Phenotypic and genetic analysis of salinity tolerance in tomato introgression lines

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Abstract

Phenotypic and genetic aspects of two different tomato inbred line populations were evaluated for salinity tolerance purposes.

The first population was the result from a cross between *Solanum lycopersicum* M82 and *S. pennellii* (LA716). A phenotypic analysis was performed and the traits that showed a significant Genotype x Environment (or Treatment) interaction in a Restricted Maximum Likelihood test and some other relevant characteristics as well were used to select which genotypes presented a higher salinity tolerance. Based on the results, a set of genotypes was proposed for further analysis: ILZ4-2, ILZ4-3, ILZ5-1, ILZ5-5, ILZ6-2 and ILZ12-2.

On the second population resulting from the cross of *S. lycopersicum* (Moneyberg) x *S. chmielewskii* (LA1840), the offspring of line 56 was analyzed to determine the presence of introgression on chromosomes 4, 6 and 12. The main reason for this research was based on the performance in the greenhouse of a line (PV091144) that apparently did not have any introgressions but it was behaving different than the control. Therefore, an analysis with Cleaved Amplified Polymorphic Sequences (CAPS) was made to verify the presence of introgressions in chromosomes 4, 6 and 12 through all the lines and only introgressions in chromosomes 4 and 12 were found. A further analysis with Single Nucleotide Polymorphisms (SNPs) determined a reduced introgression in chromosome 6 related to the line with no introgressions (in chromosome 4 and 12) and the line with an introgression only on chromosome 12 (PV091140).

Introduction

General Aspects of Salinity

Salinity is one of the main threats to the world's food production and it impairs crop production on irrigated land. Even salinity is a common phenomenon for arid and semiarid regions of the world, salt-affected soils are observed in all the climatic regions. In fact, about a half of all the existing irrigation systems of the world are under the influence of secondary salinization, alkalization and waterlogging and a lot of the irrigated lands are abandoned each year because of the unfavourable effects of secondary salinization and alkalization (Szabolcs 1987). The unfavourable soils of low fertility cause an unacceptable yield reduction. Therefore, research on plant responses to salinity has rapidly expanded in recent decades (Dajic 2006).

Dry land salinity is linked to rising water tables brought about by increased deep drainage of rainfall following forest clearance and a change from deep-rooted perennial plants to a shallow-rooted annual crop (Flowers and Flowers 2005). Besides the naturally formed saline and sodic soils, the presence of secondary salt affected soils is becoming even more visible, and one reason is different agricultural practices, mainly a bad management of irrigation. However, there are human influences that lead to adverse effects of secondary salinization, such as: overgrazing, deforestation, contamination with chemicals and accumulation of airborne or waterborne salts (Dajic 2006).

Plants differ greatly in their tolerance of salinity. The variation in salinity tolerance in dicotyledonous species is even greater than in monocotyledonous species. In the simplest analysis of the response of a plant to salinity stress, the reduction in shoot growth occurs in two phases: a rapid response to the increase in external osmotic pressure, and a slower response due to the accumulation of Na^+ in leaves (Munns and Tester 2008). Salt tolerance is the ability of plants to grow and complete their life cycle on a substrate that contains high concentrations of soluble salt (Parida and Das 2005). In the particular case of tomato, although there are comparatively salt tolerant relatives, it has proved difficult to enrich elite lines with genes from wild species that confer tolerance because of the large number of genes involved, most of them with small effect in comparison to the environment, and the high costs of recovering the genetic background of the receptor cultivar (Cuartero et al 2006).

Plants can be roughly divided into two major groups: a) halophytes, that can withstand even 20% of salts in the soil and, in most cases, successfully grow in conditions with 2-6% of salts, and b) non-halophytes or glycophytes plants exhibit various degrees of damage and limited growth in the presence of sodium salts (usually higher than 0.01%). However, there are great differences in the level of salt stress tolerance within both the halophytes and nonhalophytes, which include sensitive, moderately tolerant and very tolerant species. Although halophytes represent only 2% of the terrestrial plant species, they are present in about half the higher plant families and exhibit a great diversity of plant forms (Dajic 2006). Tomato is a moderately tolerant plant.

Plants suffer three potential effects due to salinity: i) lowering of the water potential, ii) direct toxicity of any Na and Cl absorbed and iii) interference with the uptake of essential

nutrients (Flowers and Flowers 2005). In plants, there are two response phases: the first phase (which is osmotic) starts immediately when the salt concentration around the roots increases to a threshold level, and the consequence is that the rate of shoot growth falls significantly. The second phase (ion-specific) to salinity starts when salt accumulates to 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), and they die. If the rate at which they die is greater than the rate at which new leaves are produced, the photosynthetic capacity of the plant will no longer be able to supply the carbohydrate requirement of the young leaves, which further reduces their growth rate (Munns and Tester 2008).

One common effect of salt stress in plants is the reduction in leaf growth rate which is related to the reduction of cell turgor, to cell wall rheological properties and to reduction in photosynthetic rate. Growing tomato plants with saline water produces an unbalance physiology of leaf ion contents by increasing Na and Cl concentrations and diminishing K, Ca_2, Mg_2 and NO_3. The increase of Na and Cl in leaves lowers the osmotic potential, so contributing to the maintenance of the water potential difference between the leaves and the soil required to obtain water from the saline solution. Therefore, in a simple explanation, the plants able to accumulate more Na and Cl would absorb water more easily and be more tolerant to salinity (Cuartero and Fernandez-Munoz 1999).

In summary, the mechanisms of salt tolerance are of two main types: those minimizing the entry of salt into the plant (or at least their accumulation in photosynthetic tissues) and those minimizing the concentration of salt in the cytoplasm (Munns 2002). This corresponds with two major adaptive strategies of plants to tolerate high environmental salinity: 1) stress avoidance, that are related to different physical, physiological and/or metabolic barriers with which the adverse effects of stress are ameliorated, and 2) stress tolerance, the linkage of adaptive mechanisms which enable successful survival despite the influence of stress internally. It is clear that the regulation of Na uptake and transport across the plasma membranes and tonoplast will be a key factor determining the plant cell response to salinity stress (Dajic 2006).

Effects of Salinity in Tomato

In the case of tomato, its commercial cultivars are moderately sensitive to salinity at all stages of development, including seed germination, vegetative growth and reproduction. Most crops must be grown under irrigation and inadequate irrigation management leads to salinisation of water resources and soils. For this reason, in the areas with an optimal climate for tomato, salinity becomes a serious constraint (Cuartero and Fernandez-Munoz 1999). Moderately sensitive crops, such as tomato, tolerate an EC of the saturated soil extract up to about 2.5 dS m^{-1} without any yield reduction (Maas 1986). Salinity applied during the day or in spring or summer cultivation produces a higher yield reduction than if it is applied during the night or in autumn cultivation (van Ieperen 1996) and the main reasons are the effect of higher temperatures and illumination and in addition the lower relative humidity in summer time that lower water potential in the plant by inducing faster transpiration and in the case of the fruits high salinity also decreases the water potential in the plant which leads to a reduction of the water flow into the fruit and therefore the rate of fruit expansion (Johnson et al 1992).

Regarding yield in tomato, it can be reduced by decreased average fruit weight and/or the lowering in the number of fruits produced by the plant. At relatively low ECs, the yield reduction is caused mainly by a reduction in the average fruit weight; however the fruit number remains unchanged. In the case of higher ECs, the declining number of fruits explains the main portion of yield. If yield is compared between control and salinised plants the difference becomes more marked as the harvest period progresses mainly due to reduced fruit size during the first 4 weeks of harvesting but later, fruit number also decreases (Cuartero and Fernandez-Munoz 1999)

In simulation modelling experiments performed by Heuvelink et al (2003) in tomato, salinity can reduce growth and yield because of the impact on plant water relations by increased fruit dry matter percentage, reduced leaf expansion and stomatal closure. In addition, reduced leaf elongation results in small leaf size and reduced light interception.

There are cultural strategies that can prevent the effects of salinity in tomatoes, between those alternatives the pretreatments, the modification of relative humidity and grafting seemed to provide benefits. Regarding the pretreatments, the use of them at particular growth stages increases the capacity of plants to adapt to salinity and tolerate it better than non-adapted plants. One of this treatments is seed priming, has been used during germination and early growth stages and also during fruiting. The process consist in seeds primed in 1 M NaCl for 36 h which produced a greater fruit yield at low (35 mM NaCl) and moderate (70 mM NaCl) salt levels in irrigation water (Cuartero et al 2006). In the case of relative humidity, salinity in soils or in the irrigation water also restrict water availability to plants in a similar manner to water stress, which causes reductions in growth rate and even in production (Munns 2002) and alters plant water relations (Romero-Aranda et al 2000). One strategy to alleviate water deficit imposed by salinity could be to modify relative humidity around the plants (Li et al 2004). Grafting is an alternative that seems suitable to acquire salt tolerance. Cultivars that are good producers can be grafted to rootstocks able to reduce the effect of external salt on the shoot, with the additional benefit of combining good shoot characters with good root characters. In tomato, grafting does improve plant adaptation to salt stress in cultivars of determined and undetermined growth and the results obtained by Santa-Cruz et al. (2002) and Estañ et al (2005) suggest it. The selection of adequate rootstocks could reduce the toxic effect of saline ions, which is the main deleterious effect in the long-term (Cuartero et al 2006).

Breeding for Salinity Tolerance in Tomato

Even if cultural practices could help to prevent salinity effects in tomato, it would be ideal to have lines that are tolerant to stress conditions in high electric conductivity. Although there are salt tolerant relatives of the cultivated tomato, it has proved difficult to produce elite lines that have incorporated exotic genes from wild species that confer tolerance and the main reason is the large number of genes involved. The mechanisms for salt tolerance are not fully understood and therefore, the breeding towards this trait is multi-factorial and hard to target. In addition to this, the costs of recovering the genetic background of the receptor cultivar are high (Cuartero et al 2006).

Salinity tolerance exhibits a quantitative inheritance and also is affected on a high degree by the environment, which involves a lot of traits, genes and their interaction. Because of this

complexity, the genetic variation in the wild germplasm for quantitative-agronomic traits remains largely unexploited. The first step to incorporate these physiological characters in a breeding programme is to prove the genetic variability in the available germplasm (Cuartero et al 2006).

Therefore it is very important to reduce the number of lines to be scored, which is very convenient for a proper phenotyping of traits that are difficult to evaluate, such as most physiological components of salt tolerance. Besides, with more replicates of each individual, the environmental variation can be minimized, which is a way to increase the reliability of the heritability determination. In an ideal scenario, heritabilities of the characters should be used to select the most relevant characters to evaluate the salt tolerance of segregant populations in QTL studies (Cuartero et al 2006), however it should be performed in conditions that allow having a high degree of confidence on the results and it needs a proper statistical setup.

In general, yield and quality should be the leading characteristics in any breeding program because after reduction by salinity, the crop must still be sufficient not only to cover the expenses but also to provide profit for the producer. However, salinity produces so many disturbances to plant morphology and physiology that the only way to achieve profitable yields under saline conditions might be by combining in one cultivar different morphological and physiological characteristics to make a cultivar close to the ideotype. A large number of characteristics suitable for use in breeding for salt-tolerance have emerged, and for the shoot the most outstanding are: vigor, shoot dry weight, stem growth, leaf area, leaf growth rate, leaf dry weight, succulence, water-use efficiency, Na distribution between young and old leaves, leaf K/Na, accumulation of Na, Cl, Ca_2.and NO_3 within the leaf, foliar ion regulation index, proline, myo-inositol, and stress symptoms. At fruiting the most relevant traits at the moment are: fruit size, number of fruits, pollen quantity and blossom end rot (Cuartero et al 2006).

To incorporate the traits that eventually will lead to an ideotype, it is necessary to find genetic variability; therefore genotypes with high expression of those characteristics are essential for a breeding program. In the case of tomato, it is closely related to cross compatible wild species. However, the more closely related a donor genotype to the current cultivars, even if differences in tolerance are low, the more useful the line is to the breeder. Some authors consider that as the mentioned traits related with salinity tolerance are not combined together in a single donor but in several genotypes a number of donors should then be employed in the breeding program for pyramiding all those characteristics in a single cultivar (Yeo and Flowers 1989). Tomato breeding should also resort to pyramiding characteristics since no described trait alone is likely to produce a tolerant genotype. Additionally, it should be considered that tomato hybrids have monopolized the market; therefore the traits involved in salt tolerance should then be pyramided within the parents of current hybrids in such a way that they acquire tolerance to .salinity and at the same time maintain all the traits that make a current hybrid competitive. Consequently, the introduction of the characteristics related to salt tolerance in parents of current hybrids should require separate breeding programs for each trait (Cuartero and Fernandez-Munoz 1999).

As it was mentioned before, wild relatives of tomatoes are an important source of genes for a plant breeding program because cultivated varieties of tomato are, as a whole, extremely depleted in genetic variation, whereas the related wild species are by all measures highly diverse (Chetelat et al 1995). All wild species are native to western South America and distributed from

central Ecuador, through Peru to northern Chile. Wild tomato species grow in a variety of habitats, from near sea level along the arid Pacific coast to over 3300 m in the numerous valleys of the western side of the Andes (Rick 1973; Taylor 1986). All wild relatives of tomato are diploids (2n = 2x = 24) and can be crossed to the cultivated tomato (however sometimes with difficulty). They are of great use in breeding programs as sources of disease resistance and agronomic traits (Peralta and Spooner 2005).

One of the most studied wild relatives of tomato towards salinity tolerance is *Solanum pennellii,* which originated in the arid habitats of Peru. In contrast to the cultivated tomato, *S. pennellii* accumulates more Cl and Na ions and was not impaired by the high NaCl concentrations. The *S. pennellii* plants that are more salt-tolerant seem to be more drought resistant (Dehan and Tal 1978). A genomic library was developed by Eshed and Zamir (1994) using as background the highly inbred open pollinated processing tomato variety M82 (as the female parent) and it was crossed to the highly inbred accession of *S. pennellii* LA716. A set of 50 plants provided a complete coverage of the rest of the genome. Plants were backcrossed and then selfed, and those homozygous for the desired *S. pennellii* introgression were selected. The introgression lines can be used as a highly polymorphic perpetual mapping resource and the availability of data for 350 markers for the 50 introgression lines allows screening of the population and the differences in the quantitative traits measured between M82 and the introgression lines or their hybrids with different inbred parents, can be attributed to the alien chromosome segments (Eshed and Zamir 1994).

Another wild relative of tomato that have shown potential as trait donor for salinity tolerance is *S. chmielewskii*, a plant that has been used to acquire characteristics related to sugar contents like sucrose and higher soluble sugars (Chetelat et al 1995). Kontopoulou (2009) determined that particular lines of tomato with introgressed areas of *S. chmielewskii* were tolerant to high salt concentrations. These inbred lines were developed by Keygene Inc (Wageningen, The Netherlands) by crossing *S. lycopersicum* cv. Moneyberg (a selection of Moneymaker) x *Solanum chmielewskii*. In a first screening Kontopoulou (2009) determined that line 56 did not show any reduction in fresh yield at high salinity, because of the unchangeable total dry weight and the increase of fraction to the fruits. Therefore line 56 obtained higher yields at both salinity levels, compared to Moneyberg. In the information provided by Keygene line 56 presented introgressions in chromosomes (chr) 10 and 11, however, studies performed by Grandillo (personal communication, May 6 2010) determined the presence of introgressions in chr 4 (heterozygous), 6 (homozygous) and 12 (heterozygous). Later on, Trotta (personal communication May 6, 2010) detected the introgressions in chr. 4 and 12 but the chr 6 introgression was not found.

Purpose of the Research

The aim of this research towards a better comprehension of the effect of salinity in tomato, is two different perspectives: i) a phenotypic evaluation of the Zamir lines to determine which lines can be evaluated in more detail and ii) a molecular evaluation of the *S. chmielewskii* lines to verify the presence of the introgressions in chromosomes 4 and 12 and to study what happened to the segment on chr 6.

Chapter 1

Phenotypic analysis of introgression lines of *S. lycopersicum* cv M82 x *S. pennellii* LA716 (a processing tomato with sections of salt tolerant *S. pennellii*) in two different salt treatments.

Materials and methods

Plant Material: the population studied consisted on 48 genotypes of the Eshed and Zamir (1994) inbred lines that were phenotyped for traits related to salt tolerance to determine which lines are promising for more detailed experiments.

Measurements: the plants were evaluated at different time points: at the beginning [1], middle [2] and at fruit harvest [3]. The salt treatment was applied for two months. The measurements executed were:

- Height (He), which was measured with a flexible metric tape measure from the bottom of the plant to the top meristem.
- Chlorophyll content (CC) at top (1), middle (2) and bottom (3), measured using a SPAD meter 502 (Minolta). Each measurement evaluated was an average of two measurements in the leaflet sampled.
- Stomatal conductance (Gs, sampled with a SC-1 leaf porometer (Decagon). Two measurements per plant were performed on leaflets that were in direct exposure to sunlight and they were averaged.
- Number of leaves (LNr), trusses (TNr), green fruits (GFNr), red fruits (RFNr), fruits with blossom end rot (BFNr) and total number of fruits (FNr) were counted in each plant.
- Leaf temperature (LT) was determined with an infrared laser thermometer (Extech) in two leaves that were in direct contact to sunlight.
- Relative water content (RWC) was determined calculating:

$$RWC = \frac{Fresh\ Weight - Dry\ Weight}{Saturated\ Weight - Dry\ Weight} \ x\ 100$$

  The weight was measured in a scale (Sartorious). The saturated weight was measured from lealflets that were placed in a petri dish with dionized water overnight.
- Weight of yellow leaves (Ywe), green leaves (Gwe), total leaves (TLWe), stem (Swe), green fruits (GFWe), red fruits (RFWe) and total fruits (TFWe), total fruit weight ratio (TFWeR) (percentage of the total plant weight that belonged to the fruits), total plant weight (TPWe). All weights were measured with a Sartorious scale.
- Leaf area of a leaflet (LA), measured with a ruler at the largest and widest part of the leaflet. Total leaf area (TLA) was measured from the top eight leaves with a portable leaf area meter (LI-3100C)

- Ion content (IC) was analyzed using an ion chromatographer an IC –Metrohm (881 Compact IC Pro with an 858 Professional Sample Processor) for leaves at top (T), middle (M) and bottom (B). The detailed protocols and the equipment used are explained in the Appendix.

Statistical analysis: descriptive statistics were performed in Excel (Microsoft Office) and to determine the variance in the population a Residual Maximum Likelihood (REML) was performed instead of an analysis of variance (ANOVA) because the experimental design was statistically unbalanced. The test was performed using GenStat (12^{th} Edition) to determine the variance of the traits. The following fixed model was used: Genotype + Treatment + Genotype*Treatment. The blocks were the random model.

Lines selection: the Genotype*Treatment significant interactions were used as main criteria to select a genotype and also the lines that perform better in a particular trait or the combination of them.

Results

General

In this report the genotype traits were selected based on a significant Genotype x Treatment (GxT) (by 95% confidence) and salt tolerance physiological characteristics that are considered relevant. There was a clear treatment and genetic effect in most of the traits, however only some characteristics evaluated demonstrated GxT.

In Table 1.1 it can be observed the results of the REML analysis that was obtained using Genstat. Further, the selected traits are explained in detail. The traits selected were: chlorophyll content, stomatal conductance, fruit number (green and total), fruit fresh weight (red, green and total) and total fruit weight ratio, total leaf area, and the effect of, Na, Cl and PO_4. In addition to these traits red fruit number, BER and the levels of ions K, Ca and K/Na were analyzed.

In general terms, the lines that performed better in most of the traits were ILZ4-2, ILZ4-3, ILZ5-1, ILZ5-5, ILZ6-1 and ILZ12-2; therefore in the results a major description of the traits and these lines is shown.

Chlorophyll content

In general terms, it was observed that the salt treatment increased the chlorophyll content (CC) and there is a decrease of chlorophyll through time. In addition to this, the higher amount of chlorophyll was observed in the top leaves (T) and the lower at the bottom leaves (B) for both treatments (Figure 1.1). The lines that had more CC were not necessarily lines that had a high fruit yield. The CC cannot be correlated directly with leaf area expansion and fresh weight because the measurements were done in different time points.

Regarding GxT interactions, only the measurements at the top leaves showed high significance. The selected genotypes ILZ5-1, ILZ5-5, ILZ6-1 and ILZ12-1 increased their chlorophyll content more drastically than M82, but for lines ILZ4-2 and ILZ4-3 the chlorophyll content was lower than M82 (Figure 1.2).

Table 1.1. Results of REML analysis on traits evaluated at different time points.

Trait	He[1]	He[2]	He[3]	CCT[1]	CCT[2]	CCM[1]	CCM[2]	CCB[1]	CCB[2]	Gs[1]	Gs[2]	LNr[1]	LNr[2]	LNr[3]	TNr[1]	TNr[2]	TNr[3]
Genotype	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Treatment	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
G x T	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-

Trait	FNr[2]	FNr[3]	GFNr[3]	RFNr[3]	BFR [3]	LT[2]	RWC[2]	FYLWe[3]	FGLWe[3]	TFLWe[3]	FSWe[3]	DYLWe[3]	DGLWe[3]	DTLWe[3]	DSWe[3]
Genotype	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Treatment	-	-	+	-	+	-	+	-	-	-	+	-	-	-	-
G x T	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-

Trait	FGFWe[3]	FRFWe[3]	FTFWe[3]	DGFWe[3]	DRFWe[3]	DTFWe[3]	FTPWe[3]	DTPWe[3]	TPWeR[3]	TFWeR[3]	LA[2]	TLA[3]	K(T)	K(M)	K(B)	Na(T)	Na(M)
Genotype	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+
Treatment	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	+
G x T	+	+	+	-	-	-	-	-	-	+	-	+	-	-	-	+	+

Trait	Na(B)	K/Na(T)	K/Na(M)	K/Na(B)	Ca(T)	Ca(M)	Ca(B)	Cl(T)	Cl(M)	Cl(B)	PO4(T)	PO4(M)	PO4(B)	SO4(T)	SO4(M)	SO4(B)	Mg(T)	Mg(M)	Mg(B)
Genotype	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-
Treatment	+	+	+	+	-	-	-	+	+	+	-	+	-	-	-	-	-	-	-
G x T	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-

Note: meaning for the abbreviations (there are three different time points 1,2 and 3 and T: top, M: middle and B: bottom). He: Height; CC: chlorophyll content; Gs: stomatal conductance; LNr: leaf number; TNr: truss number; FNr: total fruit number, GFNr: green fruit number; RFNr: red fruit number, BFNr: Blossom End Rot fruit number; BFR: Blossom End Rot fruit ratio; LT: leaf temperature; RWC: relative water content; FYLWe: yellow leaves fresh weight; FGLWe: green leaves fresh weight; TFLWe: total leaves fresh weight; FSWe: stem fresh seight; DYLWe: yellow leaves dry weight; DGLWe: green leaves dry weight; DTLWe: total leaves dry weight; DSWe: stem dry weight; FGFWe: green fruits fresh weight; FRFWe: red fruits fresh weight; FTFWe: total fruits fresh weight; DGFWe: freen fruits dry weight; DRFWe: red fruits dry weight; DTFWe: total fruits dry weight; FTPWe: total plant fresh weight; DTPWe: total plant dry weight; TPWeR: Total plant weight ratio; TFWeR: total fruit weight ratio; LA: leaflet area; TLA: total leaf area; K: potassium; Na: sodium; K/Na: potassium / sodium; Ca: calcium; Cl: chloride; PO_4: phosphate; SO_4: sulphate; Mg: magnesium.

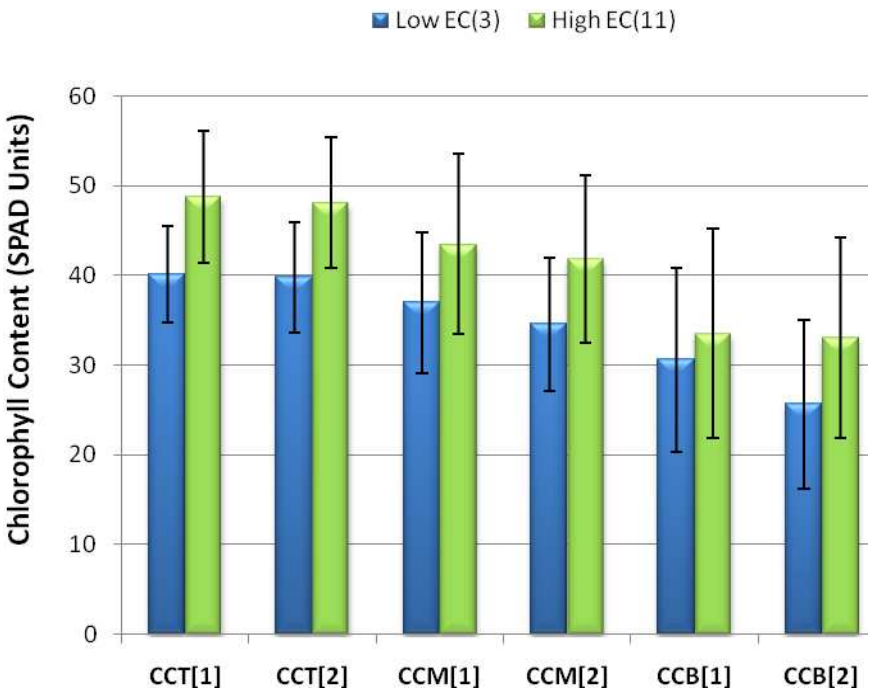

Figure1.1. Chlorophyll content average evaluated per treatment at top (T), middle (M) and bottom (B) leaves at two different time points (1 and 2).

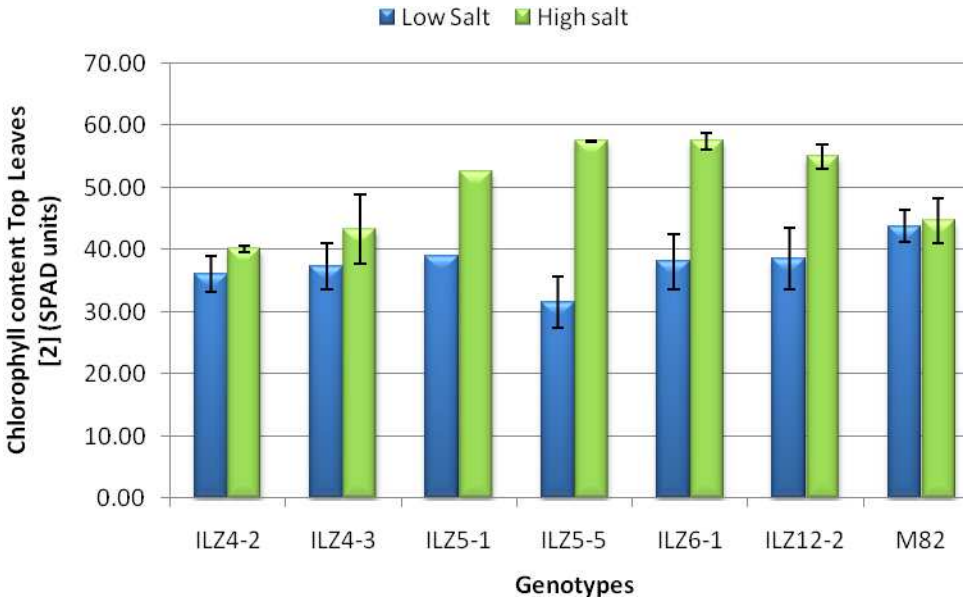

Figure 1.2. Chlorophyll content at second time point for selected lines at low and high EC treatments.

