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SALINITY TOLERANCE IN STRAWBERRY (*Fragaria spp*) AS

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SALINITY TOLERANCE IN STRAWBERRY (*Fragaria spp*) AS INFLUENCED BY GENOTYPE

By

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ABSTRACT

SALINITY TOLERANCE IN STRAWBERRY (*Fragaria spp*) AS INFLUENCED BY GENOTYPE

By

Adriana Nikoloudi

Cultivated strawberry is considered a salt sensitive crop, while its progenitors *Fragaria chiloensis* and *Fragaria virginiana* differ in sensitivity, with *F. chiloensis* being more salt tolerant than *F. virginiana.* Salinity stress influences many aspects of plant physiology including photosynthetic rate, growth rate and the accumulation of certain metabolites to counteract the osmotic imbalance initially imposed by the increased salt concentration in the root medium. We sought to investigate the effect of salinity stress and recovery on the photosynthetic performance, leaf area production, and the accumulation of specific metabolites and inorganic ions of strawberry selections that differ in salt sensitivity, in an attempt to elucidate some of the mechanisms that are involved in salinity tolerance. At early stages of salt imposition reduction in stomatal conductance limited net assimilation rate (A) in all selections tested. Later, carboxylation efficiency was strongly affected in the *F. virginiana* plants (MR 10 and NC 95-21-1), but not in *F. chiloensis* (FRA 24), and the effect was not reversible after the stress was released. Toxicity symptoms were apparent in both *F. virginiana* selections, starting approximately the second week of the treatments and the severity was correlated to the amount of salt present in the irrigation water. No symptoms developed in *F. chiloensis* until the end of the experiment. High levels of Na^+ and Cl^- were detected in leaves of both

F. virginiana selections treated with 50 and 100 mM NaCl while in *F. chiloensis* only Cl^- was increased in the 100 mM treatment. Sodium appears to accumulate much slower than chloride in the leaf tissues. Increased salt levels brought about a reduction in leaf area growth and runner production and an increase in the total soluble carbohydrates in the two selections tested. Resumption of growth and leaf area production was evident after the stress was released and it was accompanied by a decrease in total soluble carbohydrates in *F. virginiana* selection, but not in *F. chiloensis*. Glucose and fructose accounted for 70% of the total soluble carbohydrates accumulated in leaves of both selections due to salinity stress but no substantial amount of proline was detected during the stress or the recovery periods.

στον θείο μου, Παύλο

και την ανιψιά μου, Όλια

to my uncle Paul

and my niece Olia

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

CHAPTER 2

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

* These images are presented in color

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

Symbol	Parameter	Units
A	net CO_2 assimilation rate	$\mu mol\ m^{-2}\ s^{-1}$
$\mathbf{A_{max}}$	maximum CO_2 assimilation rate	$\mu mol\ m^{-2}\ s^{-1}$
$\mathbf{C_i}$	leaf intercellular CO_2 concentration	$\mu L/L$
Γ	CO_2 compensation point	$\mu L/L$
DW	dry weight	g
EC	electrical conductivity	$\mu S/cm$
$\mathbf{F_o}$	background or initial fluorescence	relative units
$\mathbf{F_v}$	variable fluorescence	relative units
$\mathbf{F_m}$	maximum fluorescence	relative units
$\mathbf{g_s}$	stomatal conductance	$mmol\ m^{-2}\ s^{-1}$
E	transpiration rate	$mmol\ m^{-2}\ s^{-1}$
IR	infrared	
IRGA	infrared gas analyzer	
k	carboxylation efficiency	$\mu mol\ CO_2\ m^{-2}\ s^{-1}\ \mu L^{-1}\ L$
L	length	cm
PPF	photosynthetic photon flux	$\mu mol\ m^{-2}\ s^{-1}$
RLER	relative leaf expansion rate	$mm^2\ cm^{-2}\ d^{-1}$
RH	relative humidity	%
SE	standard error	
TSC	total soluble carbohydrates	$\mu mol/g$

W	width	cm
WUE	water use efficiency	

INTRODUCTION

Salinity is a major problem in agriculture, particularly in the arid and semi-arid regions of the world. Approximately one third of the world's irrigated land is already affected by excess salinity (Tal, 1985). Crop productivity of irrigated land in many areas is much higher than that of non-irrigated land; however, the continuous use of poor quality irrigation water leads to the decrease in crop productivity as a result of accumulation of salts in the soil. Although no major crop species tolerate high levels of salinity, taxonomically diverse plant species are able to grow and thrive on saline soils (Amtmann and Sanders, 1999).

