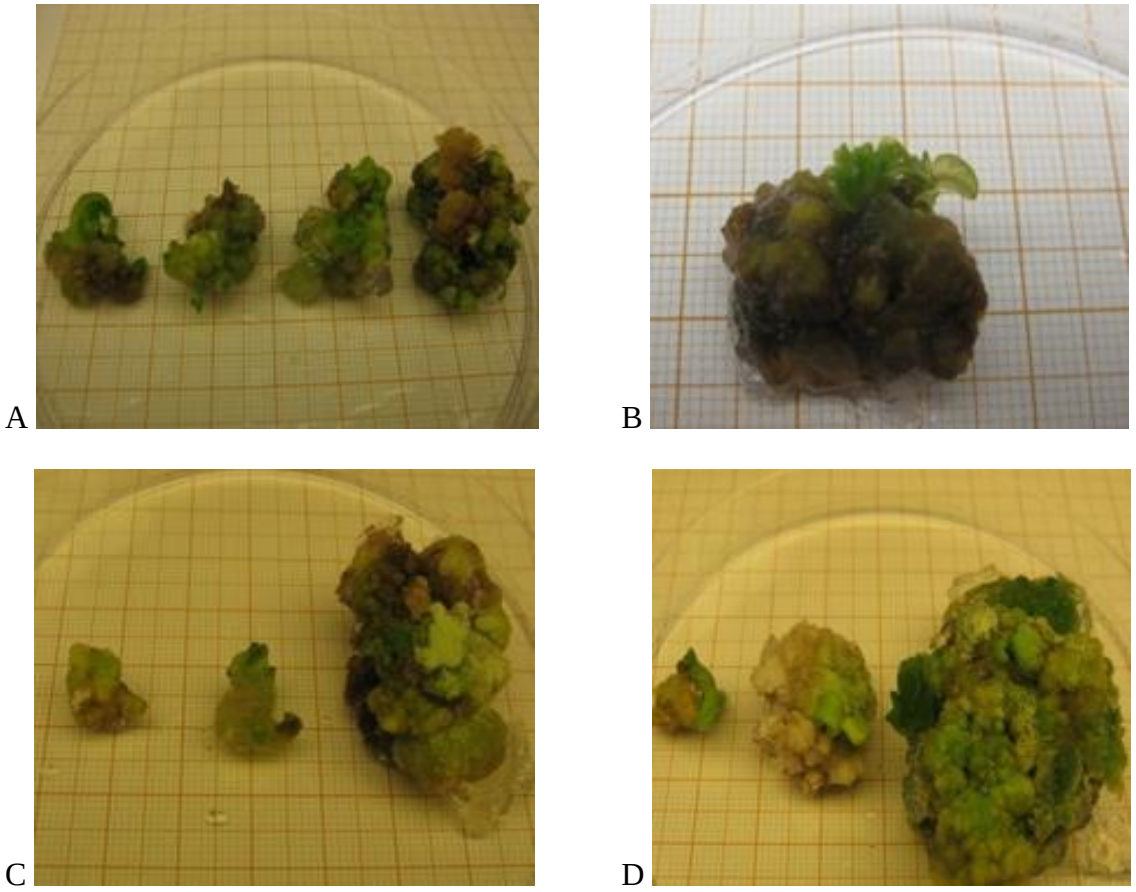

Photo 2.1: Variation in calli growth obtained of the cotyledon explants (A), epicotyl explants (B), hypocotyl explants (D) and leaf explants (D) (TDZ 0.4 µM))

2.3.2 Effect of TDZ on direct organogenesis

Direct organogenesis ranging between 90-100% was found for 0.4µM of TDZ in hypocotyl, cotyledon and leaf explants while for the epicotyl explant no organogenesis was present (Table 2.2). TDZ was necessary for direct organogenesis in the cotyledon and leaf explants. A dose effect was found, 0.4 µM was significantly better than lower concentrations to induce organogenesis (Table 2.2).

Despite a high percentage of bud initiation was observed the development to shoots was relative low. Both explant type and TDZ concentration influenced this shoot formation. If no TDZ was present in the medium epicotyl and hypocotyl explants were able to form shoots (Table 2.2). The hypocotyl explant was found to be the most responsive yielding 2.5 shoots/explant with an average weight of 2.8 g (Photo 2.2). The addition of TDZ had a negative effect on shoot formation in both epicotyl and hypocotyl explants. A concentration

of 0.4 µM TDZ is marked by the absence of shoots even when in all explants bud formation was present (Table 2.2).

Table 2.2: TDZ-induced changes in percentage of explants with buds, number of shoots per explant and organogenesis weight (g) in different type of explants in 'Bonica' genotype.

	TDZ	Epicotyl	Hypocotyl	Cotyledon	Leaf
Percentage of explant with buds	0µM	40.0±14.1[a]	36.2±18.4[b]	0±0[c]	0±0[c]
	0 .1µM	27.5±10.1[a]	26.2±6.9[b]	65.0±15.0[b]	45.0±15.1[b]
	0.2µM	42.5±20.9[a]	20.0±14.1[b]	56.2±14.6[b]	36.2±14.3[b]
	0.4µM	0±0[a]	90.0±5.8[a]	100±0[a]	90.00±6.1[a]
Number of shoots per explant	0µM	0.75±0.5[a]	2.5±0.3[a]	-	-
	0.1µM	0±0[a]	1.5±0.9[ab]	0.25±0.2[a]	0±0[a]
	0.2µM	0.75±0.5[a]	0±0[b]	0±0[a]	0±0[a]
	0.4µM	0±0[a]	0±0[b]	0±0[a]	0±0[a]
Organogenesis weight (g)	0µM	0.56±0.20[a]	2.8±0.42[a]	-	-
	0.1µM	-	0.5±0.06[b]	-	-
	0.2µM	0.60±0.19[a]	-	-	-
	0.4µM	-	-	-	-

Means followed by the same lowercase within each column are not significantly different at P = 0.05 according to the Student-Newman-Keuls test (n=4)

A  B 

Photo 2.2: Plantlets obtained without addition of TDZ of the hypocotyl explant (A) and epicotyl explant (B)

2.3.3 Effect of light quality on callogenesis:

Light quality effects were observed on callus formation and on indirect organogenesis. Light quality significantly affected the callus growth and this was tissue dependant. For the epicotyl and cotyledon explants fluorescent lamps and red light promoted callus proliferation compared to blue light (Table 2.3) (Photo 2.3). For hypocotyl explants, however, blue light resulted in higher callus weight than red light (Table 2.3) (Photo 2.3). Finally callus formed under different types of light radiation constituted a homogeneous class for the foliar explants (Photo 2.3). However, the red and blue light generated almost twice the callus weight than callus formed under fluorescent lamps (Table 2.3).

For epicotyl, hypocotyl and cotyledon explants the maximum number of shoots per explant (respectively 3, 2.5 and 2) was obtained under fluorescent light. Yet, in leaf explants the highest number of shoots was obtained under the red radiation (2.5/calli) (Table 2.3).

Table 2.3: Light quality induced changes in callogenesis weight and number of shoots for different types of explants.

	Light quality	Type of explant			
		Epicotyl	Hypocotyl	Cotyledon	Leaf
Callus weight (g)	Fluorescent	8.91 ± 1.58^{a}	10.1 ± 0.8^{ab}	21.2 ± 2.53^{a}	5.9 ± 1.11^{a}
	Blue	4.06 ± 0.80^{b}	14.6 ± 1.6^{a}	10.3 ± 1.99^{b}	10.3 ± 1.56^{a}
	Red	8.18 ± 0.52^{a}	5.8 ± 1.7^{b}	22.1 ± 2.63^{a}	9.7 ± 1.03^{a}
Number of shoots per explant	Fluorescent	2.50 ± 0.64^{a}	3.0 ± 0.91^{a}	2.0 ± 0.41^{a}	1.2 ± 0.25^{a}
	Blue	1.00 ± 0.40^{a}	1.5 ± 0.29^{a}	1.0 ± 0.41^{a}	1.2 ± 0.47^{a}
	Red	1.50 ± 0.64^{a}	0.75 ± 0.25^{a}	1.5 ± 0.86^{a}	2.5 ± 0.95^{a}

Means followed by the same lowercase within each column are not significantly different at P= 0.05 according to the Student-Newman-Keuls test (n=4)

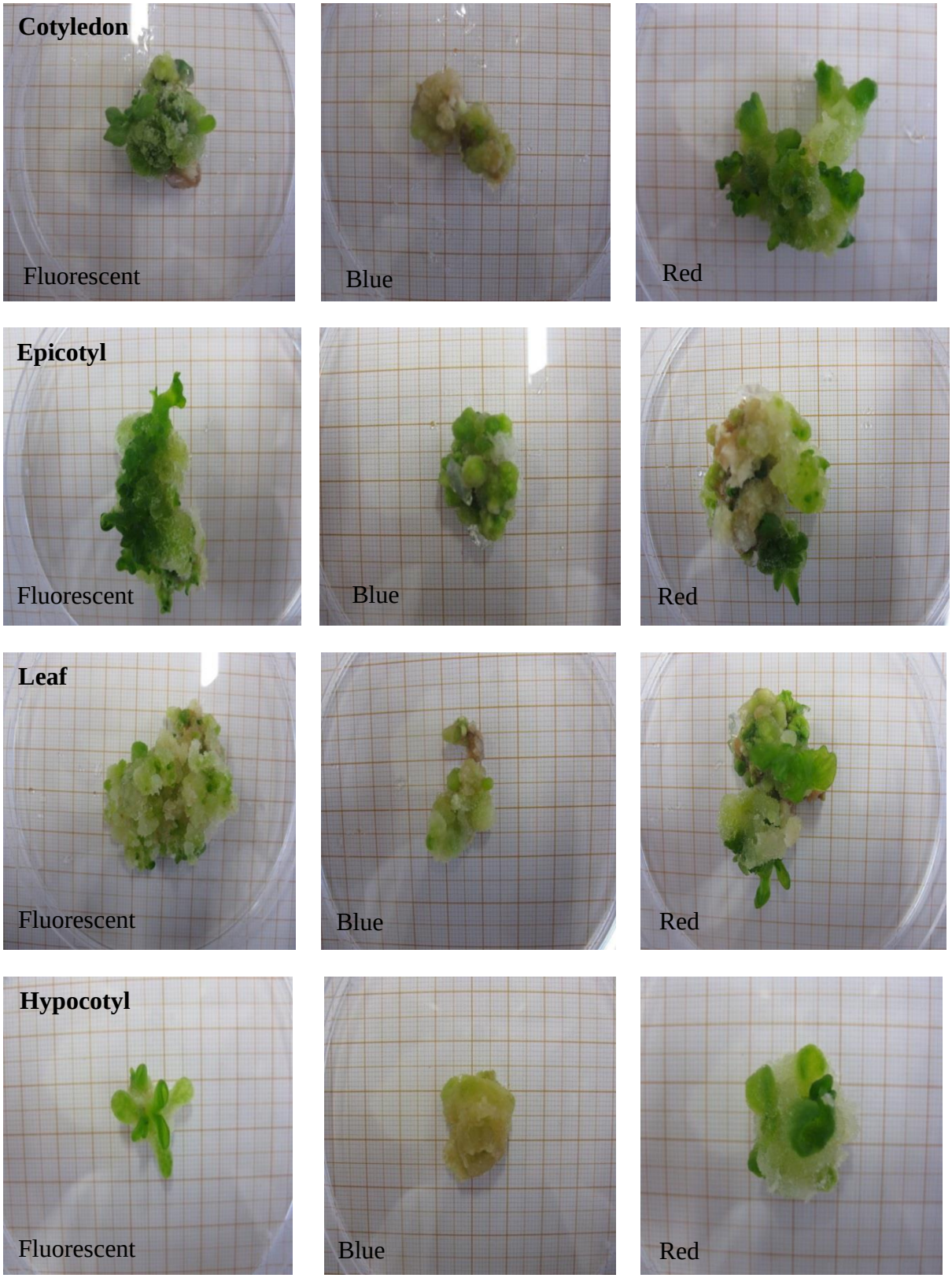

Photo 2.3: Calli and shoot from different type of explants under different light qualities

2.4 Discussions

2.4.1 Effect of TDZ

Various sources for eggplant regeneration have been reported via somatic embryogenesis using leaf explants (Gleddie et al., 1983; Mukherjee et al., 1991) or organogenesis using hypocotyl explant (Kamat and Rao, 1978; Matsuoka and Hinata, 1979) and leaf explants (Gleddie et al., 1983; Mukherjee et al., 1991). Comparative studies on the responsiveness of different types of explants were undertaken by Allichio et al. (1982) and Sharma and Rajam (1995) and from these studies it was obvious that the cultivar and explant used strongly influenced the responses.

In this study we wanted to obtain a good regeneration protocol from calli in the cultivar 'Bonica', in order to regenerate plantlets from more salt tolerant calli lines. Given the parameters studied for callogenesis (callogenesis percentage, number of shoots per callus and weight callogenesis) and for organogenesis (percentage of explants with buds) leaf and cotyledon explants were significantly more responsive in the presence of TDZ than the epicotyl and hypocotyl explants ($P < 0.05$). Similar results were reported by Magioli et al. (1998) studying the frequency of the induction of organogenic calli and the number of buds formed per explant applying low TDZ concentrations. This is in contrast with other species were much higher concentrations were needed (2-200μM) (Hüttmann and Preece, 1993). In our work the concentration of 0.4μM TDZ was the best for the in vitro regeneration of the eggplant variety 'Bonica'. Also Magioli et al. (1998) found that low concentrations of TDZ were effective for the in vitro regeneration of eggplant though he obtained an optimal response at 0.2 μM TDZ while higher concentrations generated a reduction of formed buds and the appearance of necrosis. Similar inhibitions of bud formation with high concentrations have been shown in *Pseudotsuga menziesii* (Goldfarb et al., 1991) and in pear (Leblay et al., 1991).

However, successful rooting was not achieved after shoots were transferred to root inducing media (data not shown).

2.4.2 Effect of light quality on the weight of callogenesis and the number of shoots

The impact of light quality on callus induction and regeneration of shoots were investigated by assessing the weight of callogenesis and the number of formed shoots. The fluorescent light was better than red and blue for the *in vitro* generation of shoots this for most explant

types. Similar results were obtained by Piao et al. (2002) working on the potato and Heo et al. (2002) working on herbaceous plants. This could be due to the multispectral composition of the fluorescent radiation compared to the monospectral LED light. Christiaens et al (2016) reviewed effect of LED light on the rooting capacity of ornamentals. This review also highlighte the species dependant responses, for certain species fluorescent lamps were the most optimal light source, while other species reacted better to monochromatic lights.

Likewise as for the TDZ experiment no successful rooting was obtained with these plantlets.

2.5 Conclusion

We tried to establish an *in vitro* protocol to regenerate plantlets from induced calli in order to set-up screening tests for salt tolerant calli lines. The concentration of 0.4 µM TDZ was the optimal tested concentration for the *in vitro* regeneration of the eggplant variety 'Bonica'. Leaf and cotyledon explants were significantly more responsive in the presence of TDZ this in terms of callogenesis, organogenesis and morphogenesis efficiency than the epicotyl and hypocotyl explants. The fluorescent light was better than red and blue monochromatic light for the *in vitro* propagation of the eggplant.

However, as we could not induce rooting, the *in vitro* approach to induce salt tolerant lines seemed troublesome.

Chapter 3

Effect of salt stress on germination and seedling growth in eggplant (*Solanum melongena* L.)

Chapter 3 Effect of salt stress on germination and seedling growth in eggplant (*Solanum melongena* L.)

Abstract

The effects of salinity under *in vitro* controlled conditions on germination, seedling growth and two biochemical parameters in four eggplant (*Solanum melongena* L.) cultivars were studied. Seeds and subsequent seedling growth were exposed to increasing salt stress (0, 20, 40, 80 and 160 mM NaCl). The responses of the germination, seedling growth and biochemical parameters to salt stress indicated two groups with contrasting sensitivity responses. 'Adriatica' and 'Black Beauty' were more sensitive to the applied salt stresses than 'Bonica' and 'Galine'. Germination was strongly reduced at 160 mM for all cultivars. The decline in seed germination parameters, fresh weigh, dry weigh, height and leaf number were more pronounced with the increase of NaCl concentration in the sensitive cultivars 'Adriatica' and 'Black Beauty' than in the tolerant cultivars 'Bonica' and 'Galine'. The water content decreased markedly in sensitive varieties and remained quite stable in tolerant cultivars. Higher levels of MDA and proline were detected in the leaves of the sensitive cultivars 'Adriatica' and 'Black Beauty'.

Our results suggest that germination; seedling morphology and biochemical parameters can be used as efficient indicators to detect salt stress in eggplant.

3.1 Introduction

High salt concentrations in soils largely account for the decrease in yield of a wide variety of crops worldwide (Munns and Tester, 2008). This problem is more severe in arid and semi-arid regions, where salinity is one of the major limiting factors for productivity.

Seed germination is one of the most important phases in the life cycle of plants and is highly responsive to the prevailing environment including salt stress (Saritha et al., 2007). Furthermore, the sensitivity of plants to salinity may depend on their developmental stage (Adam, 1990). Therefore, the study of salt tolerance during germination, early and late growth of plants is fundamental for detecting saline limits at each developmental stage (Zapata et al., 2004). It has been reported that salinity reduced as well as delayed germination of crop plants such as melon (Botia et al., 1998), tomato (Cuartero and Fernandez-Munoz, 1999) and eggplant (Akinci et al., 2004). Lower levels of salinity delayed germination, whereas higher levels decreased the final percentage of seed germination (Goulam and Fares, 2001; Ben Dkhil and Denden, 2010). Naz et al. (2014) working on *Pisum sativum* reported reduced germination and seedling growth (shoot length, root length, fresh and dry biomass) under saline conditions. Although it does not fully reproduce the field behaviour of plants, the percentage of germination under controlled saline conditions gives always a trend about the differential behaviour of the studied cultivars to the applied stress (Ben Naceur et al., 2001).

The inhibition of seed germination induced by salt could be generated by osmotic stress or by specific ion toxicity (Huang and Redman, 1995). Haddas (1977) showed that germination rate and final seed germination decreased with the decrease of water movement into the seeds during the imbibition phase. It is assumed that low seed moisture content under salt stress triggered cessation of metabolism or inhibition of certain metabolic steps in the germination (Younes et al., 1991).

Salt tolerance has been defined as the ability to maintain adequate growth and metabolism under stress conditions (Munns and Tester, 2008). A major factor associated to salt tolerance is the ability of plants to adjust the osmotic pressure in the cytosol, which is mediated by the synthesis of organic solutes such as proline (Ashraf and Foolad, 2007). The accumulation of this compound during stress is important for osmoregulation and cell protection from salinity (Molinari et al., 2007). However, in some species the accumulation of proline is associated to salt induced injury rather than with an osmo-protector adaptive effect (Zgallaï et al., 2005; Pattanagul and Thitisaksakul, 2008; Silveira et al., 2009; Ferreira-Silva et al., 2010).

Eggplant is considered to be moderately sensitive to salt stress (Shahbaz et al., 2012). Yet, this salt tolerance varies between eggplant varieties. The germination and seedling stages are considered as more sensitive to salt stress (Chartzoulakis and Loupassiki, 1997; Akinci et al., 2004) although information about varietal reactions in eggplant is scarce.

The present study was conducted to investigate the differential response of four eggplant varieties to increasing salinity during the germination and seedling stage. This was assessed by *in vitro* germination kinetics and seedling growth. To interpret the cultivar reactions we also studied the variation that occurs in lipid peroxidation (MDA: Malonaldehyde) and in proline accumulation.

3.2 Materials and methods

3.2.1 Plant materials and salt stress treatments

The experiment was conducted in a growth chamber in Gent University in 2011. Four commercial eggplant cultivars two open-pollinated cultivars, 'Adriatica', 'Black Beauty' and two F1 hybrids 'Bonica' and 'Galine' were used as plant material.

Seeds were surface-sterilized with 70% alcohol and rinsed with distilled water. Then, seeds were soaked in a solution of 0.02% Dreft (5-15% non-ionic surfactants, 15-30% anionic surfactants) and 5% HazTab (1,3,5 Dichloro-Triazine-Trionedihydrate-Dichlorosodium) for 20 min followed by a second soaking in a solution of mercuric chloride (0.5%) for 10 min. After three rinses with sterile distilled water the seeds were germinated on agar-solidified (0.8 %) Murashige and Skoog (1962) medium with 3% (w/v) sucrose in 0.7 L glass vessels. The pH was adjusted to 5.8 with 1 N NaOH before and then adding solidified agar (Sigma).

NaCl was added to the medium at the concentration of 0 (control), 20, 40, 80 and 160 mM. Plant material was maintained in a growth chamber at 28°C under a 16 h photoperiod regime provided by cool-white fluorescent lamps with a photon flux density of 36 $\mu mol.m^{-2}s^{-}1$ at seedling level. For each treatment and cultivar 4 seeds per vessel, this in five replications were used.

3.2.2 Plant measurements

The germination (radicle emergence) and the time to germinate were determined at 24 h intervals for 7 days. Three parameters of germination were determined which included: final germination percentage; the mean germination time (MGT) and the mean daily germination

(MDG). The mean germination time is calculated as follows: MGT = $(n_1t_1) + (n_2t_2) + \ldots\ldots + (n_xt_x)/X_n$ where n_1 is the number of germinated seeds at the first day of germination, t_1 is the number of days from start to first germination, and X_n is the total number of seeds germinated. The mean daily germination (MDG) is the final germination percentage/number of days to final germination (Jha et al., 2010).

After 6 weeks 1 seedling per vessel was randomly sampled and seedling length, number of leaves, aerial fresh (FW) and dry weight (DW) were measured. The aerial part of the plant (shoots and leaves) was weighted (FW) and then dried in a forced-draft oven at 80°C for 24 h and re-weighted (DW). Water content (WC) was calculated as the (FW-DW/DW) ratio according to Munns (2010).

3.2.3 Lipid peroxidation

Malonaldehyde (MDA), an end product of lipid peroxidation was determined according to Hodges (1999). 500 mg of leaves crushed with liquid nitrogen were homogenized in 5 mL of ethanol (80%) and centrifuged at 3,000 g for 10 min. To 1 mL of the supernatant 1 mL of thiobarbituric acid (TBA, 0.65% w/v) and 1 mL TCA (20% w/v) were added. The homogenate was incubated at 95°C for 25 min and the reaction was stopped by cooling the mixture in ice. After 10 min of centrifugation at 3,000 g the absorbance was measured by spectrophotometer at 440, 532 and 600 nm (Infinite M200, TECAN Group Ltd., Switzerland). The MDA content was calculated using the molar extinction coefficient of MDA (ε = 157 $mmol^{-1}$ cm^{-1}) and the results are expressed as nmol MDA g^{-1} FW.

3.2.4 Proline content

The determination of proline was done according to Bates et al. (1973). Plant leaves (0.5 g) were crushed in 3% sulfosalicylic acid and the homogenate filtered through filter paper. After addition of acid ninhydrin and glacial acetic acid, the resulting mixture was heated at 100°C for an hour in a water bath. The mixture was extracted with toluene and proline was quantified spectrophotometrically at 520 nm from the organic phase. Proline concentration was calculated using the following formula:

µmol proline g^{-1}fresh weight = (µg proline mL^{-1}x mL of toluene/115.5)/g of sample

3.2.5 Statistical analysis

The experimental design was a completely randomized design per cultivar. All data obtained were subjected to one way analysis of variance (ANOVA) to determine the significant

differences between the treatments or varieties using the software of SPSS Statistics 19. Tukey's HSD test (P=0.05) was used to compare the means.

3.3 Results

3.3.1 Seed germination under different salinity levels

The germination time course for the different NaCl concentrations and cultivars is given in Figure 3.1. The seed germination in the control treatment was high and reached 100% in 'Bonica' and 'Galine', 79.2 % in 'Adriatica' and 83% in 'Black Beauty'. The necessary period to reach final germination percentage of seeds was influenced by the NaCl concentrations (Figure 3.1). At the level of 20 mM of NaCl this parameter was hardly affected for all the cultivars when compared to the control. However, under a higher level of salinity the necessary period to reach the maximum seed germination increased markedly though cultivar effects were present (Figure 3.2). Seed germination attained its maximum at the fourth day for a salt concentration of 40 mM, at the fifth day for a salt concentration of 80 mM and at the sixth day for the highest salt concentration (160 m M) in 'Adriatica' and 'Black Beauty'. In contrast, the maximum seed germination is reached at the second day for a salt concentration of 40 m M and at the third day for a salt concentration of 80 and 160 mM in 'Bonica' and 'Galine' (Figure 3.1).

It is clear that the four cultivars did not react in a similar way to the applied salt stress. A salt concentration of 40 mM and 80 mM NaCl lead to a strong decrease of the final germination percentage in 'Adriatica' and 'Black Beauty' while the germination capacity of 'Bonica' and 'Galine' was hardly affected (Table 3.1). However, 160 mM NaCl was detrimental to all cultivars with hardly germinating seeds for 'Adriatica' and 'Black Beauty' and a seed germination percentage averaging 20% for 'Bonica' and 'Galine' (Table 3.1).

The mean daily germination (MDG) was not affected in the 20 mM NaCl treatment; a slight increase for the cultivars 'Bonica' and 'Galine' was even noted. 40 mM decreased already significantly MDG in 'Adriatica' (Figure 3.2). Increased salinity levels to 80 mM of NaCl caused a significant reduction in MDG in 'Adriatica', 'Black Beauty' and 'Galine' by respectively 81%, 77% and 58% when compared to their respective controls. A concentration of 160 mM strongly reduced MDG in all cultivars.

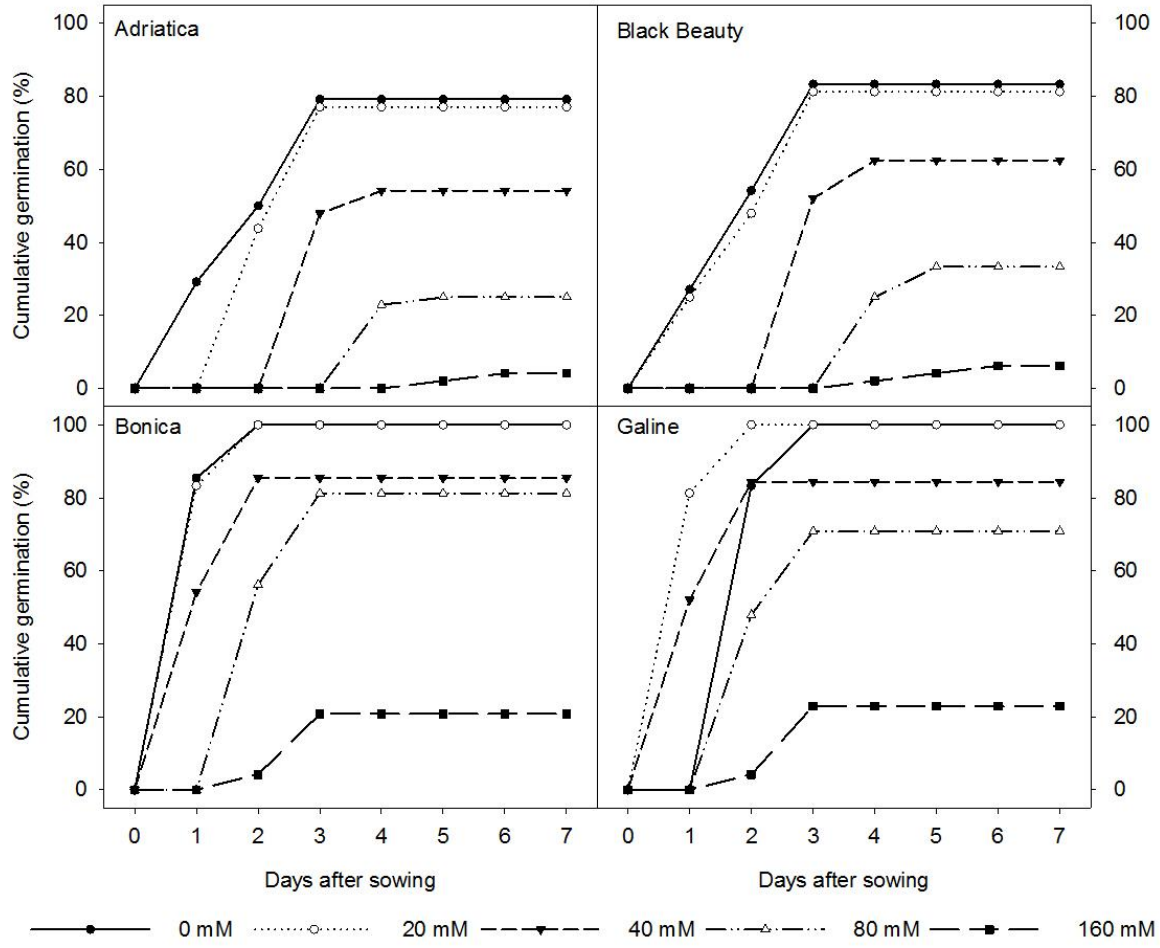

Figure 3.1Time course of seed germination under increasing saline conditions (n=20).

Table 3.1 Differential response of final seed germination (%) of eggplant cultivars to increasing NaCl levels in the medium.

Cultivar	0 mM	20 mM	40 mM	80 mM	160 mM
'Adriatica'	79.2±4.8b	77.1±7.9b	54.1±2.4c	25.0±6.8c	4.1±6.5b
'Black Beauty'	83.3±6.8b	81.2±1.5b	62.5±10.7b	33.3±6.8c	6.2±4.3b
'Bonica'	100±0a	100±0a	85.4±7.9a	81.3±7.9a	20.8±2.4a
'Galine'	100±0a	100±0a	84.4±7.1a	70.8±10.7b	22.9±1.9a

Means followed by the same lowercase within each column are not significantly different at P= 0.05 according to the Tukey's test. Data are means ± SE of 5 replications.

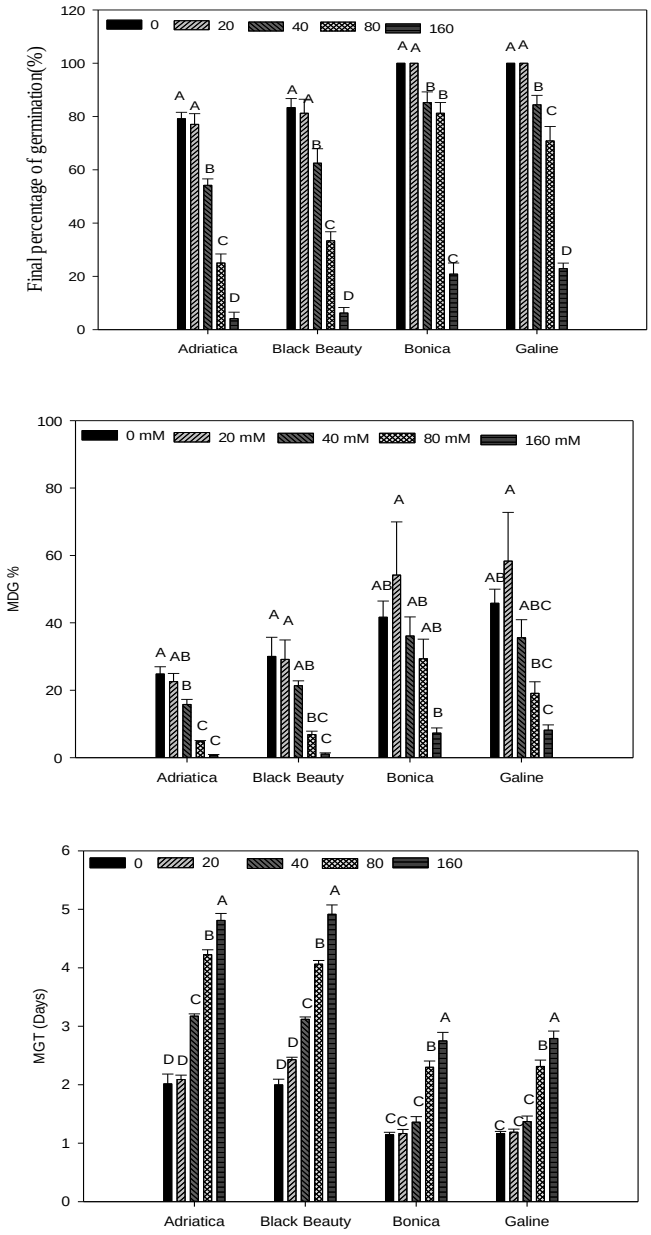

Figure 3.2 Effect of increasing NaCl on the final germination (%), mean daily germination (MDG) % and mean germination time (MGT). Comparisons between means were made with Tukey's test multiple range test (P=0.05). Values are means ± SE (n=5).

The mean germination time (MGT) is a measure of the rapidity of germination, with lower values indicating faster germination. There is a genotypic difference as 'Bonica' and 'Galine' germinate faster than 'Adriatica' and 'Black Beauty' in the control treatment. Increasing salinity significantly affected MGT of 'Adriatica' and 'Black Beauty' from 40 mM NaCl and 'Bonica and 'Galine' from 80 mM on (Figure 3.2). At 80 mM NaCl the number of days for MGT doubled, this for all cultivars.