Stomatal conductance

The stomatal conductance relates to the ability of the plant to cope with the loss of water in stress conditions. This particular trait can be affected by environmental conditions: light, relative humidity and temperature, therefore the variability between measurements is very high. In general, stomatal conductance was reduced through time under low and high EC (Figure 1.3). After the REML analysis, only the measurements of the first time point showed a significant GxT. M82 decreased their gas exchange considerably, however the lines selected showed a smaller decrease (so they were less affected) and in the case of two genotypes (ILZ5-1 and ILZ6-1) there was an increase in the gas exchange (Figure 1.4).

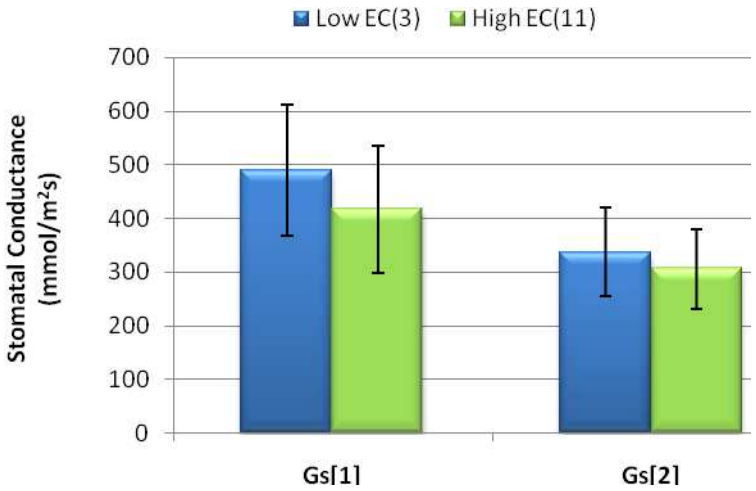

Figure 1.3. Stomatal conductance averages at two different time points per treatment.

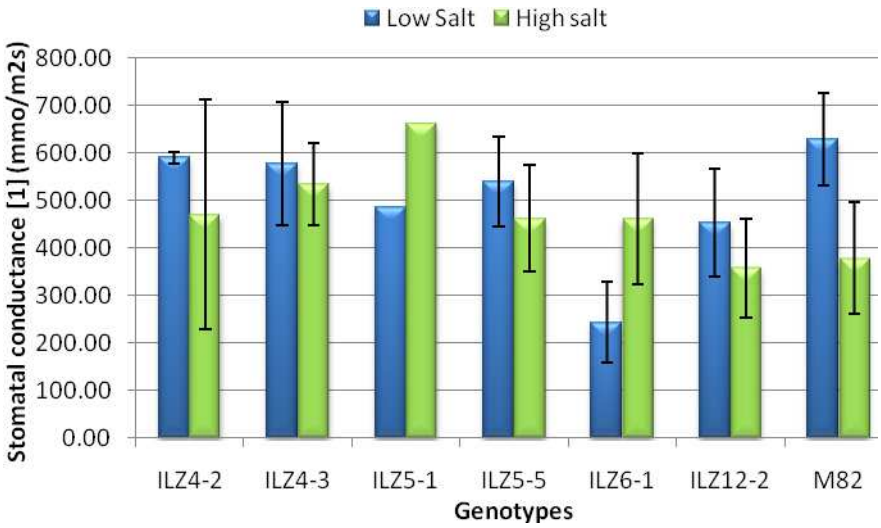

Figure 1.4. Stomatal conductance on inbred lines selected as promissory lines.

Fruit number

In general terms, there was a reduction of the number of fruits produced except for red fruits (there was an increase of 6.5%) (Figure 1.5). The more pronounced reduction of fruit number was observed in green and the total number of fruits. The red fruits were basically the same at both treatments.

For the selected lines, regarding green fruits, there was a big reduction at lines ILZ4-2, ILZ5-1 and ILZ6-1 while the other lines increased the amount of fruits. The reduction of M82 was very abrupt as on the lines previously mentioned (Figure 1.6). In the case of red fruits, even M82 increased the number, while the lines ILZ4-2 and ILZ12-2 reduced their amount (Figure 1.7). For the total fruit number, the lines ILZ4-3 and ILZ5-5 increased their amount, while the other lines did not decreased drastically. In the case of M82 the number of fruits was acceptable compared to the other lines (Figure 1.8).

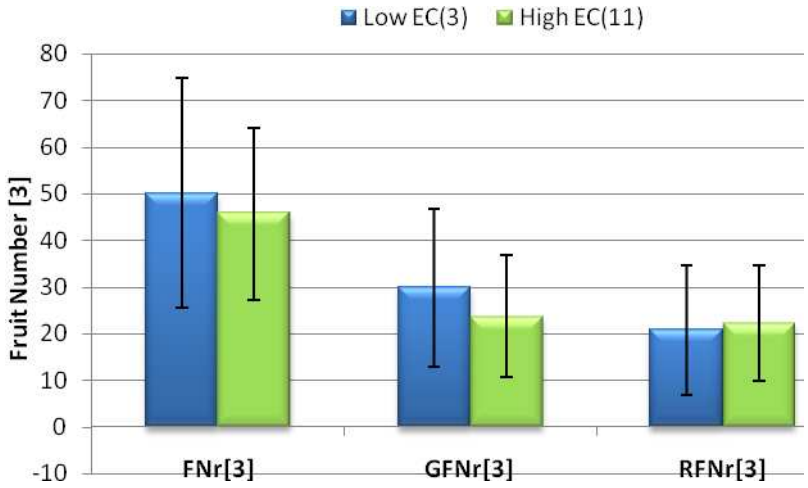

Figure 1.5. Fruit numbers averages at harvest per treatment. FNR: total fruit number, GFNr: green fruit number and RFNr: red fruit number

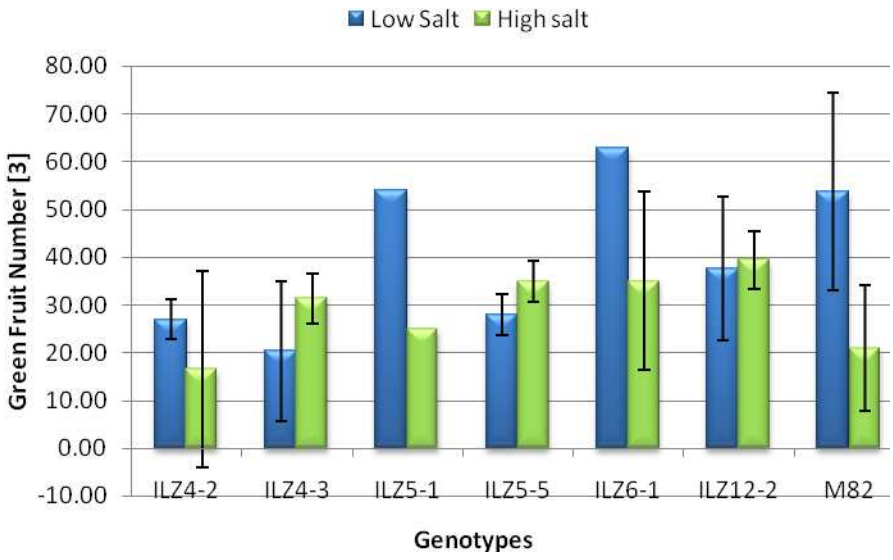

Figure 1.6. Green fruit number on low and high salt concentrations for the lines selected

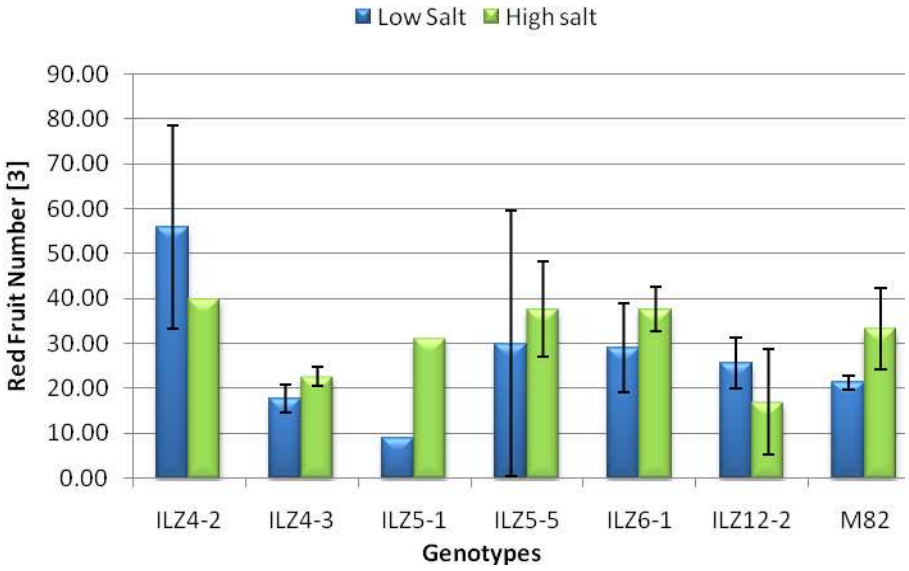

Figure 1.7. Red Fruit number for genotypes selected on low and high salt treatments.

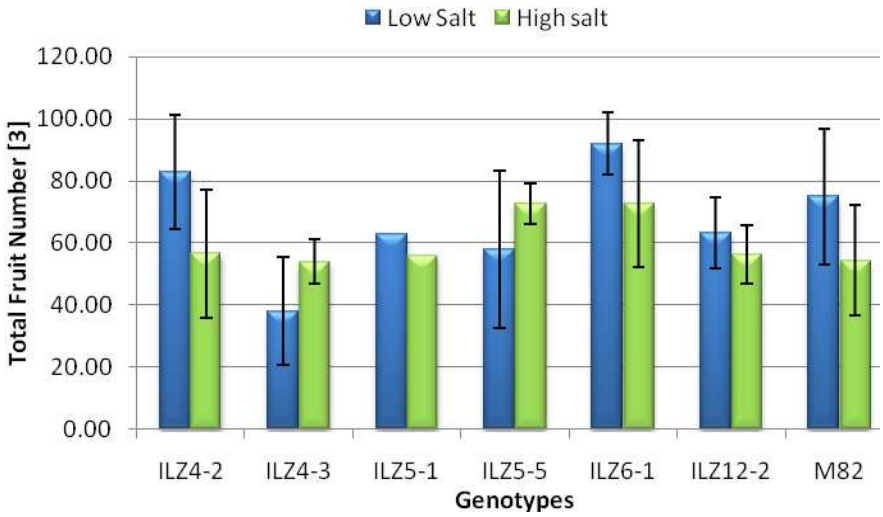

Figure 1.8. Total fruit number of selected inbred lines at third time point.

Fruit weight

This measurement was performed on the five bigger fruits and not the whole harvest. Only the fresh weight showed a significant GxT and in general there was a significant decrease from the low to the high salt treated plants on red, green and total fruits weights (Figure 1.8). The decrease was less prominent on green than red fruits. For M82, the reduction of weight was considerable in green and red fruits, while in the selected lines there is a reduction of weight in all of them, except at ILZ12-2 for green fruits (Figure 1.9 and 1.10). If this trait is compared to fruit number, the effect of the treatment is more evident for the fruit weight. Regarding the total fruit number, line ILZ4-3 showed the higher weight; however, there was high variation in the samples (Figure1.11). All the selected lines performed better than M82 under salt stress.

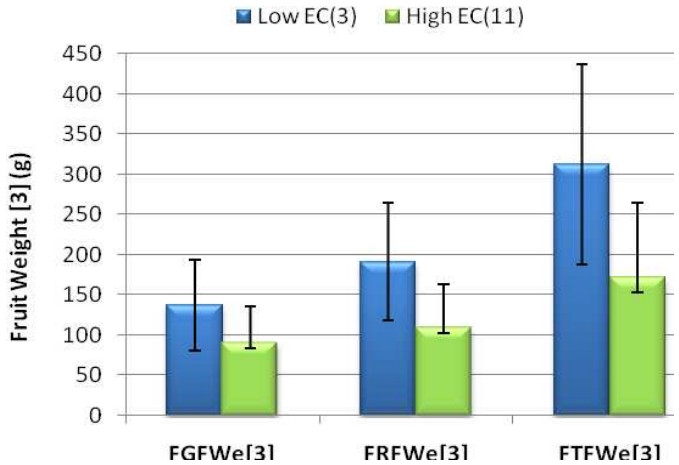

Figure 1.8. Average fruit fresh weights at harvest for freen fruits (FGFWe), red fruits (FRFWe) and the total fresh weight (FTFWe).

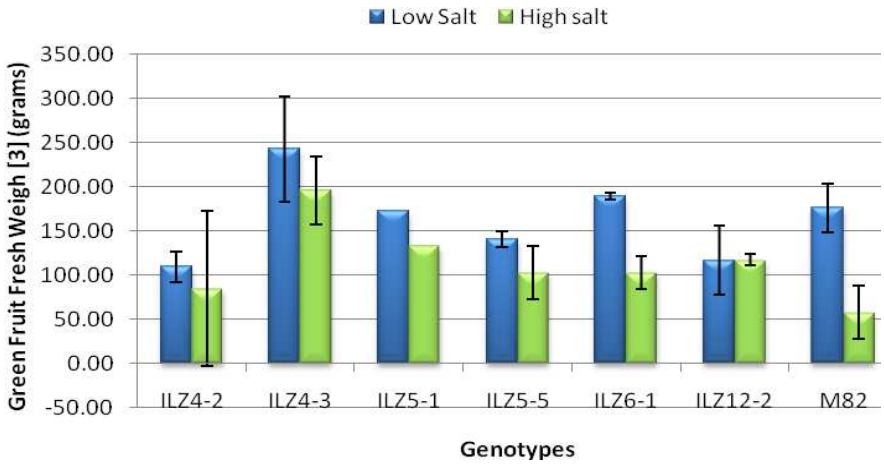

Figure 1.9. Green fruit fresh weight for genotypes selected.

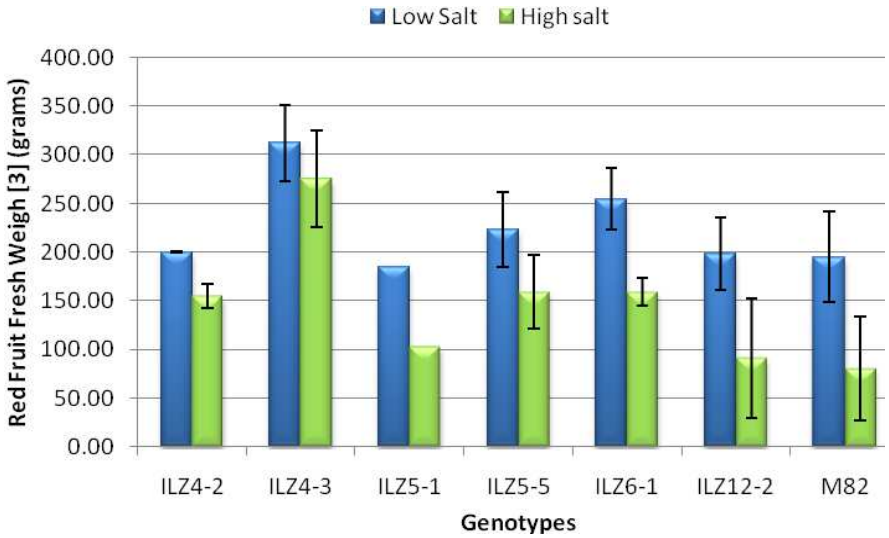

Figure 1.10. Red fruit fresh weight for selected genotypes under two salt treatments.

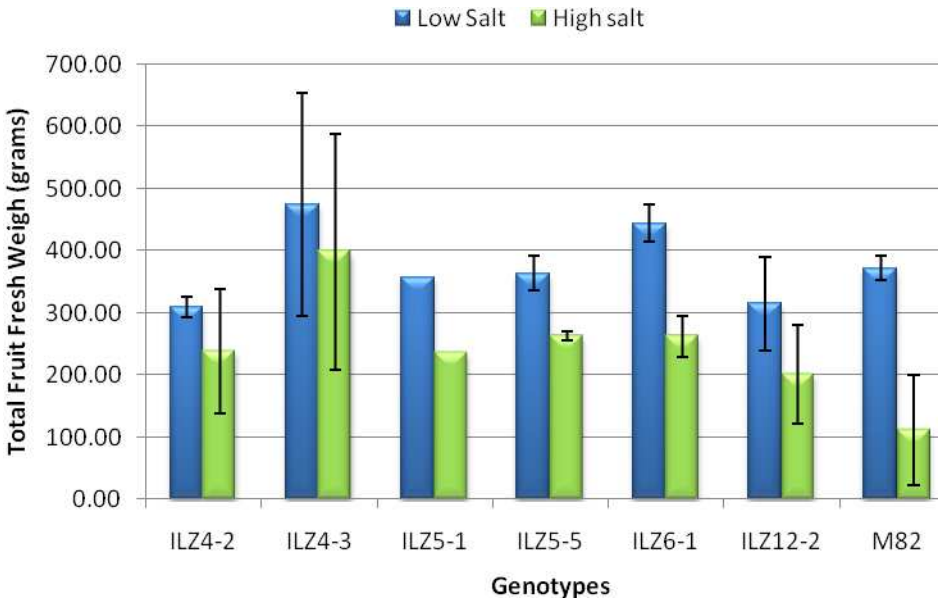

Figure 1.11. Total fruit fresh weight of genotypes at harvest under low and high salt.

Total Fruit Weight Ratio

The ratio represents what percentage of the total biomass belongs to the production of fruits. In general there was a decrease from the control to salt treatment; however it was small (Figure 1.12), inside each treatment there was high variability which demonstrates that in this trait is possible to find lines that contain a high fruit ratio. Under the lines selected, there were three lines that increased their ratio under high EC, lines ILZ4-2, ILZ4-3 and ILZ5-1, in the case of M82, there was a reduction, but it was smaller compared to the other genotypes selected (Figure 1.13).

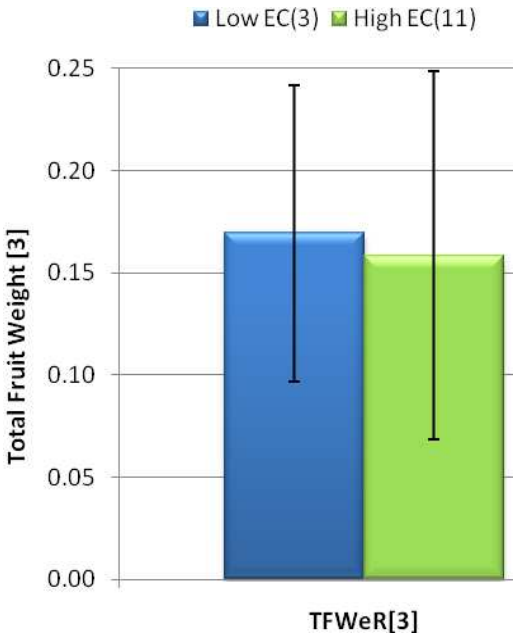

Figure 1.12. Total fruit weight ratio averages at low and high salinity.

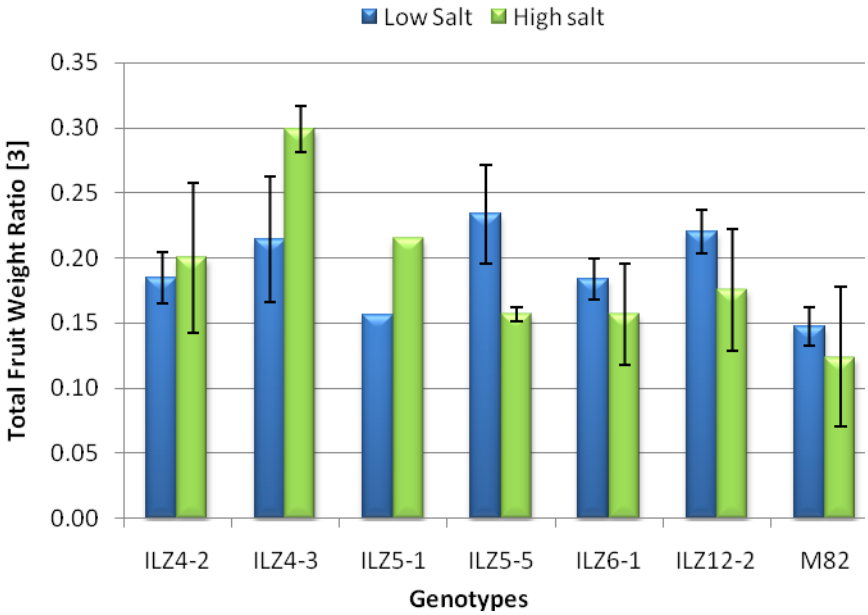

Figure 1.13. Total fruit weight ratio of genotypes at harvest.

Leaf Area Expansion

Leaf area is one of the effects that are more related to salt stress; therefore it is desirable to find a genotype that had not reduced their photosynthetic area under stress. For the statistical analysis it was observed that there was no effect of the treatment, but there was a significant GxT. The standard deviations of the averages showed high variability through the population (Figure 1.14). For the lines selected, there was an increase on the leaf area under high salt for the

lines ILZ4-2, ILZ5-1, ILZ5-5, ILZ6-1 and ILZ12-2 while the area was slightly reduced on M82 (Figure 1.15). In the case of line ILZ4-2 the increase was very drastic and for ILZ4-3, it was the only line that showed a reduction under high salt.

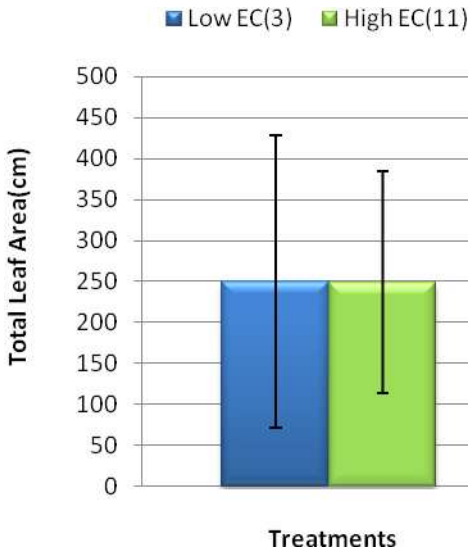

Figure 1.14. Average of total leaf area per treatment at harvest per treatment.

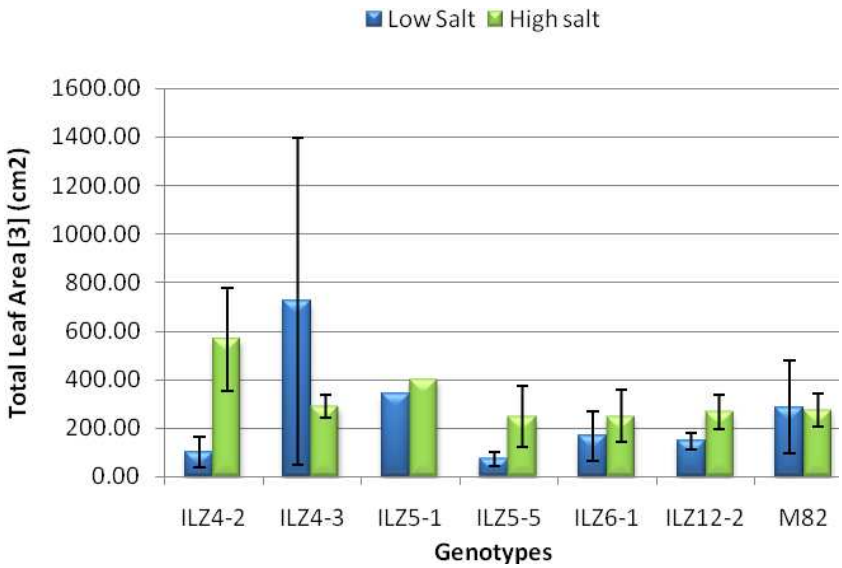

Figure 1.15. Total leaf area of genotypes at harvest time for the selected genotypes

Blossom End Rot

Blossom End Rot (BER) is a phenomenon that is usually associated with salt stress, among other factors. In the case of this experiment, there was a clear effect of the

treatment because it increased almost two times at high EC (Figure 1.15). In the experiment, there were lines with no BER at all (ILZ2-5, ILZ2-6, ILZ3-4, ILZ6-2 and ILZ11-1), however those lines usually do not show a high fruit number or weight. For the lines selected, there was an increase of BER, but not as drastic as in the case of M82 that increased 80%. Regarding the selected lines the best was ILZ4-3 (Figure 1.16).

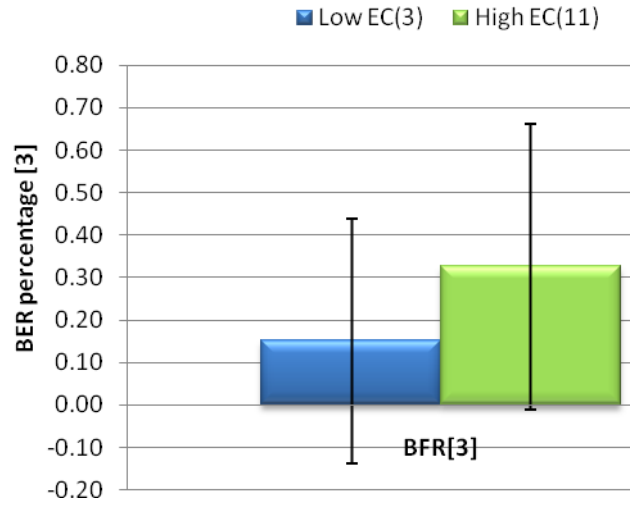

Figure 1.15. Blossom end rot averages per treatment.