Plants are often subjected to multiple stresses and it has been proposed that varieties with an increased ability to withstand one form of stress have the ability to tolerate other kind of stresses as well (Levitt, 1980). It has been hypothesized that tolerance to various stresses may be, in part, controlled by a common physiological and/or molecular mechanism. Plant responses to high NaCl have been investigated at the cellular, tissue and whole plant levels (Flowers et al., 1977; Greenway and Munns, 1980; Cheeseman, 1988); The deleterious effect of salt on plant cells has two components: osmotic stress and ion toxicity. The osmotic stress is not specific for NaCl and results from dehydration and loss of turgor, induced by external solutes; it is therefore a common component of drought and salt stress (Greenway and Munns, 1980). Ion toxicity results from the increased concentration of intracellular ions (mostly K^+) during water loss and the uptake of Na^+ and Cl^- (Serrano and Gaxiola, 1994). Excess Na^+ may disturb mineral nutrition by inhibiting the uptake of essential cations such as K^+ and Ca^{2+} (Greenway and

Munns, 1980). The physiological basis of salinity tolerance depends in part on the capacity of the plant to overcome these ion toxicities (Amzallag Nissim, 1997).

In general, hypotheses for the mechanisms by which salinity reduces shoot growth and productivity can be categorized into four general groups. 1) Salinity reduces photosynthetic rate (Robinson et al., 1983; Bongi and Loreto, 1989; Everard et al., 1994), which may limit the carbohydrate supply necessary for growth. However, the accumulation of starch observed in some species (Munns et al., 1982) in response to salt stress does not favor this possibility. 2) Salinity reduces shoot growth by reducing turgor in expanding tissues, which are not able to fully osmoregulate (Flowers et al., 1991). 3) Shoot growth is down regulated by long distance signals derived from the roots (Munns and Cramer, 1996). 4) A disturbance in mineral supply to the shoot, either excess or deficiency leads to metabolic inhibition that directly affects shoot growth (Greenway and Munns, 1980).

Cultivated strawberry is considered as salt sensitive (Maas, 1984), but there is a large variation among different wild species of the genus *Fragaria*. These differences reflect the diversity of the environment the plants originated from (Hancock and Bringhurst, 1979). Elucidation of the physiological mechanism involved in salinity tolerance by some wild *Fragaria* species will result in scientific and practical benefits. Knowledge of the photosynthetic performance of wild strawberry species during salinity could be useful in breeding programs where tolerance is a desired trait.

The present work will focus on the photosynthetic performance of some wild strawberry ecotypes during and after salt stress. In the following chapters the effect of

salt stress in the photosynthetic rate, the growth rate, and the carbohydrate and proline accumulation in salt tolerant and salt sensitive selections will be discussed.

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LITERATURE REVIEW

INTRODUCTION

The effect of salinity stress in plant growth and development has received great attention in the recent years since it is well established that reduction in productivity occurs during salinization. Munns, (1993), was the first to suggest the importance of the time frame within which salinity affects plant growth. The first phase of growth reduction is quickly apparent, and is due to the salt outside the roots. This is essentially a water stress. Then there is a second phase, that takes time to develop and results from internal injury. This is due to salt accumulating in transpiring leaves to excessive levels, exceeding the ability of the cells to compartmentalize salts in the vacuoles (Munns, 2002). Reduction in growth due to salinity stress has been reported in the literature for many species of agricultural importance (Munns et al., 1982; Awang and Atherton, 1995; Tattini et al., 1995; Lutts et al., 1996; Tattini et al., 1997; Chartzoulakis et al., 2002). When studied, resumption of growth was also evidence after the stress was released (Tattini et al., 1995; Tattini et al., 1997; Pardossi et al., 1998).