3.3.2 NaCl induced changes in seedling growth

Seedling growth as evaluated by number of leaves and shoot length is only determined for the range 0-80 mM NaCl as seed germination was too low for 160 mM NaCl. Leaf number was not affected by salt stress in 'Bonica' and 'Galine' while negative effects on shoot length were noted for 80 mM NaCl with a reduction of 59.6% and 44.2% respectively. In contrast, the number of leaves was reduced from 40 mM NaCl on and the shoot length from 80 mM NaCl on for 'Adriatica' and 'Black Beauty'; shoot length was reduced by 18.9% and 20.3% when compared to their respective controls (Photo 3.1; Figure 3.3).

The salt induced decreases in FW and DW showed an overall similar trend. The maximum decrease in FW and DW, however, was observed in 'Adriatica' and 'Black Beauty'. For instance, in the 80 mM NaCl treatment the FW decreased by 84.4% in 'Adriatica' and by 84.4% in 'Black Beauty', compared with their respective controls (Table 3.3). Likewise DW decreased by 79.2% in 'Adriatica' and by 79.4% in 'Black Beauty' for 80 mM NaCl (Table 3.3). In contrast the decline in FW between the control and 80 mM NaCl was less in 'Bonica' (35.0%) and in 'Galine' (34.1%). The same trend was observed in DW with a decrease by 35.3% in 'Bonica' and by 35.1% in 'Galine' at 80 mM NaCl (Table 3.3). Further, a significant decrease of the ratio of water in the aerial biomass relative to its dry weight (WC), was observed in the highest concentration of NaCl (80 m M) when compared to the respective control in the cultivars 'Adriatica' (47%) and 'Black Beauty' (47.54%). In contrast, the WC remained quite stable in both 'Bonica' and 'Galine' (Table 3.3).

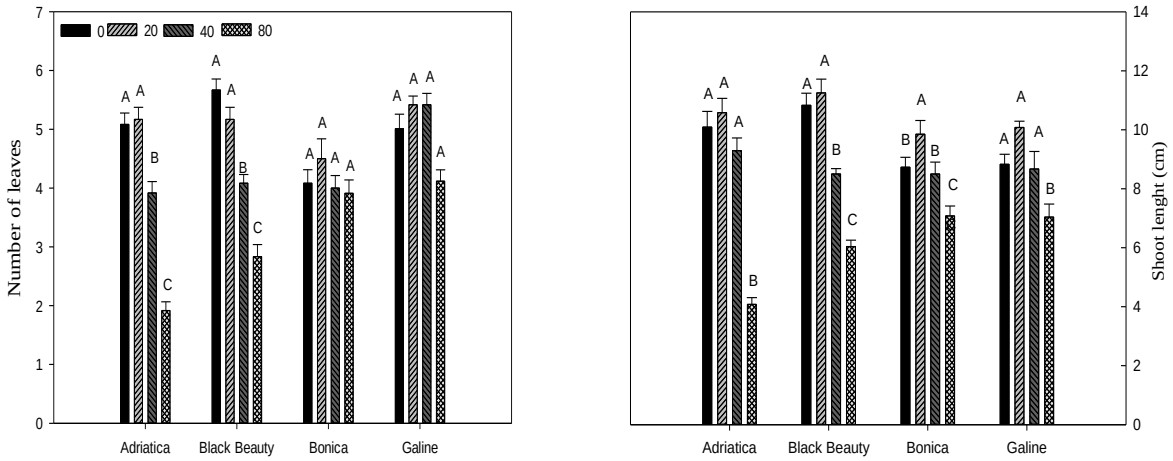

Figure 3.3: Effect of increased NaCl concentration on number of leaves and shoot length of the eggplant varieties. Comparison between means were made with Tukey's HSD test (P=0.05). Data are means ± SE of five replicates.

Table 3.3 Effect of NaCl on fresh weight (FW), dry weight (DW) and water content (WC) of the aerial biomass of eggplant seedlings after 6 weeks of growth. No data are given for 160 mM due to the high mortality for this salt level.

Cultivar	NaCl (mM)	FW (g)	DW (g)	WC (g H_2O/g DW)
Adriatica	0	2.11±0.16^{a}	0.96±0.01^{a}	1.17±0.18^{a}
	20	1.37±0.22^{b}	0.71±0.10^{b}	1.02±0.22^{ab}
	40	0.97±0.22^{b}	0.50±0.01^{c}	0.99±0.18^{b}
	80	0.33±0.04^{c}	0.20±0.01^{d}	0.62±0.15^{b}
Black Beauty	0	2.12±0.17^{a}	0.97±0.01^{a}	1.22±0.11^{a}
	20	1.38±0.22^{b}	0.70±0.10^{b}	0.98±0.17^{ab}
	40	1.00±0.21^{c}	0.55±0.01^{c}	0.94±0.18^{ab}
	80	0.33±0.04^{d}	0.20±0.01^{d}	0.64±0.04^{b}
Bonica	0	2.14±0.18^{a}	0.99±0.03^{a}	1.15±0.06^{a}
	20	1.99±0.18^{a}	0.92±0.05^{b}	1.17±0.07^{a}
	40	1.62±0.15^{b}	0.75±0.03^{c}	1.16±0.06^{a}
	80	1.39±0.13^{b}	0.64±0.06^{d}	1.21±0.18^{a}
Galine	0	2.14±0.20^{a}	1.00±0.01^{a}	1.13±0.06^{a}
	20	1.92±0.21^{ab}	0.90±0.01^{b}	1.12±0.11^{a}
	40	1.77±0.14^{b}	0.83±0.02^{c}	1.10±0.09^{a}
	80	1.41±0.24^{c}	0.65±0.04^{d}	1.16±0.09^{a}

Data are means ± SE of 5 replications Means followed by the same lowercase within each column are not significantly different at P= 0.05 according to Tukey's HSD test.

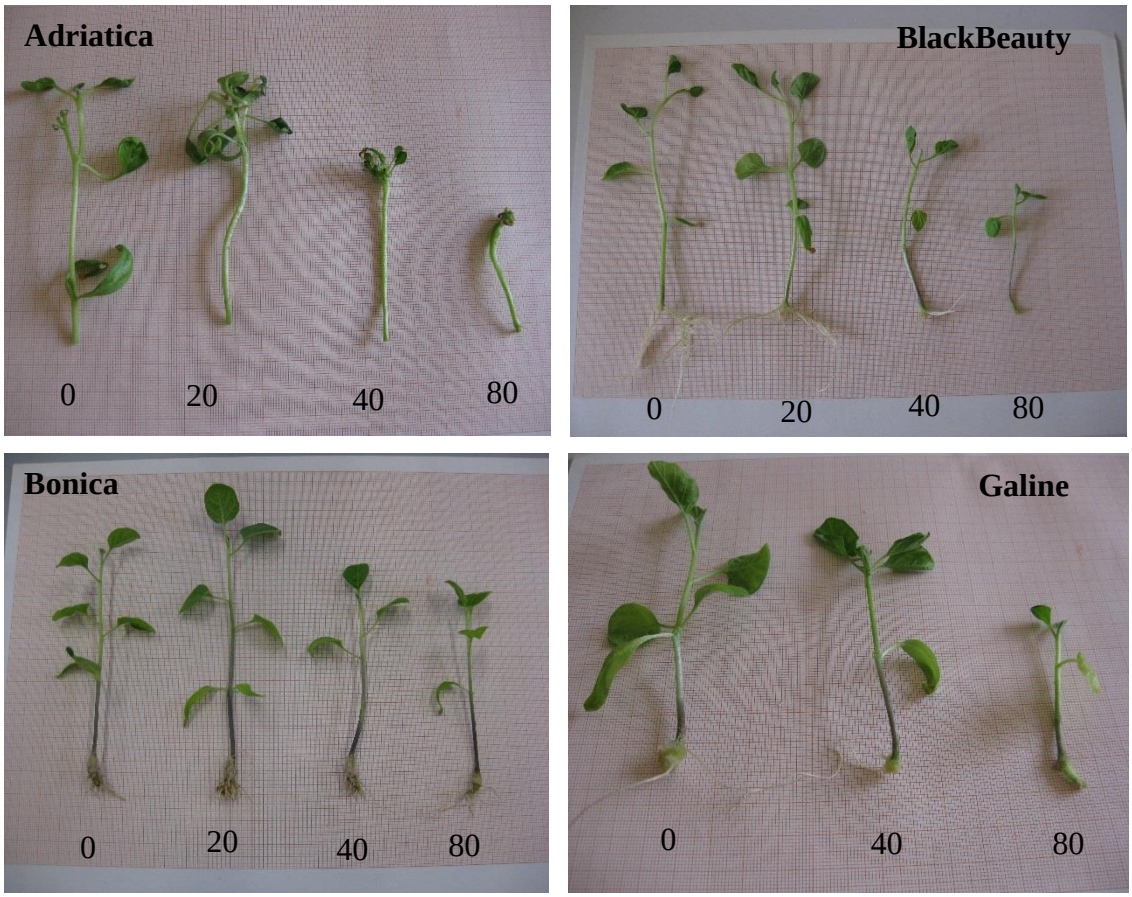

Photo 3.1: Shoot length of different eggplant cultivars under different NaCl concentrations

3.3.3 NaCl induced changes in biochemical parameters

Leaf proline increased significantly when exposed to increased NaCl levels in all the cultivars. The accumulation of proline was more pronounced in 'Adriatica' and 'Black Beauty', these cultivars showed already a significant increase at 20 mM NaCl while in 'Bonica' and 'Galine' a moderate increase was only observed at 80 mM NaCl. In 'Adriatica' and 'Black Beauty' proline increased strongly in relation to the severity of the salt stress. 'Adriatica' showed the highest proline accumulation at 80 mM NaCl.

MDA showed a similar trend as observed for proline under saline conditions. Moreover the level of lipid peroxidation in 'Adriatica' and 'Black Beauty' showed respectively 7-fold increase and 6-fold increase in the treatment 80 mM when compared to their respective controls while it showed respectively 3-fold increase and 2-fold increase in 'Bonica' and 'Galine' when compared to the respective controls. Consequently the induced lipid

peroxidation under increasing salinity was more pronounced in 'Adriatica' and 'Black Beauty' than in 'Bonica' and 'Galine'.

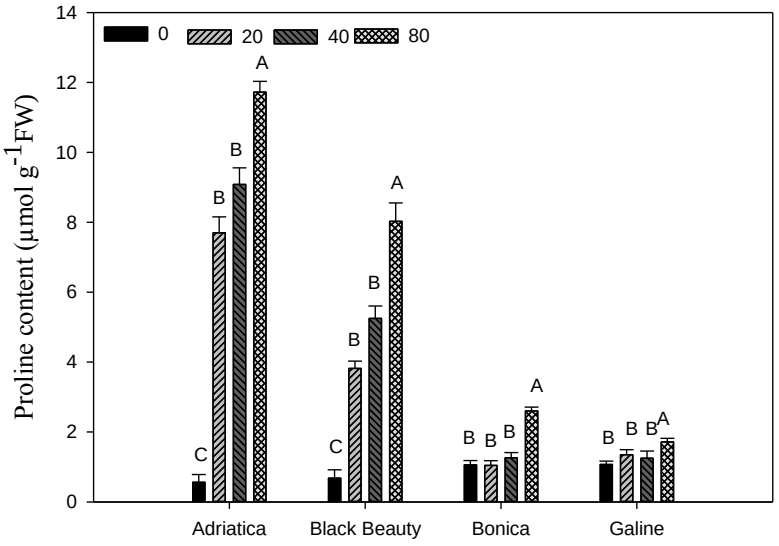

Figure 3.4 Effect of salt stress on leaf proline content (µmol g^{-1} FW). Vertical bars indicate SE. Different letters indicate significant difference between treatments based on Tukey's HSD test (P = 0.05).

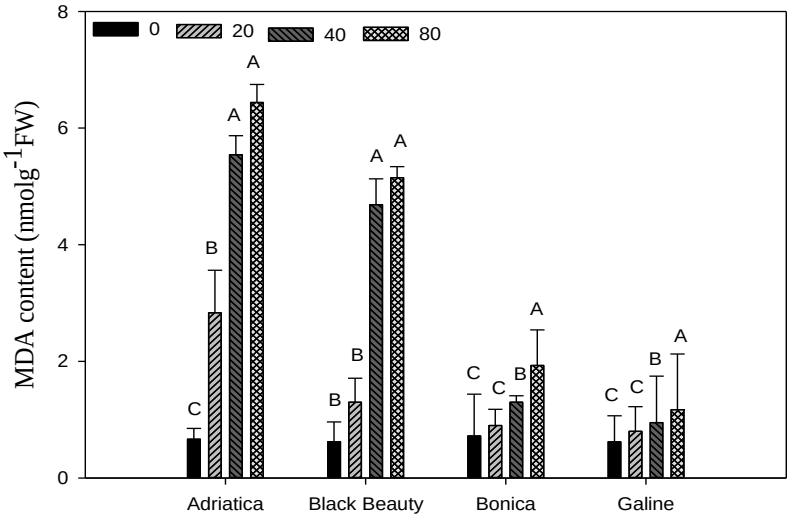

Figure 3.5 Salt stress induced changes in MDA content (nmol g^{-1}FW) in leaves of the cultivars of eggplant subjected to different NaCl concentrations. Data are means of five replicates ± SE. Different letters indicate significant difference between treatments based on Tukey's HSD test (P = 0.05).

3.4 Discussion

Seed germination is a sensitive developmental stage and most plant species germinate best in non-saline conditions. This was already shown for a number of vegetables as cucumber (Jones et al., 1989), lettuce (Coons et al., 1990; Nasri et al., 2015), beans (Goertz and Coons, 1991; Jeanette et al., 2002) and *Pisum sativum (*Naz et al., 2014) but also in wheat (Ben Naceur et al., 2001). Also in our experiment seed germination parameters (germination percentage, MGT and MDG) were adversely affected by increasing saline stress. However, two groups with contrasting sensitivity responses were found. 'Bonica' and 'Galine' behaved as more tolerant cultivars while 'Adriatica' and 'Black Beauty' were the more susceptible cultivars and already sensitive to moderate stresses. The germination process in eggplant seeds is fast in the control treatment; therefore the applied salt stress mainly affects the uptake of imbibition water by seeds due to the lower osmotic potential of germination media. Exposure to high saline concentration does not only provoke inhibition of germination but also a decrease in germination speed and rate as shown by the decrease in MDG and the increase in MGT under salt stress. This may be due to the fact that seeds seemingly develop an osmotically enforced "dormancy". This may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedlings when conditions change such as rainfall in field conditions (Gill et al., 2003; Nasr et al., 2012).

Germination of eggplant seeds is already strongly reduced at 80 mM NaCl for the susceptible cultivars. Chartzoulakis and Loupassaki (1997) found also a strong reduction of eggplant germination at 100 mM NaCl. In tomato, Cuartero and Fernandez-Munoz (1999) reported a decreasing germination trend from 80 mM NaCl and a drastic decline at 190 mM NaCl for sensitive cultivars. Similar inhibitory germination results were reported by Yildirim et al. (2002) for celery and parsley at 182 mM NaCl, by Nasri et al. (2011) for lettuce at 100 and 150 mM of NaCl. Zapata et al. (2004) found a strong inhibition of seed germination in tomato at 100 mM NaCl and in beetroot, pepper, melon and broccoli at150 mM NaCl. In *Pisum sativum* a stong reduction of germination was observed at 80 mM NaCl (Naz et al., 2014).
The effect of salinity on germinating seeds in many species is not limited to a reduced germination percentage, but also a lengthening of the time needed to complete germination is observed. The decreases in germination rate and MDG under saline conditions have been reported by Ungar (1996) on *Atriplex patula* at 34 mM NaCl, by Jeanette et al. (2002) on *Phaseolus* species at 120 mM NaCl and by Datta et al. (2009) on five *Triticum aestivum*

varieties at 150 mM NaCl. Tomato seeds needed 50% additional days to germinate at 80 mM NaCl than in a medium without salt and almost 100% more days at 190 mM (Cuartero and Fernandez-Munoz, 1999).

Salinity slows eggplant shoot growth in the seedling stage. As for the germination the effects of the salt stress in the seedling stage divided the cultivars in two groups. Growth reduction as determined by shoot height, number of leaves, FW, DW and WC was more pronounced for 'Adriatica' and 'Black Beauty' compared to 'Bonica' and 'Galine'. Leaf initiation was not affected by salt stress in 'Bonica' and 'Galine', shoot reduction at 80 mM was thus solely due to shorter internodes. Although 20 mM enhanced shoot growth in 'Bonica', the dry weight of the seedlings was already reduced at this level compared to the control. The salt sensitive cultivars ('Adriatica' and 'Black Beauty') showed, however, a pronounced decrease in FW and DW whereas the salt tolerant cultivars ('Bonica' and 'Galine') were able keep up a better dry mass production when exposed to salinity. The water content of leaf tissue (WC) may serve as an indicator of stress. A marked reduction of WC was observed in the sensitive cultivars 'Adriatica' and 'Black Beauty' thus reducing turgor and cell expansion, while the tolerant 'Bonica' and 'Galine' had the ability to maintain their WC quite stable. A decrease in FW, DW and WC of young seedlings under saline conditions has also been reported by Prado et al. (2000) on *Chenopodium quinoa* at 400 mM NaCl, by Akinci et al. (2004) on *Solanum melongena* at 100 mM NaCl and by Ben Dkhil and Denden (2010) on *Abelmoschus esculentus* at 60 mM NaCl.

The adaptation of plants to salinity is associated with the increase of osmotically active organic substances which help to alleviate the salinity-mediated osmotic stress. Osmotic regulators in plants include organic solutes such as soluble sugars and proline (Turan et al., 2009; Xu et al., 2012). In our work, proline increased in the leaves in response to salinity in all cultivars; however, more proline accumulated in 'Adriatica' and 'Black Beauty' this for the applied salinity levels. It has been reported that plants may accumulate compatible solutes such as proline under salt stress, but their relative contribution to stress tolerance varies among species or even among cultivars of a same species (Ahraf and Foolad, 2007; Abbas et al., 2014). Indeed the two cultivars with the highest proline increase were also the most susceptible cultivars with respect to shoot growth and biomass. Accumulation of proline might therefore also be due to a reduction in its use in protein synthesis which can also be severely inhibited by abiotic stresses (Stewart and Hanson, 1980). Salinity inhibits the synthesis of a majority of shoot proteins in barley (Ramagapol, 1987). Moreover salt stress

can seriously disrupt normal metabolism through oxidative damage to proteins (Imaly and Linn, 1988). Veeranagamallaiah et al (2008) identified 29 proteins that significantly up-regulated (tolerant crops) or down-regulated (susceptible crops) due to NaCl stress. Kosova et al (2013) suggested that susceptible plants can be characterised by mobilization of their energy reserves, consumption of energy reserves and enhanced protein degradation under stress. In contrast Moons et al. (1995; 1997) noticed that both ABA and ABA-responsive proteins, such as late embryogenesis abundant (LEA) protein, were present at high levels in roots of tolerant rice varieties. Kosova et al. (2013) found an increased relative abundance of proteasome subunits, indicating enhanced protein degradation upon salt stress.

It is known that free radical-induced peroxidation of membrane lipids is associated with stress induced damage at cellular level (Jain et al., 2001). Therefore the level of MDA produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage. The lower level of lipid peroxidation in leaves of 'Bonica' and 'Galine' suggests that for a same salt stress less oxidative damage occurred. The different accumulation pattern of proline might thus also be explained by the metabolic damage caused by salt stress. It might be interesting to analyse the accumulation pattern of Na^+ and Cl^- in the different cultivars as salt stress damage is partially an osmotic drought but by prolonged stress also ionic toxicity can occur.

3.5 Conclusion

This experiment under controlled *in vitro* conditions indicates that NaCl disturbed considerably the mechanism of germination at relative low salinity levels. Under increasing salt levels growth parameters in terms of fresh weigh, dry weigh, tissue water content, height and leaf number were negatively affected and increased cell damage as evaluated by MDA occurred. Our observations both on germination kinetics and young seedling development divided the cultivars in two groups. The sensitive cultivars 'Adriatica' and 'Black Beauty' supported only moderate salt stress up to 40 mM NaCl while the tolerance level of the tolerant group ('Bonica' and 'Galine') was up to 80 mM NaCl. Proline increased hugely in response to salinity in the sensitive cultivars.

Selection of genotypes able to absorb water under conditions of low soil water potential at germination and seedling stage could result in genotypes more tolerant (with enhanced growth rate) to salinity. In field cultivation, the reaction to the applied salt levels would be lower than in our experimental system as for a given salt level in the irrigation water there is

a delay in the build-up in salinity in the soil or substrate of the young seedling. Our method and all studied parameters could be used as a diagnostic tool to screen cultivars for salt stress in the seedling phase.

Chapter 4

Application of chlorophyll fluorescence to screen eggplant (*Solanum melongena* L.) cultivars for salt tolerance

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Chapter 4 Application of chlorophyll fluorescence to screen eggplant (*Solanum melongena* L.) cultivars for salt tolerance

Abstract

The objective of this study was to investigate the relative salt tolerance of four eggplant cultivars (*Solanum melongena* L.) by studying chlorophyll fluorescence parameters during the vegetative growth stage under increasing salinity levels. The plants were grown in pots filled with peat under controlled conditions and were subjected to saline stress ranging from 0 (control), 20, 40, 80 and 160 mM NaCl for 25 days. The results showed that increasing NaCl concentration hardly affected the maximum quantum yield of PSII (F_v/F_m). The quantum yield of PSII (Φ_{PSII}) decreased significantly in 'Adriatica' and 'Black Beauty' under saline stress. Photochemical quenching (q_P) decreased for 'Black Beauty' and non-photochemical quenching (NPQ) increased for 'Adriatica' under salt stress. For 'Bonica' and 'Galine' chlorophyll fluorescence parameters did not significantly change under salt stress, revealing their photochemical tolerance to salinity. After 25 days of salt stress plant growth was reduced in all cultivars, however, this decline was more pronounced for 'Adriatica' and 'Black Beauty'. Additionally, a significant correlation between biomass and Φ_{PSII} was observed for 'Adriatica' and 'Black Beauty'. Our results suggest that Φ_{PSII} can be used as a diagnostic tool to identify salt-tolerant egg-plant cultivars.

4.1 Introduction

The introduction of irrigated agriculture in arid and semi-arid regions resulted in the development of soil salinization. It is reported that already 15 million ha are saline in the Middle East and North Africa (Le Houerou et al., 1986). High salt concentrations in soil decrease yields for a wide variety of crops all over the world (Sekman et al., 2007). Indeed, salinity is regarded as one of the most important environmental constraints that affect adversely plant growth and metabolism particularly in the arid and semi-arid regions in the world (Munns and Tester, 2008).

Plants respond to salinity stress through a set of changes in basic biological functions including photosynthesis and photorespiration, synthesis of life saving molecules, fine tuning in translation and transcription, and reactive molecule scavenging (Mittler, 2002; Azevedo Neto et al., 2006; Wei et al., 2009; Turkan and Demiral, 2009). Decreased photosynthetic rate under stressful environments was generally ascribed to stomatal closure and decreased mesophyll conductance at a severe stress (Flexas et al., 2004; Bikash et al., 2005; Zribi et al., 2009). High saline concentrations contribute to leaf chlorosis, malfunctioning of the chloroplasts and photo-inhibition or photo-oxidation, which significantly affect the assimilation rate (Moradi and Ismail, 2007). Maxwell and Johnson (2000) showed that chlorophyll *a* fluorescence can constitute an important selection criterion to verify the plant sensitivity or tolerance to environmental stresses and to determine the damage of the photosynthetic apparatus caused by these stresses. Zribi et al. (2009) reported a significant increase in NPQ in salt-stressed tomato plants, without significant changes in F_v/F_m. Chlorophyll fluorescence measurements also revealed that NPQ increased whereas the electron transport rate decreased in rice plants under salt stress (Moradi and Ismail, 2007). In wheat, salt stress decreased F_v/F_m, Φ_{PSII} and q_P while increasing NPQ (Zheng et al., 2009). Chlorophyll fluorescence could thus be a non-destructive and non-invasive tool to determine effects of salt stress on photosynthetic machinery.

Eggplant (*Solanum melongena* L.) is an important vegetable crop in the Mediterranean area with Egypt, Iran and Turkey belonging to the top five eggplant producing nations (FAOSTAT, 2015). However, salinization affects irrigated agriculture in these regions and limited research has been carried out to evaluate the physiological and biochemical responses of eggplant to salt stress. Crop yield and growth parameters have been used to screen for salinity tolerance in the seedling stage (Akinci et al., 2004). Based on growth and production

parameters eggplant is considered moderately sensitive to salinity (Savvas and Lenz, 2000), with significant genotypic variation regarding salt tolerance (Akinci et al., 2004). Screening for salt tolerance should also use physiological traits in combination with growth performance. Exposure of eggplant to sub-lethal salt concentrations causes stomatal closure (osmotic stress) and reduces photosynthetic rates, which might lead to an inhibition of electron transport through photosystem II. Since hardly any information is available for eggplants, the objective of the present work was to investigate the relative salt tolerance of four eggplant cultivars by studying selected chlorophyll fluorescence parameters during the vegetative growth stage under increasing salinity levels.

4.2 Materials and methods

4.2.1 Plant material

Four commercial eggplant (*S. melongena* L.*)* cultivars, two open-pollinated ('Adriatica' and 'Black Beauty') and two F_1 hybrids ('Bonica' and 'Galine') were used as plant material. Seeds were sown into 80 mL plug trays containing a peat-based medium on 3 May 2011 in a growth chamber at a constant temperature of 25°C, RH of 70%, photon flux density of 150 µmol m^{-2} s^{-1} and photoperiod of 16 h. After 25 days eggplant seedlings at the second true leaf stage were selected for uniformity and transplanted into 2 L plastic pots. Plants were transferred to a heated glasshouse (located at 51°02'N, 03°42'E) and were fertigated with 250 mL full-strength Hoagland's solution, twice a week (Photo 4.1). The temperature in the glasshouse ranged between 22°C and 27°C while the daily maximum photon flux density averaged 340 µmol m^{-2} s^{-1}. Shading screens were used to prevent direct sunlight.

4.2.2 Salinity treatments

Five salinity treatments were applied starting 36 days after the transfer to the glasshouse. NaCl was added at 0 (control), 20, 40, 80, and 160 mM to a full-strength Hoagland's solution with electrical conductivity values of 1.18, 1.42, 2.75, 3.05, and 5.21 dS m^{-1} respectively. All solutions were prepared with distilled water. Plants were irrigated with 250 mL per pot, which was applied twice a week during 25 days.

Photo 4.1: Experimental set-up in a heated glasshouse (Left: eggplants at the start of the experiment, right plants after 4 weeks of salt stress).

4.2.3 Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence in dark- and light-adapted leaves was measured with a portable fluorometer (*PAM-2500*, *Walz*, Effeltrich, Germany). After 30 min of dark-adaptation, F_v/Fm was calculated as $(F_m - F_0)/F_m$ where F_m (induced by a short pulse (0.6 s) of saturating light (3,450 µmol m^{-2} s^{-1})) and F_0 were the maximal and minimal fluorescence (Genty *et al.* 1989). After 4 min of illumination with continuous red, non-saturating actinic light (447 µmol m^{-2} s^{-1}) and saturating pulses every 25 s, maximum (F_m') and steady state (F_s) fluorescence signals were measured in light adapted leaves. Then, the actinic light was turned off and a far red pulse was applied to obtain the minimal fluorescence after the PSI excitation (F_0'). Φ_{PSII} was calculated as $(F_m' - F_s)/F_m'$ and q_p was calculated as $(F_m' - F_s)/(F_m' - F_0')$ (Van Kooten and Snel 1990). NPQ which is proportional to the rate constant of thermal energy dissipation was estimated as $(F_m - F_m')/F_m'$ (Bilger and Björkman 1990). The electron transport rate (ETR) was calculated as $\Phi_{PSII} \times PAR \times 0.84 \times 0.5$ were the absorbed photon energy (PAR), is assumed to be equally distributed between PSI and PSII and 0.84 is the assumed light absorptance of the leaf.

The youngest fully developed leaf was selected for measurements after 5 days of saline stress (5 DSS) and was further used for the measurements taken after 10, 15, 20 and 25 DSS, this in four replicates.

4.2.4 Determination of biomass production

Plant development was assessed by fresh mass determination of the aerial parts of the plant (shoots and leaves) after 25 DSS. For each cultivar and treatment two plants per block (eight plants in total) were randomly measured.

4.2.5 Statistical analysis

Each treatment was applied to 20 plants per cultivar. The experiment was designed as a randomized complete block design, with four blocks. Each experimental unit contained five plants. Data were subjected to one way analysis of variance (*ANOVA*) followed by Tukey's HSD test (P=0.05) to distinguish differences among the treatments. Principal component analysis (PCA) was carried out on chlorophyll fluorescence parameters. Only PCAs with eigenvalues > 1, thus explaining more than a single parameter alone, were extracted. For these principal components a varimax rotation was applied on the obtained factor loadings. This rotation results in simpler factors, relating parameters mainly to one principal component axis (Manly 1994). All statistical analyses were carried out using *SPSS 19* (*IBM SPSS Statistics*).

4.3 Results

4.3.1 NaCl induced changes in chlorophyll fluorescence parameters

Up to 10 DSS no significant effects on F_v/F_m, q_p and NPQ (P>0.05) were found for any of the cultivars (data not shown). Fifteen days after applying salt stress (15 DSS) a decreasing trend for F_v/F_m was noticed for 'Bonica' (P=0.046) (Figure 4.1) although no significant negative effect was present after 25 DSS (Figure 4. 3 A). A significant decrease of F_v/F_m was found for 'Black Beauty' after 25 DSS of 160 mM NaCl (Figure 4.3*A*). F_v/F_m of 'Adriatica' and 'Galine' were not significantly affected by salt levels during the experimental period.

Significant differences in Φ_{PSII} were observed in 'Adriatica' and 'Black Beauty' (Figure 4.3*B*). Φ_{PSII} decreased progressively and significantly in 'Adriatica' from 10 DSS (P=0.010) and 'Black Beauty' from 5 DSS (P<0.001). At 15 and 25 DSS Φ_{PSII} was reduced by 41% for a level of 160 mM of NaCl compared to the respective controls in 'Adriatica'. Likewise, in 'Black Beauty' a decline of Φ_{PSII} by 39.7% after 15 DSS of 160 mM of NaCl and by 43.9% after 25 DSS was found when compared to their respective controls. In contrast Φ_{PSII} in 'Bonica' and 'Galine' was never significantly affected by the salt treatment (Figure 4.3B).

Decreases in q_p were found only in 'Black Beauty' after 25 DSS (Figure 4.3*C*). A similar but opposite trend was observed for NPQ. After 15 DSS a significant increase in NPQ for a salt stress from 40 mM (P=0.005) and 80 mM (P=0.023) on was found respectively for 'Adriatica' and 'Bonica'. For the highest salt stress level (160 mM NaCl) the increase in NPQ was respectively 26.7% for 'Adriatica' and 25.0% for 'Bonica'. However, after 25 DSS significant effects were only noted for 'Adriatica' where NPQ increased from 0.97 for control plants to 1.58 for a salt stress of 160mM NaCl or an increase of 63% (Figure 4.3*D*). After 25 DSS, ETR decreased from 23.5 for control plants to 12.5 for a salt stress of 160 mM NaCl in 'Black Beauty' (P=0.008) and from 17.7 for control plants to 8.7 for a salt stress of 160 mM NaCl in 'Adriatica' (P=0.024). No effects of salt stress on ETR were found for 'Bonica' and 'Galine'.

A scores scatter plot of the first two PCAs (explaining 80.1% of the variation) shows a good separation of 'Bonica' from the three other cultivars after 25 DSS (Figure 4.4). The loading that positively correlated with PCA1 (44.2%) was NPQ and that with PCA2 (35.8%) were Φ_{PSII} and q_P. The loading of F_v/F_m correlated negatively with PCA1. For all cultivars the scores of the PCA moved to higher NPQ values and lower F_v/F_m, Φ_{PSII} and q_P under increasing salt stress. The separation of control and salt stressed plants of 'Adriatica' along PCA1 was higher compared to the other cultivars (see arrows in Figure 4.4). For 'Black Beauty' control and salt stressed plants were well separated along PCA2.