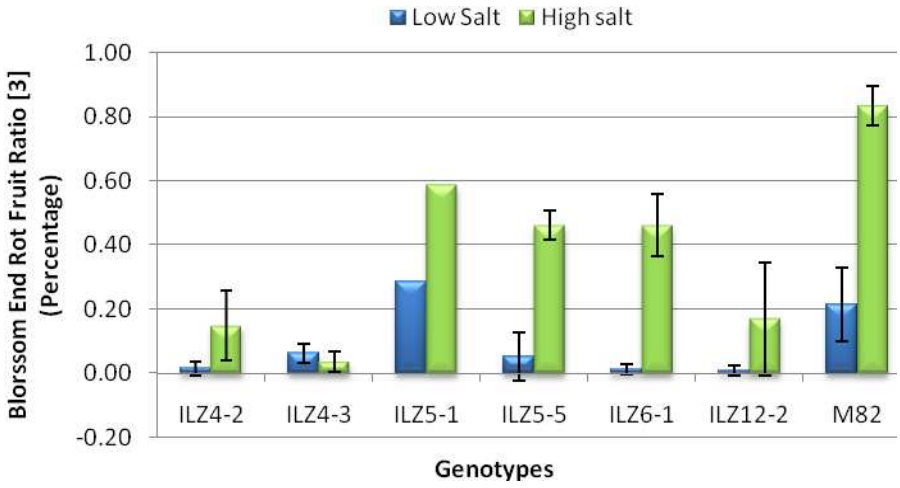

Figure 1.16. Blossom end rot percentage of the selected genotypes

Sodium

In the case of sodium, there was a clear effect of the treatment which was almost twice on the presence of salt, even considering that there was high variability at the treatments. Apparently there was no preference for the plants regarding the position of the leaf in the plant where they locate the sodium (Figure 1.17). Regarding the lines selected, all the lines showed an increase in the sodium content at top leaves (Figure 1.18) and only line ILZ12-2 decreased on middle and bottom leaves (Figure 1.19 and 1.20).

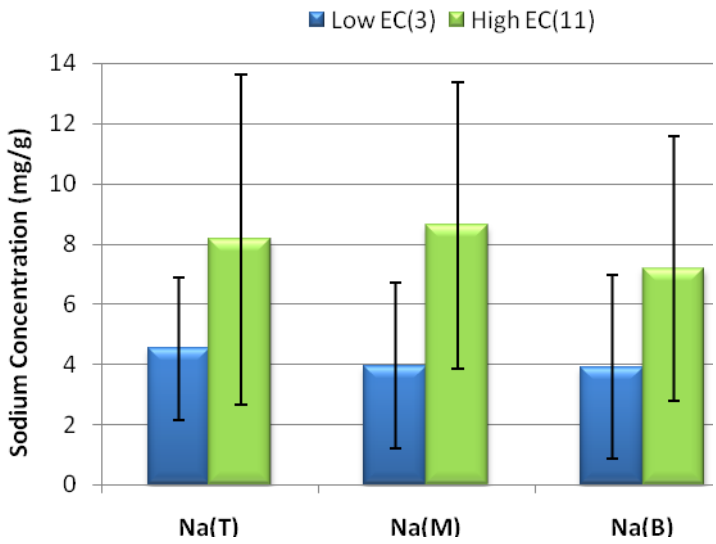

Figure 1.17. Sodium content average at top, middle and bottom leaves per treatment

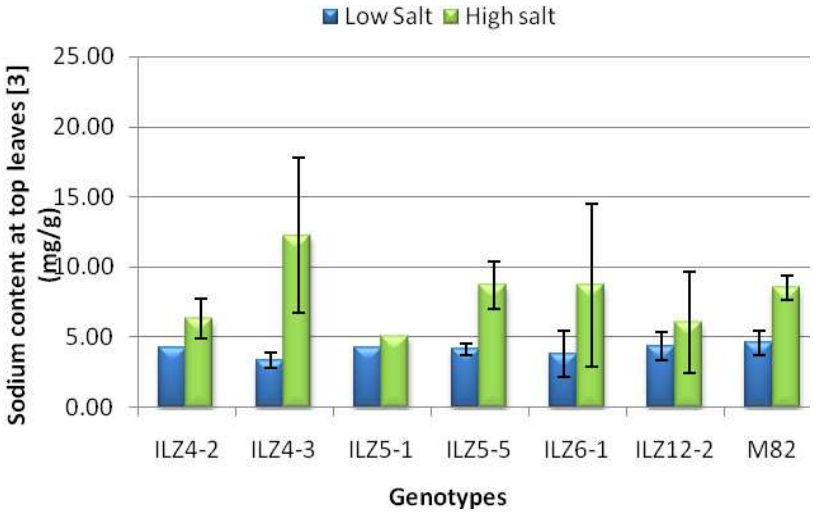

Figure 1.18. Sodium content on top leaves per genotype.

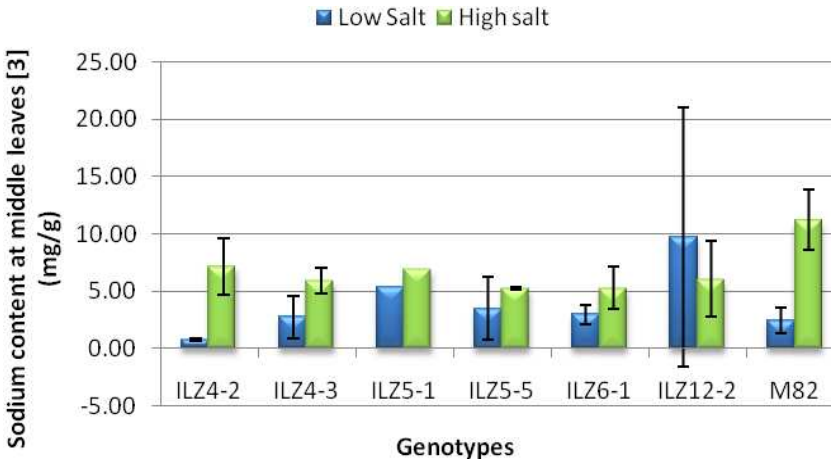

Figure 1.19. Sodium content on middle leaves per genotype.

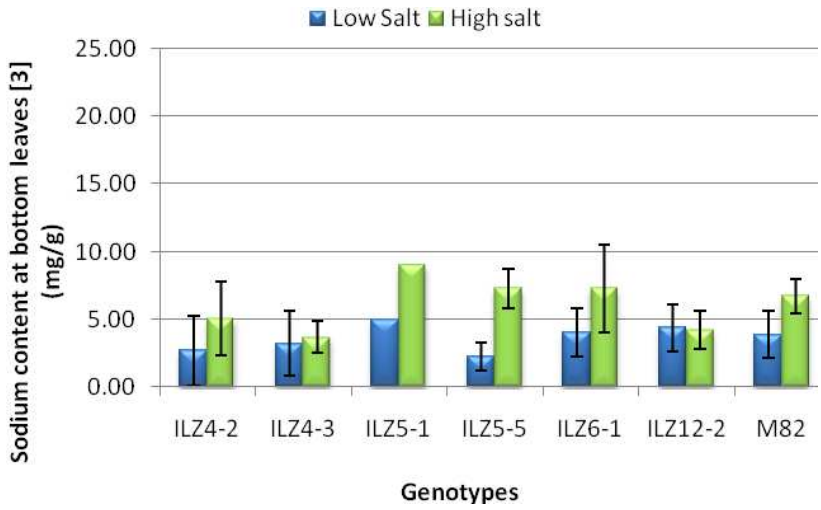

Figure 1.20. Sodium content on bottom leaves per genotype.

Potassium

The potassium content on top leaves was less than the observed in middle and bottom under stress and control plants. If the content is compared between treatments, the decrease of potassium in stressed plants was bigger in the middle and bottom (Figure1.21). It is supposed that there will be a high negative correlation between the concentration of potassium and sodium; however there was no evidence of that throughout the population. On top leaves, there was a reduction on the potassium content for M82; however there was an increase in ILZ4-2 and ILZ5-1 and almost no reduction in ILZ6-1 (Figure 1.22). For middle leaves, M82 content was almost the same in the control and all the selected lines showed a decrease except ILZ4-2 and a small decrease in ILZ4-3 (Figure 1.23). For the bottom leaves, M82 remained almost unchanged and all the selected lines showed a decrease in the potassium content, the decrease was higher in lines ILZ5-1 and ILZ5-5 (Figure 1.24).

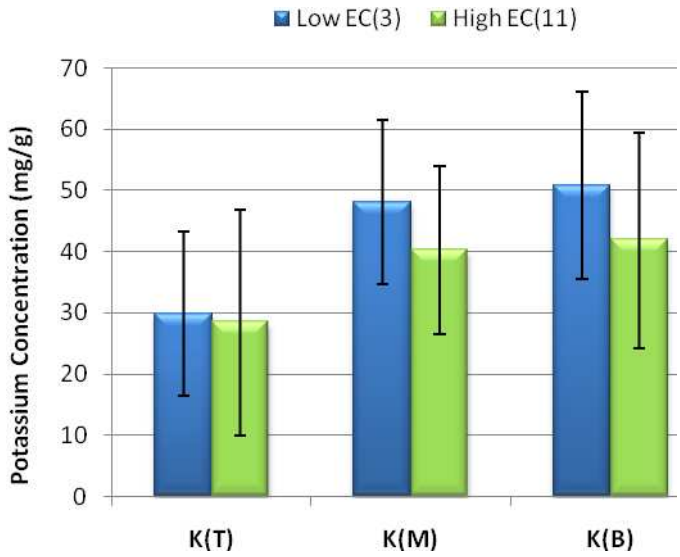

Figure 1.21. Potassium content average on top, middle and bottom leaves per treatment

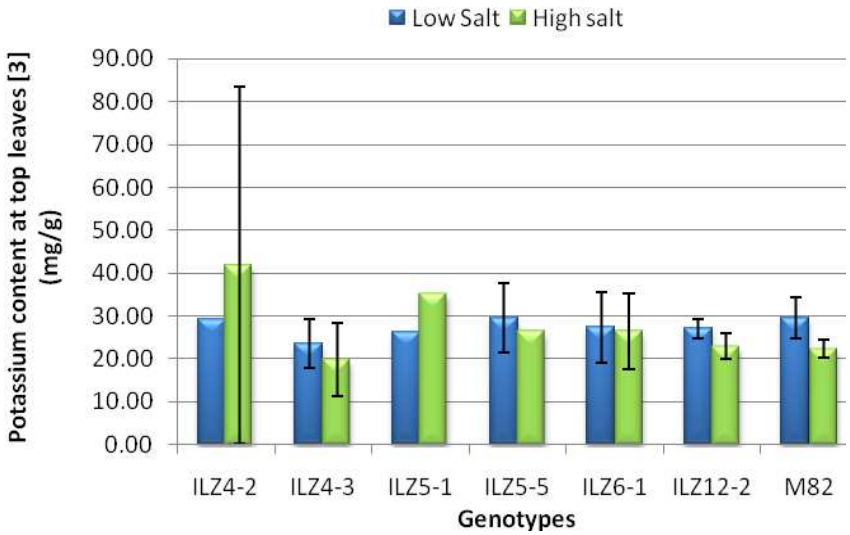

Figure 1.22. Potassium content of selected genotypes on top leaves.

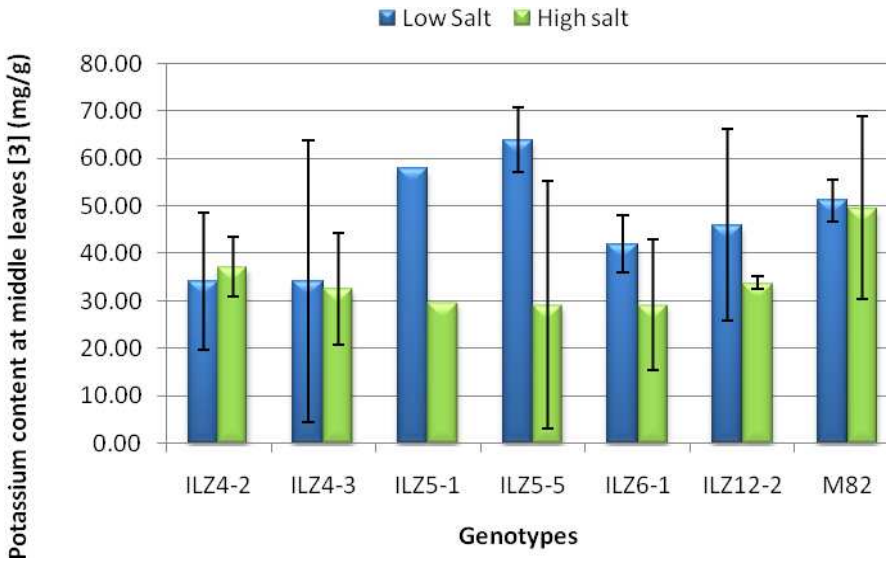

Figure 1.23. Potassium content per genotype on middle leaves

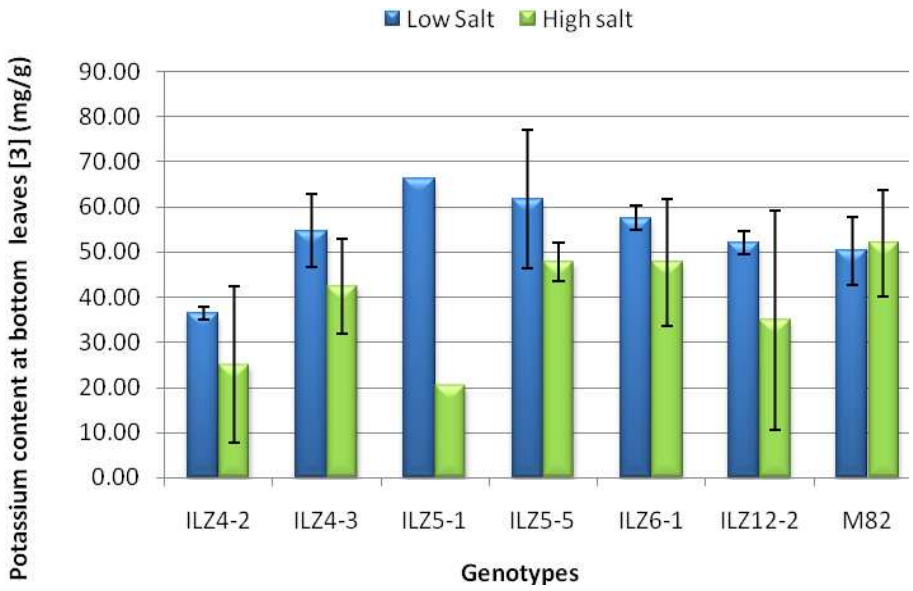

Figure 1.24. Potassium content per genotype on bottom leaves.

Potassium/Sodium

The ratio between potassium and sodium was lower at the top and it increases at middle and bottom for the plants at low salt concentration; however for the salt treated plants, the increase was much less (Figure 1.25). In the case of top leaves, M82 reduced its ratio considerably under salt stress conditions, the same behaviour was observed on lines ILZ4-3, ILZ5-5 and ILZ6-1, however the reduction was less for the other lines and in the case of ILZ5-1 it slightly increased (Figure 1.26). On middle and bottom leaves, the decrease of the potassium/sodium ratio was observed on all the lines (Figure 1.27 and 1.28) showing that the more stressed areas of the canopy under salt stress were the middle and bottom.

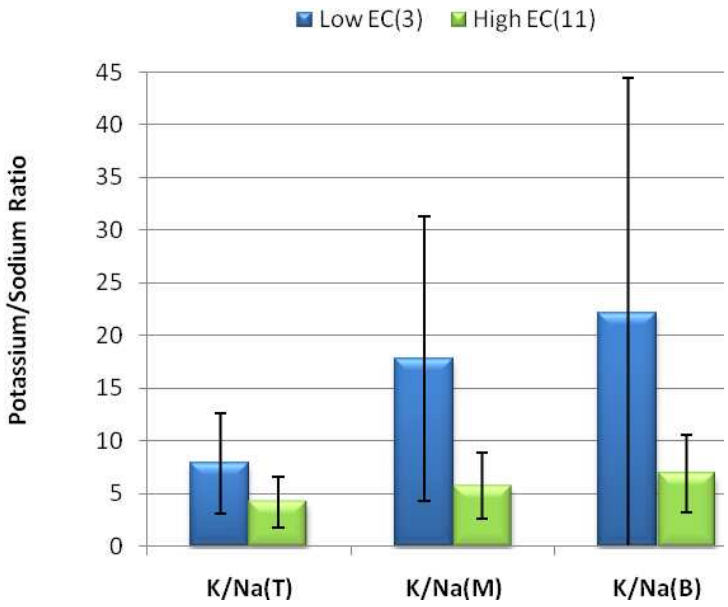

Figure 1.25. Potassium / Sodium content average on top, middle and bottom leaves per treatment

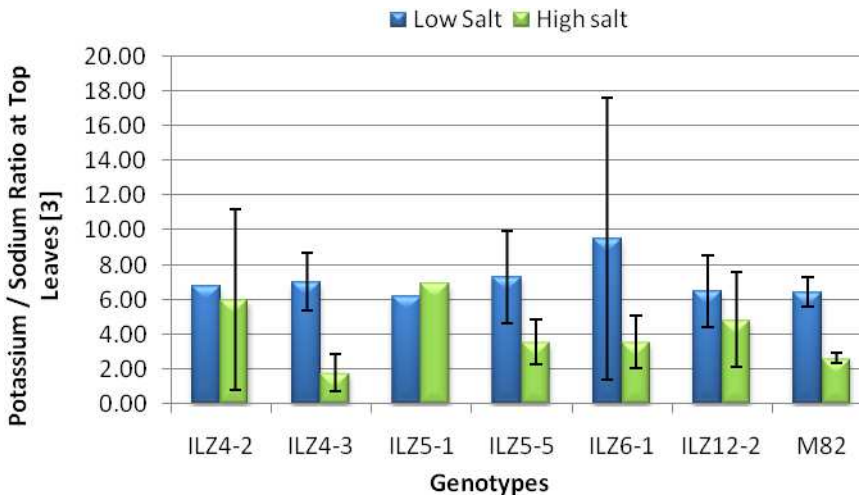

Figure 1.26. Potassium / Sodium content per genotype on top leaves

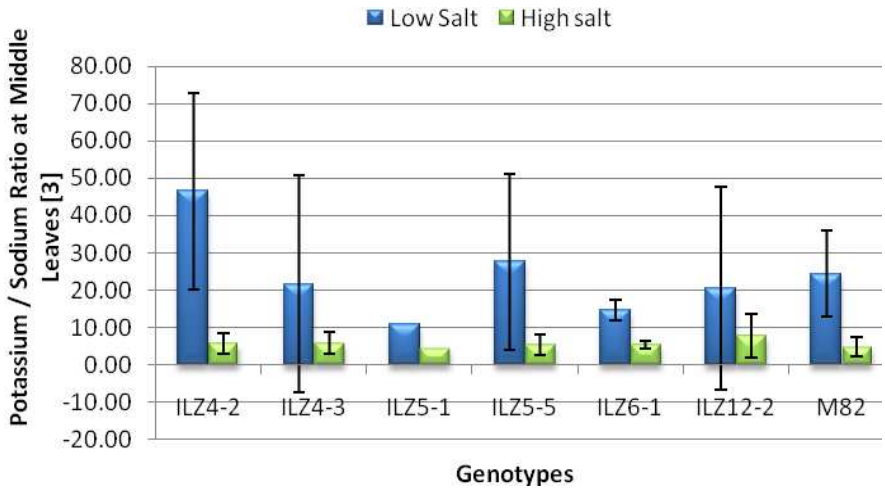

Figure 1.27. Potassium / Sodium content per genotype on middle leaves

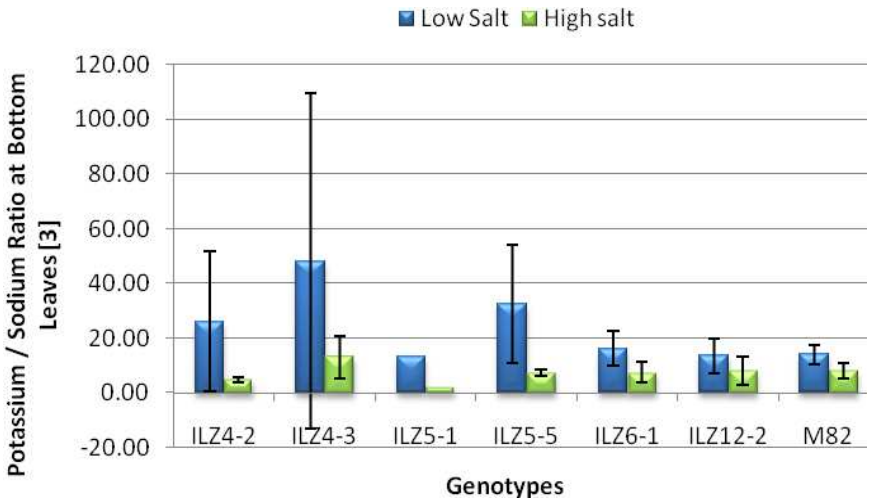

Figure 1.28. Potassium / Sodium content per genotype on bottom leaves

Chloride

The difference between control and salt stressed plants was almost three times bigger in the different canopy levels, for the plants in low EC the higher chloride content was observed on top leaves, while on high EC it was on the middle leaves (Figure 1.29). In the case of chloride content, in the REML analysis, only the middle leaves showed a significant GxT, and on this leaves it was observed that the chloride content was much less than the observed on M82 (Figure 1.30). The chloride content of the lines ILZ4-2 and ILZ4-3 was very little compared to the other lines.

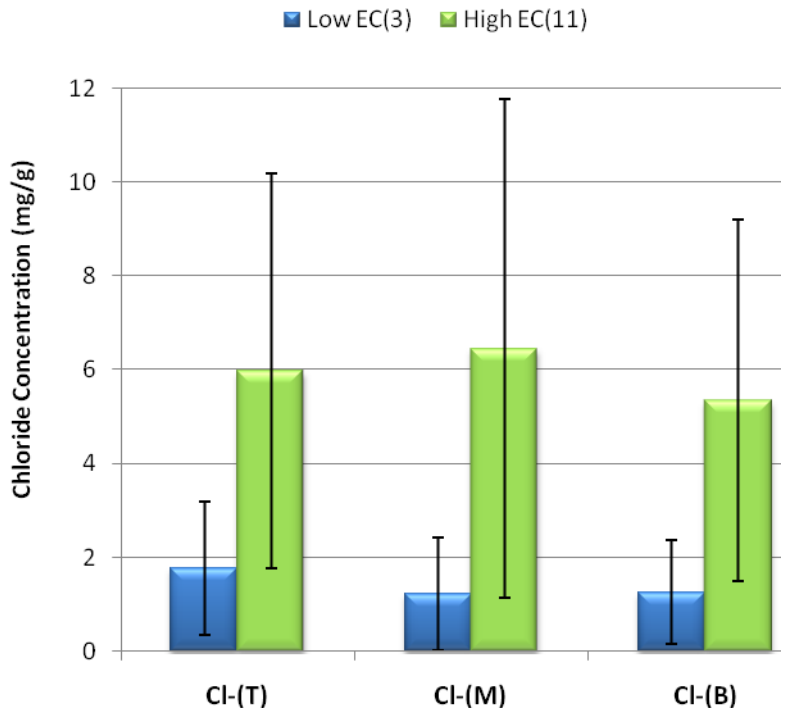

Figure 1.29. Chloride average content on top, middle and bottom leaves per treatment.

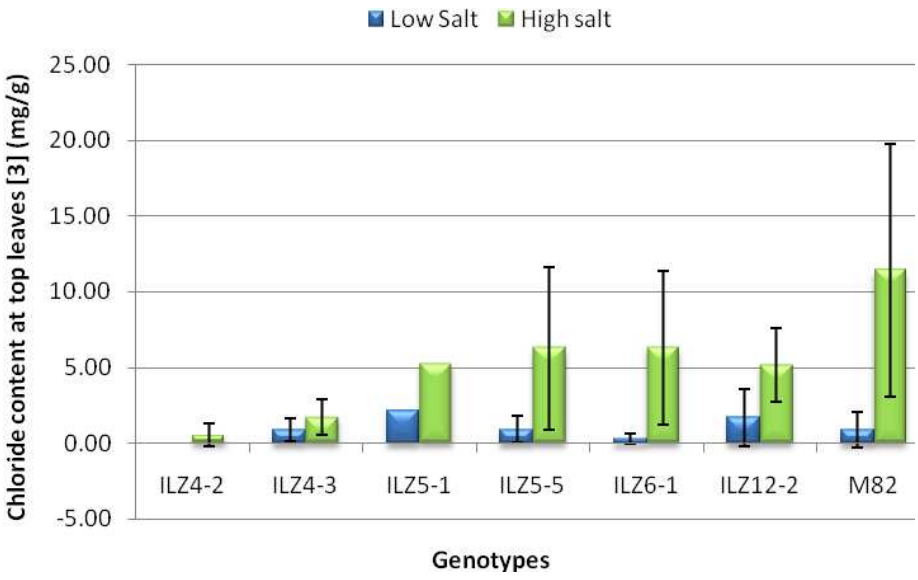

Figure 1.30. Chloride content per genotype on middle leaves.

Phosphate

In the case of the control treatment, the phosphate concentration is higher in the lower parts of the plant. For the top and middle PO_4 concentration, the control average was lower than the salt treated, but for the bottom it was slightly higher (Figure 1.31). Only the middle leaves showed a significant GxT interaction. For the selected genotypes, the concentration of all the

treatments increased except ILZ4-3 and in the case of ILZ4-2 the increase was the highest (Figure 1.32).

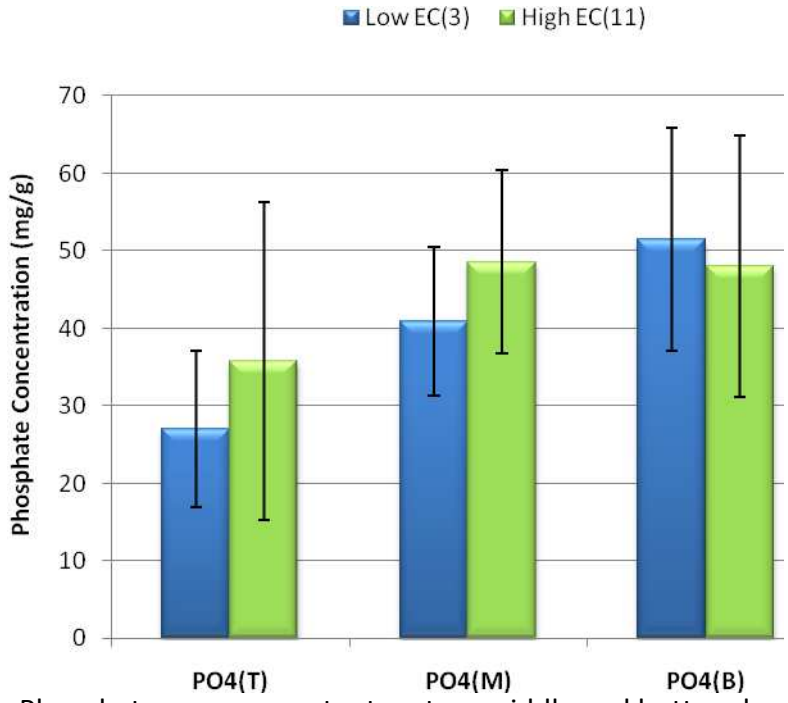

Figure 1.31. Phosphate average content on top, middle and bottom leaves per treatment.