1. PLANT RESPONSES TO SALINITY STRESS

1.1. Effect of salinity on photosynthetic performance

A. Relationship between photosynthetic rate and salinity in the root medium

An alteration in gas exchange properties has been reported as a response to moderate or high salinity stress with symptoms similar to those caused by drought stress (Bongi and Loreto, 1989). A decrease in stomatal conductance (g_s) and reduction in CO_2 assimilation and transpiration rates have been reported in species with different degrees of salt tolerance (Bongi and Loreto, 1989; Brugnoli and Lauteri, 1991); (Tattini et al.,

1995; Marler and Zozor, 1996). An increase in the CO_2 compensation point (Γ) with severity of salt stress and time of exposure was also evident in bell pepper plants when exposed to different salinity levels (Bethke and Drew, 1992). Brugnoli and Lauteri, (1991) reported a decline in stomatal conductance with increasing salinity in both salt sensitive bean and salt tolerant cotton. Although the maximum photosynthetic rate at light saturation was reduced by salt treatment, the apparent photon yield was unaffected in both species.

On the other hand, carboxylation efficiency (k) and CO_2 saturated photosynthesis were reduced in both species but not to the same degree, when measured as percent of the control. A decline in assimilation rate without a corresponding decline in internal CO_2 concentration (C_i) usually has been interpreted as a direct effect of salinity on photosynthetic machinery, but this may not be the case if uneven opening of stomata (patchiness) occurs during stress (Brugnoli and Lauteri, 1991).

In experiments with two olive cultivars that differ in salinity tolerance, (Tattini et al., 1997) reported that the inhibition of photosynthesis at high salinity was due to the direct effects of ions on photosynthetic machinery rather than on stomatal behavior, since they reported a 23-26% reduction in carboxylation efficiency of the stressed plants in both cultivars. After four weeks of stress relief, carboxylation efficiency of the salt tolerant cultivar returned to control levels, but only a 67% recovery was observed in the sensitive one. Although partial stomatal closure also occurs with salinization, the main reason for photosynthetic depression is proposed to be the loss of carboxylation and electron transport capacity in celery (Everard et al., 1994). A decrease in carboxylation capacity affects energy transduction through and from the photosystems, which may lead

to their overreduction and subsequent damage to the proteins by active radical oxygen species (Bohnert et al., 1996). Usually reduced gas exchange persists as plants continue to be exposed to high levels of salts (Marler and Zozor, 1996) while both CO_2 assimilation rate and stomatal conductance recover in many cases when the stress was released (Tattini et al., 1995; Delfine et al., 1999).

Contrasting results have been reported for sunflower in field and pot experiments (Steduto et al., 2000). The authors did not find any significant effect of mild salinity in stomatal and non-stomatal components of photosynthesis. They pointed out that under saline conditions, the control of assimilation in sunflower is dominated by modulation in the leaf area rather than by changes in photosynthetic rate. Recent studies with the cyanobacterium *Synechochoccus* sp under NaCl stress showed a rapid and reversible decline in photosynthetic activity, indicating an osmotic effect and a subsequent slow and irreversible loss of activity due to the ionic effect (Allakherdiev et al., 2000). The presence of a Na^+ channel blocker and a water channel blocker protected PSI and PSII against short and long term inactivation, demonstrating the importance of these channels on alleviating salinity stress. It should be emphasized here that a decrease in stomatal conductance and/or mesophyll capacity for CO_2 fixation do not necessarily demonstrate that the primary adverse effects of salinity are on photosynthesis since both parameters could be "feedback" inhibited due to reduced carbohydrate utilization (Greenway and Munns, 1980).