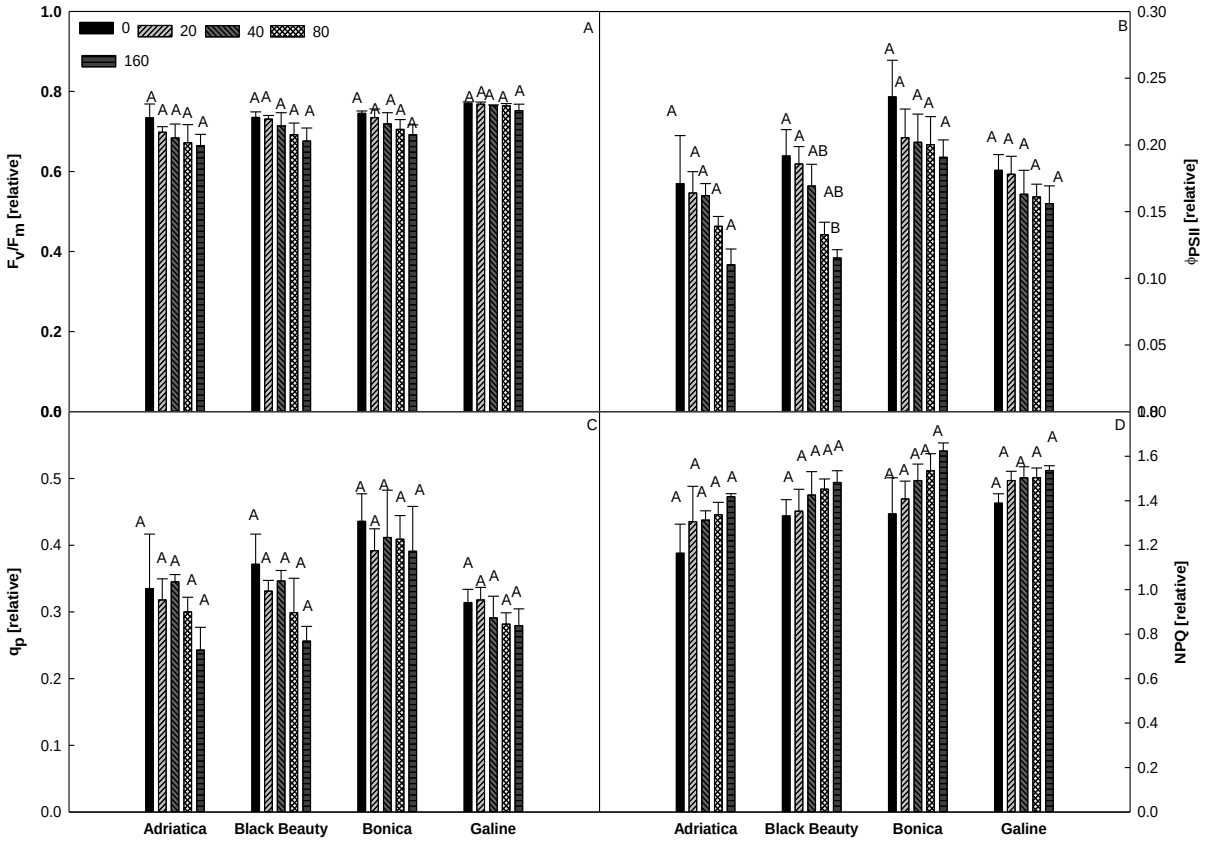

Figure 4.1. Effects of different salt treatments (0, 20, 40, 80 and 160 mM) after 5 days under NaCl stress on chlorophyll fluorescence parameters: maximum quantum yield of PSII (F_v/F_m) (A), the effective quantum yield of PSII (Φ_{PSII}) (B), the photochemical quenching (q_p) (C) and non-photochemical quenching (NPQ) (D). Different uppercase letters indicate significant differences using Tukey's test (*P*=0.05). Data are means of four replicates ± SE.

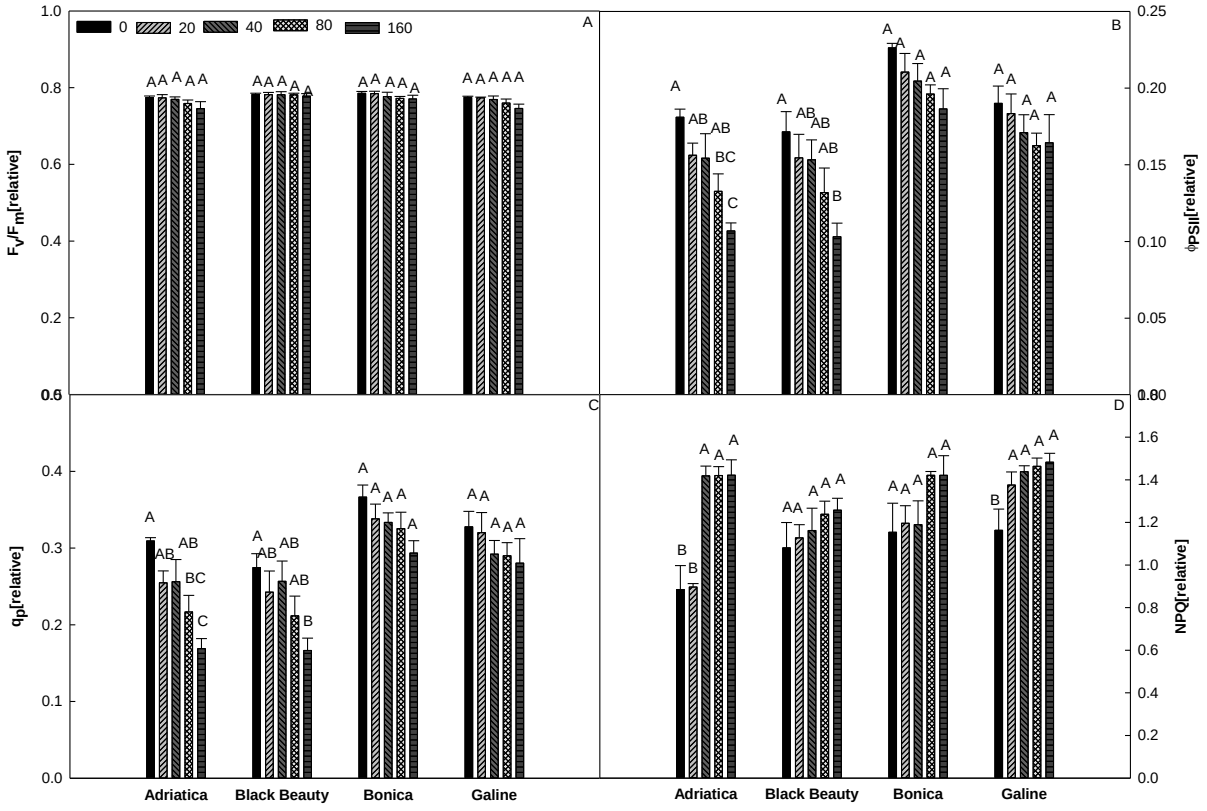

Figure 4.2. Effects of different salt treatments (0, 20, 40, 80 and 160 mM) after 15 days under NaCl stress on chlorophyll fluorescence parameters: maximum quantum yield of PSII (F_v/F_m) (A), the effective quantum yield of PSII (Φ_{PSII}) (B), the photochemical quenching (q_p) (C) and non-photochemical quenching (NPQ) (D). Different uppercase letters indicate significant differences using Tukey's test (*P*=0.05). Data are means of four replicates ± SE.

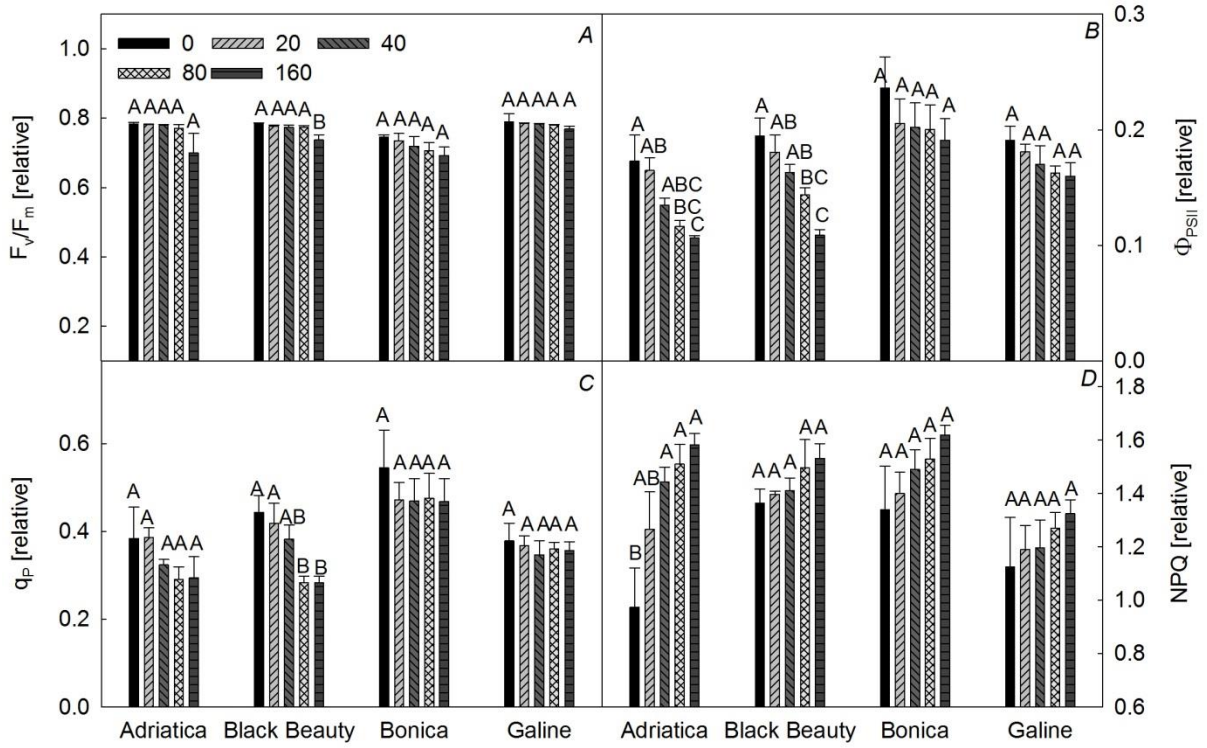

Figure 4.3. Effects of different salt treatments (0, 20, 40, 80 and 160 mM) after 25 days under NaCl stress on chlorophyll fluorescence parameters: maximum quantum yield of PSII (F_v/F_m) (A), the effective quantum yield of PSII (Φ_{PSII}) (B), the photochemical quenching (q_p) (C) and non-photochemical quenching (NPQ) (D). Different uppercase letters indicate significant differences using Tukey's test (*P*=0.05). Data are means of four replicates ± SE.

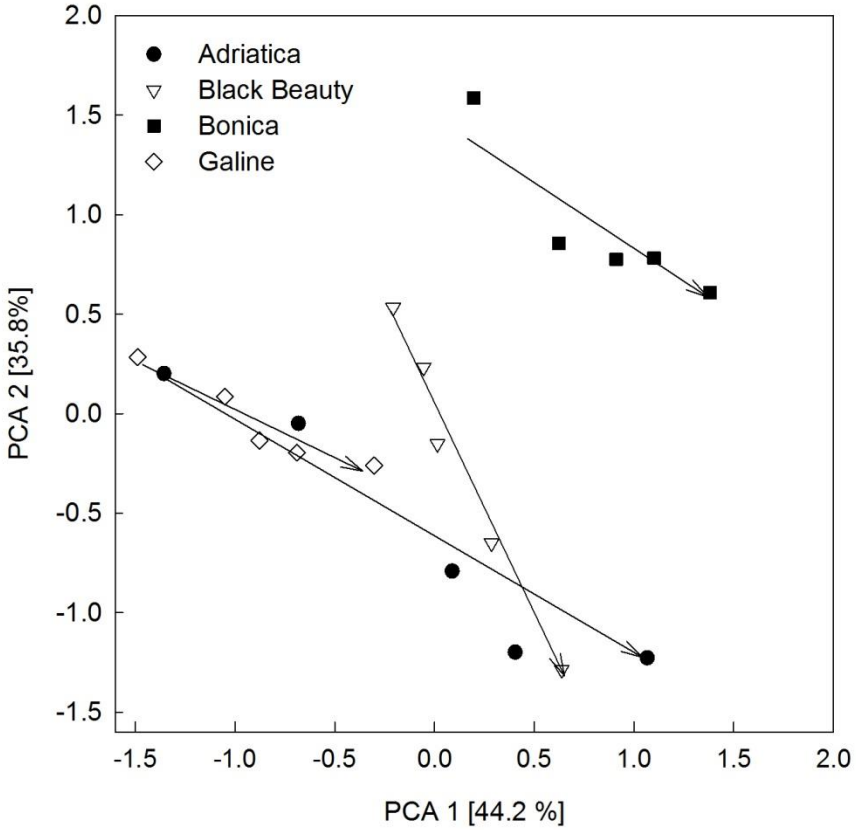

Figure 4.4. Principal component analysis (PCA) of chlorophyll fluorescence parameters of the eggplant cultivars grown for 25 days under saline stress. PCA1 is positively correlated with the non-photochemical quenching (NPQ) and negatively with the maximum quantum yield of PSII (F_v/F_m), PCA2 is positively correlated with the effective quantum yield of PSII (Φ_{PSII}) and the photochemical quenching (q_p). Each data point represents the mean of four replicates. Arrows indicate the increasing salt stress level.

4.3.2 NaCl induced changes in biomass production

Fresh aerial biomass of all cultivars decreased as the salinity increased (Figure 4.5). A significant growth reduction was already present for the lowest salt stress level (20 mM NaCl). Although plant vigour differed between the cultivars as indicated by their biomass without salt stress, the salt induced decreases in fresh biomass showed an overall similar trend. The maximum decrease in fresh biomass was observed in 'Adriatica' and 'Black Beauty'. As compared to control conditions, fresh biomass decreased by 86.6% in 'Adriatica' and by 87.8% in 'Black Beauty' at 160 mM (Figure 4.5). In contrast, the decline in fresh biomass between the control and the 160 mM NaCl level was 36.9% in 'Bonica' and 35.9% in 'Galine' (Figure 4.5). Also a significant correlation between fresh biomass and Φ_{PSII} was observed for 'Adriatica' (R=0.760, P<0.001) and 'Black Beauty' (R=0.762, P<0.001).

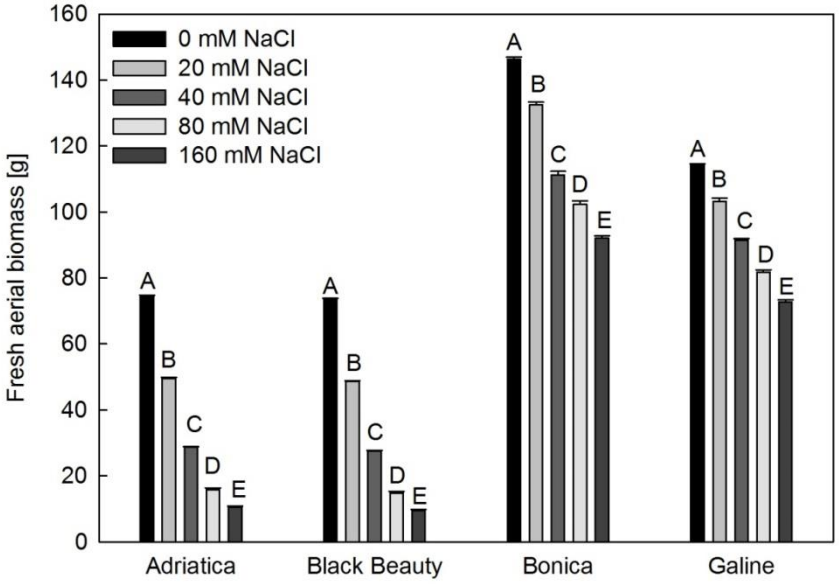

Figure 4.5. Effect of increasing salt levels on fresh aerial biomass in four eggplant cultivars. Different uppercase letters indicate significant difference using Tukey's test (*P*=0.05). Data are means of four replicates ± SE.

4.4 Discussion

In saline soil, plants experience first an osmotic stress as a consequence of a reduced osmotic potential of the soil solution. In a second stage exposure to salinity also causes accumulation of salts in the plant tissues. These salts will eventually rise to toxic levels and may cause Na^+ toxicity (ionic stress), thereby reducing nutrient acquisition or causing nutritional imbalances (Munns and Termat, 1986). Osmotic and ionic damage are interrelated and co-exist under saline conditions (Castillo *et al.*, 2007). Dry biomass measurements are considered an appropriate parameter for evaluating stress tolerance in crops (Munns et al., 2000) although fresh weight is often used in horticultural crops to describe plant biomass (Marcelis et al., 1998). Also in our experiment salinity had a significant impact on eggplant biomass. When plants were submitted to a salt stress of 160 mM NaCl for 25 days, a decrease in fresh biomass by more than 80% in 'Adriatica' and 'Black Beauty', and by more than 30% in 'Bonica' and 'Galine' were found. Important decreases of plant biomass have been reported for green beans at 100 mM (Yasar et al., 2007) and for rice at 120 mM (Demiral and Turkan, 2005), while for more salt tolerant mangroves a decrease at 250 mM was found (Ru et al.,

2009). Asch et al. (2000) reported that in rice the salt tolerant genotype had the smallest and the susceptible genotype had the greatest reduction in biomass production.

Salt stress will restrict CO_2 availability due to stomatal closure which may lead to reduced photosynthesis (Munns and Tester, 2008) and hence reduced growth. In addition non-stomatal factors also affect photosynthesis under higher saline levels (Stepien and Klobus, 2006); accumulation of NaCl in chloroplasts of higher plants will lead to inhibition of PSII and increased susceptibility to photo-damage (Sudhir and Murthy, 2004). To evaluate the direct effect of salt stress on PSII photochemistry chlorophyll *a* fluorescence was measured in four eggplant cultivars. In the present study, results showed that at 25 DSS, F_v/F_m was generally unaffected, with exception of 'Black Beauty' at the highest NaCl concentration. Similar results have been reported for wheat (Gallé et al., 2002) and tomato (Zribi et al., 2009). This slight decrease of F_v/F_m is likely to be due to the reversible inactivation or downregulation of PSII rather than to photo-damage of PSII (Demming-Adams and Adams, 1996).

PSII activity and its regulation are best studied by Φ_{PSII} (Genty et al., 1989). For two cultivars the growth inhibition was correlated to a decline of Φ_{PSII}. The salt stress induced a significant and early reduction of Φ_{PSII} indicating a decrease in electron transport through PSII in 'Adriatica' and 'Black Beauty' cultivars, which is in agreement with the findings of Zribi et al. (2009) in tomato and Lu et al. (2009) in soybean. This was also mirrored in a decrease of q_P in 'Black Beauty' under increasing salinity. This fluorescence parameter gives an indication of the ability of PSII to reduce the primary electron acceptor Q_A under the applied salt stress as well as of the number of photons used by photochemical reactions/number of absorbed photons (Govindjee et al., 1981). As Φ_{PSII} was affected after 5 and 10 DSS in 'Adriatica' and 'Black Beauty' respectively, this parameter has potential to be an early and non-destructive tool to screen eggplant cultivars for salt tolerance. The same finding has been reported for soybean by Lu et al. (2009).

Φ_{PSII} was not affected in 'Bonica' and 'Galine' up to 25 days of salt stress indicating a more optimal functioning of PSII under salt stress. Moreover the whole chain electron flow continued at an effective rate in these cultivars. Indeed, q_P was hardly affected by increasing salt stress in these cultivars, which is also obvious from the limited variation along the PCA2 axis under increasing salt stress whereas q_P was reduced in 'Black Beauty'. The photochemical quenching can contribute to protect the photosynthetic apparatus by transferring electrons to O_2 under drought or salt stress (Ort and Baker, 2002). Cornic and

Fresneau (2002) showed that oxygenation of ribulose-1,5-bisphosphate in C3 plants can efficiently replace the carboxylation when stomata close. We can assume reduced CO_2 assimilation due to salt stress as fresh biomass decreased for all cultivars. As Φ_{PSII} and ETR are not affected in 'Bonica' and 'Galine', we may argue that alternative electron sinks were more active in these cultivars. Besides photorespiration, photoreduction might also occur at the acceptor side of PSI in the Mehler reaction (Asada, 2000) resulting in a pH gradient across the thylakoid membranes and enhancing thermal dissipation of excess excitation energy (Johnson et al., 1994).

The increase in non-photochemical energy dissipation is probably the major process involved in protection against photo-damage (Krause and Weiss, 1991). This increase was more pronounced in 'Adriatica' at 25 DSS. Also changes in PCA score by salt stress were greater for 'Adriatica'. Apparently, the increase in NPQ of 'Adriatica' was effective in preventing reductions of F_v/F_m. The increased NPQ will dissipate a part of the excitation energy at the expense of photochemical utilization (Osmond, 1994; Zribi et al., 2009) thus contributing to a downregulation of PSII to avoid over-reduction of the primary electron acceptor Q_A. This response reflects a protective or regulatory mechanism to avoid photo-damage of the photosynthetic apparatus (Demming-Adams and Adams, 1996).

4.5 Conclusion

'Bonica' and 'Galine' tolerated better the applied salt stress as shown by growth performance and limited effect on primary photochemistry as compared to 'Adriatica' and 'Black Beauty'. Φ_{PSII} could distinguish the differential response to salt stress in the studied eggplant cultivars. Consequently, the light-adapted responses could be considered as an early indicator of salt induced disturbances in eggplant.

Chapter 5

Effect of salt induced stress on physiological responses in eggplant cultivars

Chapter 5 Effect of salt induced stress on physiological responses in eggplant cultivars (*Solanum melongena* L.)

Abstract

The effect of NaCl stress on physiological and biochemical parameters was investigated in four eggplant cultivars (*Solanum melongena* L.). The plants were grown in pots filled with peat under controlled conditions and were subjected during 30 days to saline stress ranging from 0 (control), 20, 40, 80 and 160 mM of NaCl. Increasing NaCl concentration increased strongly proline, malondialdehyde and carbohydrates leaf contents in sensitive cultivars 'Adriatica' and 'Black Beauty'. However the tolerant cultivars 'Bonica' and 'Galine' showed a decrease in carbohydrates accumulation and a significant increase in level of starch under saline stress. Salt stress reduced the biomass parameters in all the cultivars. The midday leaf water potential (ψ) and leaf osmotic potential (ψ_π) were significantly affected in sensitive cultivars and remained quite stable in tolerant cultivars under salt stress. Leaf Cl^- content was higher in sensitive than in tolerant cultivars. The leaf K, Ca and Mg contents were reduced under salt stress in sensitive cultivars. Increasing salinity did not change Ca and Mg content in tolerant cultivars.

The responses of the growth, physiological and biochemical parameters to salt stress were more sensitive in 'Adriatica' and 'Black Beauty' than in 'Bonica' and 'Galine'. Our results suggest that the physiological and biochemical mechanism can be adopted as an excellent tool for the diagnosis of salt stress in eggplant.

5.1 Introduction

Water scarcity and quality degradation are major constraints for agricultural development in the southern Mediterranean countries. Besides, the introduction of irrigated agriculture in arid and semi-arid regions resulted in the development of secondary soil salinization (Ritzema et al., 2008). Salinity is regarded as one of the most important environmental extremes that affects plant growth and metabolism adversely in the arid and semi-arid regions of the world (Munns and Tester, 2008). Increasing salinity causes a significant decline in biomass production (Parida and Das, 2005; Junmin et al., 2012) and thus decreases yields for a wide variety of crops all over the world (Sekman et al., 2007). Salinity of the soil affects the water uptake and thus the plant water status. One of the earliest responses under salt stress is a reduction of the leaf area and the changed plant water status most likely leads to this initial growth reduction (Dash and Panda, 2001). Plant growth suppression is directly related to the total concentration of salt ions and/or the decrease in soil osmotic potential (Silva-Ortega et al., 2008).

Under salinity stress both osmotic and ionic effects affect the metabolism of plant cells in many ways. Specific effects of salt stress on plant metabolism have been related to the accumulation of toxic ions (Na^+ and Cl^-) or to K^+ and Ca^{2+} depletion (Munns et al., 2002). In addition, accumulation of Na^+ ions changes ion balance such as Na^+/Ca^{2+} and K^+/Na^+ ratio in plant cells under saline condition. A high Na/Ca ratio results in increased cell permeability (Levitt, 1980). Ion disorder caused by salinity may also lead to changes in plant lipid metabolism (Kuiper, 1985). Lipid peroxidation, induced by free radicals, is important in membrane deterioration (Halliwell, 1987; McCord, 2000) and might lead to visible injury in leaves (Yildiztugay et al., 2011).

Plants develop an array of mechanisms to cope with salinity. Under saline conditions, plants accumulate compatible solutes such as sugars, amino-acid, protein and/or other compounds to protect themselves against the damage of the salinity and to accommodate the ionic balance in the vacuole in a process called osmotic adjustment (Yazici et al., 2007; Turkan et al., 2009; Yildiztugay et al., 2011; Xu et al., 2012). Carbohydrates contribute to 13% of the osmotic adjustment (Hu and Schmidhalter, 1998). Turan et al. (2007) reported that proline accumulation increased in plant tissue during salinity stress. Compartmentalization of toxic ions in different tissues is another possibility to enable metabolic functions and to tolerate higher amounts of salt in the soil.

Vegetables are generally considered as glycophytes and therefore susceptible to soil salinity (Colla et al., 2010; Shahbaz et al., 2012). Eggplant (*Solanum melongena* L.) is a vegetable crop of high importance in the Mediterranean region with Egypt and Turkey belonging to the top five of eggplant producing nations (FAOSTAT, 2015). This vegetable is an important greenhouse crop for out of season production, however, secondary salinization due to non-sustainable irrigated horticulture results in a decline in eggplant productivity. Few comparative studies concerning salt stress have been published on eggplant though growth parameters and horticultural performances have been used to select plants tolerant to salinity (Savvas and Lenz, 2000; Akinci et al., 2004). Chlorophyll *a* fluorescence proved to be a promising screening technique for salt tolerant eggplant cultivars (Chapter 4, Hanachi et al, 2014). Yet, no information on how physiological and biochemical traits of eggplant cultivars with an opposing salt tolerance evolve under salt stress has been published. The aim of this research is to evaluate the genotypic variation of increasing salt stress response in eggplant. Two relative salt sensitive cultivars (‘Adriatica’ and ‘Black Beauty’) and two more tolerant cultivars (‘Bonica’ and ‘Galine’) based on chlorophyll fluorescence screening were used in this study (Chapter 4, Hanachi et al., 2014). The effects of increasing salt stress on plant water relations, osmotic adaptation and foliar accumulation of sodium and chloride in these four eggplant cultivars differing in salt tolerance was addressed. This knowledge might be of further advantage to screen eggplant cultivars tolerant to salt stress.

5.2 Materials and methods

5.2.1 Plant materials

Four commercial eggplant (*S. melongena* L.) cultivars, two open-pollinated (‘Adriatica’ and ‘Black Beauty’) and two F1 hybrids (‘Bonica’ and ‘Galine’) were used as plant material. Seeds were sown into 80 mL plug trays containing a peat-based medium on 3 May 2011 in a growth chamber at a constant temperature of 25°C, RH of 70%, photon flux density of 150 µmol m^{-2} s^{-1} and photoperiod of 16 h. Eggplant seedlings were selected for uniformity and transplanted into 2 L plastic pots at the appearance of the second true leaf stage. Plants were transferred to a heated glasshouse with a minimum temperature set-point of 21°C (located at 51°02’N, 03°42’E) and were fertigated with 250 mL full-strength Hoagland’s solution (Hoagland and Arnon, 1950), twice a week. The temperature in the glasshouse ranged between 22°C and 27°C while the daily maximum photon flux density averaged 340 µmol m^{-2} s^{-1} (quantum sensor SKP215, Skye at plant canopy and connected to a data logger, type

DL3000, Delta-T, UK; data were logged every 10 min). Shading screens were used to prevent direct sunlight to the plants.

5.2.2 Salinity treatments

Five salinity treatments were applied starting 36 days (4^{th} leaf stage) after the transfer to the greenhouse. NaCl was added at 0 (control), 20, 40, 80 and 160 mM to a full-strength Hoagland's solution with electrical conductivity values of 1.18, 1.42, 2.75, 3.05, and 5.21 dS m^{-1} respectively. All solutions were prepared with distilled water. Plants were irrigated with 250 mL per pot, which was applied twice a week during 30 days.

Each treatment was applied to 20 plants per cultivar. The experiment was designed as a randomized complete block design, with four blocks. Each experimental unit contained five plants.

5.2.3 Plant water status

The midday leaf water potential (Ψ_l) (11 h – 12 h) of the youngest fully-expanded leaves was determined with a Scholander pressure chamber (model 1000, PMS Instrument Company, Albany, OR, USA). The leaf osmotic potential (Ψ_π) was determined according to the method of Callister et al. (2006). Leaves were dried in an oven at 80°C for 48 h. Then, 20 mg of dry matter was extracted with 1 ml of distilled water in a water bath of 100°C for 1 hour. The extract was centrifugated for 3 minutes at 17000 g and the osmolarity of the supernatant was determined using an osmometer (Fiske One-Ten Micro Sample Fiske Associates, Howard, USA) and the osmotic potential (ψo) was calculated with the Van't Hoff equation:

$$\psi o = - (n/V)\ RT\ /[(m/V)\ (FM - DM)/DM]$$

where ψo is the average osmotic potential of leaves (MPa); (n/V) is the measured osmolality ($mOsm \cdot kg^{-1}$); R is the gas constant ($8.314\ 472 \times 10^{-6}\ m^3 \cdot mPa\ K^{-1}\ mol^{-1}$); T is the temperature in Kelvin; (m/V) is the leaf dry weight/the water volume of the extraction; DM is dry matter weight (g) and FM is the fresh matter weight (g). The analysis was done in 4 replicates per treatment.

5.2.4 Growth parameters

After 30 days of saline stress 8 plants (2 plants per block) were taken at random for each treatment. The fresh weigh (FW) of the aerial biomass and the number of leaves were measured. Dry weight (DW) was determined after 48 h drying at 60°C. The water content of

the aerial biomass (TWC) was calculated as (FW-DW/FW). Then, leaves were milled and stored until leaf mineral analysis.

According to Jha et al. (2010) the plant tolerance index was measured as the total fresh weight (FW) of salt stressed plant ÷ total FW of control plants, and this index was related to the leaf sodium content for the salinity treatment 20 mM, 40 mM, 80 mM and 160 mM of NaCl.

5.2.5 Metabolite analysis

Fully developed upper leaves (2 leaves/replicate in a bulked sample) were harvested between 12 h and 14 h from four plants in each treatment and for each variety after 30 days of salt stress treatments. Leaf material was grounded in liquid nitrogen and stored at -80°C until analysis.

Sugars were extracted with 80% ethanol at 70°C for 10 min and further at 45°C for 3 hours, followed by centrifugation at 5,000 g for 5 min. Glucose, fructose and sucrose were analysed using high pH anion-exchange chromatography with pulsed amperometric detection (Waters; CarboPac MA1 column with companion guard column, eluent: 50 mM NaOH, 22°C). The remaining ethanol insoluble material was washed twice with ethanol 80% and the residual pellet was treated with HCl 1M for 2 hours at 95°C for starch hydrolysis. Starch was determined spectrophotometrically at 340 nm by the enzymatic reduction of $NADP^+$ (UV-VIS, Biotek Uvikon XL).

Proline was determined according to Bates et al. (1973). Plant tissue (500 mg) was extracted with 10 mL of 3% (w/v) sulfosalicylic acid. After filtration, 2 mL acid ninhydrin and 2mL glacial acetic acid were added to the extracts (2 mL) and this mixture was kept at 100°C for 1 hour in a water bath, then the reaction was stopped in an ice-bath. The formed chromophore was extracted from the acid aqueous solution by means of cold toluene (4 mL) and measured spectrophotometrically at λ = 520 nm (InfiniteM200 TECAN Group Ltd., Switzerland). The proline concentration was determined using a calibration curve and expressed as µg proline g^{-1} FW.

Lipid peroxidation wad measured as the amount of malondialdehyde (MDA) determined by the thiobarbituric acid (TBA) reaction (Hodges et al. 1999). Leaf material (1 g) was homogenized in 25 mL 80% ethanol, followed by centrifugation at 3000 g for 10 min. A 1 mL aliquot of sample extract was added to 1 mL of thiobarbituric acid (TBA, 0.65% w/v) as well as to 1 mL of trichloroacetic acid (TCA, 20% w/v) and homogenates were incubated at

95°C for 25 min, cooled and centrifuged at 3,000 g for 10 min (4°C). The MDA content was measured based on the reaction with thiobarbituric acid (TBA) and the absorbance was measured at λ=440 nm, 532 nm and 600 nm by spectrophotometer (InfiniteM200 TECAN Group Ltd., Switzerland). Malondialdehyde (MDA) equivalents were calculated as described by Hodges et al. (1999).