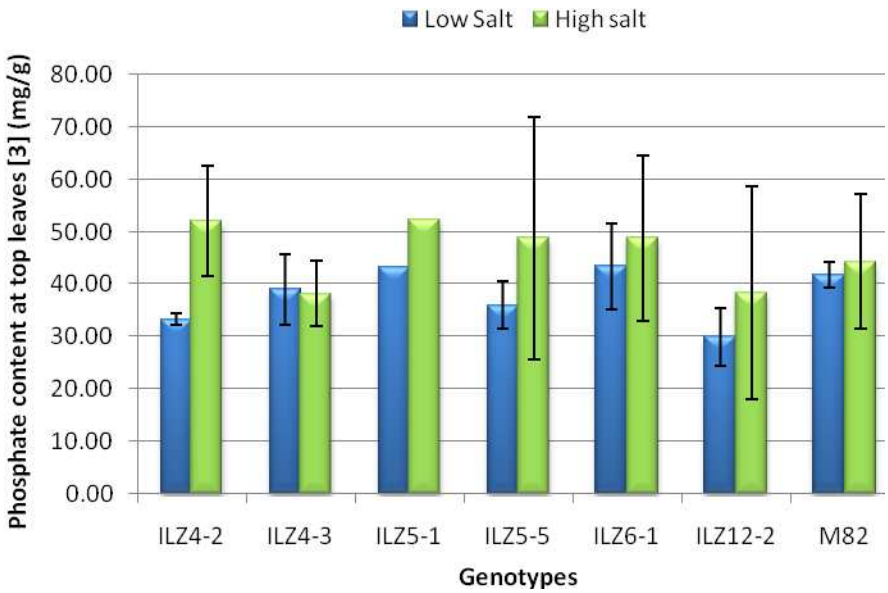

Figure 1.32. Phosphate content on middle leaves at two different salt treatments.

Calcium

Usually the calcium content is correlated with BER, but in this research it was not possible to demonstrate that in the presence of high calcium in leaves, there was less BER. The bottom calcium concentration was higher than the one located on top for the controls and it was almost the same for middle and bottom under salt stress (Figure 1.33). Interestingly, for the genotypes selected the calcium content increased for all the top leaves and in the case of genotype ILZ4-2 the increase was very drastic (Figure 1.34). In general, the calcium concentration on middle leaves was much higher than on top leaves. In the case of M82 there was a small increase as in the selected lines except ILZ5-5 and ILZ6-1 (Figure 1.35). In the case of bottom leaves, the decrease of calcium is more drastic for the selected lines while in M82 it remained unchanged and for ILZ4-3 the reduction was much less than the other selected lines (Figure 1.36).

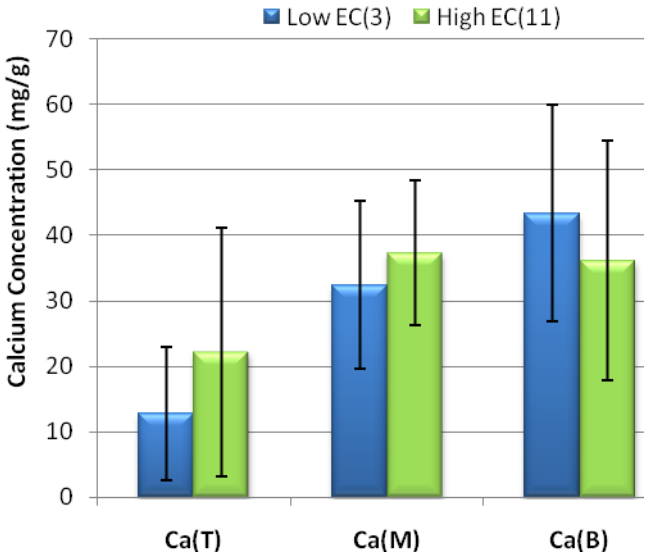

Figure 1.33. Calcium average content on top, middle and bottom leaves at two different salt treatments.

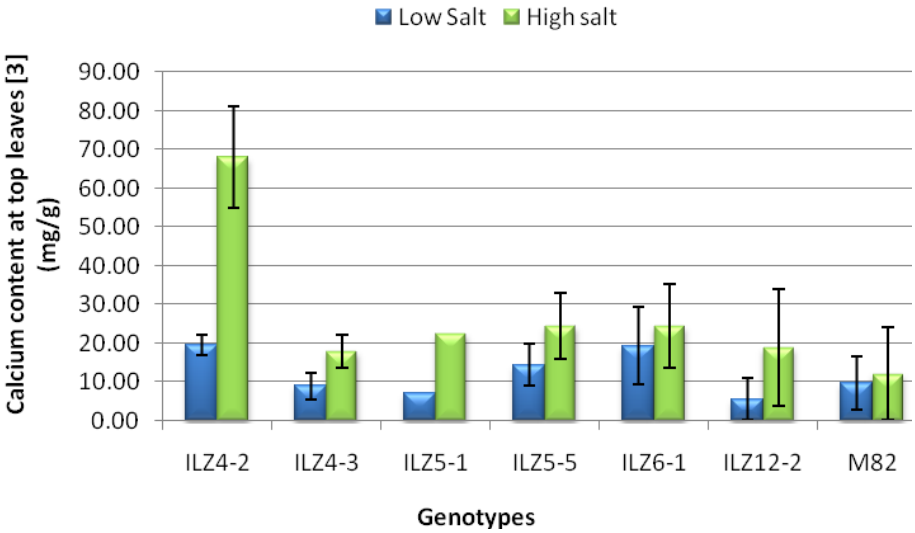

Figure 1.34. Calcium content on top leaves at two different salt treatments

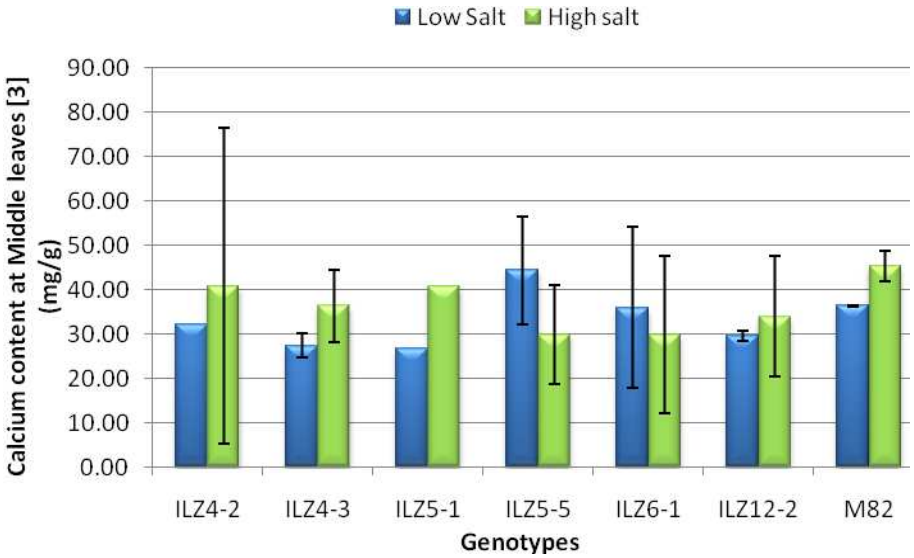

Figure 1.35. Calcium content on middle leaves at two different salt treatments

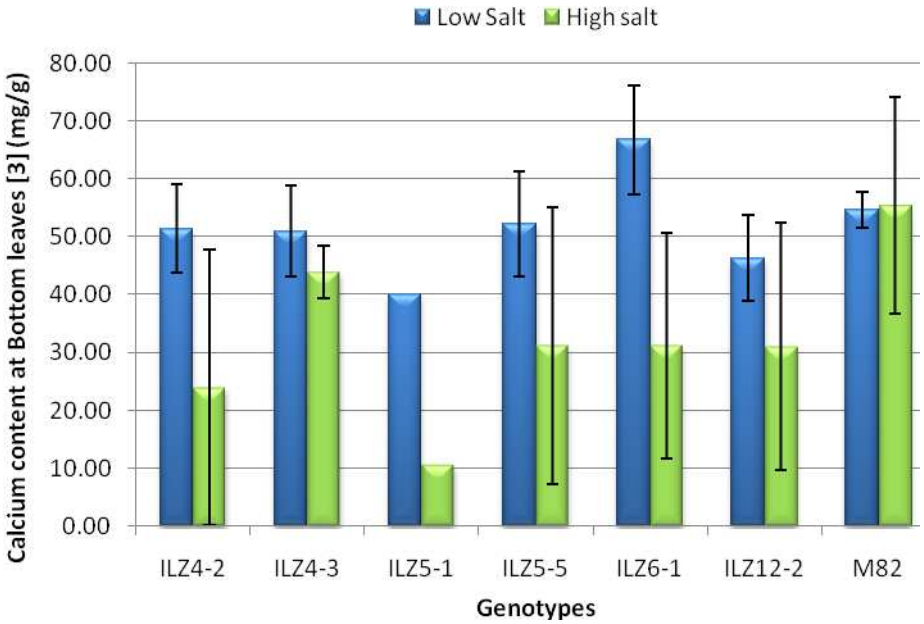

Figure 1.36. Calcium content on bottom leaves at two different salt treatments

Discussion

For this experiment, the number of replications per treatment did not allow performing an analysis of variance (ANOVA) and it was necessary to carry out the analysis of the population under a Restricted Maximum Likelihood (REML). According to Piepho and Möhring (2007), most formulas proposed for calculating heritability assume balanced data plus independent genotypic effects and both assumptions are often violated in plant breeding trials

As the number of genotypes was high, it was considered that a selection would be performed using the traits that showed a significant Genotype x Treatment (GxT - as Environment) interaction. Knowledge of the relationship between genotype and phenotype in different environments facilitate one to make accurate predictions of the response to selection in species that inhabit spatially or temporally in heterogeneous environments (Sparks 1997). In this case of inbred lines in a controlled abiotic stress trial, it was important to select on GxT interaction because of the close genetic background of the genotypes and the controlled conditions for the different treatments and the environment in greenhouse.

The performed analysis was based on the traits that showed a significant GxT interaction and significant differences in other relevant physiological traits such as: chlorophyll content, stomatal conductance, fruit number and fresh weight (green, red and total), total fruit weight ratio, total leaf area, BER and the ion content (K, Na, K/Na, Cl, PO_4 and Ca). Furthermore, the potential interactions between different factors were analyzed.

Regarding the results provided by this research, the selected lines that provide a more promising phenotype would be for fruit number and weight (ILZ4-2, ILZ5-1, ILZ5-5, ILZ6-1 and ILZ 12-2), chlorophyll content, fruit number and weight (ILZ5-5,ILZ 6-1 and ILZ12-2), high leaf area and fruit number (ILZ4-2 and ILZ5-1), K/Na and fruit number and weight (ILZ4-2, ILZ5-1 and ILZ12-2), PO_4 content (ILZ 4-2) and BER (ILZ 4-2). Special attention should be drawn to line ILZ4-2 that performed well in almost all traits evaluated and as there is an overlap with another good performer ILZ4-3, it would be convenient to assess them both.

For discussion purposes, each trait was evaluated in order to determine its relevance related to salinity tolerance processes.

Chlorophyll content

Dajic (2006) stated that increasing salinity in the growth medium in plants decreased the chlorophyll content, and therefore the net photosynthetic rate, and that this phenomenon expressed more conspicuously in salt-sensitive plants. However, in the performed experiment, chlorophyll content increased under salt stress.

In general, measurements of chlorophyll content provide more insight on senescence. Combining chlorophyll content rates in older leaves with measurements of leaf Na concentration

would provide an estimate of tolerance to Na+. Rates of photosynthesis per unit leaf area in salt-treated plants are often similar to the controls. This could be explained by the changes in cell anatomy that gave smaller, thicker leaves with an outcome of a higher chloroplast density per unit leaf area. When photosynthesis is expressed on a unit chlorophyll basis, rather than a leaf area basis, a reduction due to salinity can usually be measured.

In any case, the reduction in leaf area due to salinity means that photosynthesis per plant is always reduced (Munns and Tester 2008). Munns (2005) stated that this phenomenon is caused to compensate for the lower stomatal conductance in order to maintain the photosynthetic rate and to have high leaf transpiration efficiency.

Chlorophyll content decreased from the top to the bottom of the canopy which was also observed by Montesano and van Iersel (2007), and it is helpful to determine in which lines the older leaves become less senescent and remain active under the effects of salt; however it does not provide which lines are more effective on photosynthesis and this may be achieved with a chlorophyll fluorescence analysis that is a very powerful tool to be used *in vivo* (Baker 2008).

In studies performed by Montesano and van Iersel (2007) it was determined that low chlorophyll was 26 SPAD units, while the average was 44, for the cultivar Supersweet 100. For this experiment on inbred lines the average was 48 SPAD units. Not necessarily plants that perform well on chlorophyll content are the plants that had good yield. Between the best performers for Chlorophyll Content were lines ILZ5-5, ILZ6-1 and ILZ12-2. Lines ILZ5-5 and ILZ6-1 were also high on fruit number and fruit weight.

Stomatal conductance

The fact that stomatal conductance is lower at high EC shows that plants want to prevent dehydration. Sodium concentration thresholds trigger stomatal closure and the decrease of the transpiration flux (Maggio et al 2007). Variability is higher at low EC because environmental factors produced variability, however as closure is stimulated by high salt, the external factors reduced fluctuations reflected in the standard deviation. Tomato plants are able to adjust its osmotic potential to maintain the turgor potential and the stomatal conductance under saline conditions (Katerij et al 1998).

Stomatal density is another important aspect related to stomatal conductance that would provide insight on gas exchange and photosynthesis; however, it was not evaluated. Changes of transpiration related to salt conditions should be related to the gas exchange decrease and the lower stomatal density of developed leaves (Romero-Aranda et al 2001).

Stomatal conductance produced by the osmotic unbalance at high salt conditions, provides insight for the photosynthesis efficiency and dehydration prevention of the plant. Therefore it is an important trait to evaluate salt stress if measured under favourable conditions.

For the selected lines the fact that they were not reduced drastically (and in the case of ILZ5-1, increased) can eventually represent a benefit to endure salt stress. In addition to this, only

ILZ12-2 was not higher for stomatal conductance under high salt than M82 and as these lines had also a high fruit number and weight a high stomatal conductance may be related to it. Other relevant good traits were not related to these genotypes.

Fruit number, weight and total weight ratio

Salt stress affects tomatoes producing a reduction of yield. From low to moderate levels of salinity there is a reduction in the average fruit size (and therefore weight) and not a reduction in fruit number. However, at higher salinity levels the total number of fruits per plant is reduced (van Ieperen 1996; Cuartero and Fernandez-Munoz 1999). For this experiment fruit weight was drastically reduced by high EC.

Focusing on the selected lines, the ILZ4-3 had an outstanding behaviour, because even though that there was a reduction in weight, the performance under high salt conditions was higher than M82 at low salt. There are reports of QTLs involved in fruit weight variation that have been extensively studied in different segregating populations and advanced crosses (Villata et al 2006) and QTLs in chromosomes 4 and 12 have only been detected previously by Goldman et al (1995) on fruit weight.

Concerning fruit number, wild species have more flowers per truss and consequently more fruits per truss than the modern domesticated cultivars. In the case of *S. pimpinellifolium,* QTLs for fruit number in chromosome 5 were detected and the wild alleles were associated with more fruits and an earlier yield (Villalta et al 2006). In the case of our study, the fruit number was less reduced in the introgressions ILZ5-1 and increased in ILZ5-5 at high EC; therefore, these lines should be considered for breeding if further studies verify this assumption.

An important consideration on salt stress is that although the number of fruits is reduced their quality can be increased. It is well known that the total soluble solids content is the most important criterion for tomato paste processing which increases with salinity. Although yield reduction is also expected under salt stress, the higher quality of the tomato fruits can compensate (Carvajal et al 2000).

Leaf Area Expansion

Cuartero and colleagues (2006) determined that leaf area is a highly heritable trait; therefore it should be used in breeding programmes developing salt-tolerant tomato genotypes (Cuartero et al 2006).

For the lines with *S. pennelli* introgressions it was observed that there is a smaller reduction of leaf area of the salt treated genotypes compared to the ones on low salt. This implies that although the wild alleles increased the sodium concentration, their leaves tolerate it better (Villalta et al 2008). It is important for further experiments with a statistically balanced experimental set up to determine the heritability of leaf area, because this trait have showed the highest heritability in the experiments conducted by Cuartero et al 2006 on a cross of *S. lycopersicum* and *S. pimpinellifolium*.

Some of the selected genotypes have a large leaf area that increased under high salt conditions (ILZ4-2 and ILZ5-1) and also there were plants that presented a high fruit number and weight. The increase in leaf area of several selected lines cannot be explained; however, it is an interesting behaviour on genotypes that also succeed in fruit yield.

Blossom End Rot

The incidence of BER is related to salt stress, a phenomenon that occurs during a period of high cellular calcium demand, when fruit growth is accelerated or calcium delivery to the fruit is limited (Ho and White 2005).

Calcium movement in plants is unidirectional and the import of calcium into fruit diminishes with development and virtually stops with the onset of the rapid intake (Hanger 1979). Salt stress produces an osmotic unbalance that affects the translocation of nutrients. Therefore, it can be considered that the plants that are able to move calcium to the upper parts of the plant should suffer less BER. However, it must be considered that the calcium measurements performed were done on leaves instead of fruits.

As it was observed that some lines do not have incidence of BER (ILZ1-1, ILZ2-1, ILZ2-5, ILZ2-6, ILZ6-2, ILZ11-1 and ILZ11-3) it was considered that this trait should be related with high calcium content on the top leaves but it was not the case. Studies of calcium content should be performed for more detailed information about this phenomenon. On the other hand, some of the lines with high BER presented a high fruit number and weight (ILZ5-1, ILZ5-5 and ILZ6-1). BER can be controlled with cultural practices, such as: use of polyethylene mulch, maintaining adequate soil moisture and in a greenhouse study an increase in fruit transpiration was more effective increasing fruit calcium concentrations in the substrate (Taylor and Locascio 2004).

Taylor and Locascio (2004) stated that the greater leaf area of efficient types is more likely to deprive the fruit of calcium, particularly when the plant is under stress from low humidity or high salinity and this situation was observed on the selected lines because ILZ4-3 suffers a great reduction of leaf area but was the selected line with less incidence of BER.

Calcium

Regarding other ions, calcium may prevent some of the toxic effects of sodium on leaf photosynthesis by preventing the accumulation of this ion in leaves. Sodium flow through non-selective channels is strongly impaired by application of external calcium, and down-regulation of this channel type may be crucial for salt tolerance (Montesano and van Iersel 2007). However, for the purposes of this experiment, the calcium concentration detected in the leaves did not provide any GxT significant interactions and the correlations with traits like BER and Na was not clear.

In the particular case of tomato, the reduced calcium uptake in response to salt stress has been associated to a decreased transpiration rate rather than to competition effects with sodium (Cuartero and Fernandez-Munoz 1999). Supplemental calcium sulphate added to nutrient solution

containing salt significantly improved growth and physiological variables affected by salt stress (e.g. plant growth, fruit yield, and membrane permeability) and also increased leaf potassium, calcium, and sodium in tomato plants. In addition, it is important to mention that sodium ions may compete with calcium ions for membrane binding sites. Therefore, it has been suggested that high calcium levels can protect the cell membrane from the adverse effects of salinity (Tuna et al 2007)

In the case of tomato under salt stress conditions, the reduction in calcium uptake had been reported associated to a decreased transpiration rate rather than competition effects with sodium (Cuartero and Fernandez-Munoz 1999). Therefore, it is important to correlate this trait with stomatal conductance.

Potassium, Sodium and their Ratio (K/Na)

Salt stress produces an ionic imbalance in the cells due to excessive accumulation of sodium and chloride and reduces uptake of other mineral nutrients, such as potassium and calcium among others (Sudhir and Murthy 2004). Ion chromatography allows a single sample to be analyzed for several ions and the relation of them with other traits regarding salt stress.

In the case of potassium, its deficiency on salt stressed plants has been inversely correlated to the increased accumulation of sodium, indicating the existence of competition effects between sodium and potassium ions (Maggio et al 2007). Potassium is essential for several metabolic processes and root hair elongation and maintenance of cell turgor are two of them.

Under potassium deficiency, the stem and fruit diameter is reduced and studies by Kanai et al (2007) identified that potassium nutrition is more important for tomato plants than phosphate nutrition; thus, it is essential to monitor the potassium content under salt stress.

Potassium deficiency diminished sink activity in tomato plants (Kanai et al 2007). Regarding the inbred lines crossed with *S. pennelli*, it is assumed that as the higher salt tolerance of wild tomato species over cultivated forms has generally been associated with the halophytic character of sodium this will improve the salt stress tolerance of commercial cultivars (Estañ et al 2005).

Higher sodium accumulation in the xylem and greater sodium re-translocation through the phloem was found in *S. pennellii* when compared with *S. lycopersicum* in a study using salt tolerant wild relatives (Pérez-Alfocea et al. 2000). This study was performed on introgressed lines with segments of chromosome 7 and it indicated that the K/Na ratio in both, xylem and phloem was higher in *S. lycopersicum* than in *S. pennellii* (Villalta et al 2008). In the case of some of the selected genotypes, although there is a lower K/Na but they are able to cope with the stress, even lines with no introgressions on chromosome 7. Therefore, it will be interesting to evaluate if there are genes in chromosome 7 that are duplicated in other chromosome or they are part of a gene family.

Capacity of plants to maintain a high cytosolic K/Na ratio is believed to be a determinant trait for salt tolerance. However, the phenomenon of maintaining the capacity for an efficient production under a low K/Na conditions could be an interesting trait to be studied too.

The overall K/Na ratio is heritable in species such as wheat but not in others such as rice (Garcia et al 1995), and probably involves the contribution of different genes (Maathuis and Amtmann 1999), but it must be determined if this as a heritable trait for tomato. In the case of the selected genotypes, lines ILZ4-2 and ILZ5-1 had a high K/Na ratio and also a high fruit number and weight, while lines ILZ4-3 (with high weight) and ILZ5-5 (high fruit number) had a very low K/Na ratio.

Chloride

Previous reports of Maggio et al (2007) stated that in contrast to sodium it was observed that there is no difference in chloride concentrations between leaf ages, indicating that, at least at low-moderate salinity, these two ions follow different patterns of accumulation and/or partitioning. This information corroborates that for this particular experiment set up, it was not possible to correlate a high fruit number or weight with the chloride content and the information provided by it was not enough because it was only significant for the middle leaves. Therefore, for this experiment sodium was a more relevant selection trait.

Phosphate

Concerning to the presence of PO_4 on plants, it is known that genes for enzymes with key roles in the synthesis of osmoprotectants, like myo-inositol, are related with the PO_4 content (Munns 2005)**.** Myo-inositol synthesis begins from glucose-6- phosphate and the key step is played by the myo-inositol phosphate synthase enzyme; therefore, it may have a single genetic regulation which in theory would facilitate the development of tomato plants tolerant to salinity (Cuartero and Fernandez-Munoz 1999). In studies by Sacher and Staples (1985), myo-inositol content was highest in the most tolerant genotypes, intermediate in the normal cultivar, and lowest in the sensitive genotype after treatment with salt. In the case of this research, genotype ILZ4-2 presented the highest PO_4 content for top leaves and also was amongst the lines that had a better performance at fruit number and weight. For further experiments it would be necessary to determine the PO_4 content and myo-inositol to determine a linear correlation with both substances.

Proposed strategy to incorporate genes for salt tolerant

Several traits must be considered to eventually incorporate a promissory line to a breeding program. Gene pyramiding is a very useful approach for the introgression of genes controlling different agronomic traits to ensure that a variety may simultaneously acquire several traits (Semagn et al 2006) and for salt tolerance this might be an interesting approach when fewer lines are selected for a breeding program.

Conclusions

- A significant Genotype x Treatment interaction was the main criteria to select a trait; therefore, the most relevant traits for this research were:
    - Chlorophyll Content
    - Stomatal Conductance
    - Fruit Number
    - Fruit Fresh Weight (Green, Red and Total)
    - Total Fruit Weight Ratio
    - Total Leaf Area, and
    - Ion Content (Specially Na and Po_4).

- Chlorophyll content was increased under salt stress and its concentration decreased from top to bottom leaves.

- Stomatal conductance could not be correlated with other traits because of lack of statistical power produce by few repetitions and the influence of environmental variation.

- Fruit number and weight were reduced on high EC, however some lines produced fruits above the average of all the plants.

- Total leaf area which is considered as one of the most important traits.

- No correlation with BER and high Ca leaves content was observed.

- Lines ILZ4-2, ILZ5-1, ILZ5-5, ILZ6-1 and ILZ 12-2 were among the best lines in a lot of the traits evaluated.

Recommendations

- It would be convenient to select and evaluate lines ILZ4-2, ILZ5-1, ILZ5-5, ILZ6-1 and ILZ 12-2 in more detail with additional experiments (osmolytes, stomatal density and pollen fertiliy).

- Chlorophyll fluorescence is an additional test that combined with stomatal conductance and chlorophyll content can provide insight of the photosynthesis effectiveness under salt stress.

- Stomatal conductance should be performed on more stable environmental conditions and this trait may give insight on ion content, leaf area and photosynthesis. In addition, stomata density could be analyzed for the selected lines.

- It would be necessary to perform experiments to corroborate that the introgressions on ILZ4-2 and ILZ4-3 include the QTLs on chr 4 and 12 for fruit weight.

- It would be appropriate to determine that there are QTLs on ILZ5-1 and ILZ5-5 lines related to the QTLs determined on chr5 for fruit number on *S. pimpinellifolium* ILs.

- Regarding BER, calcium content analysis in fruits and leaves can provide insight on the correlations of BER and calcium.

- Concerning ion content, the correlation between K/Na and fruit weigh and fruit number must be analyzed.

- Phosphate and osmoprotectants (like myo-inositol) contents should be analyzed to determine if there is a linear correlation.

Chapter 2

Preliminary genetic analysis of introgression lines of MB x LA1840 (a selection of Moneymaker with genes of *S. chmielewskii*) to determine the presence of exotic segments in chromosomes 4, 6 and 12.