B. Relationship between photosynthetic rate and leaf sodium and chloride content

Several studies have been focused on the relationship between photosynthetic rate per leaf area and the accumulation of sodium and chloride ions in the leaf tissues. Differences in the absorption and accumulation of toxic ions in the leaf have been suggested responsible for differences in the rate of photosynthesis in some species. Accumulation of Na^+ and Cl^- ions has been proposed to be the primary causes of photosynthetic decline in salt stressed plants and threshold levels have been calculated for leaf tissues from various crop species (Bethke and Drew, 1992; Marler and Zozor, 1996; Tattini et al., 1997). Correlations between leaf elemental composition and photosynthetic ability showed that levels of Na^+ and Cl^- in the leaf tissue were proportional to the degree of photosynthesis reduction in bell pepper (Bethke and Drew, 1992) but not in a salt tolerant olive cultivar (Tattini et al., 1995)

Although leaf Na^+ was closely correlated with leaf scorch in salt stressed peach (Karakas et al., 2000), Ziska et al., (1990) found that a decline in CO_2 assimilation rate was related to increased leaf chloride content and independent of changes in leaf sodium status in *Prunus salicina* leaves. Cl^- accumulation rather than Na^+ was also responsible for the reduction in leaf gas exchange in sweet orange leaves when different salts were evaluated to distinguish between the effects of those two ions (Banuls and Primo-Milo, 1992).

C. Chlorophyll fluorescence

The photosynthetic process is initiated when light is absorbed by the antenna molecules within the photosynthetic membrane. The absorbed energy is then transferred as excitation energy to a reaction center and is used for chemical reactions (Strasser et al., 2000). Less than 1% of the total absorbed solar radiation is stored into photosynthetic products, while the rest is dissipated mainly as heat (latent heat, conduction/convection, and infrared radiation) or as emitted radiation (Nobel, 1991). Fluorescence is one of the several competing processes during the de-excitation of excited chlorophyll molecules in PSII. When a leaf is placed in the darkness or in dim light for several minutes and then is brightly illuminated, fluorescence rapidly rises to a peak and then it declines to reach a steady state value (Nobel, 1991). Studies of fluorescence induction kinetics have been used as stress indicators in ecophysiological studies for detecting chilling, freezing, drought and air pollution stresses in plants (Krause and Weis, 1991).

Measurement of chlorophyll fluorescence in leaves is rapid, reproducible, non-destructive, allowing periodic and repetitive sampling (Percival and Dixon, 1997). The variable component of chlorophyll fluorescence (F_v) is the difference between the maximum fluorescence signal (F_m) and the background level signal (F_o). The ratio F_v/F_m represents the maximum quantum yield of PSII that, in turn, is highly correlated with the quantum yield of net photosynthesis.

Although the F_v/F_m was not affected by salinity alone in some studies (Sharma and Hall, 1991; Jimenez et al., 1997), the addition of another stress, e.g. high irradiance (Sharma and Hall, 1991) negatively influenced the light reactions of photosynthesis as it was evident from lower F_v/F_m values. Furthermore, a number of studies have shown that

salinity alone causes a decrease in the F_v/F_m ratio in olive trees (Bongi and Loreto, 1989) and in celery (Everard et al., 1994), and this depends on the severity of the stress.

Light reaction was not significantly affected by salt stress alone in barley and sorghum, but it was impaired when salt stress was combined with photoinhibitory treatment (Sharma and Hall, 1991). A greater decrease in the photosynthetic rate than in the quantum yield indicated that the activity of Calvin cycle enzymes might be involved in the inhibition. A salinity stress exerts adverse effects on cellular metabolic activities by predisposing the photochemical apparatus to photoinhibition and decreasing the ability of cells to recover from photoinhibitory damage (Sharma and Hall, 1991). Lu and Zhang, (1998), reported that no differences were detected in the steady state fluorescence parameters and rapid fluorescence induction kinetics in salt stressed sorghum leaves supporting the idea that PSII is highly resistant to salinity stress alone. In addition, when plants were exposed to both salinity and high temperature, higher F_v/F_m values were reported for salt stressed leaves compared to the controls, indicating an enhancement in thermostability of PS II in plants experienced salt stress.

Salt accumulation in spinach leaves did not directly affect photochemistry. According to the authors' interpretation, salinity reduces photosynthesis primarily by reducing the diffusion of CO_2 to the chloroplast, both by stomatal closure and by changes in mesophyll structure and then impairs RubisCO (Delfine et al., 1998).