5.2.6 Mineral analysis

P, K, Ca and Mg were determined by inductively coupled plasma (Ultima 2, Horiba Jobin Yvon S.A.S., France) after microwave digestion of leaf material (200 mg DW) with 5 M HNO_3. The wavelengths used for determination of the elements were respectively, 177.49 nm for P, 285.21 nm for Mg, 317.93 nm for Ca and 766.49 nm for K. Nitrate and chloride were analysed by anion-exchange chromatography and monitored by suppressed conductivity detection (Ion Pac AS11 HC column, Dionex, Sunnyvale, California) after extraction of 500 mg dried leaf material in 25 mL MilliQ water for 30 min. Na was analysed by flame photometry (Solaar AA, Thermo Fisher Scientific) after dry ashing at 525°C (1 g dried leaf material) followed by extraction with 4 M HCl.

5.2.7 Statistical analysis

Data were subjected to one way analysis of variance (ANOVA) followed by Tukey's HSD test (P=0.05) to distinguish differences among the treatments using the software of SPSS Statistics 19 (IBM SPSS Statistics).

5.3 Results

5.3.1 Plant growth parameters

Increasing salt stress affected the vegetative growth of the four eggplant cultivars. Although we noted a not always significant increase in number of leaves at 20 mM NaCl in all cultivars, a further increase of the salt level reduced the number of leaves progressively (Table 5.1). The highest salinity level (160 mM NaCl) decreased the number of leaves in 'Adriatica', 'Black beauty', 'Bonica' and 'Galine' by respectively 35.9%, 28.2%, 18.0% and 12.1% when compared to their respective controls. Increased salinity level also caused a shoot length reduction in 'Adriatica', 'Black Beauty', 'Bonica' and 'Galine' by respectively 52.8%, 52.8%, 37.9% and 39.6% compared to their respective controls (Table 5.1). Thus, the

vegetative growth of 'Adriatica' and 'Black Beauty' was more affected than 'Bonica' and 'Galine' under salt treatment.

A significant decrease in dry biomass (DW) was observed for all cultivars though varietal differences were present. The highest decrease in DW was observed in 'Adriatica' and 'Black Beauty'. For 160 mM NaCl DW decreased by 74.1% in 'Adriatica' and by 72.6% in 'Black Beauty' compared to their respective controls (Table 5.1). In contrast the decline in DW between the control and the 160 mM NaCl level was limited to 35.7% in 'Bonica' and 33.8% in 'Galine'. Also a significant decrease in water content (TWC) was observed in the highest concentration of NaCl (160 mM) when compared to the respective control in the cultivars 'Adriatica' (25.5%) and 'Black Beauty' (36.8%). In contrast, TWC remained quite stable in 'Bonica' and 'Galine' (Table 5. 1).

Table 5.1: Effect of increasing levels of NaCl on morphology and plant water status of four eggplant cultivars. Plant morphological parameters include number of leaves, plant height; aerial dry weight (DW) and plant water status includes tissue water content (TWC),

Cultivar	NaCl (mM)	N° of leaves	Height (cm)	Internode length (cm)	DW (g)	TWC (%)
Adriatica	0	7.2^{a}	36.3^{a}	5.07^{a}	15.6^{a}	79^{a}
	20	8.0^{a}	35.3^{a}	4.46^{ab}	10.8^{b}	77^{a}
	40	6.3^{b}	30.1^{b}	4.83^{a}	6.7^{c}	76^{a}
	80	5.4^{c}	21.3^{c}	4.07^{bc}	5.0^{d}	67^{b}
	160	4.6^{c}	17.1^{d}	3.78^{c}	4.0^{e}	58^{c}
Black Beauty	0	7.1^{b}	36.3^{a}	5.18^{a}	16.0^{a}	78^{a}
	20	8.0^{a}	34.9^{b}	4.38^{b}	11.2^{b}	76^{a}
	40	7.0^{b}	30.2c	4.25^{b}	4.1^{c}	74^{a}
	80	6.1^{c}	21.4^{d}	3.57^{c}	5.3^{d}	61^{b}
	160	5.1^{d}	17.1^{e}	3.42^{c}	4.3^{e}	49^{c}
Bonica	0	9.4^{ab}	34.2^{b}	3.64^{a}	25.7^{a}	82^{a}
	20	10.0^{a}	39.0^{a}	3.90^{a}	23.3^{b}	82^{a}
	40	9.1^{bc}	32.4^{b}	3.56^{ab}	19.5^{c}	82^{a}
	80	8.5^{c}	27.0^{c}	3.23^{b}	17.9^{d}	82^{a}
	160	7.7^{d}	21.2^{d}	2.76^{c}	16.5^{e}	82^{a}
Galine	0	7.7^{ab}	36.5^{a}	4.73^{a}	20.9^{a}	81^{a}
	20	8.0^{a}	38.2^{a}	4.80^{a}	19.1^{b}	81^{a}
	40	7.6^{ab}	32.2^{b}	4.21^{b}	17.2^{c}	81^{a}
	80	7.2^{bc}	28.2^{c}	3.92^{b}	15.2^{d}	81^{a}
	160	6.8^{c}	22.1^{d}	3.27^{c}	13.8^{e}	81^{a}

Means followed by the same lowercase within each column and for each cultivar are not significantly different at P=0.05 according to Tukey's HSD test (n=4).

Irrespective of the salt treatments the hybrid vigour of the F1 genotypes ('Bonica' and 'Galine') compared to the open-pollinated genotypes was evident (Table 5.1).

5.3.2 Plant-water relations

Midday Ψ_l decreased significantly with increasing NaCl concentration in 'Adriatica' and 'Black Beauty' and reached respectively -1.92 MPa and -1.88 MPa after 30 days at 160 mM NaCl (Table 5.2). In contrast Ψ_l remained quite stable in 'Bonica' and 'Galine' under increasing salt stress. The levels achieved under 160 mM NaCl (-0.62 and -0.64 MPa) evidenced that the tolerant cultivars hardly encountered an osmotic stress. Likewise salt stress significantly decreased Ψ_π in 'Adriatica' and 'Black Beauty' reaching -2.52 MPa and -2.48 MPa respectively at 160 mM NaCl while no effects on Ψ_π were obtained in 'Bonica' and 'Galine' (Table 5.2).

Table 5.2: Effect of increasing levels of NaCl on midday leaf water potential (ψ_l) and leaf osmotic potential (ψ_π).

Cultivar	NaCl (mM)	ψ_l (MPa)	ψ_π(MPa)
Adriatica	0	-0.53^{d}	-0.64^{c}
	20	-0.92^{c}	-1.07^{bc}
	40	-1.32^{b}	-1.52^{b}
	80	-1.48^{b}	-1.64^{b}
	160	-1.92^{a}	-2.52^{a}
Black Beauty	0	-0.51^{c}	-0.61^{c}
	20	-0.91^{c}	-1.06^{bc}
	40	-0.93^{c}	-1.22^{bc}
	80	-1.39^{b}	-1.53^{b}
	160	-1.88^{a}	-2.48^{a}
Bonica	0	-0.47^{a}	-0.62^{a}
	20	-0.51^{a}	-0.63^{a}
	40	-0.58^{a}	-0.65^{a}
	80	-0.61^{a}	-0.66^{a}
	160	-0.62^{a}	-0.68^{a}
Galine	0	-0.49^{a}	-0.65^{a}
	20	-0.54^{a}	-0.67^{a}
	40	-0.61^{a}	-0.70^{a}
	80	-0.63^{a}	-0.72^{a}
	160	-0.64^{a}	-0.73^{a}

Means followed by the same lowercase within each column and for each cultivar are not significantly different at P=0.05 according to Tukey's HSD test (n=4).

5.3.3 Biochemical parameters

The foliar proline content increased significantly for all cultivars when exposed to increasing NaCl (Figure 5.1A). This increase was correlated to the severity of the salt stress. 'Adriatica' and 'Black Beauty' accumulated higher proline contents than 'Bonica' and 'Galine' when subjected to salt stress. 'Adriatica' showed the highest proline accumulation at 160 mM.

MDA concentration as a measure of lipid peroxidation increased in all cultivars with increasing salt stress (Figure 5.1B). In 'Adriatica' and 'Black Beauty' MDA increased respectively by 11-fold and 10-fold in the 160 mM NaCl level compared to the controls whereas the MDA increase in 'Bonica' and 'Galine' was respectively 3-fold and 2-fold for the highest NaCl concentration compared to the controls. Consequently the induced lipid peroxidation under increasing salinity was more pronounced in the cultivars 'Adriatica' and 'Black Beauty' than in 'Bonica' and 'Galine'.

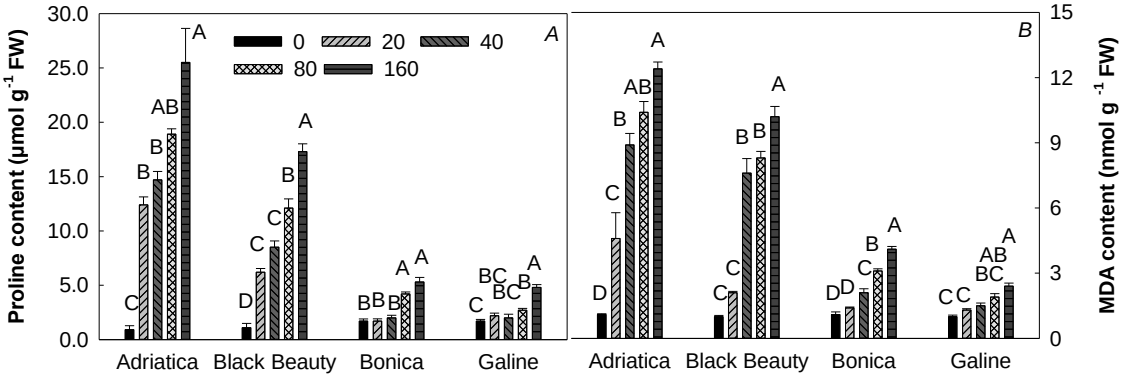

Figure 5.1: Effect of salt stress on leaf proline content (µmol g^{-1} FW) (A) and on leaf lipid peroxidation (B) of the eggplant cultivar subjected to different NaCl concentrations. Data are means ± SE. Different lowercase letters indicate the significant difference between treatments (P=0.05) based on Tukey's HSD test.

Salt stress increased significantly glucose, fructose and sucrose content in the cultivars 'Adriatica' and 'Black Beauty', this from 40 mM NaCl on (Figure 5.2). In contrast, increasing salt concentration lead to a non-significant decrease in glucose content for 'Bonica' and 'Galine', and in fructose content for 'Galine'. However the a significant decrease was noticed in fructose content for 'Bonica' and in sucrose content for the cultivars 'Bonica' and 'Galine' (Figure 5.2). Salt stress strongly increased starch accumulation in

'Bonica' and 'Galine' from 80 mM NaCl on but decreased leaf starch reserves in 'Adriatica' and 'Black Beauty' especially for 160 mM NaCl (Figure 5.2D).

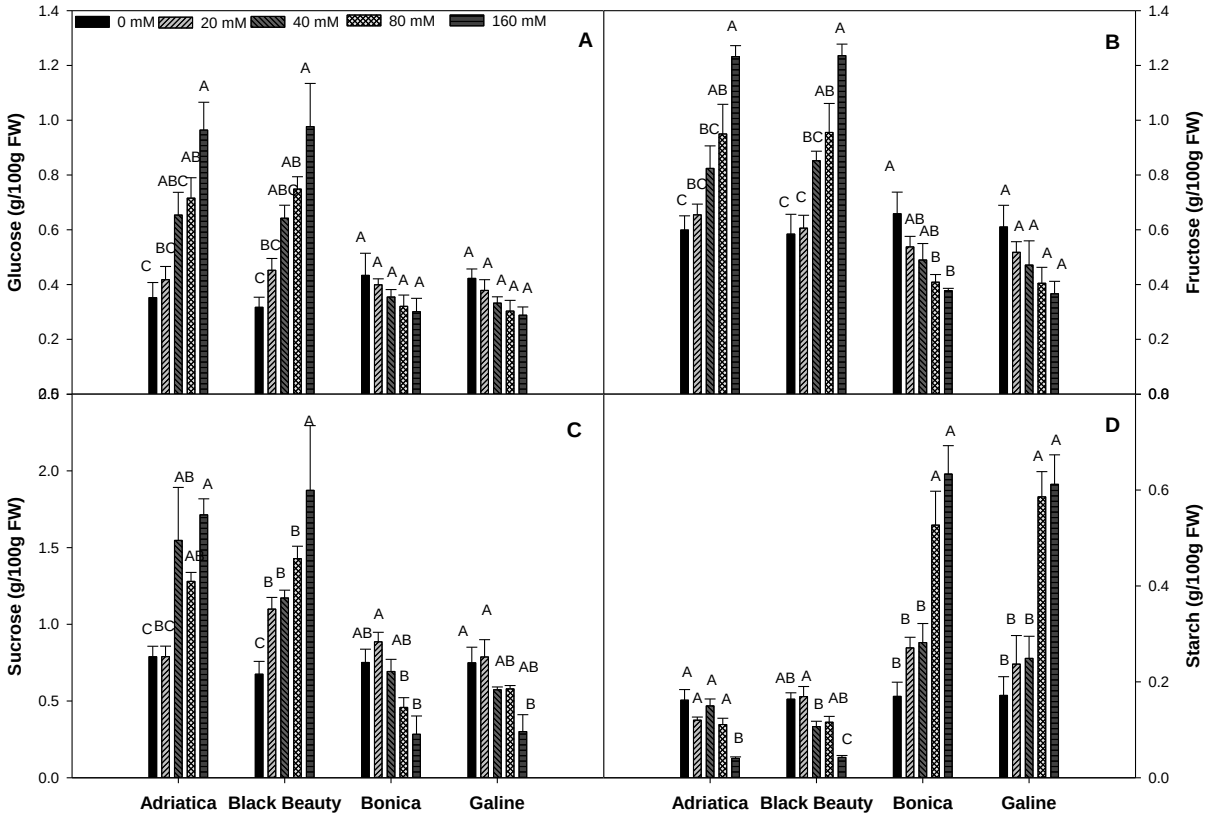

Figure 5.2 Effect of NaCl concentration on glucose (A), fructose (B), sucrose (C), and starch (D) levels in leaves of the eggplant cultivars. Data are means ± SE (not shown when smaller than the symbol). Different lowercase letters indicate the significant difference between treatments (P=0.05) based on Tukey's HSD test.

5.3.4 Mineral content

An increase of NaCl in the nutrient solution increased the foliar Na and Cl^- in all the cultivars (Table 5.3). These differences were significant from 40 mM NaCl for 'Bonica' and from 80 mM NaCl for the other cultivars. However, for the highest NaCl concentration (160 mM) the strongest increase was observed in 'Adriatica' and 'Black Beauty'. Indeed foliar Na content in 'Adriatica' was 2.7-fold higher than in 'Bonica'. Likewise foliar Na content in 'Black Beauty' was 3.4-fold higher than in 'Galine'. Leaf Cl^- accumulation was also stronger in the leaves in 'Adriatica' and 'Black Beauty' and were respectively 1.2-fold higher than Cl^- content in 'Bonica' and 'Galine', respectively.

Foliar K concentration decreased significantly from 80 mM NaCl on for 'Adriatica' and from 160 mM on for the other cultivars. However, the decline of the foliar K content at the highest salt level was more pronounced for 'Adriatica' and 'Black Beauty' (43% reduction) than for 'Bonica' and 'Galine' (28% reduction) compared to the control treatment. Increasing salt stress induced no significant change in the Ca content in both 'Bonica' and 'Galine' whereas 160 mM NaCl decreased leaf Ca content in 'Adriatica' and 'Black Beauty'. Foliar Mg content was not affected by increasing salt stress in 'Bonica' and 'Adriatica' while 160 mM NaCl decreased Mg content in 'Galine' and 'Black Beauty'.

Leaf Na/K and Na/Ca ratios were significantly higher under salt stress, this for all cultivars. There was a cultivar effect on these cation ratios resulting in higher leaf Na/K and Na/Ca ratios for 'Adriatica' and 'Black Beauty' than in 'Bonica' and 'Galine' (Table 5.2).

Also nitrate and phosphor content were influenced by the salt stress. Leaf nitrate decreased from 80 mM NaCl in 'Black Beauty' and from 160 mM in 'Adriatica' while phosphor content decreased from 160 mM on in both cultivars. No significant effects on foliar nitrate and phosphor content were found in 'Bonica'. However we noticed a significant impact on foliar nitrate in 'Galine' while increasing salinity hardly affected leaf phosphor.

Table 5.3: The effect of NaCl salinity on accumulation of, , K, Ca, Mg, Na, P, NO_3^-, Cl^- and on Na/K and Na/Ca ratios in leaves.

Cultivar	NaCl (mM)	K (g/100 g DS)	Ca	Mg	Na	P	NO_3^-	Cl^-	Na/K	Na/Ca
Adriatica	0	6.3^{a}	3.0^{a}	0.39^{a}	1.1^{b}	0.91^{a}	4.1^{a}	2.5^{c}	0.18^{c}	0.38^{c}
	20	5.9^{a}	2.9^{a}	0.38^{a}	1.4^{b}	0.84^{ab}	4.6^{a}	3.1^{c}	0.23^{c}	0.47^{c}
	40	5.7^{a}	2.9^{a}	0.37^{a}	2.5^{b}	0.81^{ab}	5.4^{a}	3.5^{c}	0.44^{c}	0.87^{c}
	80	4.4^{b}	2.4^{ab}	0.33^{a}	4.9^{a}	0.79^{ab}	3.6^{a}	6.9^{b}	1.11^{b}	2.03^{b}
	160	3.6^{b}	1.9^{b}	0.32^{a}	6.0^{a}	0.67^{b}	1.3^{b}	11.1^{a}	1.66^{a}	3.26^{a}
Black Beauty	0	6.8^{a}	3.3^{a}	0.52^{a}	1.1^{c}	0.79^{a}	9.7^{a}	2.0^{c}	0.19^{b}	0.41^{b}
	20	6.6^{a}	3.3^{a}	0.50^{ab}	1.3^{c}	0.76^{ab}	8.3^{a}	2.1^{c}	0.17^{b}	0.34^{b}
	40	6.3^{a}	3.2^{a}	0.46^{ab}	1.8^{c}	0.75^{ab}	9.5^{a}	2.8^{c}	0.28^{b}	0.54^{b}
	80	5.8^{a}	3.1^{a}	0.44^{b}	4.6^{b}	0.73^{ab}	5.2^{b}	5.6^{b}	0.79^{b}	1.46^{b}
	160	3.8^{b}	2.3^{b}	0.44^{b}	8.4^{a}	0.65^{b}	2.1^{c}	10.1^{a}	2.27^{a}	3.73^{a}
Bonica	0	5.8^{a}	3.6^{a}	0.52^{a}	0.9^{c}	0.77^{a}	1.9^{a}	3.2^{d}	0.13^{d}	0.22^{c}
	20	5.6^{a}	3.1^{a}	0.52^{a}	1.3^{bc}	0.76^{a}	1.7^{a}	4.3^{cd}	0.23^{cd}	0.42^{bc}
	40	5.4^{a}	3.1^{a}	0.50^{a}	1.9^{ab}	0.75^{a}	1.3^{a}	5.5^{c}	0.35^{bc}	0.60^{ab}
	80	5.3^{a}	3.0^{a}	0.48^{a}	2.1^{a}	0.69^{a}	1.6^{a}	7.1^{b}	0.39^{ab}	0.69^{a}
	160	4.4^{b}	2.9^{a}	0.45^{a}	2.2^{a}	0.65^{a}	0.73^{a}	8.9^{a}	0.50^{a}	0.75^{a}
Galine	0	5.9^{a}	3.1^{a}	0.50^{a}	0.83^{b}	0.91^{a}	3.4^{a}	2.7^{c}	0.14^{c}	0.26^{b}
	20	5.8^{a}	3.1^{a}	0.48^{ab}	1.02^{b}	0.89^{a}	2.8^{ab}	3.4^{bc}	0.17^{bc}	0.34^{b}
	40	5.7^{a}	3.0^{a}	0.48^{ab}	1.7^{ab}	0.86^{a}	2.6^{ab}	4.5^{b}	0.30^{bc}	0.56^{ab}
	80	5.6^{ab}	2.9^{a}	0.45^{ab}	2.1^{a}	0.83^{a}	1.8^{bc}	7.0^{a}	0.39^{ab}	0.70^{a}
	160	4.3^{b}	2.9^{a}	0.43^{b}	2.5^{a}	0.83^{a}	0.9^{c}	8.2^{a}	0.60^{a}	0.87^{a}

Means followed by the same lowercase within each column and cultivar within are not significantly different at P= 0.05 according to Tukey's HSD test (n=4).

5.3.5 Plant tolerance index of eggplant cultivars

For plants exposed to 20 mM, 40 mM, 80 mM and 160 mM NaCl the plant tolerance index (PTI) was calculated and related to the amount of Na in the leaves for all cultivars (Figure 5.3). For leaf tissue our analysis shows a positive correlation for all four cultivars: Under increasing salinity 'Bonica' and 'Galine' combine low leaf Na accumulation with high PTI thus maintaining a normal level of growth, while 'Adriatica' and 'Black Beauty' accumulating significantly higher concentrations of leaf Na failed to maintain a normal level of growth (low PTI) specially in 80 mM NaCl and 160 mM NaCl (Figure 5.3).

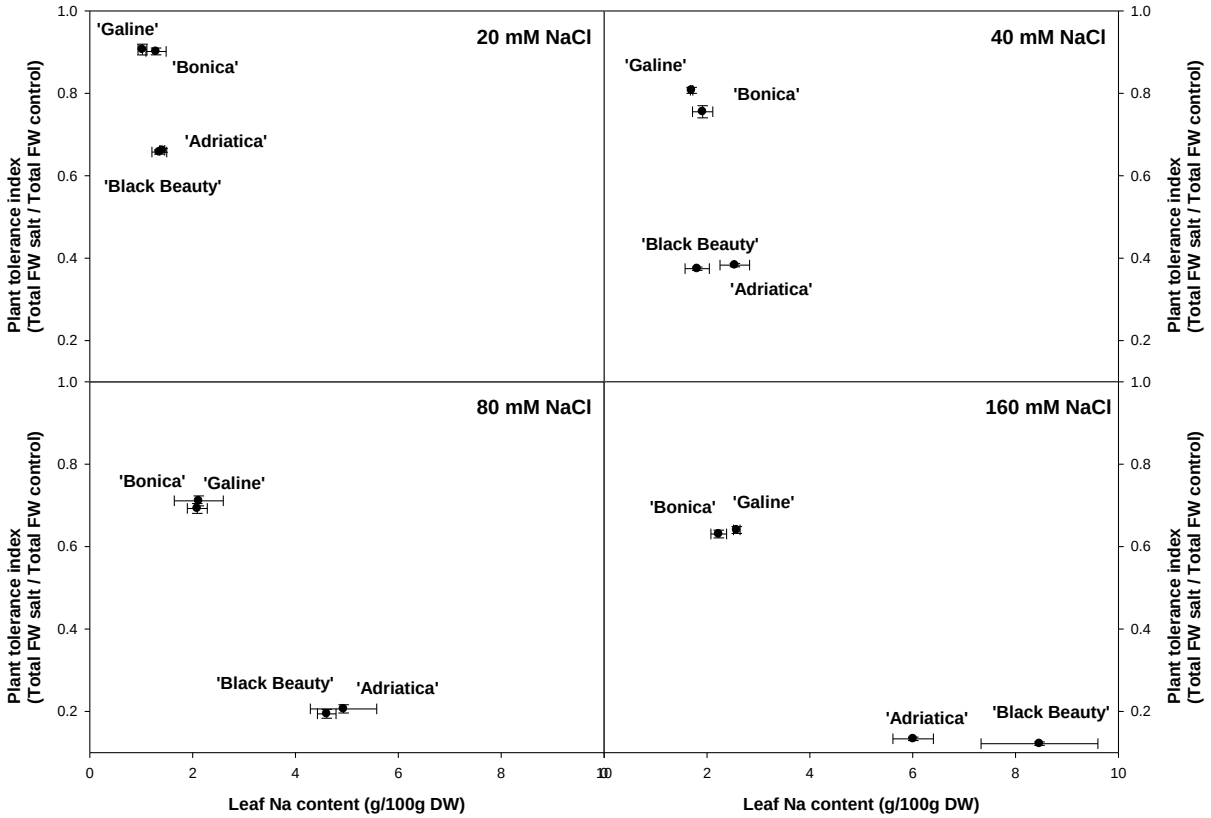

Figure 5.3 Plant tolerance index related to salinity tolerance and the sodium content. Relation between leaf sodium content and plant salinity tolerance, as measured by total fresh weight (FW) in salt stressed plant ÷ total FW in control plants, in four eggplant cultivars. Results are mean ± SE of four replications.

5.4 Discussion

Chlorophyll fluorescence screening of the studied eggplant genotypes indicated that 2 genotypes were better adapted to salt stress (Chapter 4, Hanachi et al., 2014). In this study specific morphological and physiological determinants to further identify traits of sensitivity/tolerance in eggplant genotypes were characterised.

Growth reduction is generally observed in plants exposed to salinity stress and this was reported for eggplants (Chartzoulakis and Loupassaki, 1997; Akinci et al., 2004). In this study, plant height, leaf number and internode length were affected by salinity in all cultivars. A level of 20 mM NaCl did not affect leaf initiation or was even slightly stimulating ('Black Beauty'); though dry matter production was already reduced in all cultivars. A further increase of NaCl levels resulted in a decrease of leaf initiation, internode and shoot length and dry weight. Reduction in shoot growth generally occurs in two phases (Munns and Tester, 2008), the rapid response to osmotic stress is followed by a slower response due to the accumulation of Na in the leaves. The salt sensitive cultivars ('Adriatica' and 'Black Beauty') show this response and 80mM seems to be the threshold level between osmotic and ionic stress, while for 'Bonica' and 'Galine' the threshold level for ionic stress is not reached at 160 mM indicating that Na accumulation in the leaves might be lower (see also further). In addition, levels of 80 mM NaCl decreased tissue water content (TWC) in 'Adriatica' and 'Black Beauty' while 'Bonica' and 'Galine' could maintain their TWC up to 160 mM NaCl. In sand cultures or hydroponics the threshold value for most plants is approximately 40 mM NaCl (Munns and Tester, 2008). However, in our experimental system we used a peat substrate which has a cation exchange capacity (CEC) ranging between 120-130 meq/g DW and this probably explains the higher tolerance values for ionic stress we found. Yet, irrespective the growing medium, the genetic variation within *Solanum melongena* cultivars is evident.

Leaf water potential followed the osmotic potential/EC changes in the nutrient solution resulting from NaCl addition as plants must maintain the water potential difference between leaves and solution to prevent wilting. As the leaf water potential declines, a linear decrease in osmotic potential contributes to prevent a rapid decline in turgor potential (Behboudien, 1977). The salt-induced decline in ψ was accompanied by a decrease in ψ_π in the sensitive cultivars ('Adriatica' and 'Black Beauty') thus maintaining turgor values of salt stressed plants similar or even higher than control plants. In contrast the tolerant cultivars ('Bonica'

and 'Galine') maintained relative stable values of ψ and ψ_π under increasing salinity. The strongly reduced osmotic potential of the leaves of the sensitive eggplants is lowered through the uptake of Na^+ and Cl^- and a simultaneous lower effective water uptake as seen from the decreased TWC values, which caused a greater solute concentration in the vacuole. This might indicate that 'Bonica' and 'Galine' have a tendency to act as isohydric species (Sade et al., 2012) under the applied salt stress. Isohydric plants maintain a constant midday leaf water potential (Ψ) when water is abundant, as well as under water deficiency conditions, by reducing stomatal conductance (g_s) to limit transpiration. Isohydric plant maintained their WC thus more strictly.

The adaptation of plants to salinity is associated with osmoregulation. Most plants growing in a saline environment accumulate low molecular weight water-soluble metabolites in the cytosol such as proline (Turan et al., 2007; Xu et al., 2012; Rejeb et al., 2014). In our work, proline increased in response to salinity in all cultivars; with the highest relative increase observed in the sensitive cultivars 'Adriatica' and 'Black Beauty' whereas a much lower increase was observed in 'Bonica' and 'Galine'. Although it is generally agreed upon that proline accumulation is important the relative contribution of proline to stress tolerance varies among species or even among cultivars of a same species (Ahraf and Foolad, 2007; Abbas et al., 2010). The results obtained with the eggplant cultivars suggest that the increase in proline concentration is not correlated with salinity tolerance as for the sensitive eggplant cultivars a kind of overproduction of proline is observed. This agrees with similar observations in other crops such as rice (Moradi and Ismail, 2007), tomato (Bikash et al., 2005; Zgallai et al., 2005). Stewart and Boggess (1978) showed that abiotic stress can also influence the catabolic pathway of proline, inhibiting its oxidation and thus leading to high proline levels. Proline catabolism seems to be an important mechanism to regulate proline levels in the halophyte *Thellungiella* (Kant et al., 2006). Also in potato reduced PDH expression (catabolic pathway) was found in two cultivars under salt stress (Jaarsma et al., 2013). On the other hand proline accumulation in plants acts also as a component of the non-enzymatic antioxidative defense system (Rejeb et al., 2014). MDA is widely used as an indicator of the extent of oxidation damage under stress (Jain et al., 2001). Both 'Adriatica' and 'Black Beauty' had a relative high level of MDA produced during peroxidation of membrane lipids at 160 mM NaCl. For the tolerant cultivars less salt is transferred into the leaves and this should evoke less oxidative damage in the leaves as found by the relative low increase of MDA in these cultivars.

Sugars that accumulate in response to stress can function as osmolytes to maintain cell turgor and have the ability to protect membranes and proteins from stress damage. Accumulation of sugars has been associated with salinity tolerant mechanisms in many species (Gilbert et al. 1997; Hu and Shmidhalter, 1998; Xu et al., 2012). In this experiment, increasing salt stress led to an increasing sucrose, glucose and fructose levels in the sensitive cultivars 'Adriatica' and 'Black Beauty' therefore mobilizing their starch contents to maintain these high levels. In contrast, no accumulation but rather a decline in sucrose, glucose and fructose content was observed in the tolerant cultivars 'Bonica' and 'Galine' combined with starch accumulation. It is generally accepted that the elevation in the cellular osmolarity which result from the accumulation of compatibles solutes is associated to the influx of water into, or to a reduced efflux from, cell, thus providing the turgor necessary for the expansion of cells (Hare et al., 1998). Despite the higher levels of soluble carbohydrates the sensitive cultivars could not maintain their water balance as indicated by a decreasing TWC under 80 mM and 160 mM NaCl. Also, the increasing cellular sugar accumulation could be a limiting factor for growth under salt stress and probably reduced translocation and thus utilization in the actively growing tissue will take place (Stoop and Pharr, 1994; Pattanagul and Thitisaksakul, 2008). The salt-susceptible cultivars indeed had a lower leaf initiation rate as observed by a reduced number of leaves. Furthermore reduced growth due to higher hexose levels in the cytoplasm could generate a feed-back inhibition on carbon metabolism which contributes to a lower CO_2 assimilation (Krapp and Stitt, 1995; Krapp et al., 1991). Moreover the expression of Rubisco could be inhibited by a considerable accumulation of hexoses in the cytoplasm (Koch, 1996; Sawada et al., 1992).

A different carbohydrate pattern was observed in the tolerant eggplant species. Also salt tolerant soybean (Liu and Staden, 2001), rice (Pattanagul and Thitisaksakul, 2008) and tomato lines (Balibera et al., 2000) were characterized by no sucrose accumulation under saline conditions. Salinity causes both ionic and osmotic stresses. We assume that in 'Bonica' mainly an osmotic stress is established, resulting in stomatal closure while ionic stress is hardly installed (based on Na and Cl^- content of leaves of 'Bonica', Table 5.3).

Stomatal closure will lead to lower C_i and thus reduced photosynthesis. It is also known that plants partition sugars into starch to avoid metabolic damage by lowering feedback inhibition caused by the huge amount of sucrose in cytoplasm (Krapp and Stitt, 1995) although high leaf starch contents have also a negative effect on photosynthesis. As photosynthesis was not

measured in this experiment the observed carbohydrate partitioning is not well understood for the more salt tolerant 'Bonica'.

Reasonable amounts of both K and Ca are required to maintain the integrity and functioning of cell membranes (Davenport et al., 1997; Wenxue et al., 2003). Accumulation of Na and Cl^- is a common osmoregulatory response to NaCl stress (Levitt, 1980). At the same time, NaCl causes a decrease in concentration of K and Ca in plants (Chartzoulakis and Loupassaki, 1997). Likewise, the results of the present study showed that NaCl treatment caused an increase in Na and Cl^- concentration, and a decrease in K, Ca, Mg, P and NO_3^- concentration in all cultivars. However, the tolerant cultivars, 'Bonica' and 'Galine' accumulated lower foliar amounts of Na and Cl^- and maintained higher amounts of K, Ca, Mg and PO_4^- as compared to the sensitive cultivars 'Adriatica' and 'Black Beauty'. This agrees with previous finding obtained by Akinci et al (2004) for tolerant eggplant lines. Na/K and Na/Ca ratios were reported to be associated with a relative salt tolerance in many species, where tolerant genotypes had lower Na/K and Na/Ca ratio (Perez-Alfocea et al., 1996; Ashraf, 2004; Amor et al., 2005; Yasar et al., 2006; Akram et al., 2009). In fact the low Na/K ratio in the cytosol is essential for normal cellular functions of plants. While competing with K uptake, Na may block the K specific transporters under salinity. This contributes to a toxic level of Na as well as insufficient K concentration for enzymatic reactions and osmotic adjustment (Zhu, 2003; Yasar, 2006). In other studies, it was observed that tolerant genotypes regulated the osmotic potential more effectively by avoiding the uptake of Na and Cl^- and a simultaneous absorption of more essential ions such as K (Sivritepe et al., 2003;Yasar, 2006).