Materials and methods

Plant material: The analyzed introgression lines were offspring from a cross of Moneyberg (a selection of Moneymaker) and *S. chmielewskii* accession LA1840 and it was developed by Keygene Inc. A number of lines equally growing were screened for salt tolerance by Kontopoulou (2009) in a phenotypic analysis in two salt stress treatments. The best performing line (line 56) was selfed and selected for offspring with a homozygous introgression on chr 4 (PV091143) or a homozygous introgression on chr 12 (PV091140), or with homozygous introgressions on both chr 4 and 12 (PV091130) and finally plants with no detectable introgressions (PV091144). Previous studies by Grandillo (personal communication, May 6 2010) demonstrated the presence of an introgression in chr 6, however additional research using the same markers did not probe the presence of the introgression in line 56. Additionally, a current phenotipic analysis determined that the line with no introgressions behaved differently than the Moneymaker, which was used as control. Therefore it was hypothesized that the introgression was unnoticed by the marker used or this introgression was reduced.

Methods: for all the experiments an extraction of DNA was used by the Mini-method (Appendix 1). To corroborate the presence of introgressions on chromosomes 4, 6 and 12 different markers were used with Cleaved Amplified Polymorphic Sequences (CAPS) established by Grandillo (personal communication, May 6 2010) and used by Trotta (personal communication, May 6 2010) for these lines (markers in Table 2.1 and enzymes in Table 2.2). The CAPS protocol is described in detail in Annex 1. Additionally, to determine the presence of an introgression of chr 6, primers were designed with the program Primer Select from DNAStar based on markers from the Tomato-EXPEN 2000 map at 72, 76, 83, 90 and 92.5 cM (Table 2.3). The amplified fragments were purified with an illustra Microspin G-50 column (GE Healthcare) and then they were sequenced at Greenomics using an ABI Prism 3700 sequencer. Only the forward sequence was determined using the protocol established by Greenomics after the regular PCR amplification (Appendix 2). After sequencing, the information provided was analyzed with the program SeqMan Pro (DNAStar) to determine the presence of SNPs (Single Nucleotide Polymorphisms). In addition to this, the sequences that showed SNPs were analyzed with a BLAST against the Tomato Scaffolds of the SGN-SOL database (http://www.solgenomics.net).

Table2.1. Markers used on CAPS by Grandillo (personal communication, May 6 2010) and Trotta (personal communication, May 6 2010)

Marker name	Chr.	Map position Tomato-EXPEN 2000	Forward_primer (5'-3')	Reverse_primer (5'-3')
C2_At5g42950	4	119	AGCAATGGATTTCAGAGAATGGTGTG	ACATTTTTGGCACTTGCACCAGTGAC
C2_At1g18640	6	83	AATTCCGTTGTTGCTTCAGTTCAGCC	TCGTCTATGCACACAGTGCTATCCAC
C2_At4g18593	12	59	AGGTGATTGTTATAATCGTGGAGAAAG	TTCACAATGCGCACATAAAAGCTTG

Table2.2. List of CAPS expected results with the use of the enzyme and corresponding amplified fragment. Trotta(personal communication, May 6 2010)

Marker for Chr.	Product size (bp) Moneyberg/LA1840	Polym. enzyme Moneyberg/LA1840	Polymorphic band sizes after digestion (bp) Moneyberg/LA1840
4	500	TaqI	500/300+200
6	480	TaqI	400/480
12	800	AvaII	800/600+200

Table.2.3. Primers designed with Primer Select to amplify different regions at chr 6 on tomato genotypes.

Location	Marker	Expected Size (bp)	Forward Primer	Reverse Primer
Il-Chr6-72-1	T1049-1	440	TAAAATCCGCAAGCTGGTAAGG	CATTGTGAGGGCATTGAAGAAAC
Il-Chr6-76-1	T1399-1	385	ATTCCCACTGCCTCTATCCTTTCT	TCCCGTCACCAGCAGCATC
Il-Chr6-83-1	C2_At1g18640-1	480	AATTCCGTTGTTGCTTCAGTTCAGCC	TCGTCTATGCACACAGTGCTATCCAC
Il-Chr6-85-1	cLex-2-F13-1	202	CTTCCCCATTTCAAAACCCTAACC	GAGCAGCAGCGCCACCAGAG
Il-Chr6-90-2	cLES-1-K3-2	275	AGTTATGGCCGGAAGTGGTGTCGT	CAGCCTGTTTGATTTCTTTGATAA
Il-Chr6-92.5-1	C2_At1g16870-1	360	GGCGGCGAAATCCCATCC	AGCAATGGATTTCAGAGAATGGTGTG

Results

Regarding the experiment on CAPS, all the primers amplified the right fragments and only the introgressions of chromosomes 4 and 12 were detected. Therefore the labelling of the plants was correct and the marker at 83 cM on chr. 6 did not detect the presence of any introgression. Table 2.4 summarizes the results for each genotype evaluated. This result reproduces the research executed by Trotta (personal communication, May 6 2010) instead of the one performed by Grandillo (personal communication, May 6 2010).

As the introgression in chr. 6 was not localized, markers were developed for sequencing sections at regions were the intogression was localized (between 67 an 95 cM) by Grandillo (personal communication, May 6 2010). After the SNPs analysis, 13 polymorphisms were localized only for 72 cM in the lines PV091140 and PV091144 and in most of the cases they were at a heterozygous state (Figure 2.1).

Concerning the DNA sequences obtained, a BLAST was performed against the sequence of the tomato genome (Tomato WGS scaffolds v103). The hits produced matched with scaffolds

00823 and 01157, but only the hits of 00823 were highly significant. Scaffold 00823 (Tomato WGS Scaffolds 1.03) is known to be located on chr 6 (Henri van de Geest, personal communication June 1, 2010).

Table 2.4. Result of CAPS performed with the markers used by Grandillo (personal communication, May 6 2010) and Trotta (personal communication, May 6 2010) on inbred lines and one parental line.

Genotype	Chr 4	Chr 6	Chr 12
201	-	-	-
202	-	-	-
203	+	-	+
204	+	-	+
205	-	-	+
206	-	-	+
207	+	-	-
208	+	-	-
209	-	-	-
210	-	-	-
30-1	+	-	+
30-2	+	-	+
30-3	+	-	+
40-1	-	-	+
40-2	-	-	+
40-3	-	-	+
43-1	+	-	-
43-2	+	-	-
43-3	+	-	-
44-1	-	-	-
44-2	-	-	-
44-3	-	-	-
MM-1	-	-	-
MM-2	-	***	***

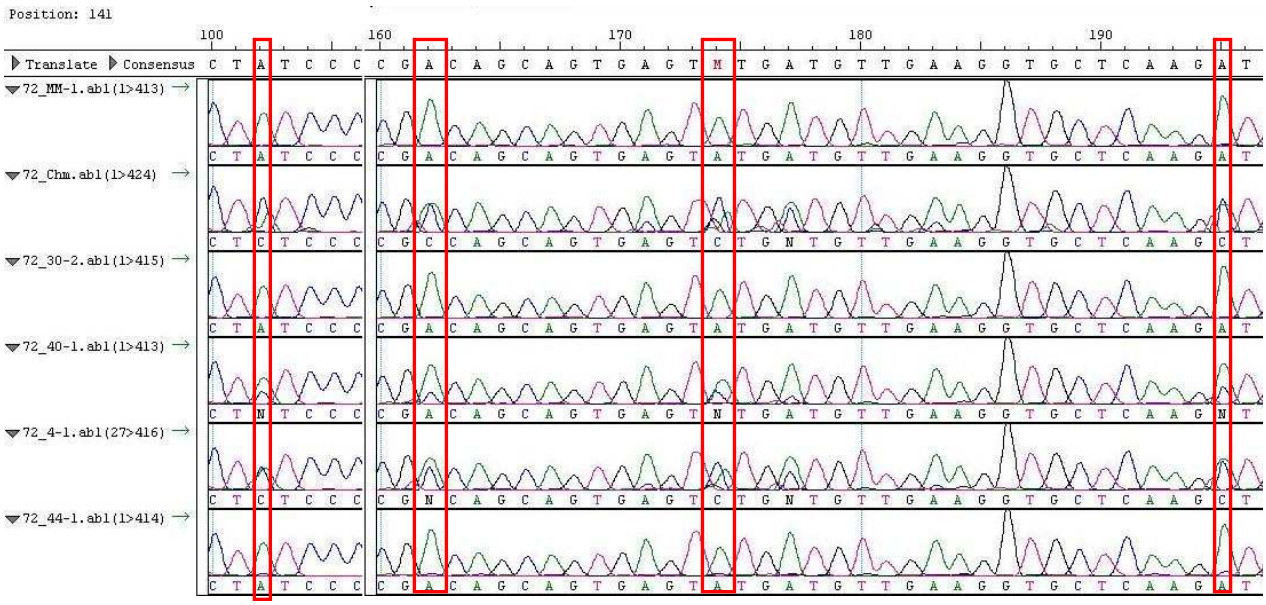

Figure 2.1. SNPs analysis using SeqMan (DNAStar) for the sequences analyzed at 72 cM with marker T1049-1.

Discussion

When breeders need to improve plants, they have to find a source of germoplasm that would supply the genes needed to undertake the breeding project (Acquaah 2007). Regarding this inbred lines, the genetic variation compared to the MB cultivar is based on the localized introgressions, therefore it is essential to determine the presence of the exotic areas in the lines evaluated.

The research performed by Grandillo (personal communication, May 6 2010) with CAPS determined the presence of introgressions in chr 4, 6 and 12 in contrary of the information provided by KeyGene that stated that introgressions were at 10 and 11; however, the research of Trotta (personal communication, May 6 2010) detected exotic segment on 4 and 12 but not on chr 6. Taking into account that the results of Trotta were reproduced in this research and that the phenotypic analysis performed by Víquez (2010 personal communication, May 4 2010) determined that PV091144 performed differently than MM it was unlikely that this genotype did not have any introgressions.

The fact that two lines presented heterozygous SNPs that matched with the ones of *S. chmielewskii* on Chr 6 and that they were not identified by marker 83 cM, provided a proof that this plants were not exactly the same as the ones analyzed by Grandillo (personal communication, May 6 2010). This implies that there is an admixture in the stock of Keygene. Additionally, if the introgression was not observed in the entire region reported (from 67 to 95 cM) and it was only observed on the marker at 72 cM, it can be concluded that the introgressed area was reduced.

In addition to this, due to the observations in the SNPs analysis, there must be a preference for the heterozygous state as LA1840. This genotype is a *S. chmielewskii* stable line that has been selfed for several generations; therefore, it is probable that the homozygous state is lethal on those loci. This will also explain why the SNPs observed in the genotypes from line 56 are heterozygous too. It is unknown how much inbreeding depression is due to lethal mutations, because homozygous embryos often die during the earliest stages of development, which makes such mutations difficult to detect (Charlesworth and Willis 2009). It is currently unclear whether changes in expression level and the abundance of proteins and metabolites observed with inbreeding are either direct effects of specific homozygous genotypes or a general indirect response to the increased homozygosity observed with inbreeding (Kristensen et al 2009).

After determining that the introgression was inside the scaffold 00823, it was observed that this area was a site of high recombination. This assumption was made deducing that the whole tomato genome consist of 950 mega bp and there is a total amount of 1200 cM, in average there is a recombination every 791.666 bp, while for the region analyzed (16 cM from 67 to 83 cM) there is a recombination every 128.618 bp. Based on an analysis of gene prediction performed by van de Geest (personal communication June 1, 2010) the introgressed area contained around 200 genes, therefore at a site of high recombination, the exotic segment can be reduced for the analysis of candidate genes. In these particular lines, even they are closer to a salt tolerant ideotype, the reduction of the introgressed area is mandatory because it is not at a

cultivar stage, therefore the region from 67 to 83 cM it is still long and further experiments with additional markers are an interesting opportunity.

Conclusions

- The determination of the introgressions on chr 4 and 12 by Trotta (personal communication, May 6 2010) using CAPS was reproduced and markers C2_At5g42950 (at 119 cM in chr 4) and C2_At4g18593 (at 59 cM in chr 12) are reliable for identification on this genotypes.

- Marker C2_At1g18640 (at 83 cM in chr 6) was not able to determine an introgression for this population because the introgressed section was reduced or fragmented.

- Several SNPs were found at 72 cM (with marker T1049-1) on the lines PV091144 and PV091140.

- The sequences amplified matched significantly only with the scaffold00823.

- The introgressed area localized is a site with high recombination.

Recommendations

- Perform an experiment using the sequence of scaffold00823 using close markers (every 500 bp for example) is needed to determine the limits of the introgression.

- Relate the phenotyping analysis of Víquez (personal communication, May 4, 2010) with the results of the experiment previously mentioned can lead to a determination of candidate genes.

- If the limits of the introgression can be determined, a fragmentation of the area would be desirable to minimize the area where candidate genes can be located.

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- To my future wife, Ana Marcela Víquez, because she had and have been my biggest support through this time and I can always count on her advice and care.

- To my friends in Costa Rica and in the Netherlands and my family for their constant support. You are a very important part of my life.

Appendix 1

Protocol for Ion Content Analysis

Ashing oven

- Weigh 25 mg of leaf and shoot, for the control and treatment (NaCl)samples
- Weigh 20mg of root samples, for the control and treatments (NaCl)samples
- Check and adjust minimum temperature of the ashing oven at room temperature
- Adjust the max temperature of the ash oven at 575oC,
- Uncap and put the samples in the ashing oven (to reach at the 575oC it will take one hour)
- Leave the samples at 575oC for 5 hrs and let the samples cool down
- Re-label the samples again

3M formic acid preparation

- Given 46.03gm (formic acid in a solution form), it is written on the bottle
- Multiply 46.03gm three times this gives 138.09gm/li (this result is for a solid form)
- To change it in to a liquid form (138.09/1.22kg) – it doesn't matter the units, this results in 113.19
- Looking at the purity level 99%, then the exact volume required is (113.19 *100/99) The result is to be in 114.3 ml/li of formic acid
- For 250ml, 114.3*250ml/1000ml = 28.60 of formic acid in 250ml solution is required

Sample preparation for the Ion chromatography (potato)

- Add 1ml of 3M of formic acid to the ash samples
- Shake it at 99oC for 15min, and allow it to cool down
- Look at the dissolvability of the samples (if the solubility is poor – add 9ml of miliQ water and put in the shaker for 30min)
- Make 10x dilution of the samples by adding 9ml of miliQ water then mix it using the vortex
- Take 100µl from the sample and make dilution of 1000X in the IC tubes (adding 9.9ml of miliQ water)
- Take 1ml from the sample and make dilution of 100X in the IC tubes (adding 9ml of miliQ water)
- Prepare 1ppm, 3ppm, 6ppm, 8ppm and 10ppm of standards for Anion (Cl, NO3, PO4, SO4) and cations (Na, K, Mg, Ca) by adding 9ml,7ml,4ml,2ml and 0ml of milliQ water in to the given anion and cation concentretions
- Prepare also the blank samples (which contains the 1ml of formic acid and 9ml of water), these blanks should have to follow the procedures as to the samples

Buffer preparation

- For the Cation column

§ Add 2120mg of Na2CO3 and 1680mg of NaHCO2 in to a glass bottle Add 900ml of milliQ water to it and stir

§ Add 100ml of acetone (now it is 10% acetone) to the solutions and stir it again

§ Take 200ml from the solution in to another glass bottle and add 1800ml of miliQ water hence the acetone is 100X diluted

- For the Anion columns

§ Add 2080µl of 65% HNO3/L in a glass bottle

§ Add 900ml of milliQ water to it and stir the solution

§ Add 100ml of acetone (now it is 10% acetone) to the solutions and stir it again

§ Take 200ml from the solution in to another glass bottle and add 1800ml of miliQ water hence the acetone is 100X diluted

Table A. REML analysis outcome from the traits analyzed on phenotypic analysis of M82 x LA716

Trait	Genotype	Treatment	G x T
He[1]	<0.001	0.024	0.555
He[2]	0.001	0.018	0.209
He[3]	<0.001	0.004	0.188
CC1[1]	<0.001	<0.001	0.016
CC1[2]	<0.001	<0.001	0.009
CC2[1]	<0.001	0.004	0.157
CC2[2]	<0.001	0.002	0.118
CC3[1]	<0.001	0.026	0.324
CC3[2]	<0.001	0.02	0.192
Gs[1]	<0.001	0.08	0.042
Gs[2]	0.017	0.129	0.999
LNr[1]	<0.001	0.121	0.708
LNr[2]	<0.001	0.059	0.969
LNr[3]	<0.001	0.136	0.995
TNr[1]	<0.001	0.114	0.597
TNr[2]	<0.001	0.035	0.352
TNr[3]	<0.001	0.332	0.547
FNr[2]	<0.001	0.323	0.311
FNr[3]	<0.001	0.153	0.043
GFNr[3]	<0.001	0.039	<0.001
RFNr[3]	<0.001	0.612	0.747
BFNr[2]	<0.001	0.006	0.002
BFNr[3]	<0.001	0.013	0.018
BFR[3]	<0.001	0.011	0.117
LT[2]	0.908	0.972	1.000
RWC[2]	<0.001	0.018	0.334
FYLWe[3]	<0.001	0.561	0.891
FGLWe[3]	<0.001	0.466	0.068
TFLWe[3]	<0.001	0.469	0.764
FSWe[3]	<0.001	0.005	0.403
DYLWe[3]	<0.001	0.53	0.967
DGLWe[3]	<0.001	0.334	0.123
DTLWe[3]	<0.001	0.659	0.797
DSWe[3]	<0.001	0.115	0.367
FGFWe[3]	<0.001	0.006	0.001
FRFWe[3]	<0.001	0.007	0.004
FTFWe[3]	<0.001	0.005	<0.001
DGFWe[3]	<0.001	0.947	0.201
DRFWe[3]	<0.001	0.226	0.108
DTFWe[3]	<0.001	0.493	0.101
FTPWe[3]	<0.001	0.013	0.376
DTPWe[3]	<0.001	0.47	0.663
TPWeR[3]	0.001	0.061	0.249

TFWeR[3]	<0.001	0.749	0.027
LA[2]	<0.001	0.066	0.482
TLA[3]	0.035	0.495	0.039
K(T)	0.33	0.824	0.478
K(M)	0.252	0.064	0.254
K(B)	0.064	0.169	0.708
Na(T)	<0.001	0.001	<0.001
Na(M)	<0.001	0.002	0.001
Na(B)	0.001	0.004	0.001
K/Na(T)	0.088	0.007	0.35
K/Na(M)	0.341	0.005	0.582
K/Na(B)	0.235	0.007	0.456
Ca(T)	0.136	0.208	0.958
Ca(M)	<0.001	0.172	0.944
Ca(B)	0.142	0.489	0.246
Cl-(T)	0.345	0.002	0.138
Cl-(M)	<0.001	<0.001	0.006
Cl-(B)	0.515	<0.001	0.275
PO4(T)	0.194	0.261	0.707
PO4(M)	<0.001	0.036	0.019
PO4(B)	0.329	0.566	0.683
SO4(T)	0.077	0.298	0.955
SO4(M)	<0.001	0.668	0.548
SO4(B)	0.07	0.287	0.302
Mg(T)	0.897	0.799	0.965
Mg(M)	0.006	0.711	0.179
Mg(B)	0.108	0.282	0.644

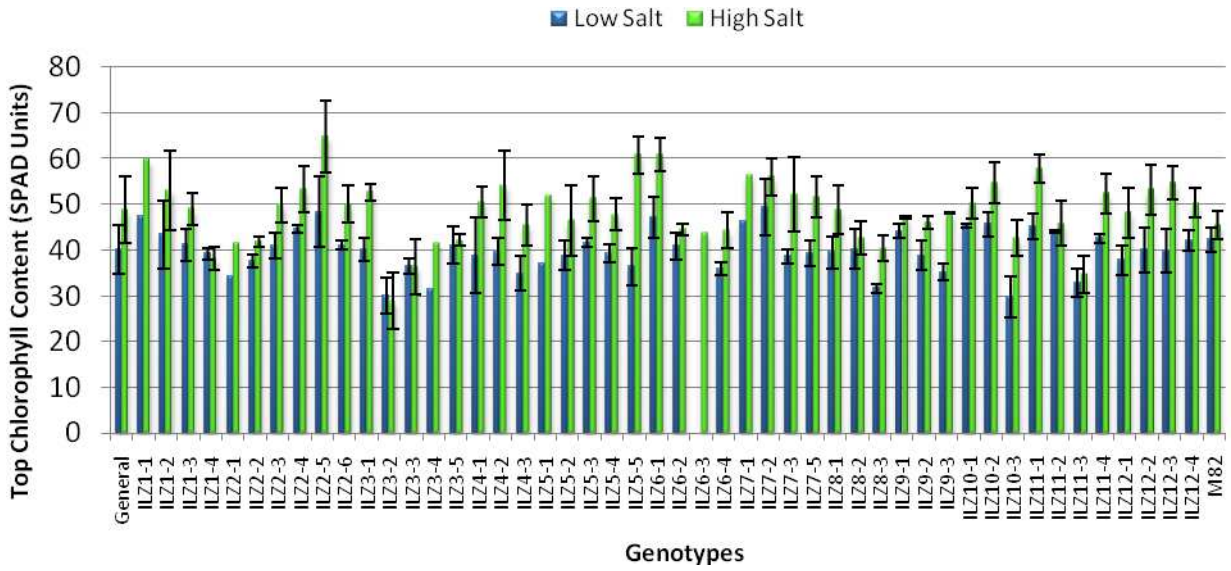

Figure A. Chlorophyll content of top leaves of all genotypes analyzed at first time point.

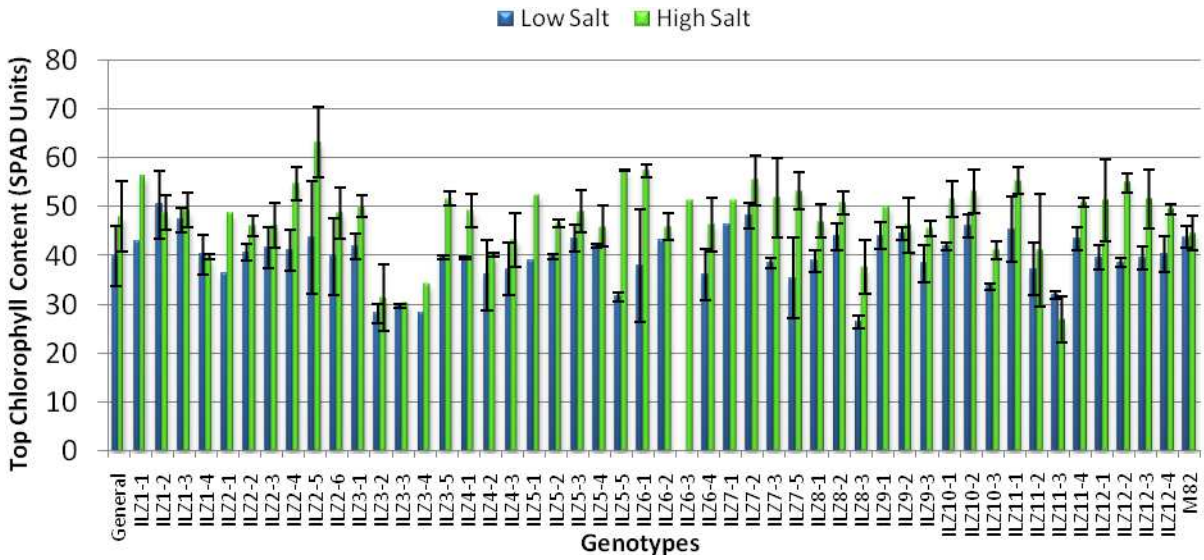

Figure B. Chlorophyll content of top leaves of all genotypes analyzed at second time point