1.2. Effects of salinity on carbon balance

A number of researchers have suggested that salinity reduces growth to a greater extent than photosynthetic rate per se, causing a subsequent carbohydrate built up (either

as starch and /or soluble sugars) due to the lack of utilization which in turn affects photosynthetic rate (Greenway and Munns, 1980) and references therein. Data supporting the opposite view comes from experiment with peach trees (Ziska et al., 1990) where mean soluble and insoluble carbohydrate content decreased with increased salinity, suggesting that there is no evidence for carbohydrate feedback inhibition of CO_2 uptake.

On the other hand, active carbohydrate accumulation when present in plant tissues has been seen as a response mechanism through which the plant uses sugar components in a stress adaptive process. Glucose, sucrose, fructose, and fructans have been implicated in drought and salt tolerance in wheat cultivars (Kerepesi and Galiba, 2000). The authors argue that tolerant wheat genotypes accumulated more mono-saccharides and fructans than the sensitive ones. Furthermore the amount of fructans was better indicator of salinity tolerance, while monosaccharides were better correlated to the degree of drought tolerance.

1.3. Ion accumulation

A complication of salt tolerance studies in higher plants is the differentiation into tissues and organs that may result in different ion concentration in different plant parts. It has been proposed that root cells are exposed to much lower salt concentrations than leaf cells because ions carried by the xylem would accumulate in the cell walls of leaf cells due to transpiration (Flowers et al., 1991).

Accumulation of salts in the cytoplasm generates low external solute potential that in turn leads to the need of osmotic adjustment within the protoplast, or, if it fails, to cellular death due to dehydration (Flowers and Yeo, 1988). Another important organ

specific factor for ion accumulation is the selectivity for xylem loading at the root central cylinder. According to (Lauchli, 1984) this process is more selective in terms of K^+/Na^+ discrimination than uptake from the soil solution at the root epidermis. The mechanism that operates is resorption of Na^+ from the xylem sap and accumulation of Na^+ by xylem parenchyma cells in the root and the stem base.

Older leaves of non-halophytes grown under high salinity conditions have higher ion concentrations than the younger leaves. This could be explained by a rapid volume increase in young expanding leaves and the prolonged ion intake by the older fully expanded leaves (Greenway and Munns, 1980). In contrast, ion concentration in young and old leaves of halophytic species is similar (Flowers et al., 1977).

Use of isoosmotic concentration of salts or polyethylene glycol (PEG) have been tested to distinguish between water stress and/or ion excess caused by high salinity. Less growth in salt than in PEG would indicate that ion toxicity rather than an osmotic effect is responsible for the growth inhibition (Greenway and Munns, 1980). Comparing the inhibition of growth caused by high NaCl at high and low relative humidities may help resolve this distinction (water stress vs. ion excess). At high rates of transpiration (i.e. low relative humidity) a higher growth inhibition has been reported in some species (Greenway and Munns, 1980 and ref. therein) presenting evidence in favor of adverse water relations at high external NaCl. However, this sometimes can be misleading because at low relative humidity an ion excess may occur since net ion uptake to the shoots is often greater at high than at low rates of transpiration, especially when both internal and external ion concentration are high (Scott-Russell and Barber, 1960)

1.4. Osmotic adjustment

In response to salt and drought stress, plants accumulate organic solutes that allow the cells to maintain their osmotic balance increasing intracellular osmotic pressure, and leading to water retention. (Tarczynski et al., 1993; Bohnert et al., 1996; Nelson et al., 1998). These compounds (named also compatible solutes) accumulate in the cytosol without damaging proteins and membranes and may stabilize these structures when challenged with adverse conditions such as dehydration, high temperature or denaturing chemicals (Galinski, 1993; Serrano, 1996). These metabolites are generally connected to the main flow of carbon and nitrogen in plant cells by short pathways (Bohnert et al., 1996). Compatible solutes include sugar alcohols, quaternary ammonia compounds, proline, and tertiary sulfonic compounds (Bohnert et al., 1996). Besides the function of keeping cell osmotic balance, many of these molecules have other roles in stress protection (Nelson et al., 1998). A decrease in stomatal conductance caused by the osmotic effect of salts in the external medium could increase the production of free radicals and the damage associated with photoinhibition (Loescher and Everard, 1996). It has been reported that the presence of the polyol mannitol in the chloroplast of transgenic tobacco plants conferred increased capacity to scavenge active oxygen species produced under oxidative stress (Shen et al., 1997; Shen et al., 1997).