Moreover, the ability of the plant to exclude Na and Cl^- from the cytosol via compartmentalization into vacuoles has been frequently reported as a salt tolerance mechanism employed by several glycophytes (Martinez Rodriguez et al., 2008; Paranychianakis and Angelakis, 2008). The high salt sensitivity in the cultivars 'Adriatica' and 'Black Beauty' is strongly associated with a considerable accumulation of salt ions in leaves especially Cl^-. This could be due to a salt exclusion system less effective in leaves of sensitive cultivars compared to tolerant cultivars (Silveira et al., 2012). The accumulation of this ion alters osmoregulation, the stability of the membrane potential and the maintenance of turgor (Richards et al., 2010) and will lead to an ultimate cease of cell division and elongation and plant biomass. We found indeed a positive correlation between the PTI and leaf Na^+ concentration and this was also reported by Jha et al. (2010) on *Arabidopsis*, by Jaarsma et al. (2013) on potato and by Chaaban et al. (2015) on barley cultivars. These

authors suggested that *Arabidopsis* and potato may use mechanisms involved with Na^+ tissue tolerance, such as intracellular compartmentation and increased accumulation of compatible solutes. This was hypothesized by Munns and Tester (2008) as a possible tolerance mechanism in glycophytes and apparently Bonica and Galine respond in this way.

5.5 Conclusion

The metabolic responses to salt stress indicate two different responses. Proline increased hugely in response to salinity in sensitive cultivars while it hardly increased in the tolerant cultivars. However, the accumulation of this amino acid (proline) is more due to the metabolic damage caused by water stress rather than a resistance factor. Furthermore, salinity stress affects adversely carbohydrate metabolism in sensitive and tolerant cultivars. In sensitive ones, salinity caused a considerable accumulation of sugars (sucrose, glucose and fructose) in the leaves. However, under saline conditions, tolerant cultivars showed a decline of sugar content and starch accumulation.

The tolerant cultivars, 'Bonica' and 'Galine' accumulated lower foliar amounts of Na and Cl^- and maintained higher amounts of K, Ca, Mg and PO_4^- as compared to the sensitive cultivars 'Adriatica' and 'Black Beauty'. Summing up that in our study, Na/K and Na/Ca ratios appeared to determine salinity tolerance also in eggplant.

Chapter 6

Contrasting responses of photosynthesis and stress adaptation in two eggplant cultivars (*Solanum melongena* L.) under different salt stress levels

Chapter 6 Contrasting responses of photosynthesis and stress adaptation in two eggplant cultivars (*Solanum melongena* L.) under different salt stress levels

Abstract

The effect of NaCl stress on gas exchanges, metabolic adaptation and mineral content of shoots and roots was invested in eggplant (*Solanum melongena* L.). Two cultivars, 'Bonica' and 'Black Beauty', differing in their tolerance to salt stress were used.

Significant decrease in net photosynthesis (A_n) was noticed in both cultivars under increasing salt stress though respiration rates (R_n and R_d) were not affected. Photorespiration (R_l), was more reduced in 'Black Beauty' than in 'Bonica' at 160 mM NaCl. The increase of the ratio R_l/A_n indicated that photorespiration was an important electron sink for 'Bonica' under salt stress. The ratio J_c/J_t was not affected by increasing salt levels except for 'Black Beauty' at 160 mM NaCl. Under 160 mM NaCl level less than 40% of the total electron flow was used for carboxylation of RuBP in 'Black Beauty' and 'Bonica'. The ratio A_n/A_t decreased under increasing salinity in both cultivars while the ratio R_d/A_t increased.

A concentration of 40 mM NaCl significantly reduced g_s in Black Beauty, this for both 13 and 21 DSS. Significant lower g_s was only found for 160 mM NaCl in Bonica. Transpiration rate (E) was significantly reduced in 'Black Beauty' and 'Bonica'. After 21 DSS, E decreased by 72.1% and 67.2% respectively in 'Black Beauty' and 'Bonica'.

No significant effects were found on F_v/F_m for any of the cultivars. The highest salinity stress (160 mM NaCl) decreased Φ_{PSII} and q_p in both cultivars however 'Bonica' maintained higher values than 'Black Beauty'. At the highest salt stress level (160 mM NaCl) the increase in NPQ was respectively 18% for 'Black Beauty' and 8.0% for

'Bonica'. Significant correlations between fresh biomass and Φ_{PSII} and between fresh biomass and q_p were observed for 'Black Beauty' and 'Bonica'.

No significant effects on Chl*a*, Chl*b*, total Chl, Chl*a*/*b* and carotenoids (*P*>0.05) were found in any of the cultivars. Proline and MDA increased significantly in response to salinity in both cultivars. However proline and MDA increase was more pronounced in 'Black Beauty'. Furthermore, Black Beauty accumulated high levels of soluble carbohydrates in the leaves. On the other hand, Bonica accumulated high amounts of starch under increasing salt stress.

Leaves of 'Bonica' accumulated lower concentration of Na than 'Black Beauty'. At 160 mM NaCl sodium accumulation was higher in the roots than in the leaves of 'Bonica'. In contrast 'Black Beauty' accumulates higher Na in leaves than in roots. Leaf and root K contents were reduced respectively by 41.1% and by 27.9% in 'Black Beauty' at 160 mM NaCl while no effect on K uptake was found for 'Bonica'. Significant increases for the Na/K ratio were only observed in 'Black Beauty'.

The salt induced decreases in number of leaves, height, DW and FW of the two cultivars showed an overall similar trend, however, 'Black Beauty' was more affected than 'Bonica'.

6.1 Introduction

Salinity is one of the most serious abiotic stresses limiting plant growth and development, especially in salt sensitive crop species (Pitman and Läuchli, 2002). The detrimental effect of high salinity on plants can be observed at the whole-plant level as the death of the plant and/or decreases in productivity. Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within the cells. During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism are affected. The earliest response is a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress intensifies (Parida and Das, 2004). Carbohydrates, which are needed for cell growth, are supplied mainly through the process of photosynthesis, and photosynthesis rates are usually lower in plants exposed to salinity and especially to NaCl.

Photosynthesis, together with cell growth, is therefore one of the primary processes to be affected by salinity (Munns et al., 2006). Indeed, photosynthesis is known to be very sensitive to environmental stresses. Salinity reduces net photosynthetic rate, transpiration rate, and stomatal conductance in many plant species (Lakshmi et al., 1996; Tezara et al., 2002; Gibberd et al., 2002; Burman et al., 2003). Salt induced reduction of photosynthesis rate can be caused by partial stomatal closure caused by an associated osmotic stress (De Pascale and Barbieri, 1995; Goldstein et al., 1996;), non-stomatal limitations caused by excessive salt build-up and/or an ionic imbalance in the leaves (Yeo et al., 1985; Drew et al., 1990) or both limitations (Downton et al., 1990; Yeo et al., 1991).

When NaCl directly inhibits photosynthesis the plant will likely suffer from oxidative stress. Oxidative stress will lead to damages of the plant cell membranes (Koca et al., 2007) and combined with high levels of salt ions to disintegration of organelles, with chloroplasts being the most sensitive organelles to salt stress (Demetriou et al., 2007). Salinity tolerance is highly related to the maintenance of net photosynthetic rates and stomatal conductance (Lakshmi et al., 1996) and elevated chlorophyll concentration (Krishna Raj et al., 1993; Salama et al., 1994). Low salinity maintains chlorophyll content (Winicov and Button, 1991) and high salinity degrades chlorophyll content (Malibari et al., 1993; Salama et al., 1994). Excess salt leads to a change in the ionic composition of the stroma of the chloroplasts which in turn can cause shrinkage of the thylakoids and stacking of adjacent membranes in grana (see review by Ashraf 2004). An irreversible impairment of the photosynthetic apparatus,

associated with a reduction of Rubisco activity, occurs when the stress is prolonged and salt continues to accumulate in the leaves (Delfine et al., 1999).

As an indirect consequence of stomatal closure, lower intercellular CO_2 concentration will increase the susceptibility to photochemical damages as excessive light energy at PSII level will increase due to low CO_2 assimilation rates. Wang et al. (2007) showed that NaCl stress (200 mM) inhibited the electron transport activity of PSII more than that of PSI. Also in susceptible eggplant cultivars (chapter 4) chlorophyll fluorescence measurements, showed a considerable decrease in the efficiency of PSII and ETR under increasing salt stress. The absorbed energy non-utilized in the photochemical pathway was dissipated as heat and this was confirmed by the concomitant increase in NPQ. Such increase was suggested to cause a down-regulation of PSII in order to avoid over-reduction of Q_A, the first quinone electron acceptor of PSII (Krupa et al., 1993; Ramalho and Nunes, 1999). As proposed by Lima et al. (2002) and Damatta et al. (2002) under drought stress, the reduction of O_2 via the Mehler-peroxidase pathway and possibly photorespiration might provide photo-protection by acting as an alternative sink for excess energy in the photosynthetic apparatus. Photorespiration may thus be an alternative sink for light induced electron flow, and it is often presented as a process that may help consume an appreciable electron flow during periods of restricted CO_2 availability in the chloroplasts and high irradiance (Krause and Cornic, 1987; Stuhlfauth et al., 1990). According to Valentini et al (1995) the ratio J_C/J_O is a good indicator of relative rates of carboxylation versus oxygenation and may be directly controlled by the kinetic properties of Rubisco. However no data on the role of this alterative sink under stress conditions are available in eggplants.

In this study we investigate the effects of salt stress on the regulation of photosynthesis in the salt tolerant eggplant cultivar ‘Bonica’ and the salt susceptible cultivar ‘Black Beauty’. This study was further supplemented with measurements of typical reactions to salt stress such as lipid peroxidation of the cell membranes, proline content, carbohydrate and mineral content. Furthermore we tried to get insight if a different distribution of sodium and chloride ions in roots and aerial parts existed between these cultivars and if this was correlated with photosynthetic efficiency.

6.2 Materials and methods

6.2.1 Plant materials

Two eggplant (*Solanum melongena* L.*)* cultivars, 'Bonica' and 'Black Beauty' were used as plant material. Seeds were germinated in trays filled with peat in a growth chamber at a constant temperature of 25°C, RH of 70 % and photon flux density of 150 µmol m^{-2} s^{-1}. After 25 days eggplant seedlings at the appearance of the second true leaf stage were selected for uniformity and transplanted to 2 L plastic pots filled with peat. Plants were transferred to a heated glasshouse (located at 51°02'N, 03°42'E) and supplied with a full-strength Hoagland's solution (Hoagland and Arnon, 1950). During the salt stress experiment the mean temperature was 22°C and the mean air humidity was 72%.

6.2.2 Salinity treatments

Four salinity treatments were applied starting 36 days after the transfer to the greenhouse (4^{th} leaf stage). NaCl was added at 0 (control), 40, 80 and 160 mM to a full-strength Hoagland's solution. Plants were irrigated with 250 mL/pot which was applied twice a week during 30 days. Each treatment was applied to 20 plants per cultivar (5 plants/block). The experiment was a randomized complete block design with five replications for each treatment.

6.2.3 Gas exchange and chlorophyll fluorescence measurements

To determine the effect of salt on foliar gas exchange, measurements were conducted once a day (09:00h and 12:00h) on four randomly selected plants from each of the four treatments. This was done on 13 DSS (13^{th} of June 2012) and 21 DSS (21^{st} of June 2012). Measurements were performed on sunny days. All measurements were conducted on the youngest fully developed leaves. Gas exchange and chlorophyll fluorescence parameters were measured using a portable photosynthesis system (model LI-6400; Li-Cor Biosciences, Lincoln, NE, USA) fitted with fluorescence head (6400-40 Leaf Chamber Fluorometer, Li-Cor Biosciences, Lincoln, NE, USA). Light saturated net photosynthesis (A_n, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and transpiration rate (E, mmol H_2O $m^{-2}s^{-1}$) were measured on the selected plants. Mitochondrial respiration during the night was measured on dark covered leaves early in the morning (R_n, µmol m^{-2} s^{-1}). The mitochondrial respiration during the day (R_d, µmol $m^{-2}s^{-1}$) was estimated from R_n according to Valentini et al. (1995).

The chamber temperature of the fluorescence head was set to match the actual temperature measured in the treatment environment at the start of the measurement (25°C). The light

source of the fluorescent head was maintained at 1,500 µmol photons $m^{-2}s^{-1}$ and CO_2 was maintained at treatment conditions 400 µmol CO_2 mol^{-1}.

To evaluate the presence of photo-inhibitory processes, the maximum quantum yield of PSII (F_v/F_m) was measured on dark adapted leaves (30 min). Red actinic light (1,500 µmol $m^{-2}s^{-1}$) was then switched on and the quantum yield of PSII electron transport (Φ_{PSII}), the efficiency of energy capture by the open PSII reaction centre were determined by measuring steady-state fluorescence and maximum fluorescence during a light saturation pulse of 7,000 µmol $m^{-2}s^{-1}$ recorded on the adaxial surface of the same leaves used for gas exchange measurements. F_v/F_m, Φ_{PSII}, photochemical quenching (q_p,), and non-photochemical quenching NPQ were calculated as follows:

$$F_v/F_m = (F_m\text{-}F_0/_{Fm})$$

$$\Phi_{PSII} = (F_m\text{'}\text{-}F_s)/F_m\text{'}$$

$$q_p = (F_m\text{'}\text{-}F_s)/(F_m\text{'}\text{-}F_0\text{'})$$

$$NPQ = (F_m\text{-}F\text{'}_m)/F_m$$

Where F_0 is the initial fluorescence in leaves submitted to a period of darkness; F_m= maximum fluorescence in leaves acclimated to darkness; F_v = variable fluorescence in leaves acclimated to darkness ($F_v = F_m\text{-}F_0$); F_0'= initial fluorescence in leaves submitted to ambient light, F_m' is the maximum fluorescence in leaves submitted to ambient light, F_s is the steady fluorescence in leaves acclimated to ambient light.

According to Krall and Edwards (1992) the total electron flow (J_t) can be derived from the quantum yield of PSII (Φ_{PSII}), the light intensity incident on the leaf (PAR), the fractional absorption of light by leaf (a) and the absorptance of PSI + PSII (f):

$$J_t = \Phi_{PSII} \times PAR \times a \times f \ (\mu mol\ m^{-2}\ s^{-1})$$

where 'a' equals 0.84 and 'f' equals 0.5 (Schreiber, 1997).

The partitioning of electrons between photosynthesis (J_c) and photorespiration (J_o) was obtained using the values of the electron transport rate (J_t), A_n and mitochondrial respiration rate in the light (R_d), as follows (Epron et al. 1995):

$$J_c = 1/3\ [J_t + 8(A_n + R_d)] \text{ and } J_o = 2/3\ [J_t - 4(A_n + R_d)]\ (\mu mol\ m^{-2}\ s^{-1})$$

Photorespiration (R_l, µmol CO_2 m^{-2} s^{-1}), was calculated according to Valentini et al. (1995):

$$R_l = 1/12\ [J_t - 4(A_n + R_d)]\ (\mu mol\ m^{-2}\ s^{-1})$$

Total assimilation rate was calculated as $A_{tot} = (A_n + R_d + R_l)$. Light use efficiency (LUE) was calculated as A_n/PAR (Long et al., 1993) (PAR=1500 µmol $m^{-2}s^{-1}$), the apparent carboxylation efficiency (CE) was calculated as A_n/C_i (Flexas et al., 2001) and water use efficiency (WUE) was calculated as A_n/T_r (Hamid et al., 1990).

Photo 6.1: Dark (a) and light (b) measurments of gas exchange and chlorophyll fluorescence parameters.

6.2.4 Metabolite analysis

Fully developed upper leaves (2 leaves/replicate in a bulked sample) were harvested between 12 h and 14 h from four plants in each treatment (1 plant/block) and for each variety after 30 days of salt stress. Leaf material was grounded in liquid nitrogen and stored at -80°C until analysis.

Pigments (150 mg FW) were extracted with 80% acetone. Pigments were measured spectrophotometrically ($UVIKON_{XL}$, BIO-TEK Instrument, USA), this in four replicates. Chlorophyll a (Chl*a*), chlorophyll b (Chl*b*), and carotenoids (µg g^{-1} FW) were calculated according to Lichtenthaler (1987):

$$\text{Chl}a = 12.25\, A_{663.2} - 2.79\, A_{646.8}$$

$$\text{Chl}b = 21.5\, A_{646.8} - 5.1 A_{663.2}$$

$$\text{Carotenoids} = (1000\, A_{470} - 1.82\ \text{Chl}\ a - 85.02\ \text{Chl}\ b) / 198.$$

Sugars were extracted with 80% ethanol at 70°C for 10 min and further at 45°C for 3 hours, followed by centrifugation at 5000 g for 5 min. Glucose, fructose and sucrose were analysed using high pH anion-exchange chromatography with pulsed amperometric detection (Waters; CarboPac MA1 column with companion guard column, eluent: 50 mM NaOH, 22°C). The remaining ethanol insoluble material was washed twice with ethanol 80% and the residual pellet was treated with HCl 1M for 2 hours at 95°C for starch hydrolysis. Starch was determined spectrophotometrically at 340 nm by the enzymatic reduction of $NADP^+$ (UV-VIS, Biotek Uvikon XL).

Proline was determined according to Bates et al. (1973). Plant tissue (500 mg) was extracted with 10 mL of 3% (w/v) sulfosalicylic acid. After filtration, 2 mL acid ninhydrin and 2mL glacial acetic acid were added to the extracts (2 mL) and this mixture was kept at 100°C for 1 hour in a water bath, then the reaction was stopped in an ice-bath. The formed chromophore was extracted from the acid aqueous solution by means of cold toluene (4 mL) and measured spectrophotometrically at λ = 520 nm (Infinite M200 TECAN Group Ltd., Switzerland). Proline was determined using a calibration curve and expressed as µg proline g^{-1} FW.

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) determined by the thiobarbituric acid reaction (Hodges et al. 1999). Leaf material (1 g) was homogenized in 25 mL 80% ethanol, followed by centrifugation at 3,000 g for 10 min. A 1 mL aliquot of sample extract was added to 1 mL of thiobarbituric acid (TBA, 0.65% w/v) as well as to 1 mL of trichloroacetic acid (TCA, 20% w/v) and homogenates were incubated at 95°C for 25 min, cooled and centrifuged at 3,000 g for 10 min (4°C). The MDA content was measured based on the reaction with thiobarbituric acid (TBA) and the absorbance was measured at λ=440 nm, 532 nm and 600 nm by spectrophotometer (InfiniteM200 TECAN Group Ltd., Switzerland). Malondialdehyde (MDA) equivalents were calculated as described by Hodges et al. (1999).

6.2.5 Mineral analysis

Eight leaves were harvested from four plants in each treatment (1 plant/block) and for each variety and combined into a composite sample, dried at 70 °C for 48 h and then grounded. After dry-ashing at 550°C P, K, Ca, Mg, S, and Na were measured using ICP-OES (Iris Intrepid IIXSP, Thermo Scientific, USA). A potentiometric analysis using an ion-selective electrode (VWR, Belgium) for chlorides was performed.

6.2.6 Growth parameters and plant water status

At the end the experiment (30 DSS) eight plants were taken at random of each treatment (2 plants x 4 blocks). The fresh weigh (FW) of the aerial part of the plant (shoot and leaves) and the number of leaves were measured. The samples were then dried in a forced draft oven at 70°C for 48 h and dry weight (DW) was determined. The tissue water content (TWC) was calculated as the (FW-DW/FW) ratio.

The leaf water potential (ψ_{midday}) of the youngest fully-expanded leaves was determined with a Scholander pressure chamber (model 1000, PMS Instrument Company, Albany, OR, USA). The leaf osmotic potential (ψ_{π}) was determined according to Callister et al. (2006). Measurements were done in four replicates (See chapter 5).

6.2.7 Statistical analysis

All analyses were carried out on a completely randomized design. All data obtained were subjected to one way analysis of variance (ANOVA) to determine the significant differences between the treatments or cultivars using the software of SPSS Statistics 21. Tukey's multiple range test (P=0.05) was used to compare the means. Principal component analysis (PCA) was carried out on FW, DW, TWC, total chlorophyll content, Φ_{PSII}, ETR, q_p, osmotic potential, leaf water potential, F_v/F_m, proline, starch, Na/K ratio in leaves, Na/K ratio in roots and MDA of the eggplant cultivars . Only PCAs with eigenvalues > 1, thus explaining more than a single parameter alone, were extracted. For these principal components a varimax rotation was applied on the obtained factor loading. This rotation results in simpler factors, relating parameters mainly to one principal component axis (Manly 1994). All statistical analyses were carried out using SPSS 21 (IBM SPSS Statistics).

6.3 Results

6.3.1 NaCl induced changes in gas exchange parameters

The temporal change in photosynthetic capacity under increasing salinity is shown in Figure 6.1. At 13 DSS a significant decrease in net photosynthesis (A_n) was noticed in both cultivars under increasing salt stress (Figure 6.1A). At 160 mM NaCl A_n was reduced by 69.2% and 78.4% respectively in 'Black Beauty' and 'Bonica' when compared to their respective controls. A similar trend was found in both cultivars at 21 DSS (Figure 6.1B). The mitochondrial respiration (R_n and R_d) was not affected by increasing salt stress in 'Black

Beauty' and 'Bonica' after 13 and 21 DSS (Figure 6.1C and E). However at 13 DSS, although no significant impact is shown, 'Bonica' maintained higher R_n (P = 0.08) and R_d (P = 0.121) than 'Black Beauty' for the highest salinity level salinity (160 mM NaCl) (Figure 6.1E). Overall Bonica had a significant higher R_d (P=0.001) and R_n (P < 0.003) than Black Beauty at 13 DSS.

Thirteen days after applying salt stress (13 DSS) the photorespiration (R_l) started to decrease significantly in both cultivars. However the reduction of R_l was more pronounced in 'Black Beauty' than in 'Bonica' in the 160 mM NaCl (Figure 6.1G). After 21 DSS 'Black Beauty' had lower values of R_l than 'Bonica' for the highest level of salinity treatment (P= 0.033; Figure 6.1H).

At 13 DSS salinity decreased significantly the gross photosynthesis (A_t) in both cultivars. A_t was reduced by 55.1% and by 51.3% respectively in 'Black Beauty' and 'Bonica' (Figure 6.2A). The same finding observed at 13 DSS, was noticed at 21 DSS (Figure 6.2B).

Total electron flow (J_t) was negatively affected in both 'Black Beauty' and 'Bonica' under saline conditions. Significant negative effects of salt stress in J_t were noticed in 'Black Beauty' (P=0,003) and 'Bonica' (P=0.003) from 13 DSS on. J_t was reduced by 50.8% and 51.9% respectively in 'Black Beauty' and 'Bonica' for 160 mM NaCl compared to their respective controls. Also at 21 DSS a reduction of 51.3% (P=0.017) and by 48.2% (P=0.021) was observed respectively in 'Black Beauty' and in 'Bonica' for 160 mM of NaCl compared to their respective controls. Moreover 'Black Beauty' tended to lower values of J_t than 'Bonica' after 13 DSS (P=0.09).

To gain insight into the relative importance of the photorespiratory pathway as a mechanism for dissipating excess energy, we estimated the carboxylative and oxygenative electron flows as well as the ratio J_o/J_t and J_c/J_t. The carboxylative electron flux (J_c) decreased significantly from 80 mM NaCl in 'Black Beauty' and from 40 mM NaCl in 'Bonica' at 13 DSS (Figure 6.2C). The decrease at 160 mM NaCl reached 51.3 % in 'Bonica' and 54.7% in 'Black Beauty' though 'Bonica' maintained higher absolute values of J_c (42.84 µmol m^{-2} s^{-1}) than 'Black Beauty' (34.32 µmol m^{-2} s^{-1}) (Figure 6.2C). A similar trend for J_c was observed in 'Bonica' and 'Black Beauty' after 21 DSS (Figure 6.2D).

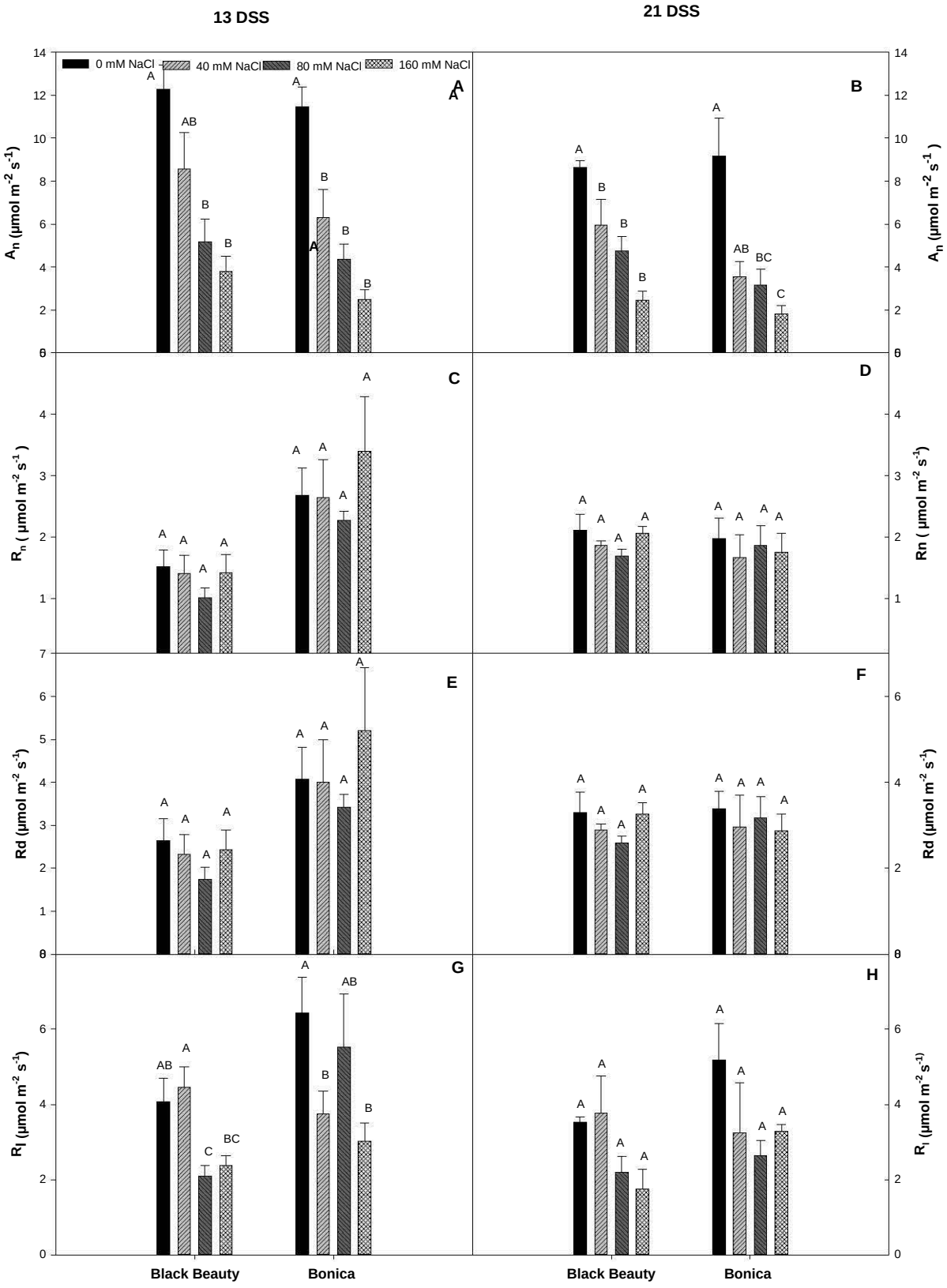

Figure 6.1: Changes in net assimilation (A_n), mitochondrial respiration during the night (R_n) and day (R_d) and photorespiration (R_l) in leaves of the eggplant cultivars ('Black Beauty' and 'Bonica') subjected to different NaCl concentrations (date 1: June 13, 2012; date 2= June 21, 2012). Data are means ± SE. Different lower case letters indicate the significant difference between treatments (*P*=0.05) based on Tukey's HSD test.

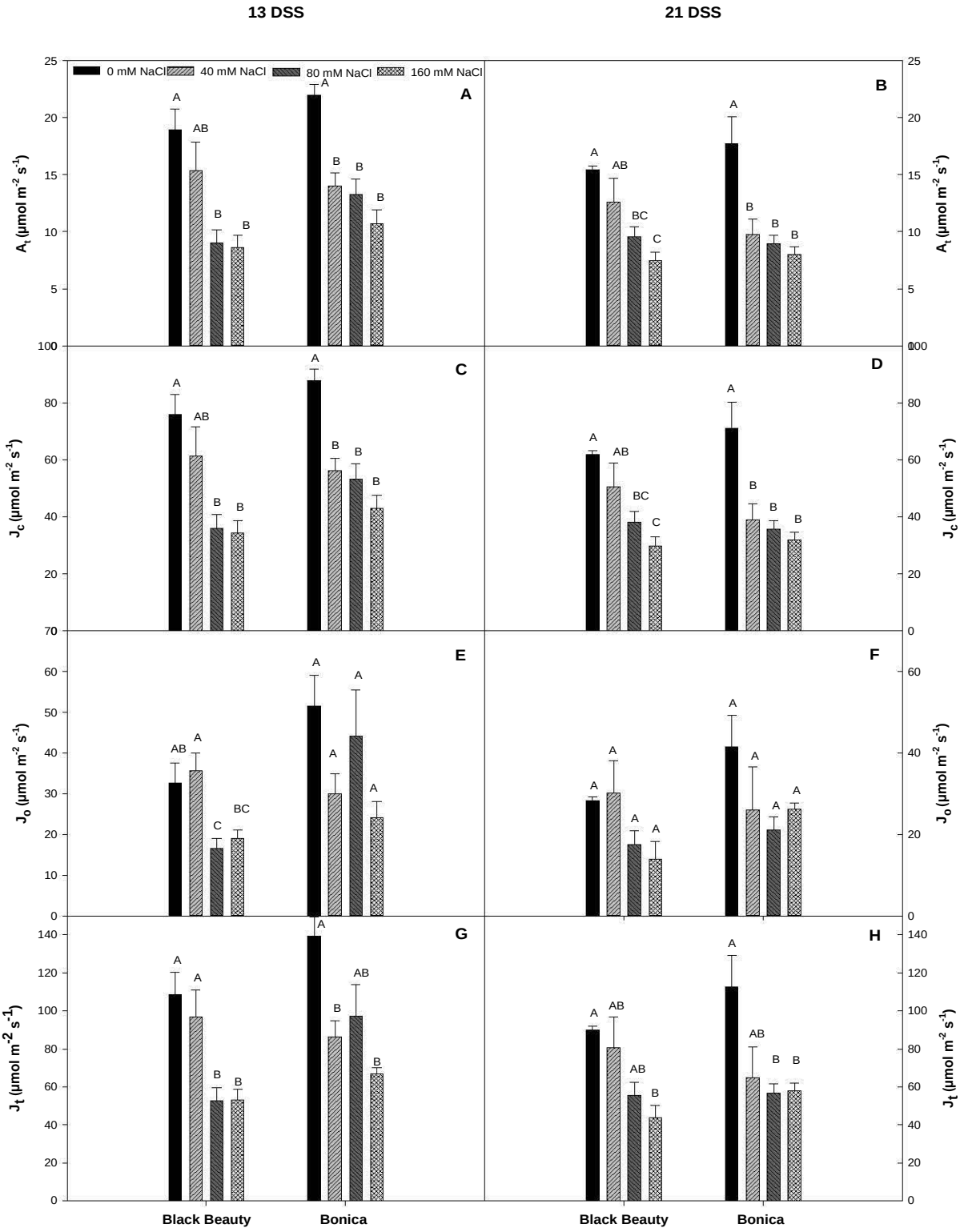

Figure 6.2: Changes in A_t, J_c, J_o and J_t in leaves of the eggplant cultivars ('Black Beauty' and 'Bonica') subjected to different NaCl concentrations in two dates (date 1: June 13, 2012; date 2= June 21, 2012). Data are means ± SE. Different lower case letters indicate the significant difference between treatments (P=0.05) based on Tukey's HSD test

At 13 DSS, oxygenative electron flux (J_o) decreased significantly in 'Black Beauty' from 80 mM NaCl on, while for Bonica relative high within treatment fluctuations were observed and therefore no significant effect on J_o was observed (Figure 6.2E). Overall J_o was higher in Bonica (P=0.033). Twenty one days after applying salt stress a decreasing though not significant trend for J_o was noticed in 'Black Beauty' and 'Bonica' from 80 mM NaCl on (Figure 6.2F). Also at 21 DSS 'Bonica' maintained higher values of J_o (26.3 µmol m^{-2} s^{-1}) than 'Black Beauty' (13.9 µmol m^{-2} s^{-1}) at 160 mM NaCl.