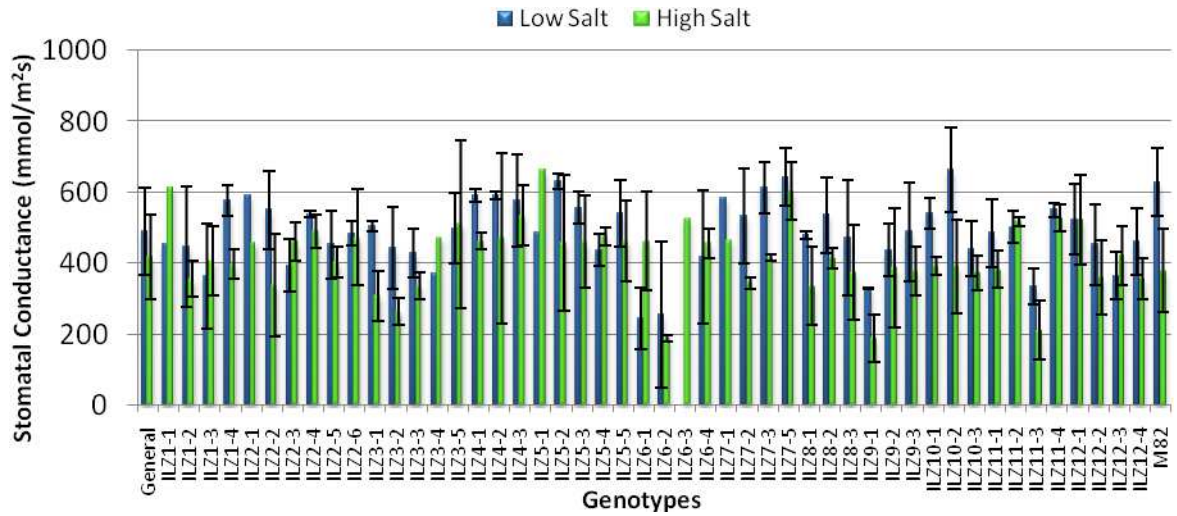

Figure C. Stomatal conductance of all genotypes analyzed at first time point

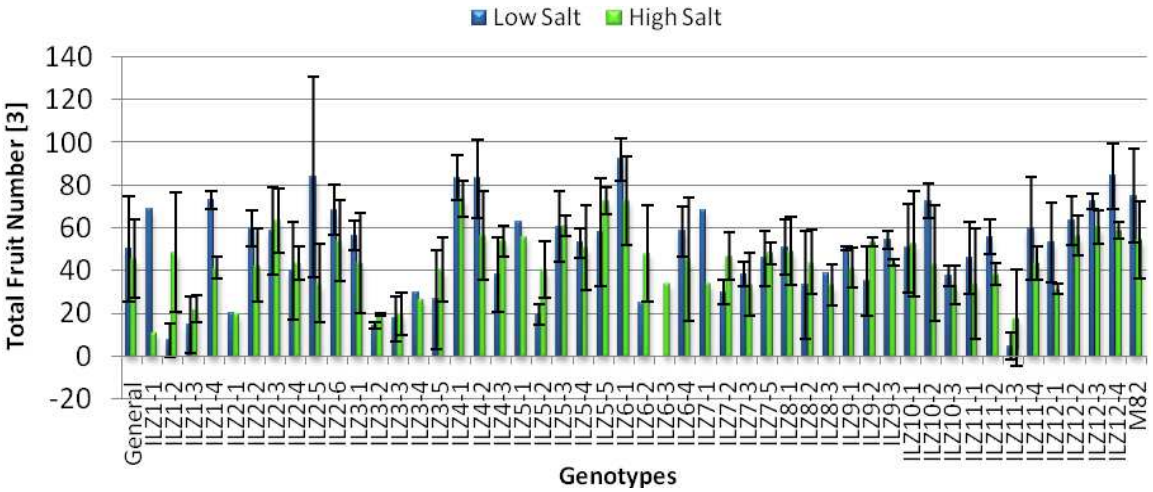

Figure D. Total fruit number of all genotypes analyzed at third time point

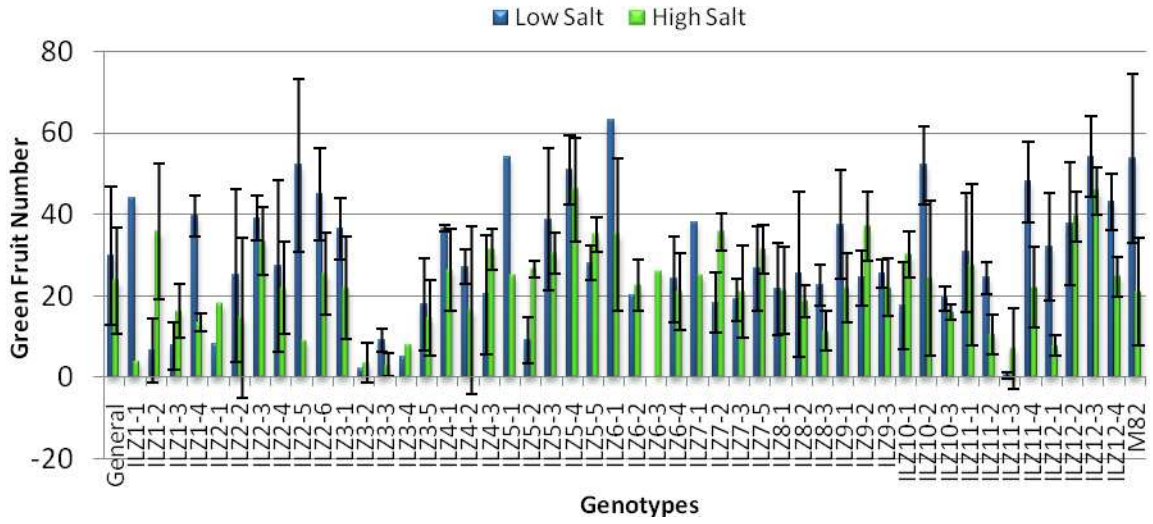

Figure E. Green fruit number of all genotypes analyzed at third time point

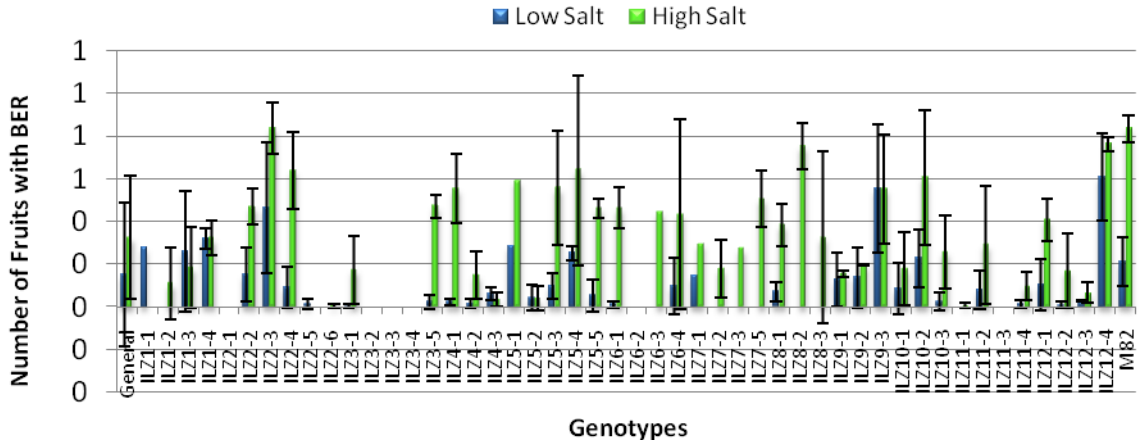

Figure F. Blossom End Rot Ratio of all genotypes analyzed at third time point.

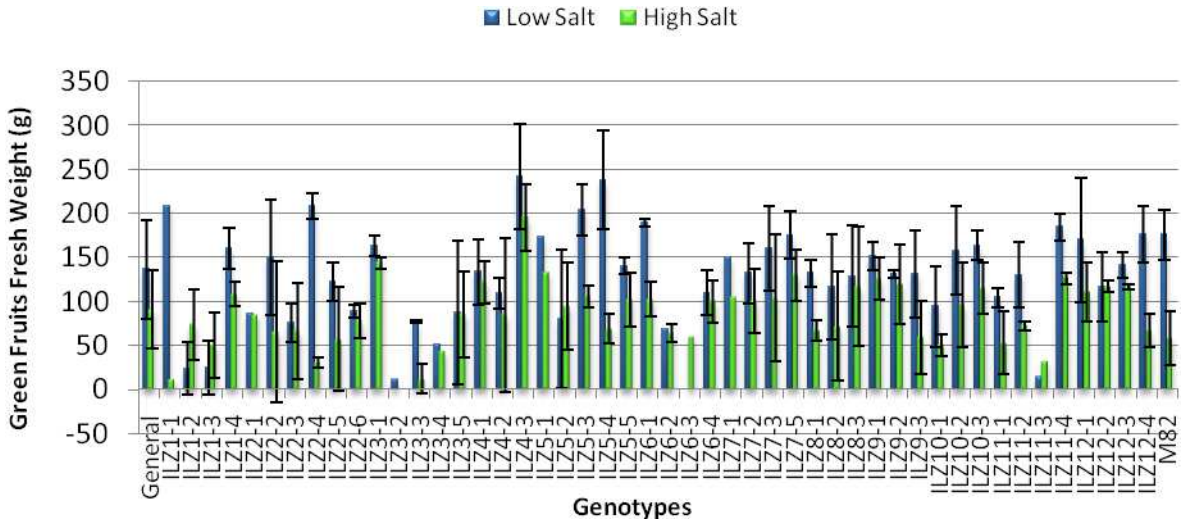

Figure G. Green fruit weight of all genotypes analyzed at third time point.

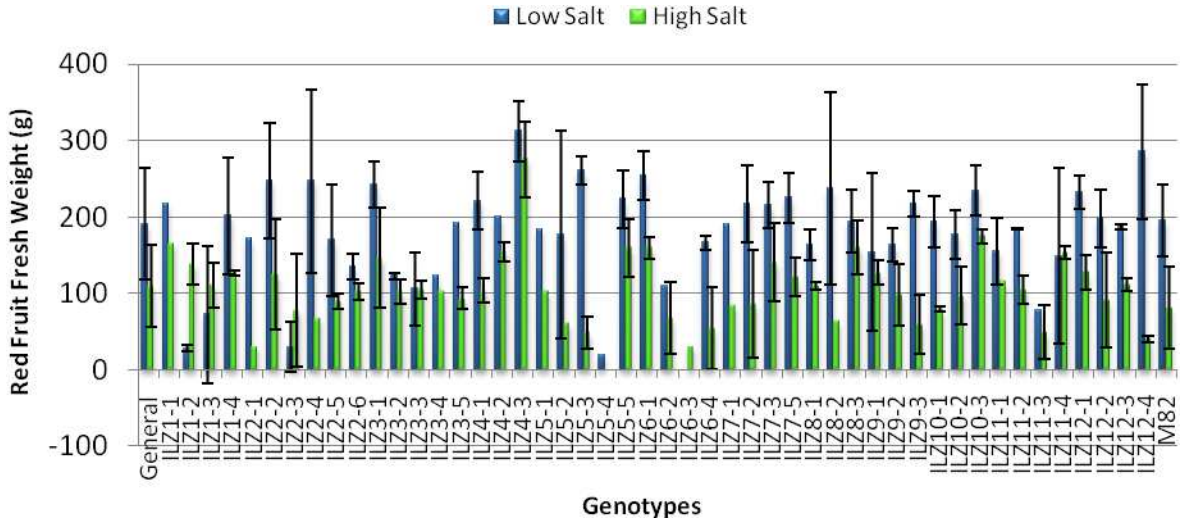

Figure H. Red fruit weight of all genotypes analyzed at third time point.

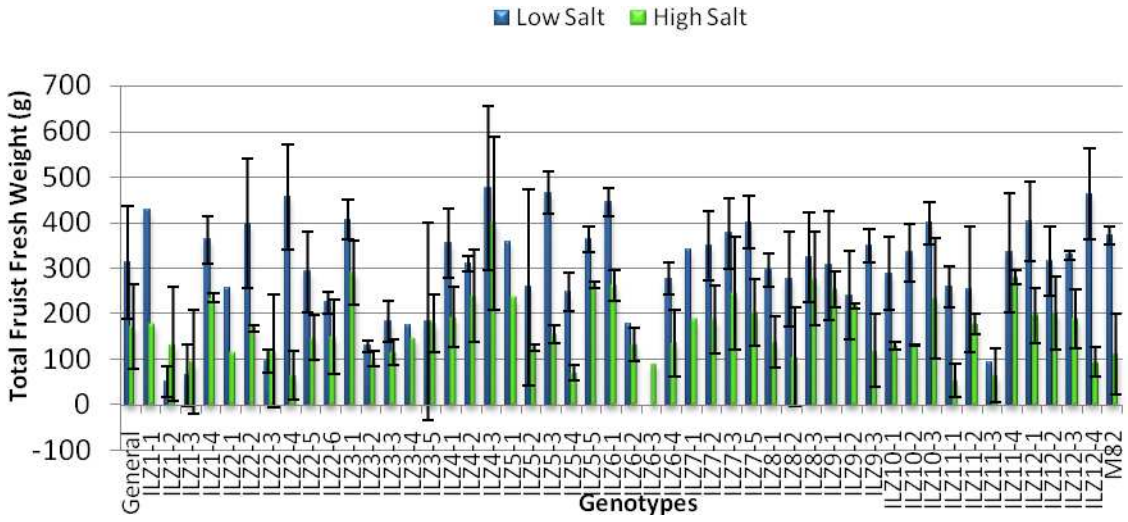

Figure I. Red fruit weight of all genotypes analyzed at third time point.

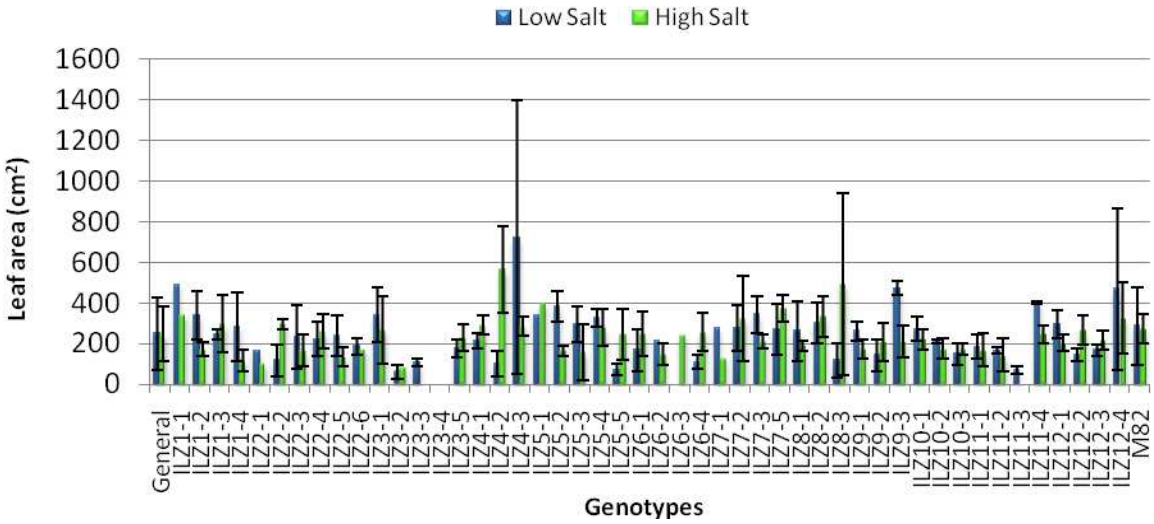

Figure J. Leaf area of all genotypes analyzed at third time point.

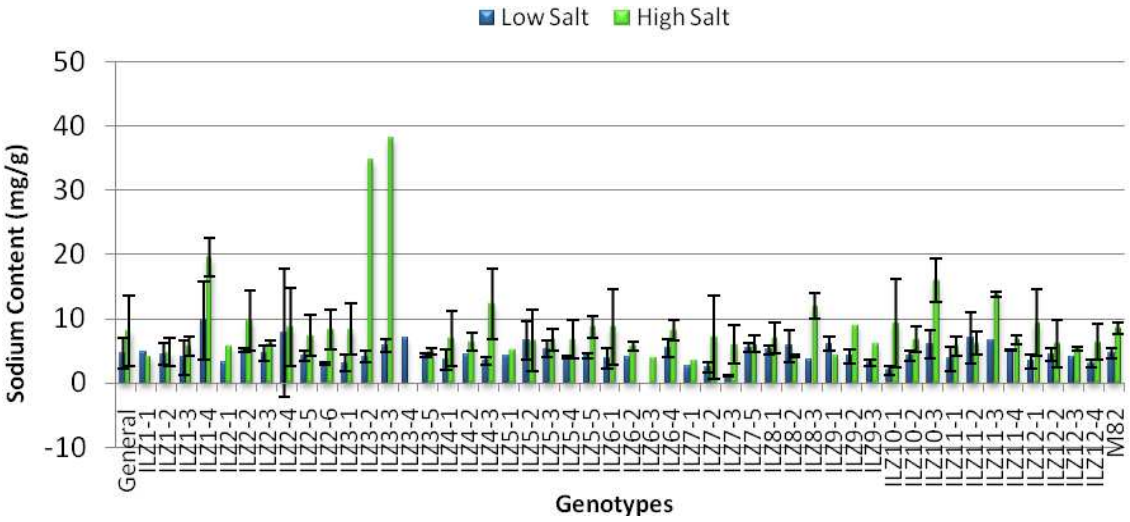

Figure K. Sodium content of top leaves on all genotypes analyzed at third time point.

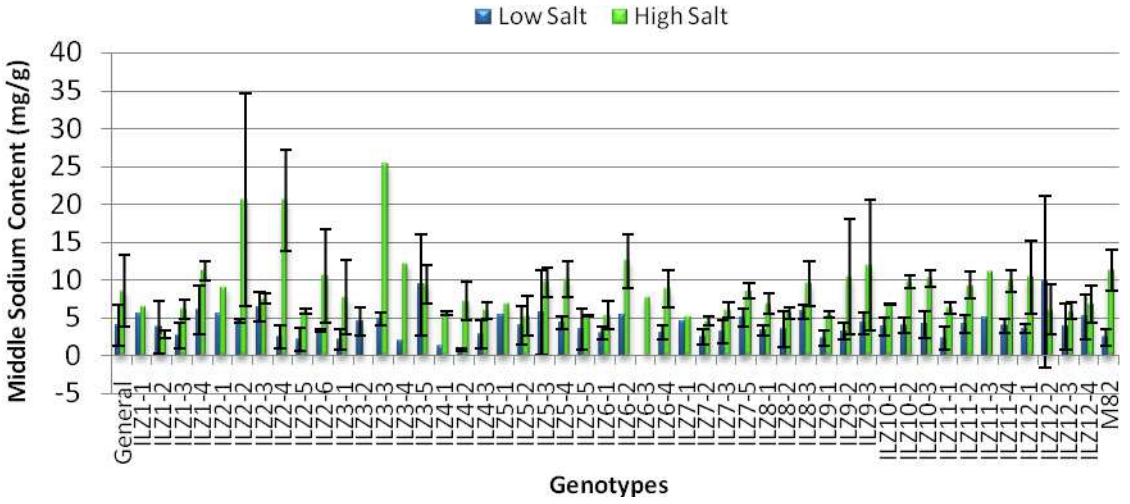

Figure L. Sodium content of middle leaves on all genotypes analyzed at third time point.

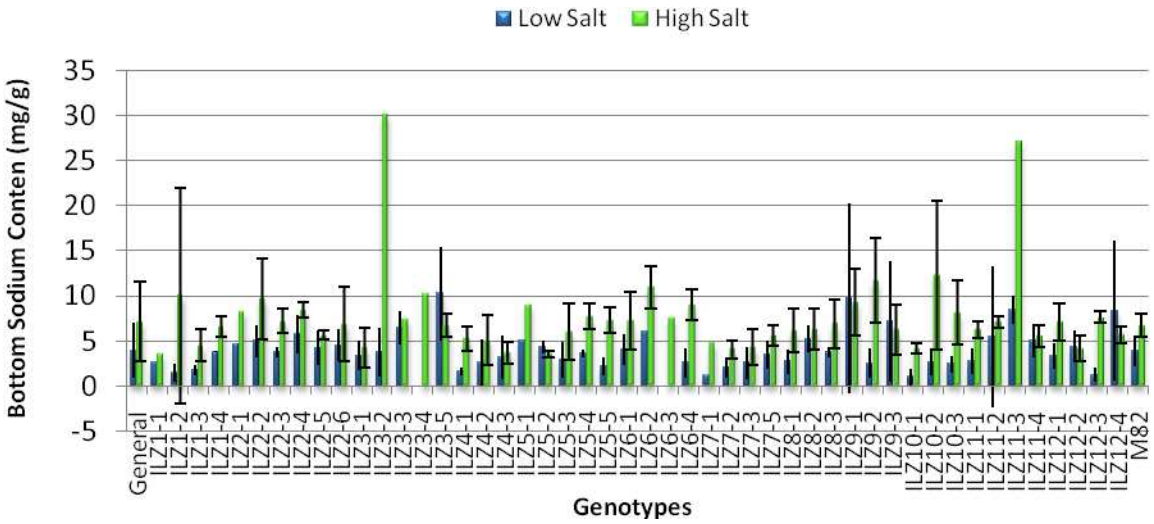

Figure M. Sodium content of bottom leaves on all genotypes analyzed at third time point.

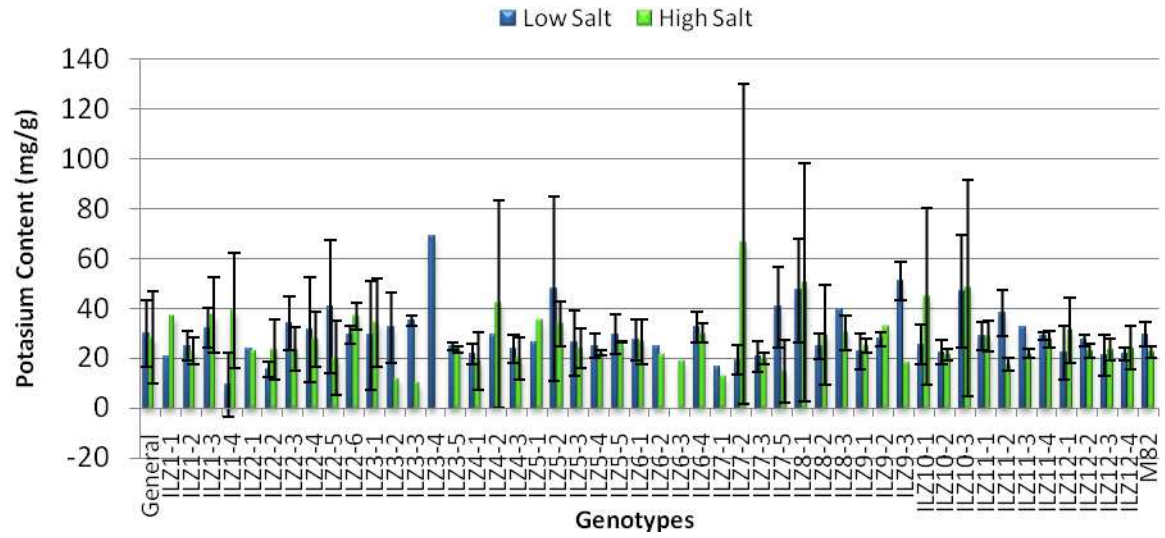

Figure N. Potassium content of top leaves on all genotypes analyzed at third time point.

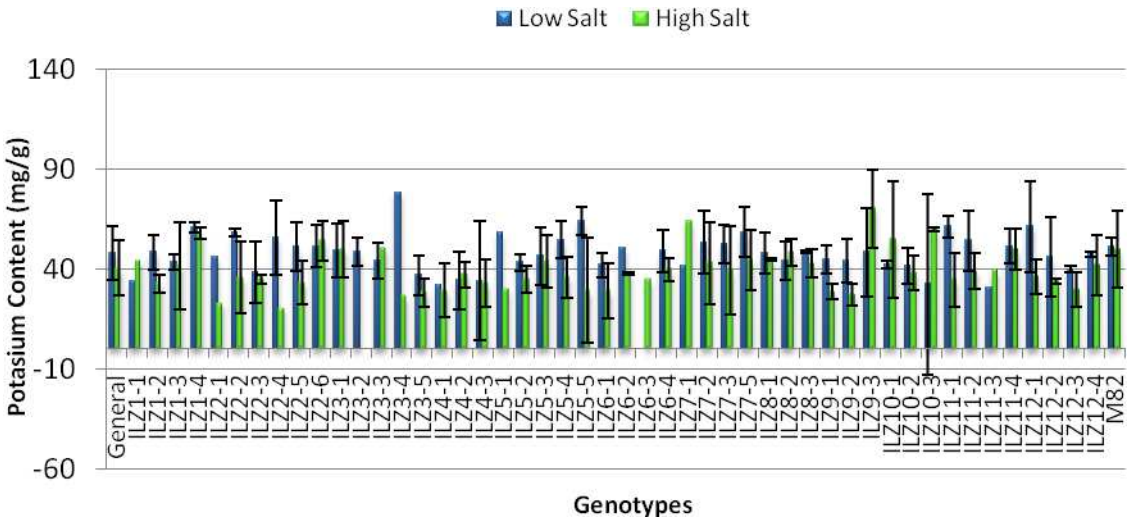

Figure O. Potassium content of middle leaves on all genotypes analyzed at third time point.

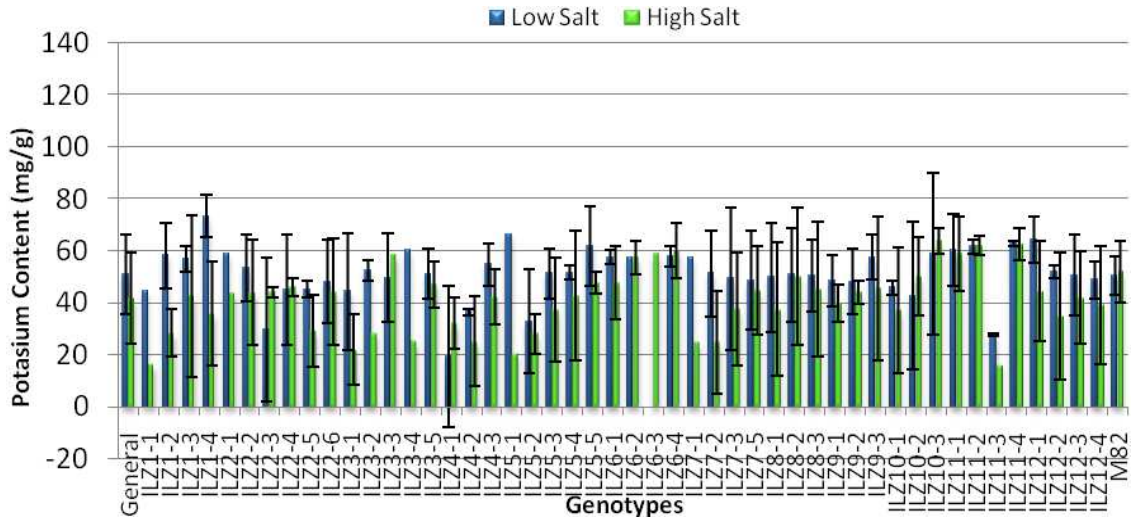

Figure P. Potassium content of bottom leaves on all genotypes analyzed at third time point.

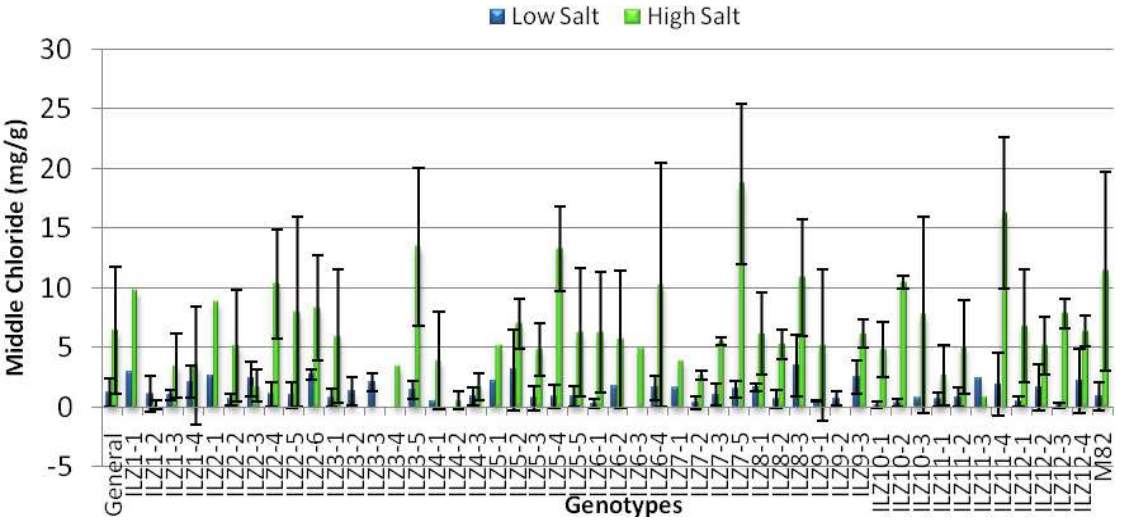

Figure Q. Chloride content of middle leaves on all genotypes analyzed at third time point.