The relative importance of J_c and J_o are indicated by the ratio J_c/J_t and J_o/J_t. The ratio J_c/J_t was not affected by increasing salt levels (Figure 6. 3E and F). In general more than 60% of the total electron flow was used for carboxylation of RuBP in both cultivars. Similar, no significant effect of salinity was present at 13 DSS in 'Black Beauty' and 'Bonica' for J_o/J_t ratio. In general less than 40% of the total electron flow was used for oxygenation of RuBP in 'Black Beauty' and 'Bonica' (Figure 6.3H).

At 13 DSS, the ratio A_n/A_t decreased under increasing salinity in both cultivars (Figure 6. 3A). A significant lower ratio was found for 160 mM NaCl. Likewise, at 21 DSS the ratio A_n/A_t was significant lower at 160 mM NaCl for 'Black Beauty' and 'Bonica' (Figure 6.3B).

The ratio R_d/A_t increased under increasing salt stress in both cultivars and for both measuring dates (13 DSS and 21 DSS). This increase was more pronounced in 'Bonica' than in 'Black Beauty' at 13 DSS while one week later the R_d/A_t ratio further increased for 'Black Beauty' but stabilized in 'Bonica' (Figure 6.3D).

Furthermore we evaluated the correlation between gas exchange parameters at 21 DSS and leaf sodium content (measured at 30 DSS). High negative correlations ($r \geq -0.68$) were found between A_n, A_t, J_c, and J_t, with Na accumulation in leaves of 'Black Beauty' and 'Bonica' (Table 6.1; Table 6.2). A moderate negative correlation was found between R_l and J_o and the Na content of the leaves ($-0.56<r<-0.68$).

With respect to the gas exchange parameters, higher values of A_t and A_n in Black Beauty are correlated with higher values of R_l, J_o, J_c, J_t, g_s and E. Significant correlations were also observed in 'Bonica'. Higher values of A_t and A_n were correlated with higher values of R_l, J_c, g_s, J_o, J_t and E.

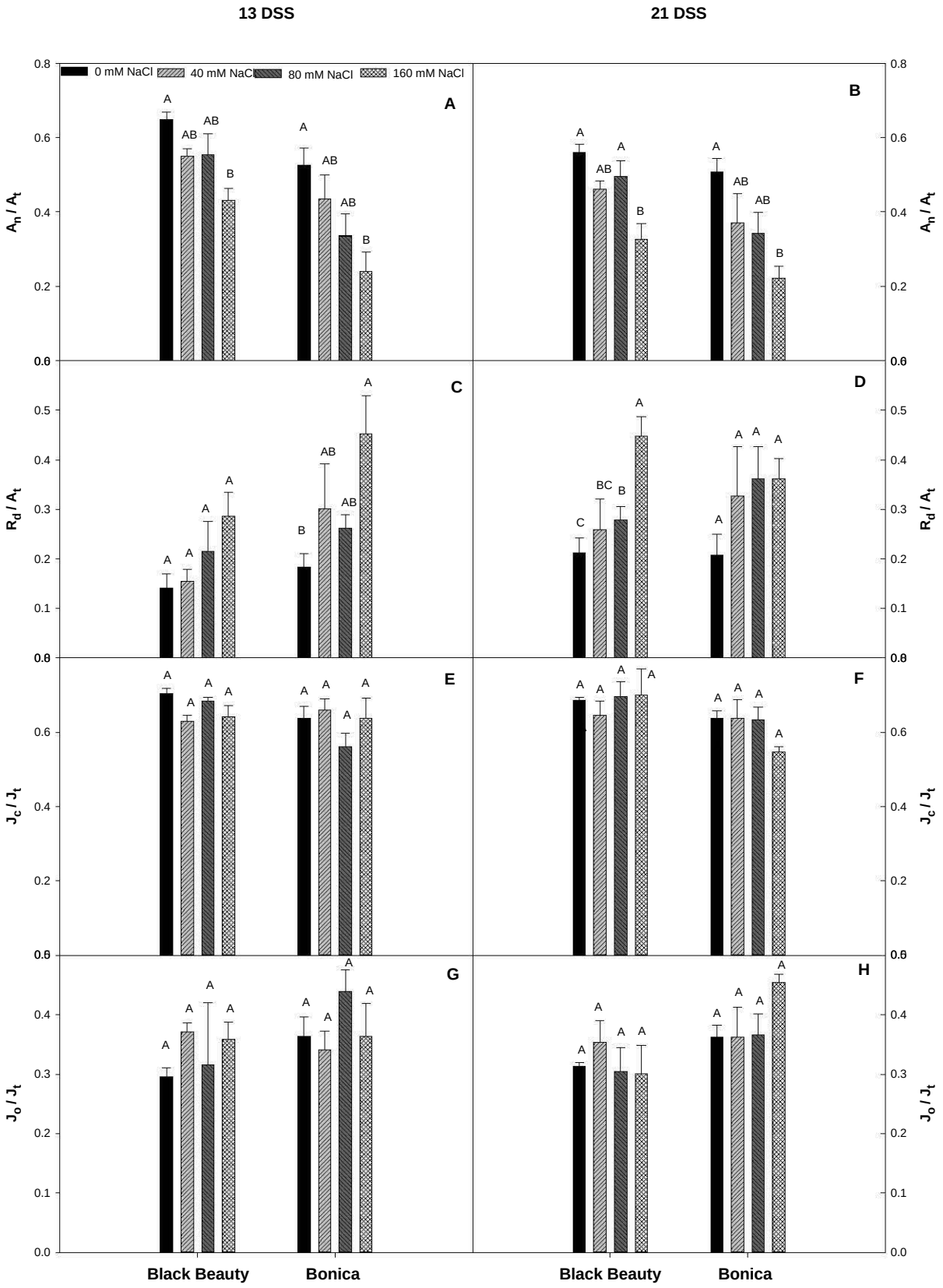

Figure 6.3: Changes in A_n/A_t, R_d/A_t, J_c/J_t and J_o/J_t ratio in leaves of the eggplant cultivars ('Black Beauty' and 'Bonica') subjected to different NaCl concentrations in two dates (date 1: June 13, 2012; date 2= June 21, 2012). Data are means ± SE. Different lower case letters indicate the significant difference between treatments (P=0.05) based on Tukey's HSD test.

Table 6.1: Correlations between gas exchange parameters, chlorophyll content, parameters of the plant water balance and leaf Na contents in 'Black Beauty' after 21 days of salt treatment. The significant correlations are indicated with * or ** for significance levels of 0.05 and 0.01, respectively.

	NaCl	Chl*a*	Chl*b*	Total Chl	A_n	R_l	A_t	J_c	J_o	J_t	g_s	E
NaCl	-	-	-	-	-0,79**	- 0,56*	-0,74**	-0,74**	-0,56*	-0,69**	-0,77**	-0,78**
Chl*a*	-	-	0.87**	0.98**					-	-	-	-
Chl*b*	-	0,87**	-	-					-	-	-	-
Total Chl	-	0,98**	-	-					-	-	-	-
A_n	-0,79**	-	-	-	-	0.72**	0.95**	0.95**	0.72**	0.89**	0.89**	0.89**
R_l	-0,56*	-	-	-	0.72**	-	0.86**	0.86**	1**	0.95**	-	-
A_t	-0,74**	-	-	-	0.95**	0.86**	-	1**	0.86**	0.97**	0.81**	0.82**
J_c	-0,74**	-	-	-	0.95**	0.86**	1**	-	0.86**	0.97**	0.81**	0.82**
J_o	-0,56*	-	-	-	0.72**	1**	0.86**	0.86**	-	0.95**	-	-
J_t	-0,69**	-	-	-	0.89**	0.95**	0.97**	0.97**	0.95**	-	0.68**	0.69**
g_s	-0,77**	-	-	-	0.89**	-	0.81**	0.81**	-	0.68**	-	0.99**
E	-0,78**	-	-	-	0.89**	-	0.82**	0.82**	-	0.69**	0.99**	-

The bold numbers, in Table 7 are used to emphasize the correlation between leaf Na content and gas exchange parameters.

Table 6.2: Correlations between gas exchange parameters, chlorophyll content, parameters of the plant water balance and leaf Na contents in 'Bonica' after 21 days of salt treatment. The significant correlations are indicated with * or ** for significance levels of 0.05 and 0.01, respectively.

	NaCl	Chl*a*	Chl*b*	Total Chl	A_n	R_l	A_t	J_c	J_o	J_t	g_s	E
NaCl	-	-	-	-	-0.75**	-0.52*	-0.75**	-0.75**	-0.52*	-0.68**	-0.76**	-0.77**
Chl*a*	-	-	0.94**	0.99**	-	-	-	-	-	-	-	-
Chl*b*	-	0.94**	-	0.97**	-	-	-	-	-	-	-	-
Total Chl	-	0.99**	0.97**	-	-	-	-	-	-	-	-	-
A_n	-0.75**	-	-	-	-	0.67**	0.95**	0.95**	0.67**	0.87**	0.88**	0.86**
R_l	-0.52*	-	-	-	0.67**	-	0.82**	0.82**	1**	0.94**	0.62**	0.63**
A_t	-0.75**	-	-	-	0.95**	0.82**	-	1**	0.82**	0.96**	0.82**	0.81**
J_c	-0.75**	-	-	-	0.95**	0.82**	1**	-	0.82**	0.96**	0.82**	0.81**
J_o	-0.52*	-	-	-	0.67**	1**	0.82**	0.82**	-	0.94**	0.62**	0.63**
J_t	-0.68**	-	-	-	0.87**	0.94**	0.96**	0.96**	0.94**	-	0.77**	0.77**
g_s	-0.76**	-	-	-	0.88**	0.62**	0.82**	0.82**	0.62**	0.77**	-	0.99**
E					0.86**	0.63**	0.81**	0.81**	0.63**	0.77**	0.99**	-

The bold numbers, in Table 7 are used to emphasize the correlation between leaf Na content and gas exchange parameters.

NaCl decreased significantly light use efficiency (LUE) in both cultivars this as well at 13 DSS and 21 DSS (Figure 6.8A and B). At 13 DSS no significant effects of increasing salt stress on carboxylation efficiency (CE) were noticed. However, after 21 DSS a significant decrease was found for 'Bonica' from 40 mM on (Figure 6.4D) and a decreasing tendency in Black Beauty from 80 mM on.

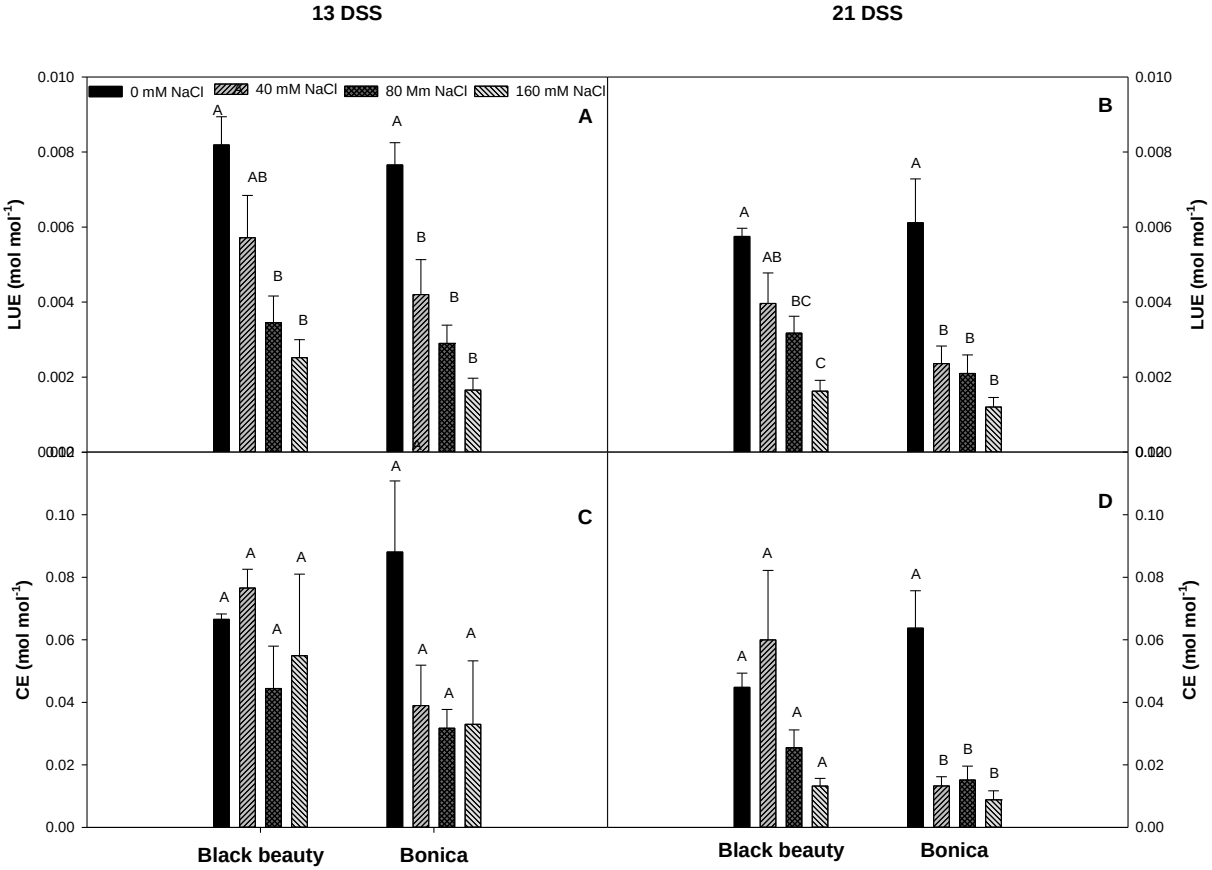

Figure 6.4: Changes in light use efficiency (LUE) and carboxylation efficiency(CE) in leaves of the eggplant cultivars ('Black Beauty' and 'Bonica') subjected to different NaCl concentrations at 13 DSS and 21 DSS (date 1: June 13, 2012; date 2= June 21, 2012). Data are means ± SE. Different lower case letters indicate the significant difference between treatments (P=0.05) based on Tukey's HSD test.

6.3.2 NaCl induced changes in chlorophyll fluorescence parameters

No significant effects were found on F_v/F_m for any of the cultivars, this for both 13 DSS and 21 DSS (Figure 6.5A and B).

Significant differences in Φ_{PSII} were observed in 'Black Beauty' and 'Bonica' (Figure 6.5C and D). Φ_{PSII} was reduced progressively and significantly from 13 DSS in 'Black Beauty' (*P*=0.003) and in 'Bonica' (*P*=0.003). The decrease of Φ_{PSII} in 'Black Beauty' was slightly more pronounced than in 'Bonica'. In fact Φ_{PSII} was reduced in 'Black Beauty' by 51.4% at 21 DSS for a level of 160 mM NaCl (*P*=0.017) compared to the control. Also in 'Bonica' we noticed a decline of Φ_{PSII} by 47.6% at 21 DSS for a level of 160 mM NaCl (*P*=0.041) compared to the control. Significant correlations between fresh biomass and Φ_{PSII} (r =0.863 , *P*=0.010) and between fresh biomass and q_p (r =0.880, *P*=0.016) were observed for 'Black Beauty'. Likewise, significant correlations between fresh biomass and Φ_{PSII} (r =0.979, *P*=0.000) and between fresh biomass and q_p (r=0.983, *P*=0.000) were observed for 'Bonica'.

At 13 DSS, salt stress affected q_p similarly in 'Black Beauty' (*P*=0.05) and 'Bonica' (*P*=0.016). For the level of 160 mM NaCl q_p decreased by 52% both cultivars compared to their respective control (Figure 6.4E). After 21 DSS only for 'Black Beauty, a significant decrease in q_p was found (*P*=0.042) (Figure 6.4E). In contrast, q_p in 'Bonica' was not significantly affected by the highest salt treatment although a decreasing trend was noticed (Figure 6.4F).

A similar but opposite trend was observed for NPQ though effects were non-significant (Figure 6.4G and H).

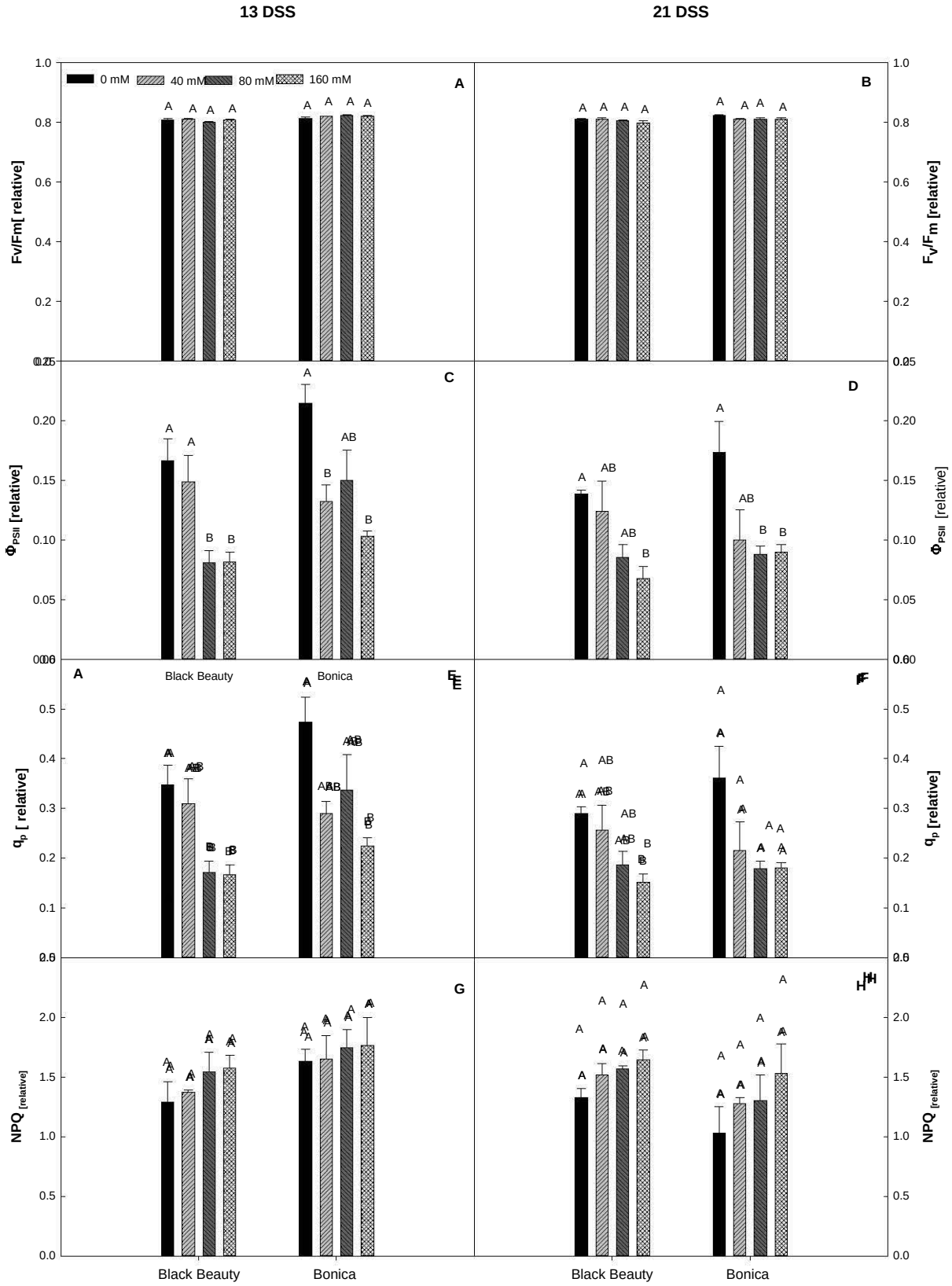

Figure 6.5: Changes in chlorophyll fluorescence parameters in leaves of the eggplant cultivars ('Black Beauty' and 'Bonica') subjected to increasing NaCl concentrations in two dates (13 DSS: June 13, 2012; 21DSS= June 21, 2012). Data are means ± SE. Different lower case letters indicate the significant difference between treatments (P=0.05) based on Tukey's HSD test.

We also correlated chlorophyll fluorescence characteristics (21 DSS) and leaf sodium content (Table 6.3; Table 6.4). For Black Beauty, higher values of Na content are correlated with lower values of q_p (*P*=0.007) and Φ_{PSII} (*P*=0.003) (Table 6.4). Also, a weak but significant negative correlation between Na content and F_v/F_m (*P*=0.017) was found. Moreover a negative correlation between the photochemical processes and NPQ were observed in 'Black Beauty' (*P*=0.002) (Table 6.4). Also for 'Bonica', higher values of Na content are correlated with lower values of q_p (*P* =0.004) and Φ_{PSII} (*P*=0.004) (Table 6.4). No significant correlations between Na content and F_v/F_m (P=0.119) or NPQ (*P*=0.440) were found. Also no correlation between the photochemical processes and NPQ (*P*=0.092) were observed in 'Bonica' (Table 6.5).

Table 6.3: Correlation matrix between chlorophyll fluorescence parameters and leaf Na contents in 'Black Beauty'. The significant correlations are indicated with * or ** for significance levels of 0.05 and 0.01, respectively.

	Na	F_v/F_m	q_p	NPQ	Φ_{PSII}
Na	-	-0.58*	-0.64**	0.58*	-0.69**
F_v/F_m	-0.58*	-	0.74**	-0.68**	0.77**
q_p	-0.64**	-0.74**	-	-0.71**	0.98**
NPQ	-0.58*	-0.68**	-0.71**	-	-0.78**
Φ_{PSII}	-0.69**	0.77**	0.98**	-0.78**	-

Table 6.4: Correlation matrix between chlorophyll fluorescence parameters and leaf Na contents in 'Bonica'. The significant correlations are indicated with * or ** for significance levels of 0.05 and 0.01, respectively.

	Na	F_v/F_m	q_p	NPQ	Φ_{PSII}
Na	-	-	-0.67**	-	-0.68**
F_v/F_m	-	-	-	-	-
q_p	-0.67**	-	-	-	0.97**
NPQ	-	-	-	-	-
Φ_{PSII}	-0.68**	-	0.97**	-	-

6.3.3 NaCl induced changes in plant water relations.

Stomatal conductance to water vapour (g_s) of both cultivars was greatly affected by the increasing salt stress. A concentration of 40 mM NaCl already significantly reduced g_s in Black Beauty, this for both 13 and 21 DSS. Significant lower g_s was only found for 160 mM NaCl in Bonica. After 13 DSS g_s was reduced by 81% and 78% respectively in 'Black Beauty' and 'Bonica' at 160 mM NaCl compared to their controls (Figure 6.6A). Similar, at 21 DSS g_s decreased by 75% and 70% respectively in 'Black Beauty' and 'Bonica' at 160 mM NaCl compared to their controls (Figure 6.6B). We found a close correlation between stomatal conductance and net assimilation in 'Bonica' and in 'Black Beauty' (Figure 6.7, Table 6.1, Table 6.2).

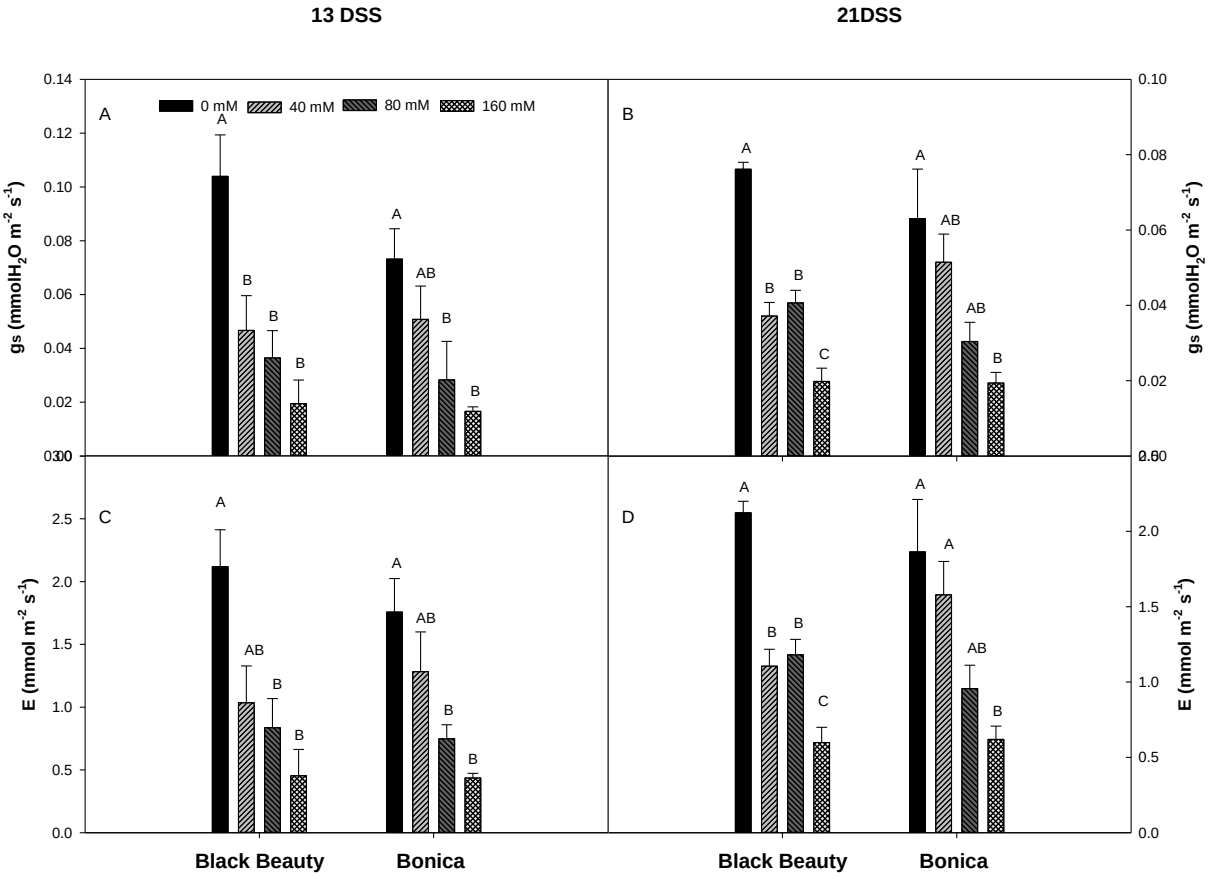

Figure 6.6: Changes in stomatal conductance (g_s) and transpiration (E) in leaves of the eggplant cultivars ('Bonica' and 'Black Beauty') subjected to different NaCl concentrations in two dates (13 DSS, 21 DSS). Data are means ± SE. Different lower case letters indicate the significant difference between treatments (P=0.05) based on Tukey's HSD test.

Stomatal closure also affected the transpiration rates. Significant differences in transpiration rate (E) were observed in the two cultivars. At 13 DSS, E was significantly reduced from 80 mM NaCl on for both cultivars while at 21 DSS E was significantly reduced at 40 mM NaCl in Black Beauty. A salinity level of 160 mM NaCl reduced E by 78.6% and 75.4% respectively in 'Black Beauty' and 'Bonica' compared to their controls (Figure 6.6C). Likewise after 21 DSS E decreased by 72.1% and 67.2% respectively in 'Black Beauty' and 'Bonica' compared to respective controls (Figure 6.6D). We also found a positive and statistical significant correlation was between A_n and E in 'Black Beauty' and 'Bonica' (Figure 6.6, Table 6.1, Table 6.2).

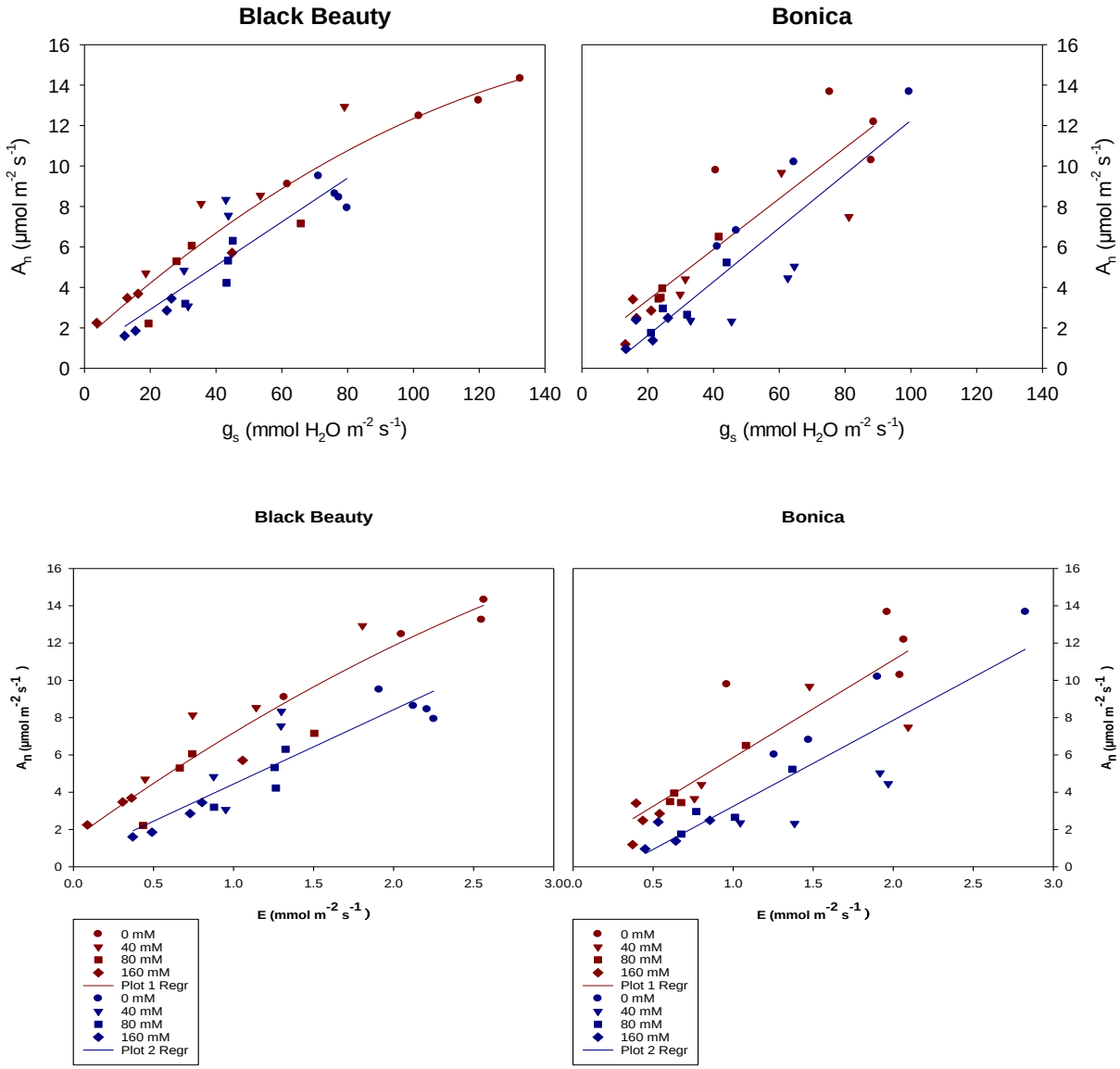

Figure 6.7: Relation between net photosynthesis (A_n) and stomatal conductance (g_s) measured after13 DSS (red colour) and after 21 DSS (blue colour) in 'Black Beauty' and 'Bonica'.

After 13 DSS WUE was not affected by increasing salt stress in 'Black Beauty' and 'Bonica' (Figure 6.8), though a trend to a better WUE at 160 mM NaCl as present in 'Black Beauty'. However, 21 days after the imposition of salt stress WUE in salinized plant were not significantly different from non-salinized plants in 'Black Beauty'. Surprisingly WUE decreased by the salt stress in 'Bonica' at 21 DSS (Figure 6.8).

As the NaCl concentration increased, both the midday ψ_l and ψ_π decreased significantly in 'Black Beauty' and reached values of -1.9 MPa and -2.5 MPa for 160 mM NaCl. Furthermore, in 'Black Beauty' g_s and ψ_π were significantly correlated: the more stomata were closed the more negative the osmotic potential (Figure 6.9). However, the leaf water potential remains quite stable in 'Bonica' and also the leaf osmotic potential is hardly affected by increasing salt stress (Table 6.7). Also no correlation between g_s and ψ_π was found for Bonica (R^2=0.14, P= 0.59).

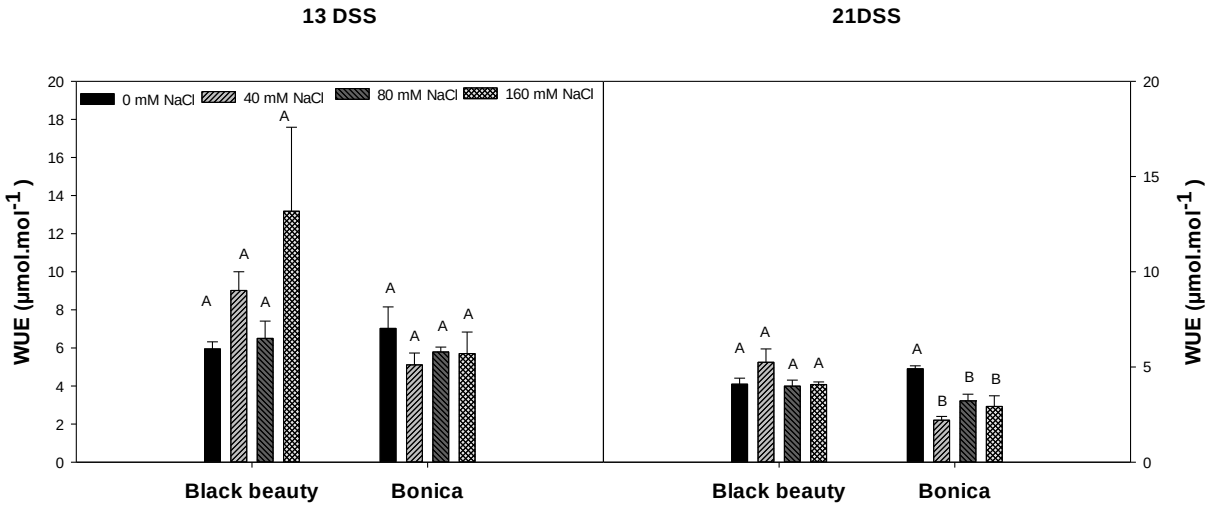

Figure 6.8: Changes water use efficiency (WUE) in leaves of the eggplant cultivars ('Black Beauty' and 'Bonica') subjected to different NaCl concentrations in two dates (date 1: June 13, 2012; date 2= June 21, 2012). Data are means ± SE. Different lower case letters indicate the significant difference between treatments (P=0.05) based on Tukey's HSD test.

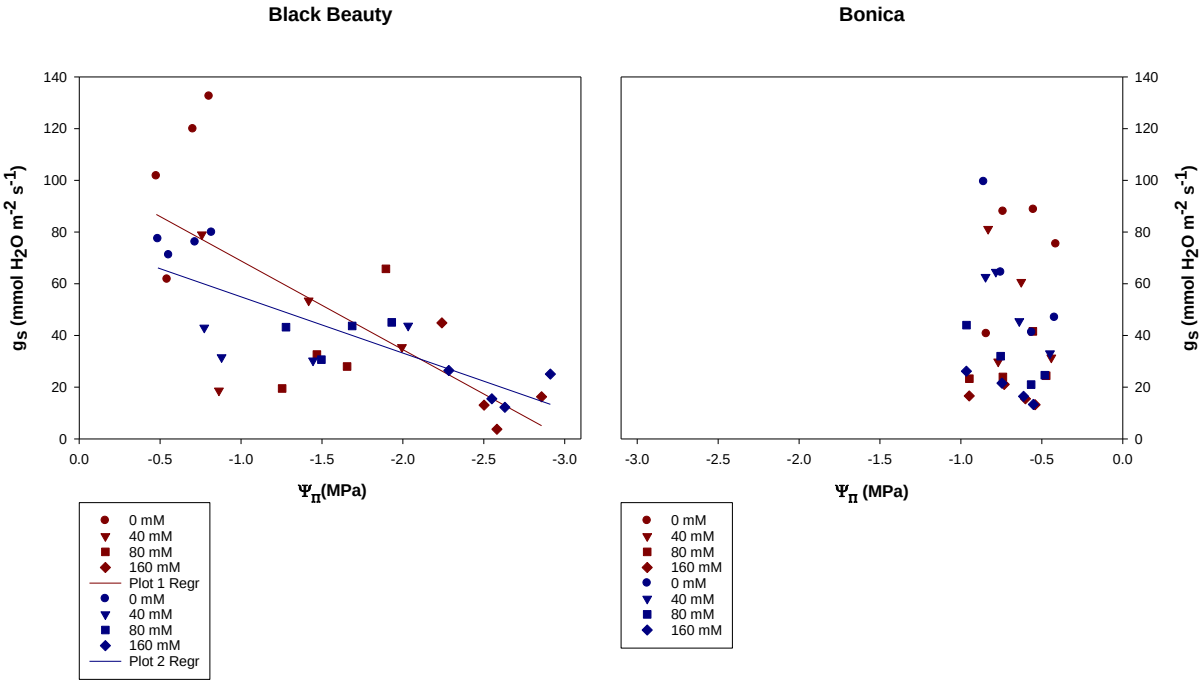

Figure 6.9: Relationship between (between g_s and ψ_π measured after13DSS (red colour) and after 21 DSS (blue colour) in 'Black Beauty' and 'Bonica'.

6.3.4 NaCl induced changes in pigments and metabolites

No significant effects on Chl*a*, Chl*b*, total Chl, Chl*a/b* and carotenoids (P>0.05) were found in any of the cultivars. For the 40 mM NaCl level we noticed a slight increase in Chl*a* in both cultivars. However a decreasing trend for all pigments was noticed from 80 mM NaCl on (Table 6.5). As compared to the control conditions Chl*a* decreased by 26.9% in 'Bonica' and by 5.1% in 'Black Beauty' at 160 mM NaCl while the decline in Chl*b* between the control and the 160 mM NaCl level was 25.6% in 'Black Beauty' and 21.3% in 'Bonica' (Table 6.5). The highest though not significant Chl*a/b* ratio was found for 80 mM in both cultivars.

No significant correlations between leaf Na and chlorophyll content were found (Table 6.1, Table 6.2).

Table 6.5: Effect of increasing NaCl concentration on chlorophyll a (Chl*a*), chlorophyll b (Chl*b*), Chl*a*/*b*, total chlorophyll (Chl*a*+*b*) and carotenoids in leaves of the eggplant cultivars.

cv	NaCl (mM)	Chl*a* (µg g^{-1} FW)	Chl*b* (µg g^{-1} FW)	Chl*a*/*b*	Chl*a*+*b* (µg g^{-1} FW)	Carotenoids (µg g^{-1} FW)
'Black Beauty'	0	689.9[a]	340.6[a]	2.3[a]	994.5[a]	140.9[a]
	40	758.9[a]	294.4[a]	2.6[a]	1053.3[a]	173.6[a]
	80	726.8[a]	250.3[a]	2.9[a]	977.1[a]	164.9[a]
	160	654.8[a]	253.4[a]	2.6[a]	908.2[a]	137.1[a]
'Bonica'	0	485.3[a]	188.4[a]	2.5[a]	673.7[a]	136.6[a]
	40	511.5[a]	198.0[a]	2.6[a]	709.6[a]	129.8[a]
	80	385.2[a]	139.3[a]	2.7[a]	524.6[a]	113.8[a]
	160	354.5[a]	148.3[a]	2.4[a]	502.9[a]	95.7[a]

Means followed by the same lowercase within each column and for each cultivar are not significantly different at P= 0.05 according to the Tukey's HSD test (n=4).

The level of lipid peroxidation increased significantly when exposed to increased NaCl in the two cultivars. 'Black Beauty' accumulated more MDA than 'Bonica'; the level of lipid peroxidation in 'Black Beauty' increased 10-fold for 160 mM NaCl compared to the control whereas a 3-fold increase in 'Bonica' was found. Consequently, the induced lipid peroxidation under increasing salinity was more pronounced in the cultivar 'Black Beauty' than in 'Bonica' (Figure 6.10).

The leaf proline content increased significantly under increasing salinity level in both cultivars, though proline increase was much more pronounced in 'Black Beauty'. Under the 160 mM NaCl, the leaf proline content showed 13-fold increase in 'Black Beauty' and 2.5-fold increase in Bonica when compared with their respective controls (Figure 6.10).

Figure 6.11 shows the negative relationship between Φ_{PSII} and MDA (at 21 DSS) and q_p and MDA in both cultivars (linear regression, 'Black Beauty': R^2=0.74, P< 0.001 and non-linear quadratic regression, 'Bonica': R^2=0.66, P< 0.001)). The same trend was observed when plotting q_p against MDA a negative and statistical significant correlation was observed in 'Black Beauty' (linear regression, R^2=0.72, P<0.001) and in 'Bonica' (non-linear quadratic regression, R^2=0.68, P<0.001).

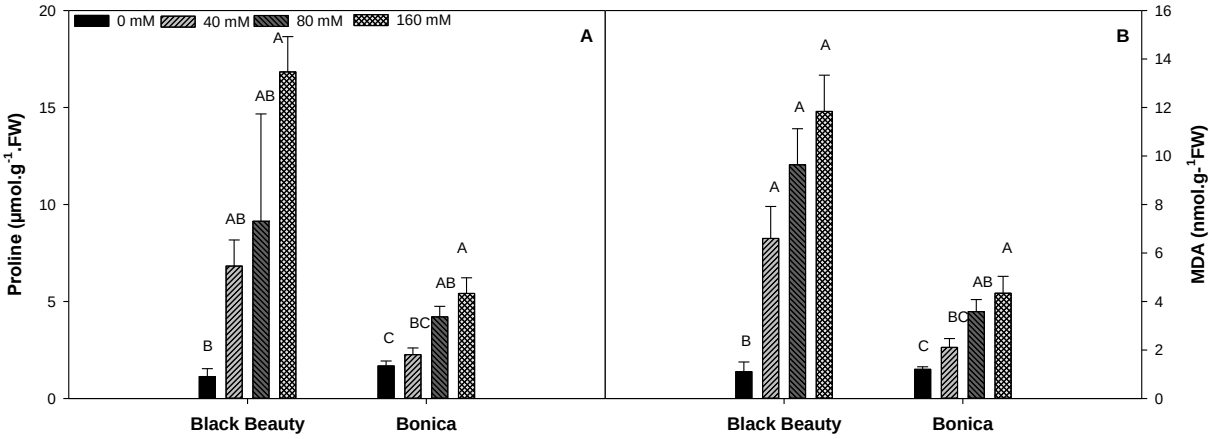

Figure 6.10: Effect of salt stress on leaf proline content (µmol g^{-1} FW) (A) and on leaf lipid peroxidation (B) at 30 DSS of the eggplant cultivar subjected to different NaCl concentrations. Data are means ± SE. Different lowercase letters indicate the significant difference between treatments (P=0.05) based on Tukey's HSD test.

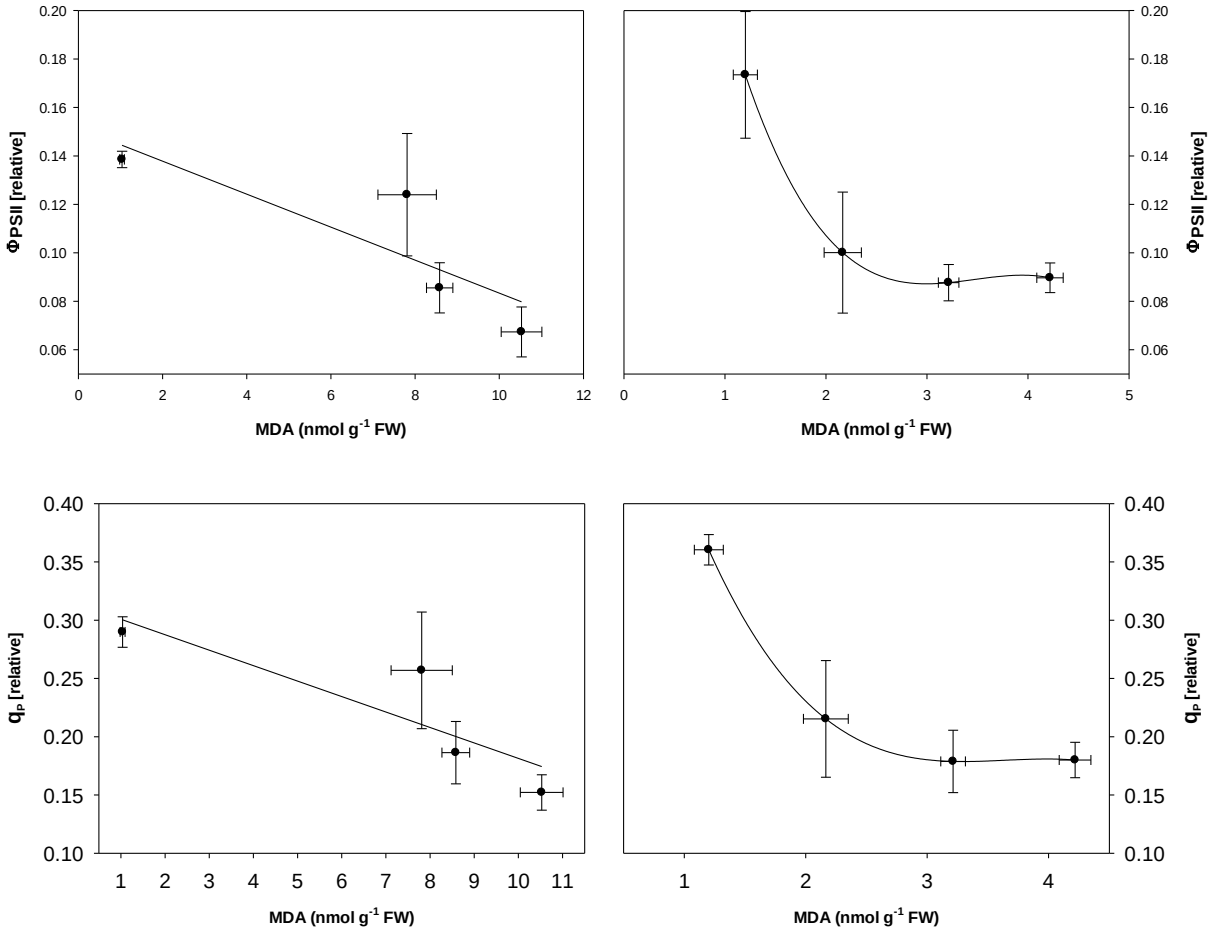

Figure 6.11: Relationship between Φ_{PSII} and MDA and between q_p and MDA measured after 21 DSS in 'Bonica' (left) and 'Black Beauty' (right). Each point represents the mean value of 5 replicates.

Under increasing salt stress glucose, fructose and sucrose content increased significantly in the cultivar 'Black Beauty' (Figure 6.12A, B and C). In contrast, increasing salt concentration lead to a significant decrease in fructose and sucrose content in the cultivar 'Bonica' (Figure 6.12B and C). Salt stress strongly increased starch accumulation in 'Bonica' while a tendency to lower leaf starch reserves was observed in 'Black Beauty'.

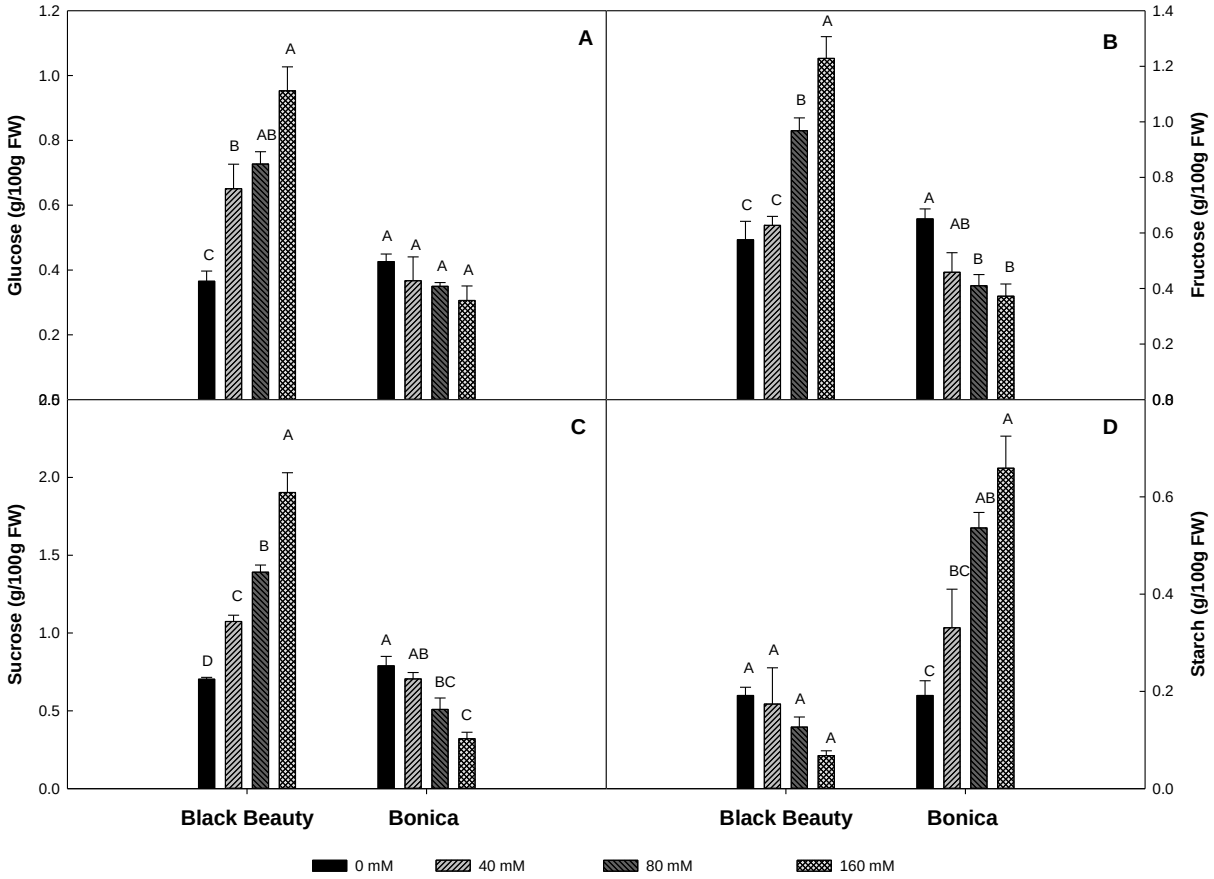

Figure 6.12: Effect of NaCl concentration on glucose (A), fructose (B), sucrose (C), and starch (D) levels in leaves of the eggplant cultivars. Data are means ± SE (not shown when smaller than the symbol). Different lowercase letters indicate the significant difference between treatments (P=0.05) based on Tukey's HSD test.

6.3.5 NaCl induced changes in mineral content

NaCl treatments increased the Na in the leaves and in the roots as compared to the controls in both cultivars (Table 6.6).

Leaf Na increased significantly in leaves and roots of 'Black Beauty' under saline conditions though Na levels were higher in the leaves than in the roots for 160 mM NaCl. Na increase was also significant in leaves and in roots in 'Bonica'. A 13-fold increase was found in leaves in 'Bonica' for 160 mM NaCl when compared to the control.

Furthermore, the sodium accumulation was higher in the roots than in the leaves of 'Bonica'. For 'Bonica' lower concentration of leaf Na was found compared to 'Black Beauty'. Indeed at 160 mM NaCl 'Black Beauty' accumulated 2.9-fold Na leaf content than 'Bonica'.
Although leaf Cl^- content increased by increasing salt concentrations in the two cultivars, the Cl^- accumulation was significant in 'Bonica' and not significant in 'Black Beauty'. The Cl^- concentration in the leaves in 'Black Beauty' was 1.13 fold higher than that of Cl^- in 'Bonica' at 160 mM NaCl treatment (Table 6.6).

K concentration decreased significantly in leaves (P=0.001) and non-significant in roots (P=0.757) under salt stress in 'Black Beauty'. Leaf K and root K contents were reduced respectively by 41.1% and by 27.9% in 'Black Beauty' at 160 mM NaCl. In contrast, no effect on K content in leaves and roots was found for 'Bonica' at increasing salt stress (Table 6.6). Increasing salinity gave no significant changes in the leaf and root Ca and Mg content in 'Bonica' and 'Black Beauty' (Table 6.6).

Increasing salinity decreased significantly the P leaf content in both 'Black Beauty' (P=0.002) and 'Bonica' (P=0.052). No effect on root P content was observed under saline conditions. (Table 6.6). Also S content in leaves and roots was hardly affected by an increasing salt stress in 'Black Beauty' and 'Bonica'.

The Na/K and Na/Ca ratios in leaves and roots were higher in salinized plants for both cultivars than those in the control plants. However, significant differences for the Na/K ratio were only observed in 'Black Beauty' (Table 6.6). Moreover the increased Na/K and Na/Ca ratios in the leaves and roots was more pronounced in 'Black Beauty' than in 'Bonica' at 160 mM NaCl compared to their respective controls. For example leaves Na/K and Na/Ca ratios increased at 160 mM of NaCl respectively more than 16-fold and 11-fold when compared to their respective controls in 'Black Beauty'. However, leaves Na/K and Na/Ca ratio increased at 160 mM of NaCl respectively 15-fold and 10-fold when compared to their respective controls in 'Bonica'. Besides, roots Na/K and Na/Ca ratios increased at 160 mM of NaCl respectively more 3-folds and 2-folds when compared to their respective controls in 'Black Beauty'. In contrast, roots Na/K and Na/Ca ratios increased at 160 mM of NaCl less than 2-fold when compared to their respective controls in 'Bonica' (Table 6.6). It is noteworthy that the salt induced increase of Na/K and Na/Ca ratios was more pronounced in leaves than in roots.

Table 6.6: Effect of NaCl concentration on accumulation of K, Ca, Mg, Na, P, S, Cl^-, Na/K and Na/Ca in leaves and roots of the eggplant cultivars.

Cultivar	Tissue	NaCl (mM)	K (g/100g)	Ca (g/100g)	Mg (g/100g)	Na (g/100g)	P (g/100g)	S (g/100g)	Cl^- (g/100g)	Na/K	Na/Ca
'Black Beauty'	Leaves	0	6.44^{a}	3.11^{a}	0.55^{a}	0.44^{c}	0.74^{a}	0.20^{a}	2.81^{a}	0.07^{b}	0.13^{a}
		40	6.71^{a}	3.23^{a}	0.58^{a}	0.89^{c}	0.69^{a}	0.20^{a}	2.81^{a}	0.13^{b}	0.28^{a}
		80	5.30^{ab}	3.17^{a}	0.56^{a}	1.86^{b}	0.66^{ab}	0.20^{a}	4.53^{a}	0.34^{ab}	0.69^{a}
		160	3.79^{b}	2.84^{a}	$0.5.^{a}$	3.80^{a}	0.57^{b}	0.17^{a}	6.95^{a}	1.20^{a}	1.50^{a}
	Roots	0	1.86^{a}	1.33^{a}	0.26^{a}	1.10^{b}	0.49^{a}	0.28^{a}	*	0.44^{b}	0.87^{a}
		40	1.60^{a}	1.21^{a}	0.24^{a}	1.19^{b}	0.44^{a}	0.26^{a}	*	0.78^{ab}	0.96^{a}
		80	1.15^{a}	1.37^{a}	0.25^{a}	1.18^{b}	0.46^{a}	0.24^{a}	*	0.91^{ab}	0.86^{a}
		160	1.34^{a}	1.11^{a}	0.23^{a}	2.38^{a}	0.44^{a}	0.28^{a}	*	1.70^{a}	2.20^{a}
'Bonica'	Leaves	0	4.77^{a}	2.85^{a}	0.62^{a}	0.09^{c}	0.72^{a}	0.25^{a}	1.25^{c}	0.02^{a}	0.04^{a}
		40	5.02^{a}	3.35^{a}	0.61^{a}	0.56^{b}	0.59^{ab}	0.19^{a}	3.28^{bc}	0.11^{a}	0.16^{a}
		80	4.58^{a}	3.50^{a}	0.66^{a}	1.29^{a}	0.60^{ab}	0.19^{a}	4.67^{ab}	0.34^{a}	0.44^{a}
		160	4.99^{a}	3.52^{a}	0.68^{a}	1.37^{a}	0.55^{b}	0.18^{a}	6.11^{a}	0.30^{a}	0.41^{a}
	Roots	0	1.19^{a}	1.44^{a}	0.34^{a}	1.55^{b}	0.68^{a}	0.33^{a}	2.61^{a}	1.27^{a}	1.3^{a}
		40	0.81^{a}	1.61^{a}	0.25^{a}	1.84^{b}	0.67^{a}	0.29^{a}	2.86^{a}	2.41^{a}	1.3^{a}
		80	1.00^{a}	1.24^{a}	0.27^{a}	1.99^{b}	0.47^{a}	0.22^{a}	2.24^{a}	1.73^{a}	1.4^{a}
		160	1.21^{a}	1.29^{a}	0.23^{a}	2.55^{a}	0.73^{a}	0.26^{a}	6.49^{a}	2.19^{a}	2.4^{a}

Means followed by the same lowercase within each column and cultivar within are not significantly different at P= 0.05 according to the Tukey's HSD test (n=4).

*:No data because of the lack of sufficient plant material.

6.3.6 NaCl induced changes on growth parameters

The effects on biomass were evaluated in term of number of leaves, height, aerial FW and DW. The salt induced decreases in number of leaves, height, DW and FW showed an overall similar trend, however, 'Black Beauty' was more affected than 'Bonica'.

Number of leaves and plant height was slightly enhanced by 40 mM NaCl in both cultivars; however a further increase of the salt level significantly decreased number of leaves and plant height for 'Black Beauty' and plant height in 'Bonica'. In 'Bonica' leaf initiation was only reduced at 160 mM NaCl (Table 6.7). The highest salinity level (160 mM NaCl) decreased the number of leaves in 'Bonica' and 'Black Beauty' respectively by 17.6% and 27.3% and the plant height in 'Bonica' and 'Black Beauty' respectively by 34.6% and 87.8% when compared to their respective controls (Table 6.7). Consequently, the impact of the salinity was more pronounced in 'Black Beauty' than in 'Bonica'.

A very pronounced decrease in FW and DW was observed in 'Black Beauty' under increasing salt stress (Table 6.7). For instance, in the 160 mM treatment, the FW decreased by 87.8% and DW decreased by 72.2%, compared to their respective controls (Table 6.7). In contrast, the decline in FW and DW between the control and the 160 mM NaCl level was less in 'Bonica' (36.9% and 35.7%).

Table 6.7: Effect of increasing levels of NaCl on morphology and plant water status of two eggplant cultivars. Plant morphological parameters include number of leaves, plant height, aerial fresh weight, aerial dry weight (DW) and plant water status includes tissue water content (TWC), midday leaf water potential (ψ_l) and leaf osmotic potential (ψ_π).

cv	NaCl (mM)	N° of leaves	Height (cm)	FW (g)	DW (g)	TWC (g/g)	ψ (MPa)	ψ_π (MPa)
'Black Beauty'	0	7.52^{ab}	31.4^{b}	74.7^{a}	16.1^{a}	0.78^{a}	-0.54^{a}	-0.63^{a}
	40	8.50^{a}	37.0^{a}	27.9^{b}	7.10^{b}	0.74^{a}	-0.98^{ab}	-1.22^{ab}
	80	6.51^{b}	22.9^{c}	14.5^{c}	5.41^{c}	0.62^{b}	-1.42^{b}	-1.50^{b}
	160	5.41^{c}	18.4^{d}	$9.1b^{d}$	4.42^{d}	0.50^{c}	-1.90^{c}	-2.51^{c}
'Bonica'	0	9.92^{ab}	34.7^{b}	149.8^{a}	25.9^{a}	0.82^{a}	-0.49^{a}	-0.63^{a}
	40	10.70^{a}	38.0^{a}	113.1^{b}	19.6^{b}	0.82^{a}	-0.61^{a}	-0.66^{a}
	80	9.00^{b}	29.5^{c}	103.7^{c}	18.0^{c}	0.82^{a}	-0.64^{a}	-0.67^{a}
	160	8.23^{c}	22.7^{d}	94.4^{d}	16.6^{c}	0.82^{a}	-0.65^{a}	-0.70^{a}

Means followed by the same lowercase within each column and for each cultivar are not significantly different at P=0.05 according to Tukey's HSD test (n=4).

A significant decrease in TWC, as a measure of expansion growth, was observed for both 80 mM NaCl and 160 mM NaCl in 'Black Beauty'. In contrast, the TWC remains quite stable in 'Bonica' (Table 6.7).

A scores scatter plot of the first two PCAs (explaining 68.28% of the variation) shows a clear separation of 'Bonica' from 'Black Beauty' after 21 DSS (Figure 6.11). The loading that positively correlated with PCA1 (37.40%) were FW, DW and TWC and that with PCA2 (30.88%) were Φ_{PSII}, ETR, q_p , ψ_π, ψ, and Fv/Fm. The loading of MDA, proline and starch correlated negatively with PCA1. The loading of Na/K in leaves and Na/K in roots correlated negatively with PCA2. For both cultivars the scores of the PCA moved to higher MDA, Proline, Starch, Na/K ratio in leaves and Na/K ratio in roots values and lower FW, DW, TWC, ψ_π, ψ, Φ_{PSII}, ETR and q_p under increasing salinity.

The separation of control and salt stressed plants of 'Black Beauty' along PCA1 was higher compared to 'Bonica' (Figure 6.13). For 'Black Beauty' control and salt stressed plants were well separated along PCA2.

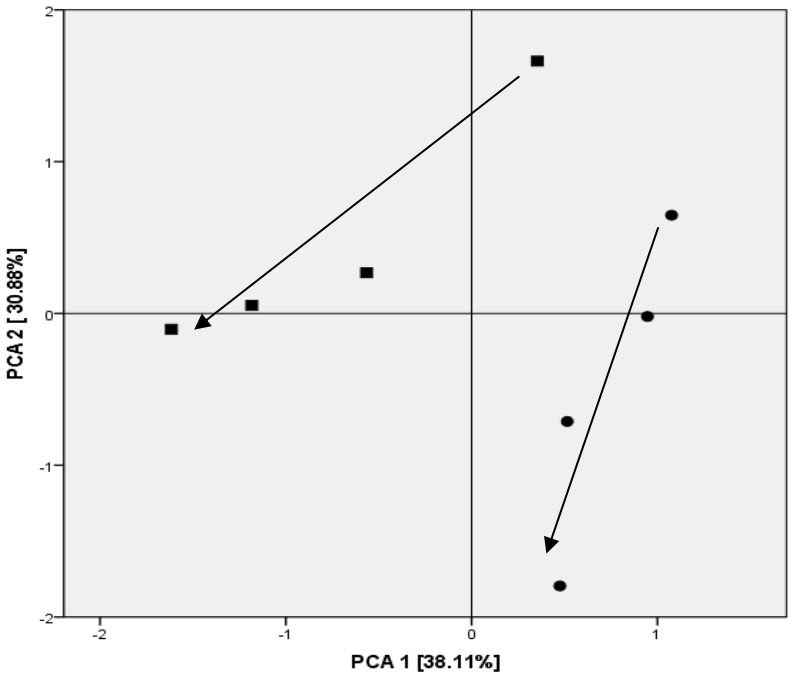

Figure 6.13: Principal component analysis (PCA) of FW, DW, TWC, Φ_{PSII}, ETR, qp, $\psi\pi$, ψ, Fv/Fm, proline, starch, Na/K ratio in leaves, Na/K ratio in roots and MDA of the eggplant cultivars grown for 21 days under saline stress. PCA1 is positively correlated with FW, DW and TWC and negatively with MDA, proline and starch. PCA2 is positively correlated with Φ_{PSII}, ETR, q_p , ψ_π, ψ, and Fv/Fm and negatively with Na/K in leaves and Na/K in roots. Each data point represents the mean of four replicates. Arrows indicate the increasing salt stress level (●: Black Beauty; ■ Bonica)

6.4 Discussion

Results of previous chapters indicated that differences in salt stress tolerance exist in eggplant cultivars. In this chapter we submitted two cultivars (one tolerant and one susceptible) to increasing salt stress. In this chapter we strongly focused on photosynthesis though mineral content in shoot and roots and other adaptive metabolite strategies and plant growth parameters were also included.

Salt stress affects CO_2 availability through stomatal closure which may lead to reduction of photosynthesis (Munns and Tester, 2008) and consequently contribute to the reduction of growth. In our research increasing salt stress lead to reduced stomatal conductance directly affecting gas exchanges (Figure 6.7). Both the rate of gross assimilation (A_t = net photosynthesis + total respiration in the light) and net assimilation decreased with increasing salt stress/closing of the stomata in both eggplant cultivars. Low leaf water potentials have been found to be closely related to both stomatal closure and non-stomatal reduction of photosynthesis (Matthews and Boyer, 1984; Kaiser, 1987). Yet, only 'Black Beauty' reduced its leaf water potential to levels below -2.5 MPa, while no correlation between leaf water potential and stomatal conductance was observed in 'Bonica' (Figure 6.9). This again reveals a tendency for isohydric behaviour in Bonica where a relative constant midday leaf water potential (Ψ) is observed while the control of the plant water balance under increasing salt stress acts through reduced stomatal conductance (gs).