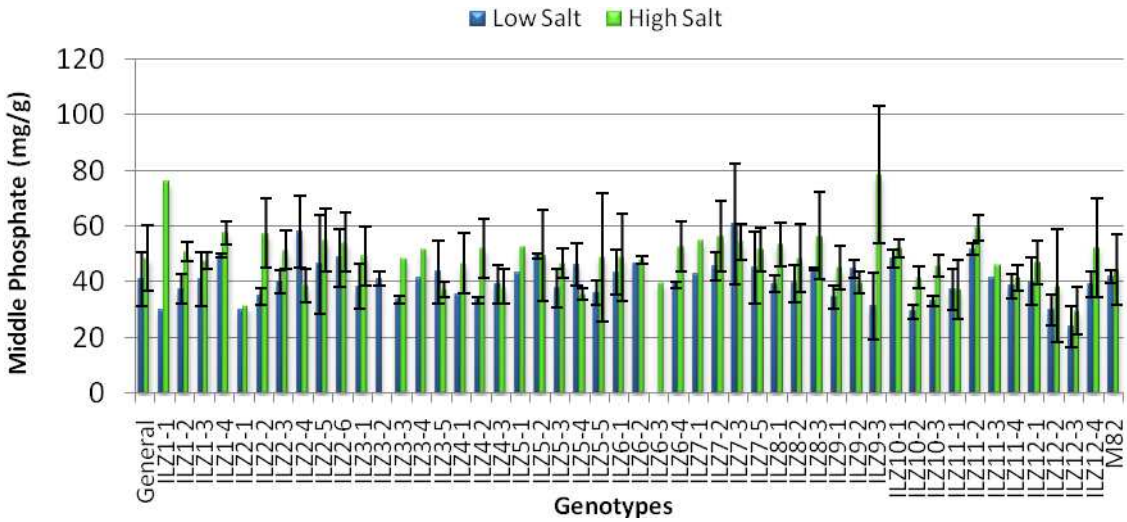

Figure R. Phosphate content of middle leaves on all genotypes analyzed at third time point.

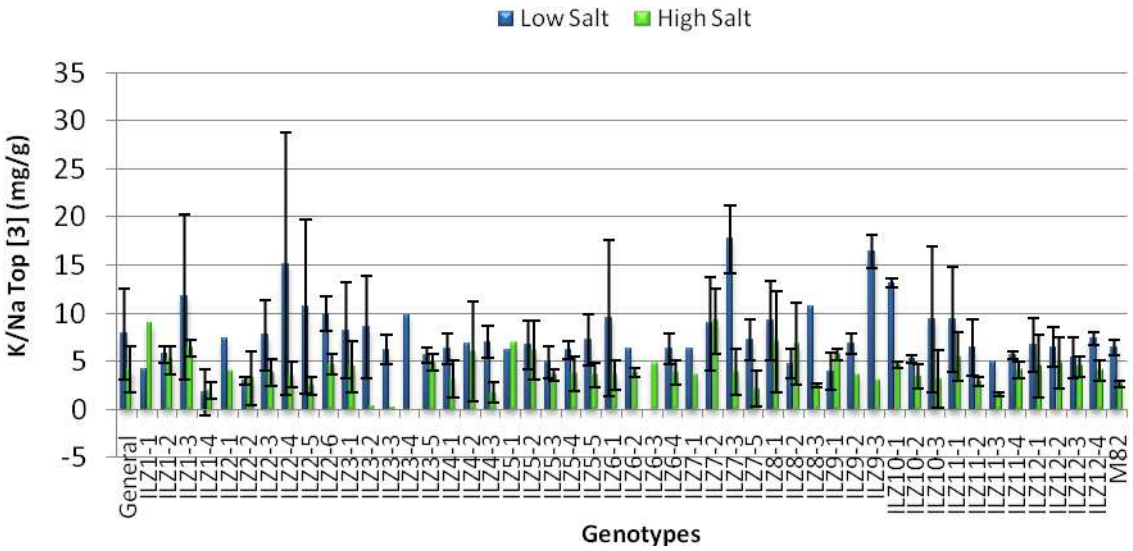

Figure S. Potassium/Sodium ratio of top leaves on all genotypes analyzed at third time point.

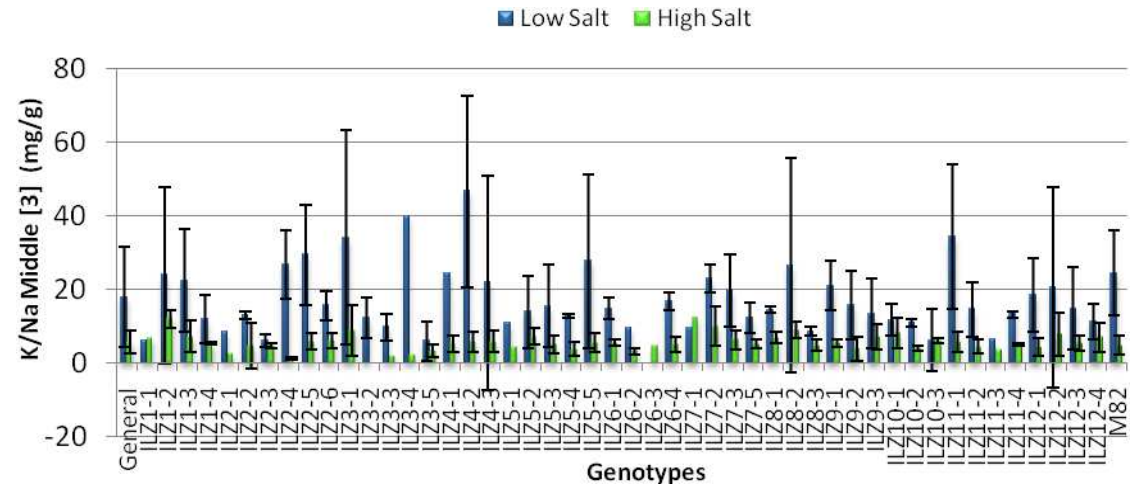

Figure T. Potassium/Sodium ratio of middle leaves on all genotypes analyzed at third time point.

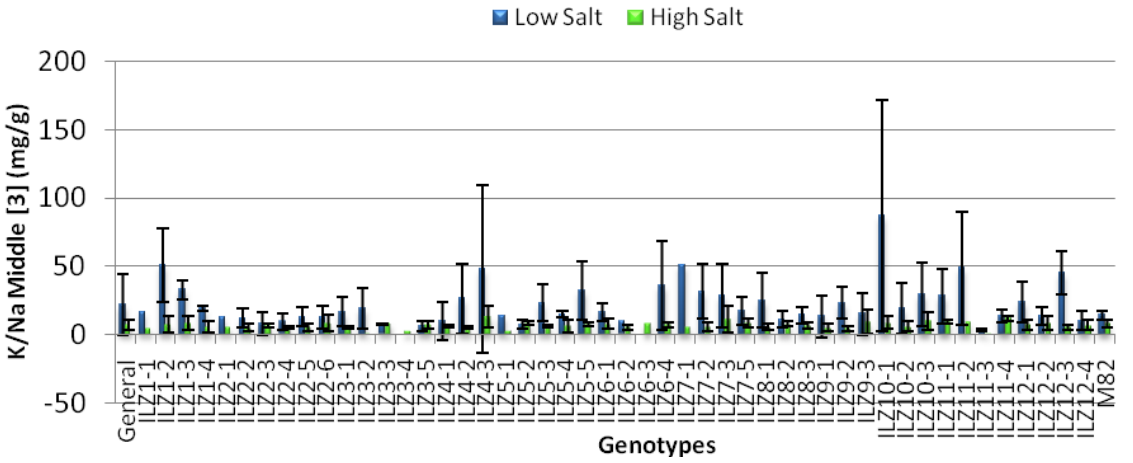

Figure V. Potassium/Sodium ratio of bottom leaves on all genotypes analyzed at third time point.

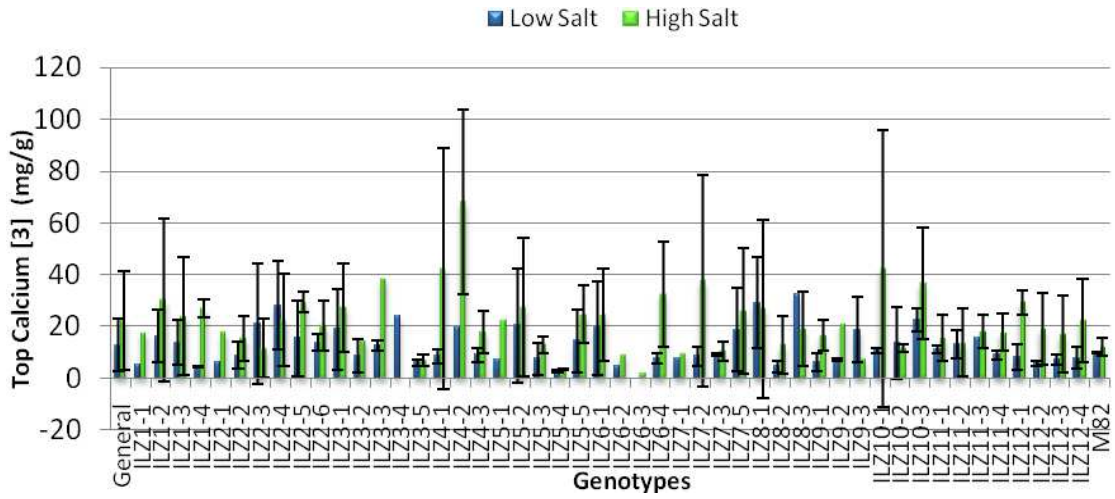

Figure W. Calcium content ratio of top leaves on all genotypes analyzed at third time point.

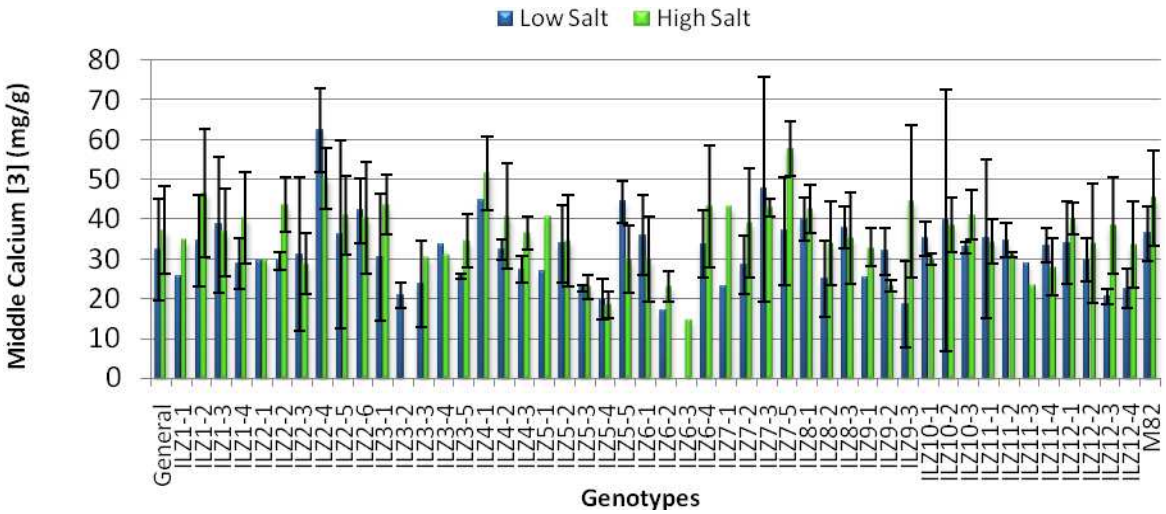

Figure X. Calcium content of middle leaves on all genotypes analyzed at third time point.

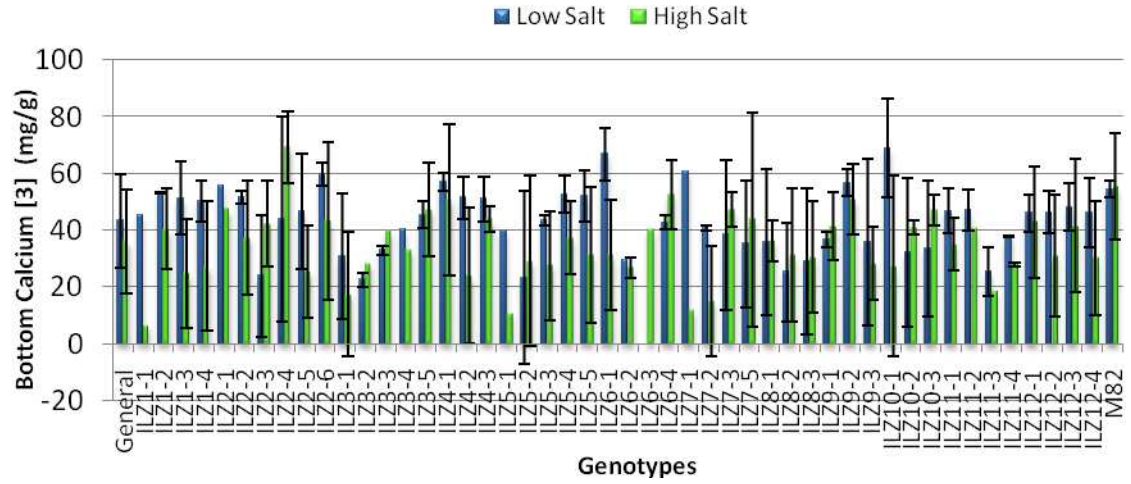

Figure Y. Calcium content of bottom leaves on all genotypes analyzed at third time point.

Appendix 2

Method 1.
Isolation of DNA from leaves (Mini Method).

- Collect about 2-3 cm2 leaf material in a 2 ml screw eppendorf tube. Freeze it in liquid nitrogen.
- Crush it and keep the tubes on ice or in the liquid nitrogen until you have 24 samples.
- Add 400 µl extraction buffer
  500 µl nuclei lysis buffer
  50 µl sarkosyl
- Vortex very well and incubate in a water bath at 65oC for at least one hour.
- Add 800 ul chloroform/isoamylalcohol (24:1) and mix by keeling the tubes.
- Centrifuge 5 min., speed 15000 rpm at room temperature.
- Pipette the supernatant (about 800 µl) in a new 2 ml tube. Add 800 µl isopropanol. Mix carefully. The important part is to have a 1:1 ratio.
- Centrifuge 1 min., speed 15000 rpm. The DNA will then be as a pellet in your tube.
- Rinse the pellet for 20 min. in 250 µl 76% EtOH with 10 mM NH4Ac.
- Dry the pellet (at 37C for 15 min) and dissolve in 100 µl TE. (1 µl 2 mg/ml stock RNase; 30 min. RT)

Rnase can be added to the extractionbuffer (2 µl of the 2 mg/ µl solution)

DNA extraction Buffer:
0.35 M Sorbitol
100 mM Tris
5 mM EDTA,
pH 7.5
When you want to autoclave it , add the Sorbitol afterwards.

Nuclei lysis Buffer:
200 mM Tris-Hcl
50 mM EDTA
2M NaCl
2 % CTAB

Sarkosyl:
10 g N-lauroyl sarcosine in 100 ml water

CAPS methods

PCR

	1 x
Taq Buffer	2.00
dNTPs	0.80
Forward Primer	0.50
Revers Primer	0.50
Dream Taq	0.05
dH_2O	15.15
DNA	1.00
Total	20.00

Electrophoresis

	1x
PCR sample	5.00
RedGel	2.00
dH2O	5.00
total	12.00

Digestion

	1x
Enzyme Buffer	2.00
Enzyme	0.50
DNA	5.00
dH2O	12.50
total	20.00

Preparation for Sequencing

Greenomics method before sequencing:

Plasmid DNA: 250 ng/reaction (100 ng/µl or more in water)
Purified PCR product: 100 ng/reaction (50 ng/µl or more in water)

Sequencemix:

5 µl PCR-product or plasmid
4 µl mix (DETT+buffer)
1 µl primer (5 pmol/µl)

Sequenceprogram voor DETT (Dye van Amersham)

25 x:	94 ° C	20 “
	55 ° C	15 “
	60 ° C	1’

DNA sequences assembled in contigs for chromosome 6.

72 cM

Moneymaker

CA-AGTGCTTCAGGGACAAG-A-TGCCAGCCAAGGNAANGT-GCAACTTGT-GCTTGCCATCATCAGTCAGGA-GGAAGCTTTGGCTCGAGA-
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Chmielewskii

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30-2

CACA-AGTGCTTNAGG-ACAAG-A-TGACAGCCAAGGNAAAGT-GCAACTTGT-GCTTGCCATCATCAGTCAGGA-GGAAGCTTTGGCTCGAGA-
ACTCTATCCCGATCGCTGTCCACCTTTGTCCTCAGCTGGTGTTAGTGGAAATTTCATGTTGAACGACAGCAGTGAGTATGATGTTGAAGGTGCTCAAGATGAGCCTAACTTTGATGTTCATGAGCAAAAACCAAACCATCTCAAT
CTGTTGAACATCAGTGCTGAGAGATTCAAGGAGACGATGCCTCTTCAGCAACAATCTCATCCAAACAAGGATGAACTGGTCACAAACTTAGACTTCAGTCTGAAGAGGAAGCAAGCTAATGAACCTACTGTGATGATGGATCAAA
AGATATACACATGCGAGTTTCTTCAATGCCCTCAC-AAT

40-1

NCACA-AGTGCTTC-GGGACN-G-ACTGCAGCC-AAGG-AAAGT-GCAACTTGT-GCTTGCCATCATCAGTCAGGN-GGNAGCTTTGGCTCGAGC-
ACTCTNTCCCGATCGCTGTCCACCTTTGTCCTCAGCTGGTGTTAGTGGAAATTTCATGTTGAACGACAGCAGTGAGTNTGATGTTGAAGGTGCTCAAGNTGAGCCTAACTTTGATGTTCATGAGCAAAAACCAAACCATCTCAAT
CTGTTGAACATCAGTGCTGAGAGATTCAAGGAGACGATGCCTCTTCAGCAACAATCTCATCCAAACAAGGATGAACTGGTCACAAACTTAGACTTCAGTCTGAAGAGGAAGCAAGCTAATGAACCTACTGTGATGATGGATCAAA
AGATATACACATGCGAGTTTCTTCAATGCCCTCAC-AA

43-3

CA-AGTGCTTCAGGGACAAG-A-TGCCAGCCAAGGNAANGT-GCAACTTGT-GCTTGCCATCATCAGTCAGGA-GGAAGCTTTGGCTCGAGA-
ACTCTATCCCGATCGCTGTCCACCTTTGTCCTCAGCTGGTGTTAGTGGAAATTTCATGTTGAACGACAGCAGTGAGTATGATGTTGAAGGTGCTCAAGATGAGCCTAACTTTGATGTTCATGAGCAAAAACCAAACCATCTCAAT
CTGTTGAACATCAGTGCTGAGAGATTCAAGGAGACGATGCCTCTTCAGCAACAATCTCATCCAAACAAGGATGAACTGGTCACAAACTTAGACTTCAGTCTGAAGAGGAAGCAAGCTAATGAACCTACTGTGATGATGGATCAAA
AGATATACACATGCGAGTTTCTTCAATGCCCTCAC-AAT

44-1

AGCCAAGGTAAAGT-GCAACTTGT-GCTTGCCATCATCAGTCAGGC-
GGNAGCTTTGGCTCGAGACACTCTCTCCCGATCGCTGTCCACCTTTGTCCTCAGCTGGTGTTAGTGGAAATTTCATGTTGAACGNCAGCAGTGAGTCTGNTGTTGAAGGTGCTCAAGCTGAGCCTAACTTTGATGTTCATGAGCA
AAAACCAAACCATCTCAATCTGTTGAACATCAGTGCTGAGAGATTCAAGGAGACGATGCCTCTTCAGCAACAATCTCATCCAAACAAGGATGAACTGGTCACAAACTTAGACTTCAGTCTGAAGAGGAAGCAAGCTAATGAACCT
ACTGTGATGATGGATCAAAAGATATACACATGCGAGTTTCTTCAATGCCCTCAC-AA

76 cM

Moneymaker

ANCTCTCTAACGGGTATATTTTTCTGATCTTAT-CCCGTT-GGGATCTTTACACTTGNTGATCTTG-TTCTTATTTGT-TGGGATCTGCATAAAGTTATGATCTTTAG-
CTATTTTTTGGTCTATTGGGGTGTTAATGCAGGTGGGGTTGAAAGGAAGAAGGGTGAACCAATTGCAGGG-
ACAGAAATTCAATAATAAGGCTTCAAAGAGCCGATTAGTAGTAAGGGCGAATGCTAAAGATATTGCATTTGACCAGAAATCAAGAGCTGCCCTTCAAGCAGGAATTGATAAGCTCGTTAATGTTGTCGGTGTCACTCTTGGTCCT
AGGGGTATGTATGTTTCCTAAGCTTCATCTTCCAAATCAATGGAAGTTCTTTTGTTTTTATTTTCATGTAAAGTGTCTTGCAATGAATGAAGTCATTTATCAGTGGTGTT-
GGTTAGGAAAAAGTACAAGATTAAACTATAATACGTAGCGAATCAGAGAGTGCTTTGATGCAATTTTTGAGTATTGGAGATTAATAATAATGTCTTAGTGTTAGTTGAAACAAGTACATAGGAAGTTTAAAGTTTAAGATAATTG
TAAGGATAGCTATTTTCCCTCTTCTGGAGGCGAAGACTTTAAACATGAGATATTTTGCAGATGTATCAATGTTATGTGTTAGTCATCC

Chmielewskii

CTCTAACGG-TN-A-TTTTCTGATCTTAT-CC-GTTAA-GATCTTTACACTTG-TGATCTTG-TTCTTATTTGT-TGGGATCTGCATAAAGTTATGATCTTTAG-
CTGTTTTTTGGTCTATTGGGGTGTTAATGCAGGTGGGGTTGAAAGGAAGAAGGGTGAACCAATTGCAGGG-
ACAGAAATTCAACAATAAGGCTTCAAAGAGCCGATTAGTAGTAAGGGCGAATGCTAAAGATATTGCATTTGACCAGAAATCAAGAGCTGCCCTTCAAGCAGGAATTGATAAGCTCGTTAATGTTGTCGGTGTCACTCTTGGTCCT
AGGGGTATGTATGTTTCCTAAGCTTCATCTTCCAAATCAATGGAAGTTCTTTTGTTTTTATTTTCATGTAAAGTGTCTTGCAATGAATGAAGTCATTTATCAGTGGTGTTTGGTTAGGAAAAAGTACAAGATTAAACTATAATGC
GTAGCTAATCGGAGAGTGCTTTGATGCAATTTTTGAGTATTGGAGATTAATGATAATGTCTTAGTGTTAGTTGAAACAAGTATATAGGAAGTTTAAAGTTTAAGATAATTGTAAGGATAGCTATTTTCCCTCTTCTGGAGGCGAA
GACTTTAAACATGAGATATTTTGCAGATGTATCAATGTTATGTGTTAGTCATCCTTCCTGTAGAAATGTGAAATTGCCAAAATATAA

30-2

NCTCTCTAACGGGTN-ATTTTTCTGATCTTAT-CN-GTTCGGGATCTTTACACTTG-TGATCTTGCTTCTTATTTGT-TGGGATCTGCATAAAGTTATGATCTTTAG-
CTATTTTTTGGTCTATTGGGGTGTTAATGCAGGTGGGGTTGAAAGGAAGAAGGGTGAACCAATTGCAGGGCACAGAAATTCAATAATAAGGCTTCAAAGAGCCGATTAGTAGTAAGGGCGAATGCTAAAGATATTGCATTTGACC
AGAAATCAAGAGCTGCCCTTCAAGCAGGAATTGATAAGCTCGTTAATGTTGTCGGTGTCACTCTTGGTCCTAGGGGTATGTATGTTTCCTAAGCTTCATCTTCCAAATCAATGGAAGTTCTTTTGTTTTTATTTTCATGTAAAGT
GTCTTGCAATGAATGAAGTCATTTATCAGTGGTGTT-
GGTTAGGAAAAAGTACAAGATTAAACTATAATACGTAGCGAATCAGAGAGTGCTTTGATGCAATTTTTGAGTATTGGAGATTAATAATAATGTCTTAGTGTTAGTTGAAACAAGTACATAGGAAGTTTAAAGTTTAAGATAATTG
TAAGGATAGCTATTTTCCCTCTTCTGGAGGCGAAGACTTTAAACATGAGATATTTTGCAGATGTATCAATGTTATGTGTTAGTCATCCTTCCTGTAGAAATGTG

40-1

ANCTCTCTAACGG-TN-N-TTTTCTGATCTTAT-CC-GTT-GGGATCTTTACACTTG-TGATCTTG-TTCTTATTTNT-TGGGATCTGCATAAAGTTATGATCTTTAG-
CTATTTTTTGGTCTATTGGGGTGTTAATGCAGGTGGGGTTGAAAGGAAGAAGGGTGAACCAATTGCAGGG-
ACAGAAATTCAATAATAAGGCTTCAAAGAGCCGATTAGTAGTAAGGGCGAATGCTAAAGATATTGCATTTGACCAGAAATCAAGAGCTGCCCTTCAAGCAGGAATTGATAAGCTCGTTAATGTTGTCGGTGTCACTCTTGGTCCT
AGGGGTATGTATGTTTCCTAAGCTTCATCTTCCAAATCAATGGAAGTTCTTTTGTTTTTATTTTCATGTAAAGTGTCTTGCAATGAATGAAGTCATTTATCAGTGGTGTT-
GGTTAGGAAAAAGTACAAGATTAAACTATAATACGTAGCGAATCAGAGAGTGCTTTGATGCAATTTTTGAGTATTGGAGATTAATAATAATGTCTTAGTGTTAGTTGAAACAAGTACATAGGAAGTTTAAAGTTTAAGATAATTG
TAAGGATAGCTATTTTCCCTCTTCTGGAGGCGAAGACTTTAAACATGAGATATTTTGCAGATGTATCAATGTTATGTGTTAGTCAT

43-3

ATTTTTCTGATCTTATNCC-GTT-GGGATCTTTACACTTG-TGATCTTGCTTCTTATTTGN-TGGGATCTGCATAAAGTTATGATCTTTAG-
CTATTTTTTGGTCTATTGGGGTGTTAATGCAGGTGGGGTTGAAAGGAAGAAGGGTGAACCAATTGCAGGG-
ACAGAAATTCAATAATAAGGCTTCAAAGAGCCGATTAGTAGTAAGGGCGAATGCTAAAGATATTGCATTTGACCAGAAATCAAGAGCTGCCCTTCAAGCAGGAATTGATAAGCTCGTTAATGTTGTCGGTGTCACTCTTGGTCCT
AGGGGTATGTATGTTTCCTAAGCTTCATCTTCCAAATCAATGGAAGTTCTTTTGTTTTTATTTTCATGTAAAGTGTCTTGCAATGAATGAAGTCATTTATCAGTGGTGTT-
GGTTAGGAAAAAGTACAAGATTAAACTATAATACGTAGCGAATCAGAGAGTGCTTTGATGCAATTTTTGAGTATTGGAGATTAATAATAATGTCTTAGTGTTAGTTGAAACAAGTACATAGGAAGTTTAAAGTTTAAGATAATTG
TAAGGATAGCTATTTTCCCTCTTCTGGAGGCGAAGACTTTAAACATGAGATATTTTGCAGATGTATCAATGTTATGTGTTAGTCATCCTTCCTGTAGAAATGT