The ratio A_n/A_t decreases significantly under increasing salt stress in both cultivars which indicates that the respiration losses become more important. Mitochondrial respiration was not affected by salt stress, even under high salinity. Both eggplant cultivars maintained similar respiration rates as the control plants. Jacoby et al (2011) reviewed the role of mitochondrial respiration under salt stress and found a high variability in the respiratory responses amongst the studied species but sensitive species showed a trend towards increased respiratory rates. If we consider the ratio of R_d/A_t then indeed a higher fraction of the total assimilation goes to respiration under higher saline stress. High respiration rates are linked to higher ATP production; therefore both cultivars could maintain sufficient energy levels under increasing salt stress levels. However, this energy is probably used for different purposes such as osmotic adjustment in 'Black Beauty' or for sodium exclusion and tissue tolerance in 'Bonica'.

Moreover non-stomatal factors also severely affect photosynthesis under higher salinity levels (Stepien and Klobus, 2006). Chlorophyll fluorescence parameters can be used for observing stress and damage of the photosynthetic apparatus. No effects of salinity stress on F_v/F_m were observed though a significant reduction of Φ_{PSII} indicated a decrease in electron transport through PSII which is in accordance with results that have been reported for tomato (Zribi et al., 2009); for soybean (Lu et *al*., 2009) and for eggplant (Chapter 4, Hanachi et al., 2014). Likewise a decrease in photochemical quenching (q_p) and an enhanced thermal dissipation of excess excitation energy (NPQ) was observed in both cultivars. However, in contrast to the results of Chapter 4, the chlorophyll fluorescence parameters did not differentiate between Black Beauty and Bonica for the applied salt stresses.

These results of the chlorophyll fluorescence analysis might indicate that decreases in PSII efficiency were acting as a down-regulation to maintain a balance between light-driven linear electron flow (J_t) and requirements of reducing power for both carboxylation and oxygenation of RuBP as suggested by Kraus and Weis (1991). Indeed, carboxylation can effectively be replaced by oxygenation of ribulose-1,5-biphosphate in C3 plants in case of closed stomata. The relative importance of the electron flow to carboxylation and oxygenation is indicated by the ratio J_c/J_{tot} and J_o/J_{tot}. These electron flows were not affected by increasing salt stress and ranged between 60 % -70 % for carboxylation of RuBP. As such also the ratio J_c/J_o which represents the balance between RuBP carboxylation and oxygenation was not significantly affected.

Yet, photorespiration (R_l) in C3 plants may be considered as an alternative electron sink for the light-induced electron flow, and is often presented as a process that may help consume an appreciable electron flow during periods of restricted CO_2 availability in the chloroplast and high irradiance (Krause and Corinc, 1987; Stuhlfauth et al., 1990). Early reports stated that R_l should represent only 15% of A_n in non-stressed conditions (Ogren, 1984), but later work described levels close to 30-40% for *Nicotiana tabacum* (Zelitch, 1992), 35-40% for *Helianthus annuus* (Jacob and Lawlor, 1993) and more than 50% for *Triticum aestivum* (Gerbaud and André, 1987) at temperatures of around 20°C and saturating irradiance. Our observations for non-stressed eggplants showed that R_l reaches levels of ± 36 % of A_n in Black Beauty and 57 % in Bonica. These data are in concordance with the literature, if we take into account the higher temperature (30-35°C) experienced during our measurements. Under salt stress this ratio increased considerably up to 79% for 160 mM in Black Beauty (40 % of the total assimilation rate) and up to 204% in Bonica (32% of the total assimilation rate)

at 21 DSS. Photorespiration is clearly an important electron sink for 'Bonica' under salt stress.

Also specific ion effects of salt stress on photosynthesis have been described in several plant species such as tomato (Heuer at al., 1993), bell pepper (Bethke and Drew, 1992) and pea plants (Fedina et al., 1994). Both direct effects of Na and Cl^- or decreases in K contents might explain the photosynthetic impairment. Reduced chlorophyll content due to potassium deficiency may limit photosynthesis (Battie-Laclau et al., 2014) but reduced chlorophyll content might also arise due to ionic stress and subsequent oxidative stress (Seeman and Critchely, 1985; Abdullah and Ahmed, 1990; Hamada and El-Enany, 1994). In this study no significant effects of salt stress were observed on the content of photosynthetic pigments in both cultivars, nevertheless chlorophyll content tended to decrease at 160 mM NaCl in 'Black Beauty' and 'Bonica'. Not only the pigment content is important, salinity also causes chloroplasts to aggregate. This then leads to ultrastructural changes of the assimilating organs (Glagoleva et al., 1992) and include dilatation of thylakoid membranes and enlarged mesophyll cells (Brugnoli and Bjorkmann, 1992; Mitsuya et al., 2000). Another tendency found in both species is that low salt levels (40 mM NaCl) increased the chlorophyll content in both eggplant cultivars. As for this level K content is not affected yet, Na is not substituting for K as observed in K-deficient plants (Battie-Laclau et al., 2014). This tendency at low salinity is not well understood though it was also observed in tomato (Romero-Aranda et al., 2001) and other crops (Winicov and Button, 1991; Locy et al., 1996).

Salt stress causes an oxidative stress to many cellular components (Halliwell et al., 1989; Yasar et al., 2008). Oxidative damage caused by ROS as a consequence of salt stress contributes to membrane lipid peroxidation thus reducing membrane fluidity and selectivity (Sairam et al., 2002; Xu et al., 2012). Membranes are thus vulnerable targets for stress induced cellular damage, and the extent of damage is commonly used as a measure of tolerance to imposed stress (Dhindsa et al., 1981; Wise et al., 1987; Zhao et al., 1992; Gadallah, 1999; Jain et al., 2001; Yasar et al., 2008). The extent of lipid peroxidation differed hugely between Black Beauty and Bonica when grown under increasing levels of salinity. The lower lipid peroxidation in 'Bonica' reflected that this cultivar has a higher capability of cellular protection against oxidative damage caused by the applied salt treatment (Noctor and Foyer, 1988; Jain., 2001; Demiral and Turkan, 2005;Yasar et al., 2008; Yildiztugay et al.,2011; Abbas et al., 2014). Even the huge increase in proline developed by 'Black Beauty'

failed to contribute to the alleviation of salt-induced peroxidative damage and maintaining the homeostasis of reactive oxygen species under stress and this was also observed in Chapter 5.

The adaptation of plants to salinity is associated with osmoregulation. To accommodate the ionic balance in the vacuoles, cytoplasm accumulates low-molecular-mass compounds termed compatible solutes because they do not interfere with normal biochemical reactions (Hasegawa et al., 2000; Zhifang and Loescher, 2003). These compatible solutes include proline, a multifunctional amino acid. In addition to its role as a cytoplasmic osmoticum under stress, proline may function as a carbon and nitrogen source for post stress recovery and growth (Fukutaku and Yamada, 1984), a stabilizer for membranes, protein synthesis (Kardpol and Rao, 1985) and cytoplasmic enzymes (Paleg et al., 1984), a scavenger for free radicals (Smirnoff and Cumbes, 1989; Saradhi et al., 1995) and as sink for energy to regulate redox potential (Alia and Saradhi, 1993). It has been reported that salt sensitive cultivars accumulated significantly higher levels of free proline compared to tolerant ones (Lutts et al., 1999; Vaidyanathan et al., 2003; Xu et al., 2012). In our work, proline increased in response to salinity in both cultivars; however, more proline accumulated in 'Black Beauty' at various salinity levels as also observed in Chapter 5. Current observations confirm that the accumulation of this amino acid is more due to the metabolic damage caused by salt stress rather than to a function as tolerance factor (Hanson et al., 1994; Bikash et al., 2005; Zgallai et al., 2005).

The accumulation of soluble carbohydrates in plants as osmotic regulator has been widely reported as response to salinity (Hu and Shmidhalter, 1998; Murakeozy et al., 2003; Xu et al., 2012). These osmolytes also help to tolerate dehydration by improving their ability to maintain the osmotic balance within cells (Da Silva Lobato et al., 2008). Additional benefits of these solutes have been described including buffering cellular redox potential, protecting the cell from dehydration by stabilizing membranes and proteins structure and providing possible energy source under severe stress (Hasegawa et al., 2000). According to Cram (1976), sugars contribute up to 50% of the total osmotic potential in glycophytes subjected to saline conditions. In this study, salinity increased sucrose, glucose and fructose content in the sensitive cultivar 'Black Beauty'. However, increasing salt concentration lead to the decline in sucrose, glucose and fructose content in the tolerant cultivar 'Bonica'. This response was also reported by Dubey and Sing (1999) and by Pattanagul and Thitisaksakul (2008) in rice and by Balibrea et al. (2000) in tomatoes.

It is generally accepted that the elevation in the cellular osmolarity (ψ_π) which result from the accumulation of compatibles solutes is associated to the influx of water into, or to a reduced efflux from, cell, thus providing the turgor necessary for the expansion of cell (Hare et al., 1998). Salt stress caused an apparent decline in ψ and ψ_π in the sensitive cultivar 'Black Beauty' while the tolerant cultivar 'Bonica' maintained stable values of ψ and ψ_π under increasing salinity confirming the results of chapter 5. In this respect Bonica is more successful to maintain sufficient water influx than Black Beauty as for Bonica the tissue water content is hardly affected even under 160 mM NaCl.

Carbohydrate accumulation can also induce a feed-back inhibition on photosynthesis. According to Krapp and Stitt (1995) and Krapp et al. (1991) higher concentrations of sucrose in the cytoplasm could generate a negative feed-back inhibition on carbon metabolism which contributes to a lower CO_2 assimilation. Moreover the expression of Rubisco could be inhibited with a considerable accumulation of sugar in cytoplasm (Koch, 1996; Sawada et al., 1992). Therefore the differences in sugars accumulation in cultivars differing in salt tolerance, *in casu* Black Beauty and Bonica, may explain tolerance differences between these cultivars. However, Bonica accumulates starch and also starch stacked in the grana may result in a negative feed-back on photosynthesis due to possible mechanical damage by large starch grains (Cave et al., 1981). In any case no differences in photosynthetic activity (A_n and A_t) for each salt level were observed between the cultivars ($P > 0.05$).

Reduced stomatal conductance not only influences photosynthesis but will also affect the transpiration rates. Transpiration rate was indeed reduced under increasing salt stress in both cultivars as reported for many other species (Lakshmi et al., 1996; Marler and Zozor, 1996; Mickelbart and Marler, 1996; Tezara et al., 2002; Gibberd et al., 2002; Burmann et al., 2003). Surprisingly effects on WUE were limited while we expected an increase of WUE (Parida and Das, 2005; Sun et al., 2011).

Munns and Tester (2008) defined that the ion-specific phase of plant response to salinity starts when salt accumulates toxic concentrations in the old leaves (which are no longer expanding and so no longer diluting the salt arriving in them as younger growing leaves do). The NaCl treatments caused an increase in Na and Cl concentration in both leaves and roots in 'Bonica' and' Black Beauty' and a decrease in foliar K in the sensitive cultivar 'Black Beauty'. Indeed, the uptake of NaCl competes with that of others nutrient ions especially K leading eventually to potassium deficiency (Ball and Farquhar, 1984). As a result Bonica had lower Na/K and Na/Ca ratios compared to Black Beauty. These ion ratios are associated with

the relative salt tolerance in many species, where tolerant genotypes had lower Na/K and Na/Ca ratio (Perez-Alfocea et al., 1996; Yasser et al., 2006). A low Na/K ratio in the cytosol is essential for normal cellular functions of plants. While competing with K uptake, Na may block the K specific transporters under salinity. This contributes to a toxic level of Na as well as insufficient K concentration for enzymatic reactions and osmotic adjustment (Zhu, 2003; Yassar, 2006).

Regulation of Na uptake by cells and long distance Na transport seems to be a crucial adaptation of plants to salt stress (Munns et al., 2000). When exposed to salinity plants accumulate Na in their roots and exclude it from the shoots they are referred to as Na excluders. In contrast, some species efficiently accumulate high amount of Na in the shoots and are known as Na includers (Ashraf, 2004). Comparative analysis of Na accumulation revealed that roots of Bonica accumulated approximately twofold more Na compared to the shoots suggesting that a mechanism of controlled long distance transport from roots to shoots may exists. In contrast, especially at 160 mM NaCl higher Na contents were observed in the shoots of Black Beauty. As 'Bonica' maintained a lower Na concentration in the leaves than 'Black Beauty', this cultivar may control long- distance more effectively than the salt sensitive 'Black Beauty'. The exclusion of sodium from the leaves is the most common and important mechanism of salt tolerance in monocotyledonous plants. Na exclusion is a result of restricted Na uptake by the roots and low rates of transport in the xylem from the root to shoot (Munns et al., 2005; Xu et al., 2012). This Na exclusion system can be achieved by restricted loading of the xylem, or efficient removal of Na from the upper part of the root system and the base of the shoot (Xu et al., 2012). However, when these toxic ions start to accumulate in the leaves another salt tolerance mechanism of many glycophytes is to exclude Na and Cl^- from the cytosol via compartmentalization into vacuoles (Martinez Rodriguez et al., 2008; Paranychianakis and Angelakis, 2008; Silveira et al., 2012). Although we have no data about a partial ion exclusion mechanism in our plants it might be possible that the capacity of 'Black Beauty' with respect to this salt exclusion system of the cytosol is less effective compared to Bonica.

Salinity stress has been reported to affect a variety of morphological traits and to decrease almost all growth parameters, including shoot and root, leaf area, fresh and dry weight, plant height, yield and some yield quality attributes (Ersalan et al., 2008; Li, 2009; Tantawy et al., 2009). The morphological observations in this study are very similar to those described in chapter 5 for 'Black Beauty' and 'Bonica'. A level of 40 mM NaCl did not affect leaf

initiation and height or was even slightly stimulated in both cultivars, indicating that slightly older plants support already better salt stress as for younger plants (chapter 5) this effect was only seen for 20 mM and not 40 mM (chapter 5). However, at 40 mM salt stress affected already the fresh and dry matter production in 'Black Beauty' and 'Bonica'. By increasing NaCl concentration, leaf initiation, and plant height and fresh and dry weight were strongly affected in the salt sensitive cultivar 'Black Beauty' and moderately affected in the salt tolerant 'Bonica'. Besides, 80 mM NaCl level decreased tissue water content (TWC) in 'Black Beauty' while 'Bonica' could maintain its TWC up to 160 mM NaCl as already observed in chapter 5. Munns and Tester (2008) showed that threshold for maintaining a constant TWC in most plants is approximately at 40 mM NaCl. Yet, depending on the salt stress trial (hydroponics or soil) and halophyte or glycophyte character of the species, threshold values range 200 mM NaCl in mangrove (*Brugiera parviflora*) (Parida et al., 2004), 200 mM NaCl on jute species (*Corchorus olitorius*) (Chaudhuri and Chouhuri, 1997), 90 mM on *Atriplex griffithii* (Khan et al., 2000) and 40 mM on okra (*Abelmoschus esculentus*) (Ben Dkhil and Denden, 2010).

6.5 Conclusion

Photosynthesis is strongly affected by increasing salt stress in both cultivars though mitochondrial respiration remains unaffected in absolute values and the ratio R_d/A_n increases. Photorespiration is an alternative electron sink and at higher salt levels 'Bonica' use this pathway to a higher amount than 'Black Beauty'.

A differential reaction to increasing salt stress was found in the studied biochemical parameters. Higher oxidative stress as assessed by MDA levels was found in 'Black Beauty'. Osmotic adjustment included higher proline and soluble carbohydrate levels in response to salinity in the sensitive cultivar 'Black Beauty'. This might be explained by a different distribution of sodium and chloride in the shoots and roots. The tolerant cultivar 'Bonica' could better maintain lower salt levels in the shoots, restricting them to the root system compared to 'Black Beauty'. The more tolerant character of Bonica to salt stress was also reflected in the superior growth performance for a similar salt level compared to 'Black Beauty'.

Chapter 7

General conclusion and perspectives

Chapter 7 General conclusion and perspectives

The water resources in Mediterranean countries are limited and are subjected to an increasing competition between agricultural, domestic and industrial uses. Climate warming will further enhance the need for irrigation but the high evapotranspiration rates, irrigation and reduced rainfall will contribute to salinization in these regions. The progression of this salinization is increasingly threatening agricultural production.

Vegetables are typically crops that are irrigated as they have a high cash value. The vegetable sector has become one of the strategic sectors of the Tunisian economy. In this research we focused on salt acclimation strategies in eggplants as these are potentially promising crops for the Tunisian horticultural industry. Human and natural selection lead to a wide genetic diversity among cultivated and wild species of eggplants. Looking for varieties which are more tolerant to abiotic factors becomes an urgent and crucial need. Improving salt tolerance of genotypes is often inhibited by the lack of effective evaluation methods for salt tolerance among genotypes (Zeng et al., 2003). The use of biotechnological methods may speed up the selection process. Salt tolerance, however, shows the features of a multigenic trait, and quantitative trait loci (QTLs) associated with tolerance were identified in some major agronomic crops as barley, citrus, rice, and tomato. However, the fact that a QTL represents many genes remains a problem to find key loci within a QTL. *In vitro* selection of salt tolerant cell lines is used in tolerance programs in various species including solanaceous plants. To generate variation within existing eggplant cultivars the first approach in this work was to exploiting somatic variation by tissue culture. Therefore we started with the development of a reliable regeneration system. We evaluated different explant types, increasing concentrations TDZ up to 0.4 µM as well as light quality. Although callogenesis, indirect and direct organogenesis were obtained shoot proliferation was not very high and the rooting phase was not successful. As this strategy proved to be a lengthy and troublesome tactic the research continued with an *in vivo* approach.

However, in field conditions the co-existence of multiple stresses is common. A tolerance strategy of plants to a specific stress can therefore better not be assessed in the field and the use of (semi) controlled conditions reduces the randomness of stress observed in field

conditions. In this study morphological, physiological and biochemical responses to increasing salinity levels was studied in four genotypes without prior knowledge of their tolerance level. Salt stress had a depressive effect on the studied parameters in all varieties though its degree depended on the variety and stress intensity. The observed responses lead to a better understanding of salt tolerance mechanisms in eggplant genotypes.

It is generally recognized that the sensitivity of a plant to salinity is influenced by its developmental stage. The majority of species are more tolerant at germination, the young seedling stage is more sensitive during emergence and as plants develop more they become increasingly more tolerant to salt stress. Also in this study different developmental stages of eggplant and their susceptibility to salt stress were studied.

Seed germination is one of the most crucial and decisive phases in the growth cycle of plant species as it determines plant establishment and the final yield of the crops. Germination is characterized by three phases. The first, imbibition involves rapid water uptake. In the second phase (lag phase), hydration of cotyledons and activation of preexisting enzymes may take place. In the third phase a further increment in water uptake marks the starting point of the growth phase, with enhanced cell division and radicle emergence. Therefore screening of salt tolerance at an early growth stage can be based on germination parameters and this we studied for four eggplant cultivars. Exposure to increasing NaCl concentrations did not only reduce the germination percentage but also decreased germination speed and rate as shown by the decrease in MDG and the increase in MGT under salt stress. Considering the germinations parameters under controlled saline conditions two groups with contrasting sensitivity responses were found. ‘Bonica’ and ‘Galine’ behaved as more tolerant cultivars while ‘Adriatica’ and ‘Black Beauty’ were the more susceptible cultivars and already sensitive to moderate stresses. The sensitive cultivars supported only moderate salt stress up to 40 mM NaCl while the tolerance level of the tolerant group (‘Bonica’ and ‘Galine’) was up to 80 mM NaCl though small variation depending on the parameter are present (Table 7.1). These initial germination observations were further confirmed by the **young seedling growth** under controlled conditions: increasing salt negatively affected fresh weigh, dry weigh, tissue water content, height and leaf number. In general the young seedling growth parameters were already influenced at slightly lower salt levels compared to the germination parameters. This reflects an avoidance strategy of seeds to prevent germination under stressful environment.

Table 7. 1: Sensitivity of the studied *in vitro* germination and growth parameters to salt stress (+++: very high sensitivity; ++:high sensitivity; +: moderate sensitivity; 0: no sensitivity). Values between brackets give the salt level (mM NaCl) where the studied parameter was significantly reduced compared to the control treatment (Chapter 3 and 5)

	Germination parameters				**Seedling biomass**					**Biomass vegetative phase**			
	Adriatica	Black Beauty	Bonica	Galine		Adriatica	Black Beauty	Bonica	Galine	Adriatica	Black Beauty	Bonica	Galine
Critical salt level for significant decrease					*Critical salt level for significant decrease*					*Critical salt level for significant decrease*			
Final seed germination	++ (40)	++ (40)	+ (40)	+ (40)	FW	++ (20)	++ (20)	+ (40)	+ (40)	++ (20)	++ (20)	+ (20)	+ (20)
MDG	+++ (40)	++ (80)	+ (160)	+ (160)	DW	++ (20)	++ (20)	+ (40)	+ (40)	++	++	+	+
MGT	++ (40)	++ (40)	+ (80)	+ (80)	WC/TWC	++ (40)	++ (80)	0	0	+++	+++	0	0
					Shoot length	++ (80)	++ (40)	+ (160)	+ (160)	++ (40)	++ (20)	+ (80)	+ (80)
Effect at 160 mM salt stress					Number of leaves	++ (40)	++ (40)	0	0	++ (40)	++ (80)	+ (80)	+ (80)
Final seed germination	+++	+++	++	++									
MDG	+++	+++	++	++									
MGT	+++	+++	++	++									

Although this germination and young seedling experiment under controlled conditions does not fully reproduce the field behaviour of plants, it discriminates tolerance levels between the cultivars. With respect to the observed effects on biomass susceptibility levels are very similar to those of the pot experiment during the **vegetative phase** (Table 7.1). It might therefore be a very useful screening technique to test relative cultivars tolerances. As Bonica is the main cultivar used in Tunisia, new introductions could be screened in comparison with Bonica and only cultivars with a similar behaviour or higher tolerance should be withhold for further field testing.

The first weeks of the production cycle of eggplants is the **vegetative phase**. Good leaf development (source) is necessary to support the following generative phase (sinks). In this thesis we focused strongly on effects of salt stress during the vegetative phase. For the experiments we used pot experiments, so that the salt stress was gradually imposed to the plants and adaptive strategies could be established.

It is generally accepted that increasing NaCl level, especially in the sensitive cultivars is expected to greatly disrupt **metabolic homeostasis** and therefore, could induce alterations at the physiological and biochemical levels (Bray et al., 2000). These alterations may be generated by three major hazards: (1) water stress caused by more negative water potential (elevated osmotic pressure) in the root environment; (2) specific ion toxicity associated with excessive Cl^- or Na^+ uptake (Ashraf and Harris, 2004); and (3) nutrient ion imbalance when the excess of Na^+ or Cl^- leads to a decline of the uptake of K^+, Ca^{2+}, $Mg^{2+}$and PO_4^- or to the impaired internal distribution of one or other of these ions (Gorham et al., 1985).

Chlorophyll fluorescence was used to evaluate photosynthesis dysfunction and to discriminate salt tolerance levels between four eggplant cultivars (Chapter 4). The salt stress induced a significant and early reduction of Φ_{PSII} indicating a decrease in electron transport through PSII in 'Adriatica' and 'Black Beauty' (respectively 6 and 8.4 g Na/100 g leaf DW at 160 mM NaCl). Φ_{PSII} was not affected in 'Bonica' and 'Galine' (respectively 2.2 and 2.5 g Na/100 g leaf DW at 160 mM NaCl) up to 25 days of salt stress indicating a more optimal functioning of PSII under salt stress. Moreover the linear electron flow rate continued at an effective rate in these cultivars.

Photosynthesis is one of the primary processes in plant metabolism (Chapter 6). Under increasing salt stress both Black Beauty and Bonica closed progressively their stomata to avoid excessive water loss. This in turn resulted in comparable lower assimilation rates despite the fact that Black Beauty and Bonica have a different accumulation pattern for leaf sodium. For both cultivars high correlations were found between leaf sodium and both total

and net photosynthesis. Comparing leaf sodium levels of chapter 4 and 5 (CF measurements) and chapter 6 (photosynthesis and CF measurements) leaf sodium levels were lower for the second experiment and differences between cultivars were less pronounced (respectively 3.8 and 1.3 g Na/100 g leaf DW for Black Beauty and Bonica at 160 mM NaCl). This might explain why in chapter 6 gas exchanges and also chlorophyll fluorescence parameters did not discriminate between these two cultivars. To have a better insight in the response of photosynthesis to salt stress it might be interesting to study responses of both the effect of salt levels and PEG treatment (osmotic stress) on photosynthesis as we cannot exclude that the reduction in photosynthesis was more due to osmotic effects than to ionic effects.

Both cultivars could maintain their mitochondrial respiration rates (chapter 6) similar to the control plants. Maintenance of ATP provision and oxidation of redox equivalents to support photosynthesis is a crucial role for mitochondria under stress conditions (Jacoby et al., 2011). Photorespiration in C3 plants may be considered as an alternative sink for light-induced electron flow during periods of restricted CO_2 availability in the chloroplast due to closed stomata. Furthermore there is a close cross-regulation with other metabolic pathways so as to maintain redox homeostasis under oxidative stress conditions (Foyer et al., 2009). Based on our observations Bonica enhanced more the photorespiratory pathway, which is one way to regulate oxidative stress, than Black Beauty under the highest salinity level.

Free radical-induced peroxidation of membrane lipids is associated with oxidative stress and MDA is widely used as an indicator of the extent of oxidative damage. We used this biochemical indicator both at seedling stage and at the vegetative stage. The responses were similar in the three salt stress experiments: susceptible cultivars (‘Adriatica’ and ‘Black Beauty’) accumulated high levels of MDA and tolerant cultivars (Bonica and Galine) did hardly increase MDA levels for a same salt stress level. This response reflect the capacity of the tolerant cultivars to better exclude Na and Cl^- from the cytosol via compartmentalization into vacuoles in comparison to the sensitive ones (‘Adriatica’ and ‘Black Beauty’). Besides tolerant genotypes regulated their osmotic potential more effectively by mitigating the uptake of Na and Cl^- and a simultaneous uptake of more essential ions such as K (Table 5.3). Also tolerant cultivars maintained a lower Na concentration in the leaves than sensitive ones while the inverse was found in the roots, this suggest that the tolerant cultivars have a more effective long-distance control and prevent translocation to the leaves.

Increasing salinity affects the soil matrix potential and plants need to maintain their internal water potential below that of soil to maintain turgor. On the one hand the inorganic ions that

accumulate in the cells will contribute to the cellular osmolarity though plants will also by their biochemical pathways accumulate compatibles solutes to maintain the influx of water into, or to a reduced efflux from cells, thus providing the turgor necessary for the expansion of cell. Most plants growing in a saline environment accumulate low molecular weight water-soluble metabolites in the cytosol such as proline. Proline accumulation is a common physiological response and this response was also found in the salt susceptible eggplant cultivars this as well at the seedling stage as in the vegetative stage. Proline accumulation was not found in the tolerant cultivars. A major function of proline is osmoprotection but also radical scavenging.

Also sugars that accumulate in response to stress can function as osmolytes to maintain cell turgor and have the ability to protect membranes and proteins from stress damage. Sugar accumulation has been associated with salinity tolerant mechanisms in many species. In this experiment, increasing salt stress led to an increasing sucrose, glucose and fructose levels in the sensitive cultivars 'Adriatica' and 'Black Beauty' therefore mobilizing their starch contents to maintain these high levels. In contrast, no accumulation but rather a decline in sucrose, glucose and fructose content was observed in the tolerant cultivars 'Bonica' and 'Galine' combined with starch accumulation. Especially this starch accumulation is not well understood as when reported in plants it is mainly linked with source-sink imbalances and resulting negative feed-back effects on photosynthesis. Growth reduction is also observed in the tolerant cultivars although not to the same extend as in the susceptible cultivars and this is reflected in a relative high plant tolerance index. Therefore it cannot be excluded that this reduced vegetative growth/reduced sink activity lead to the accumulation of starch as no need to increase the osmotic potential and to convert starch to soluble sugars was present. Yet, further research is needed to underbuild this hypothesis.

This study clearly showed that variation in salt tolerance in eggplant cultivars is also associated with variation of different physiological traits. Consequently, different parameters need to be investigated to understand how salt tolerance is established in the eggplant genotypes and to discriminate their relative salt tolerance levels.

A specific methodology depending on the growth stage (germination or vegetative stage) can be used. Certain of our assessed methods could be an easy and economical way for large scale screening as for instance in breeding and or selection programs. Yet for a good understanding combined physiological and biochemical traits should be considered in screening salt tolerance of eggplant genotypes rather than only a single specific trait. Our research showed that efficient screening procedures for germplasm evaluation could be based

on germination or on chlorophyll fluorescence but also that tolerance of eggplants is related to the concentration of sodium in the shoot. Another easy to measuring parameter is the leaf water content under increasing salt stress which stability or decrease was linked to tolerant or susceptible cultivars. This last aspect is to our knowledge not described in salt stress research though these responses are known with respect to drought stress responses (isohydric, anisohydric behaviour).

Although this study gives already a good insight in the responses of eggplant genotypes to salt stress, the experiments were conducted in controlled and semi-controlled environments where the interaction with other climatic variables was limited. The next step should therefore be to study the behaviour of the same varieties in the field conditions to interprete the interactions with high light intensities and/or elevated temperatures.

Although during the vegetative phase already a clear distinction between tolerance levels can be made further research should also investigate the effect of salt stress on the production potential in eggplant varieties. This requires assessing the impact of salinity on flowering and fruiting stages more specifically on the pollen fertility and abortion/necrosis of young fruits which could adversily affect yield. More in-depth studies could assess the impact of salt on the pollen tube growth and stigma receptivity. Precocious flowering under limited salt stress might also be interesting to study as this might be a tool to control the vegetative/generative balance in soilless culture systems. The study of the yield component is agronomically important as it will allow identifying the reduction rate caused by salinity in tolerant varieties and susceptible varieties. In addition not only total production but also fruit quality parameters (fruit development, average fruit weight) should be evaluated.

A more fundamental perspective could be the study of how sodium enters and is transported in eggplants as our study clearly discriminated in two different uptake and distribution patterns indicated by absolute Na values on the one hand and by the K/Na ratio on the other hand. If molecular markers could be defined for this trait then this would open possibilities for mass selection in breeding programmes as most breeding companies have the potential for high-throughput screening.

Breeding companies could also invest in high throughput phenotyping for salt tolerance in eggplant by analysing the seed germination parameters using leaf image analysis (RGB cameras) or to evaluate the effect on the photosynthesis by using image chlorophyll analysis.

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Curriculum Vitae

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Education

February 2008	Master of Science in Agriculture and improvement of crop production (second year of masters is the thesis); National Institute of Agriculture of Tunisia in collaboration with National Institute of Agronomic Research of Tunisia, INAT, Tunis, Tunisia.
June 2001	Diploma of Engineer (Five years of higher education); High Institute of Horticulture and Cattle Breeding of Chott Meriam Sousse Tunisia.
June 1996	Diploma of Baccalaureate experimental science.

Training

Avril 2001	Physico-chemical analysis of the soil .
May 2001	Determination of nitrogen content according to the Kjeldahl method.
January 2006	Chlorophyll index measurements with SPAD-502, National Institute of Agronomic Research of Tunisia.
April 2006	Measurement of the peroxidase activity
March 2007	Molecular Biology (PCR and Micro-satellites techniques), National Institute of Agronomic Research of Tunisia.

October 2012	Modern method of olive harvest.
December 2012	Work psychology and human resource management and organization.
January 2013	Compost production and method of use.
March 2013	Quality of vegetable products and marketing methods.
May 2013	Performance and merit techniques.
Jun 2013	Good governance and anti-corruption.
January 2015	Phytosanitary maintenance of fruit trees.
February 2015	Budget management by objective.
April 2015	Methodology of work in agricultural extension.

PROFESSIONAL CAREER

2008 – 2010	High Institute of Horticulture and Cattle Breeding of Chott Meriam Sousse Tunisia. Realization of research work within a research unit on 'Conservation and Plant Resource Development through the creation of botanical gardens'. Function: PhD student.
2008 – 2011	High Institute of Horticulture and Cattle Breeding of Chott Meriam Sousse Tunisia. Practical teaching of vegetable crops, spice crops and seeds and Horticultural Plants. Function: Assistant.
2012-2015	Tunisian Ministry of Agriculture. Function: research engineer.
2016	Ghent University from 01/01/2016 to 30/06/2016 (BOF scholarship granted by University of Ghent).

SCHOLARSHIPS

2011	Research stay at Ghent University from 01/05/2011 to 31/08/2011 (scholarship granted by Tunisian government).
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PUBLICATIONS

M'Hamdi, M., **Hannachi, S**., & Mehouachi, T. (2011). Effets de huits porte-greffes sur la croissance végétative et la production en fruits chez deux variétés de pastèque (*Citrillus lanatus*). Revue de l'INAT, Volume 26(1), 111-120, category: AGRONOMY, Tunisie.

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ORAL PRESENTATION