44-1

ANCTCTCTAACGG-TA-ATTTTTCTGATCTTAT-CCCGTT-
GGGTNNNTTACACTTGNTGATCTTGCTTCTTATTTNCCTGGGATCTNCATAAAGTTATGATCTTTAGNCTATTTTTTGGTCTATTGGGGTGTTAATGCAGGTGGGGTTGAAAGGAAGAAGGGTGAACCAATTGCAGGGCACAGAA
ATTCAATAATAAGGCTTCAAAGAGCCGATTAGTAGTAAGGGCGAATGCTAAAGATATTGCATTTGACCAGAAATCAAGAGCTGCCCTTCAAGCAGGAATTGATAAGCTCGTTAATGTTGTCGGTGTCACTCTTGGTCCTAGGGGT
ATGTATGTTTCCTAAGCTTCATCTTCCAAATCAATGGAAGTTCTTTTGTTTTTATTTTCATGTAAAGTGTCTTGCAATGAATGAAGTCATTTATCAGTGGTGTT-
GGTTAGGAAAAAGTACAAGATTAAACTATAATACGTAGCGAATCAGAGAGTGCTTTGATGCAATTTTTGAGTATTGGAGATTAATAATAATGTCTTAGTGTTAGTTGAAACAAGTACATAGGAAGTTTAAAGTTTAAGATAATTG
TAAGGATAGCTATTTTCCCTCTTCTGGAGGCGAAGACTTTAAACATGAGATATTTTGCAGATGTATCAATGTTATGTGTTAGTCATCCTTCCTGTAGAAATGTGAAATTGCCAAAATATAACAAGGTAAAATTTTCATTTTTTCA
TCAAAAGAGACTTTCCTTTTGAAGCCAGAATTGATTTTCCTCCTCCAGCATTGCTGCAGGTTTCATGCTATACCAGTAAATCAGTCATTTATTTGATCAACTCTGTNATGATGCTTTNGT

83 cM

Moneymaker

TACA-TGGNCTCTGCTGTA-CCCCTTTTGCCCCACATTGGCCATCG-CAAGGTCAACATCNGGCTCTGNTC-
TNGTTCTTCGTCGGGCTGNTCAACTCAAATTTGAATCTTGTTTTCTCAACTCGTCTGGGGCTCATCTGTTTTGTTTTGTTATCGCTTGGTTGTTTTGTGAATCTTTACTGTCATGCTTGTAAAATGAAATGAACCCGAGTGAGCT
GAACCTATGTAGNGGATTCATGTAGCTGACCCCAGNCTAATTTGGGATTTTGATGATGCTGATTGATTGGTACCTGTAAAACGGAACACGGGATAGGTTAAAATATAGATGGAATTGGTTGTAGTATTGTGGCTGACTGATGGTG
TTTTGAATCTTGTTAGAGGTTCTTGATGTTTGGCATAATGCGAATGCTGTATGCTTTGATGTGGATAGCACTGTGTGCATAAGACN

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TACA-TGGA-CCTGCTGTA-CCC-TTTTGNCACACATTG-CCATCT-CAAGGTCAACATCAGGNTCTGAGC-TTGTTCTTGGTCGGGCTGATCAACTAAAATTTGAATCTTGTTTTCTAAACTAGN-
TGGGGCTAATCTGTTTTGTTTTGTTATAGNTTGGTTGTTTTGTGAATATTTACTGTCATGCTTGTAAAATGAAATGAACCCGAGTGAGCTGAACCTATGTAGAGGATTCATGTAGCTGACCCCAGACTAATTTGGGATTTTGATG
ATGCTGATTGATTGGTACCTGTAAAACGGAACACGGGATAGGTTAAAATATAGATGGAATTGGTTGTAGTATTGTGGCTGACTTATGGTGTTTTGAATCTTGTTAGAGGTTCTTGATGTTTGGCATAATGCGAATGCTGTATGCT
TTGATGTGGATAGCACTGTGTGCATA-GACGA

30-2

ACAATGGAGCCTGCTGTACCCC-TTTTGACACCCATTG-CCTTNNGCAAGGTAAACATCAGGATCTGAGA-TTGCTCTTCGTATGGCTGATAAACTAAAATTTGAATCTTGTTTTCTAAACTAGA-
TGGGGCTAATCTGTTTTGTTTTGTTATAGATTGGTTGTTTTGTGAATATTTACTGTCATGCTTGTAAAATGAAATGAACCCGAGTGAGCTGAACCTATGTAGAGGATTCATGTAGCTGACCCCAGACTAATTTGGGATTTTGATG
ATGCTGATTGATTGGTACCTGTAAAACGGAACACGGGATAGGTTAAAATATAGATGGAATTGGTTGTAGTATTGTGGCTGACTGATGGTGTTTTGAATCTTGTTAGAGGTTCTTGATGTTTGGCATAATGCGAATGCTGTATGCT
TTGATGTGGATAGCACTGTGTGCATA

40-1

NCAATGGAGCCTGCTGTA-CCC-TTTTGACACCCATTG-CCTTCNNCAAGGTAAACATCAGGATCTGAGA-TTNCTCTTCGTATGGCTGATAAACTAAAATTTGAATCTTGTTTTCTAAACTAGA-
TGGGGCTAATCTGTTTTGTTTTGTTATAGATTGGTTGTTTTGTGAATATTTACTGTCATGCTTGTAAAATGAAATGAACCCGAGTGAGCTGAACCTATGTAGAGGATTCATGTAGCTGACCCCAGACTAATTTGGGATTTTGATG
ATGCTGATTGATTGGTACCTGTAAAACGGAACACGGGATAGGTTAAAATATAGATGGAATTGGTTGTAGTATTGTGGCTGACTGATGGTGTTTTGAATCTTGTTAGAGGTTCTTGATGTTTGGCATAATGCGAATGCTGTATGCT
TTGATGTGGATAGCACTGTGTGCATA-GACNAANN

43-3

NTAC-ATGGAGCCTGCTGTA-CCCCTTTTGNCACACATNG-CCATCGA-AAGGTCA-CATCAGGNTCTGAGC-TTGTTCTTCGTNGGGCTGATCAACTAAAATTTGAATCTTGTTTTCTAAACTAGN-
TGGGGCTAATCTGTTTTGTTTTGTTATAGATTGGTTGTTTTGTGAATATTTACTGTCATGCTTGTAAAATGAAATGAACCCGAGTGAGCTGAACCTATGTAGAGGATTCATGTAGCTGACCCCAGACTAATTTGGGATTTTGATG
ATGCTGATTGATTGGTACCTGTAAAACGGAACACGGGATAGGTTAAAATATAGATGGAATTGGTTGTAGTATTGTGGCTGACTGATGGTGTTTTGAATCTTGTTAGAGGTTCTTGATGTTTGGCATAATGCGAATGCTGTATGCT
TTGATGTGGATAGCACTGTGTGCATAAGAC

44-1

NTACA-TGGNCTCTGCTGTA-CCCCTTTTGCCCCACATTGGCCATCGA-AAGGTCAACATCAGGCTCTGNGACTTGTTCTTCGTCGGGCTGATCAACTCAAATTTGAATCTTGTTTTCTNAACTNGC-
TGGGGCTAATCTGTTTTGTTTTGTTATNGCTTGGTTGTTTTGTGAATCTTTACTGTCATGCTTGTAAAATGAAATGAACCCGAGTGAGCTGAACCTATGTAGAGGATTCATGTAGCTGACCCCAGACTAATTTGGGATTTTGATG
ATGCTGATTGATTGGTACCTGTAAAACGGAACACGGGATAGGTTAAAATATAGATGGAATTGGTTGTAGTATTGTGGCTGACTGATGGTGTTTTGAATCTTGTTAGAGGTTCTTGATGTTTGGCATAATGCGAATGCTGTATGCT
TTGATGTGGATAGCACTGTGTGCATAAGAC

85 cM

Moneymaker
CAAGTTCTCGTCATCGATTNAGGATC-TGGTGAACGNTGCGAACTTTGATTGTTCCGCCACTGG-ATTCTCTCTGCAAGCCATGGACTCCAGTCACGTGGCTCTGGTGGCGCTGCTGCTCCAA

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CTANTCCGCA-ACNG-CNAATGTTGGACACTACGGCTTG-TTCAG-GTCAGTCTTGCTGAATCAAGTTCTCNTCATCGNTTCAGGNTC-TGGTGNACGNTGCGNACTTTGATTGTTCCGCCACTGG-
ATTCTCTCTGCAAGCCATGGACTCCAGTCACGTGGCTCTGGTGGCGCTGCTGCTCCAAAN

30-2
AAATGTTGGA-ACTACGTCTTG-TTCAG-GTAAGTCT-GCTGAAGAAAGTTCT-ANAATCGATTAAGGATC-TGGTGAACGATGCGAACTTTGATTGTTCCGCCACTGG-
ATTCTCTCTGCAAGCCATGGACTCCAGTCACGTGGCTCTGGTGGCGCTGCTGCTCCAA

40-1
CAAGTTCTCGTNATCNATTCAGGATCTTGGTGAACGATGCGAACTTTGATTGTTCCGCCACTGGTATTCTCTCTGCAAGCCATGGACTCCAGTCACGTGGCTCTGGTGGCGCTGCTGCTCCAA

43-3
NCTANTCCGCAGAATGACAAATGTTGGA-ACTACGTCTTGNTTCAGTGTCAGTCTTGCTGAATCAAGTTCTCGTCATCGATTCAGGATC-TGGTGNACGNTGCGAACTTTGATTGTTCCGCCACTGG-
ATTCTCTCTGCAAGCCATGGACTCCAGTCACGTGGCTCTGGTGGCGCTGCTGCTCCAAA

44-1
AAGTTCTN-TAATCNATTAAGGATCTTGGTGAACGATGCGAACTTTGATTGTTCCGCCACTGG-ATTCTCTCTGCAAGCCATGGACTCCAGTCACGTGGCTCTGGTGGCGCTGCTGCTCCAA

90 cM

Moneymaker
CA-ATCCCCCTTTCTCT-GAT-GAAAAGTGGGTCTCGCATCA-TCT-GCTTCGTGGGTNG-GCGTT-A-TCA-T-GG-A-CGT-T-GTTAATGCTGAA-CA-GGCTCG-TATAGCCGA-GG-
AGGCCGGTGCGTGTGCTGTCATGGCTCTTGA-GCGTGTCCCTGCCTGATATACGCGCTCAGGGCGGCGTTGCACGT-ATGTCGGATCCCCAGCTTATCAAAGAAATCAAACAGGCTGANN

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T-CAGGTTCG-GAA-ATGG-TGC-CTCACAG-A--ANACACCAAGCA-ATCCCCCTT-CTCT-GA-TGAAAAGTGGGTCTCGC-TCA-NAT-GCTTCGGGG--CG-GCGTTA--TCA-T-GG-A-CGT-TG-TTAATGCTGAA-
CA-GGCTCG-TATAGCCGA-GG-AGGCCGGTGCGTGTGCTGTCATGGCTCTTGA-GCGTGTCCCTG-CTGATATACGCGCTCAGGGCGGCGTTGCACGT-ATGTCGGATCCCCAGCTTATCAAAGAAATCAAACAGGCTGAN

30-2

TACAGGTACGNGACCATGGGTGCTCTNACAATANTACACACCAAGCA-ATCCCCCTTTCTCTTGATTGAAAAGTGGGTCTCGCATCANTCTTGCTTCGGGG-ACG-GCGTTNN-TCA-T-GG-AACGT-TGGTTNATGCTGAA-
CA-GGCTCG-TATCGCCGA-GG-NGGCCGGTGCGTGTGCTGTCATGGCTCTTGA-GCGTGTCCCTG-CTGATATACGCGCTCAGGGCGGCGTTGCACGT-ATGTCGGATCCCCAGCTTATCAAAGAAATCAAACAGGCTGANNN

40-1

GTACAGGTACGTGACCATGGTTGC-CTN-CANTACTACACAC-AAGCACATCCCCCTTTCTCTTGATTGCAAAGTGGGTCTCGC-TCAATCTTGCTTCGGGGTANG-GCGTTNN-TCA-T-GG-A-CGT-TGGTTNATGCTGAA-
CA-GGCTCG-TATCGCCGA-GG-AGGCCGGTGCGTGTGCTGTCATGGCTCTTGA-GCGTGTCCCTG-CTGATATACGCGCTCAGGGCGGCGTTGCACGT-ATGTCGGATCCCCAGCTTATCAAAGAAATCAAACAGGCTGANNN

43-3

GGG-
ACGNGCGTTNACTCAATTGGGAACGTGTGTATNATGCTGAAACAAGGCTCGNTCTCGCCGAAGGAAGGCCGGTGCGTGTGCTGTCATGGCTCTTGAAGCGTGTCCCTGNCTGATATACGCGCTCAGGGCGGCGTTGCACGTTATG
TCGGATCCCCAGCTTATCAAANAAATCAAACAGGCTGANNN

44-1

CGTGTGTTCATTGCTGAAACAAGGCTCG-TCTCGCCGAAGGNAGGCCGGTGCGTGTGCTGTCATGGCTCTTGA-GCGTGTCCCTGNCTGATATACGCGCTCAGGGCGGCGTTGCACGT-
ATGTCGGATCCCCAGCTTATCAAANAAATCAAACAGGCTGANNN

92.5 cM

Moneymaker

CNTGGNTACCTCGGGCAAACTCTATCCAGCTCTCCCCTCCTGTTCNGCAACATTTCATGAGTACAGACACAAACCNCCTTAGCT-
GTAAATCCAAAACTTTAATTCCACGTGTAAGAGACAACACTACACCCACTAAAGCTTTGTTACAATTTGCACAGGTTAAGTATAAGCATATTTTTCACTCCAATATATAGTTTGAGCCGATCAAAAACATTTTCCTAAGATACAG
TTCAAGCTAAAGGCAATTGGTACATTCACTACTCGAAAGATGTACCTGAATTTCATGTAAGTACATGTATCTTTTTTGGTAAGCTCAGAAATAGAAGCTGCCGAAGTGAATAGTGAACGACCATCTGGACCTATATCCAGTGATT
TATCCTTCTCGTCTTCTTTGAGAAAGCGTGCACGAAGATCCTTGTTGAGCGTTTCAGCAGCTTTGGCACCTGCTGCAGCATTTTCGTGTTTTATATCCGGTCTGAAGTTGGGTTTCTTCGGGTGTGTGCTTGTCGCGAAGGTTCT
GGAAGAAGAGACCTTAGAAGATGCTGAGATTGCTGCCTGTATACGGATATCTCCGGTGGTGGGATGGGATTTCGCCGCCGTAGCGGCTGATCGGAGTATTGACCGGAGCATTTCTTCAGGGTANNNN

Chmielewskii

It was nos assembled by SeqMan

30-2

CNTGGNTACCTCGGNCAAACTCTATCCAGCTCTNCCCTCC-
TATCACAAACATTTCATGAGTACAGACACAAACCACCTTAGCAAGTAAATCCAAAACTTTAATTCCACGTGTAAGAGACAACACTACACCCACTAAAGCTTTGTTACAATTTGCACAGGTTAAGTATAAGCATATTTTTCACTCC
AATATATAGTTTGAGCCGATCAAAAACATTTTCCTAAGATACAGTTCAAGCTAAAGGCAATTGGTACATTCACTACTCGAAAGATGTACCTGAATTTCATGTAAGTACATGTATCTTTTTTGGTAAGCTCAGAAATAGAAGCTGC
CGAAGTGAATAGTGAACGACCATCTGGACCTATATCCAGTGATTTATCCTTCTCGTCTTCTTTGAGAAAGCGTGCACGAAGATCCTTGTTGAGCGTTTCAGCAGCTTTGGCACCTGCTGCAGCATTTTCGTGTTTTATATCCGGT
CTGAAGTTGGGTTTCTTCGGGTGTGTGCTTGTCGCGAAGGTTCTGGAAGAAGAGACCTTAGAAGATGCTGAGATTGCTGCCTGTATACGGATATCTCCGGTGGTGGGATGGGATTTCGCCGCCGTAGCGGCTGATCGGAGTATTG
ACCGG

40-1

CC-GG-TACCTCGGGCAAACTCTATCCAGCTCTTCCCTCC-
TATAACAAACATTTCATGAGTACAGACACAAACCACCTTAGCAAGTAAATCCAAAACTTTAATTCCACGTGTAAGAGACAACACTACACCCACTAAAGCTTTGTTACAATTTGCACAGGTTAAGTATAAGCATATTTTTCACTCC

AATATATAGTTTGAGCCGATCAAAAACATTTTCCTAAGATACAGTTCAAGCTAAAGGCAATTGGTACATTCACTACTCGAAAGATGTACCTGAATTTCATGTAAGTACATGTATCTTTTTTGGTAAGCTCAGAAATAGAAGCTGC
CGAAGTGAATAGTGAACGACCATCTGGACCTATATCCAGTGATTTATCCTTCTCGTCTTCTTTGAGAAAGCGTGCACGAAGATCCTTGTTGAGCGTTTCAGCAGCTTTGGCACCTGCTGCAGCATTTTCGTGTTTTATATCCGGT
CTGAAGTTGGGTTTCTTCGGGTGTGTGCTTGTCGCGAAGGTTCTGGAAGAAGAGACCTTAGAAGATGCTGAGATTGCTGCCTGTATACGGATATCTCCGGTGGTGGGATGGGATTTCGCCGCCGTAGCGGCTGATCGGAGTATTG
ACCGGA

43-3

CC-GGNTACCTC-GGCAAACTCTATCCAGCTCTTCCCTCC-
TATAACAAACATTTCATGAGTACAGACACAAACCACCTTAGCAAGTAAATCCAAAACTTTAATTCCACGTGTAAGAGACAACACTACACCCACTAAAGCTTTGTTACAATTTGCACAGGTTAAGTATAAGCATATTTTTCACTCC
AATATATAGTTTGAGCCGATCAAAAACATTTTCCTAAGATACAGTTCAAGCTAAAGGCAATTGGTACATTCACTACTCGAAAGATGTACCTGAATTTCATGTAAGTACATGTATCTTTTTTGGTAAGCTCAGAAATAGAAGCTGC
CGAAGTGAATAGTGAACGACCATCTGGACCTATATCCAGTGATTTATCCTTCTCGTCTTCTTTGAGAAAGCGTGCACGAAGATCCTTGTTGAGCGTTTCAGCAGCTTTGGCACCTGCTGCAGCATTTTCGTGTTTTATATCCGGT
CTGAAGTTGGGTTTCTTCGGGTGTGTGCTTGTCGCGAAGGTTCTGGAAGAAGAGACCTTAGAAGATGCTGAGATTGCTGCCTGTATACGGATATCTCCGGTGGTGGGATGGGATTTCGCCGCCGTAGCGGCTGATCGGAGTATTG
ACCGG

44-1

CC-GG-TACCTC-
GGCAAACTCTATCCAGCTCTTCCCTCCTTATAACAAACATTTCATGAGTACAGACACAAACCACCTTAGCAAGTAAATCCAAAACTTTAATTCCACGTGTAAGAGACAACACTACACCCACTAAAGCTTTGTTACAATTTGCACA
GGTTAAGTATAAGCATATTTTTCACTCCAATATATAGTTTGAGCCGATCAAAAACATTTTCCTAAGATACAGTTCAAGCTAAAGGCAATTGGTACATTCACTACTCGAAAGATGTACCTGAATTTCATGTAAGTACATGTATCTT
TTTTGGTAAGCTCAGAAATAGAAGCTGCCGAAGTGAATAGTGAACGACCATCTGGACCTATATCCAGTGATTTATCCTTCTCGTCTTCTTTGAGAAAGCGTGCACGAAGATCCTTGTTGAGCGTTTCAGCAGCTTTGGCACCTGC
TGCAGCATTTTCGTGTTTTATATCCGGTCTGAAGTTGGGTTTCTTCGGGTGTGTGCTTGTCGCGAAGGTTCTGGAAGAAGAGACCTTAGAAGATGCTGAGATTGCTGCCTGTATACGGATATCTCCGGTGGTGGGATGGGATTTC
GCCGCCGTAGCGGCTGATCGGAGTATTGACCGG

Gene code, chromosome number with main introgression and fruit colour for each of the 62 genotypes used in the experiments.

Genotype	Gene code	Main introgression on chromosome	fruit colour
1	Moneyberg	parent	red
2	LA1840	wild type	n.a.[a]
3	TKM6U 0025	6	orange
4	TKM6U 0051	6	red
5	TKM6U 0062	3	yellow
6	TKM6U 0070	6	red
7	TKM6U 0080	6	red
8	TKM6U 0088	2	red
9	TKM6U 0092	9	red
10	TKM6U 0099	7	red
11	TKM6U 0112	11	red
12	TKM6U 0171	1	red
13	TKM5U 0637	10	red
14	TKM5U 0648	2	red
15	TKM5U 0659	8	red
16	TKM5U 0669	2	red
17	TKM5U 0674	7	red
18	TKM5U 0810	12	orange
19	TKM5U 0945	6	orange
20	TKM5U 1055	2+3	red
21	TKM5U 1190	4	red
22	TKM6U 0016	1	red
23	TKM5U 0439	1+5	pink
24	TKM5U 0455	11	red
25	TKM5U 0475	3	red
26	TKM5U 0486	4	red
27	TKM5U 0540	10	red
28	TKM5U 0571	4	red
29	TKM5U 0580	4	red
30	TKM5U 0595	8	red
31	TKM5U 0618	12	red
32	TKM5U 0623	7	red
33	TKM6U 0193	2	red+orange+yellow+pink
34	TKM6U 0190	12	red
35	TKM6U 0206	2	red
36	TKM6U 0217	3	red
37	TKM6U 0229	12	red
38	TKM6U 0277	5	red
39	TKM7U 0005	8	red
40	TKM7U 0016	1+2	orange
41	TKM7U 0083	5	red
42	TKM7U 0116	6	pink
43	TKM5U 0009	8	red
44	TKM5U 0030	9	red
45	TKM5U 0057	5	red
46	TKM5U 0103	12	orange
47	TKM5U 0142	4	red
48	TKM5U 0168	9	red
49	TKM5U 0225	8	red
50	TKM5U 0234	6	orange
51	TKM5U 0283	12	red
52	TKM5U 0306	3	red
53	TKM5U 0308	12	red
54	TKM5U 0338	9	red
55	TKM5U 0372	9	red
56	TKM5U 0398	10+11	red
57	TKM5U 0422	7	red
58	TKM5U 0438	1	pink
59	06SG259	n.a.[b]	orange
60	06SG260	n.a.	pink
61	06SG261	n.a.	n.a.
62	06SG262	n.a. o	range

[a]) Not available since no ripe fruits were formed during the experiment; [b]) not available.

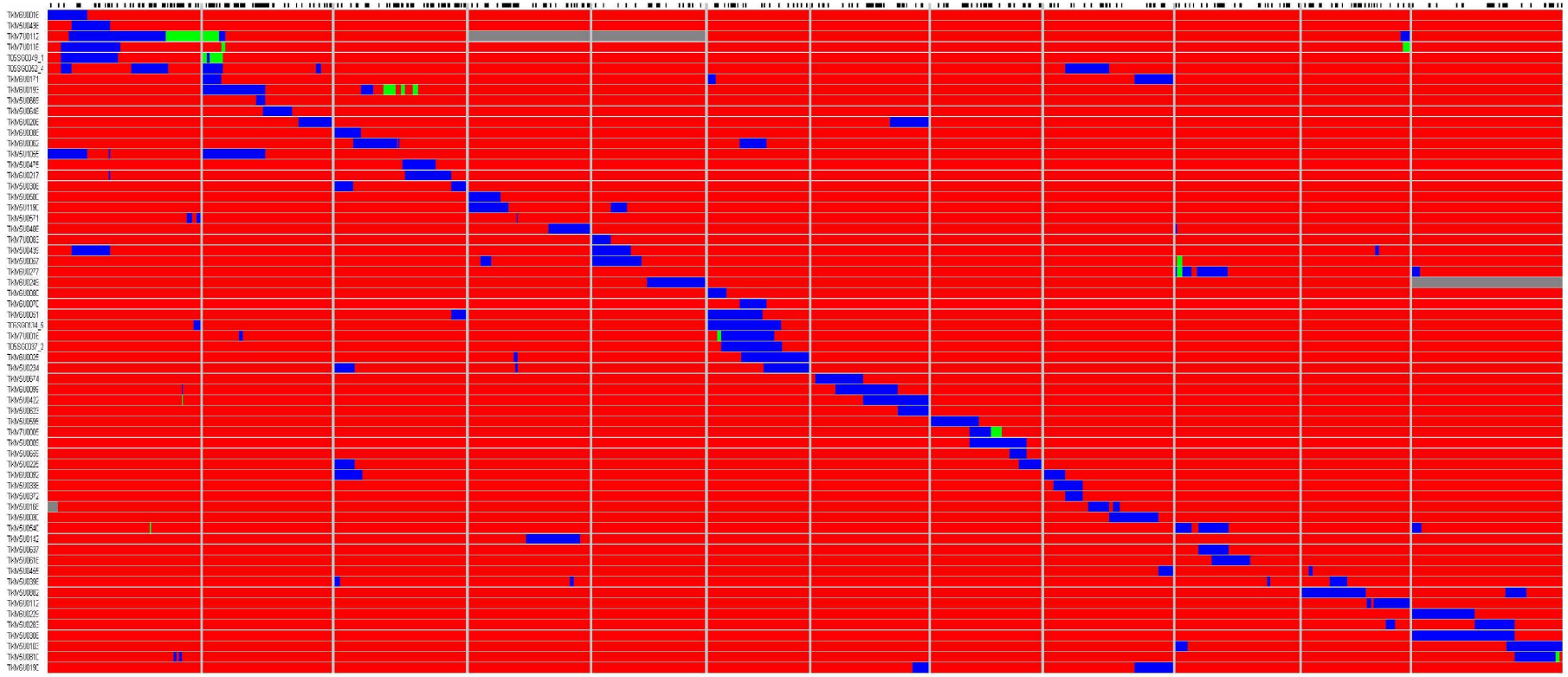

Figure 2.1. Gene map of the used genotypes. Each genome of the different genotypes is divided into 12 chromosomes (grey lines). Red lines are 'Moneyberg' DNA segments. Blue lines are DNA introgression segments of *L. chiemelewski*. Green lines are heterozygous DNA segments. The black dots above the gene map are the polymorphic markers.