Accumulation of proline has been implicated in osmotic stress tolerance by some authors, but it remains a matter of debate whether or not it actually contributes to tolerance or it is a consequence of other stress-induced changes in metabolism. Genotypic variation exists in the amount of proline accumulated due to osmotic stress in rice (Lutts et al., 1996), barley (Hanson et al., 1977), citrus (Nolte and Hanson, 1997) and many

other species. However, salt–induced proline synthesis is not always rapid and begins only when cell injury is evident (Hanson et al., 1977). Proline accumulation may provide protection when there is imbalance between photosynthetic energy capture and NADPH utilization in carbon fixation under stress (Delauney and Verma, 1993). This hypothesis supports the idea that a particular pathway leading to an osmolyte may be more important than the accumulation per se (Nelson et al., 1998)

Sugar alcohols are major photosyntetic products that are stored in the vacuoles in many higher plant species (Loescher et al., 1992). In order to serve as compatible solutes during salt stress these molecules accumulate in the cytoplasm (Flowers et al., 1977; Greenway and Munns, 1980). This may indicate that salt stress causes a re-allocation of the sugar alcohol to the cytoplasm (Loescher and Everard, 1996). Although the concentration of sugar alcohols increases after exposure to salinity, their accumulation could be the result of increased production or reduced transport and utilization resulting from salt-induced growth inhibition (Loescher and Everard, 1996). Evidence supporting the hypothesis of increased mannitol production during salt stress has come from studies with celery. Alteration of carbon partitioning under salt stress, (where a shift in photosynthates from sucrose to mannitol occurred) indicated that this is an adaptive response rather than a consequence of the stress (Everard et al., 1994).

Important to the understanding of plant response to salinity is the allocation of carbon to storage, resulting in increases in carbohydrate accumulation with salt stress. For example, storage would include accumulation of non-structural carbon, which facilitates osmotic adjustment and turgor maintenance (Cheeseman, 1988). Though this does not

result in loss of carbon from the plant, (e.g. respiration) it may remove it from the pool available for immediate metabolism or growth.

2. PHOTOSYNTHETIC PERFORMANCE OF STRAWBERRY

Several factors affect maximum photosynthetic rate (A) in strawberry including light level, temperature, nutrient availability, developmental stage, and method of propagation (Hancock, 1999). In general, values in the range of 7-15 μmol m^{-2} s^{-1} for *F. virginiana* and 20-30 μmol m^{-2} s^{-1} for *F. chiloensis* have been reported in the literature, while for cultivated strawberry (*Fragaria* x *ananassa*) grown in the field, the rates are intermediate between the progenitor species (15-25 μmol m^{-2} s^{-1}), although those species have not been compared in a common environment (Hancock et al 1989a; Hancock, 1999).

Several reports exist that evaluate gas exchange characteristics of strawberry species and cultivars in relation to yield potential (Hancock et al., 1989b; Cameron and Hartley, 1990). High photosynthetic potentials in cultivated strawberry are not associated with high productivity unless these high rates are maintained during critical periods. In addition, since strawberries have a number of active sinks, carbon allocation to fruits is a very important factor affecting yield (Hancock, 1991). Micropropagated plants have higher CO_2 assimilation rates than conventionally propagated ones (Cameron et al., 1989). The presence of flowers and fruits influences leaf photosynthetic rate and stomatal conductance (Forney and Breen, 1985). Higher photosynthetic rates are associated with periods of flowering and runner production (Hancock et al., 1989b), while fruit removal results in a decline in CO_2 assimilation rate (Schaffer et al., 1985; Schaffer et al., 1986a).

Fully expanded leaves that are 10-20 days old and have been acclimated in high light intensity have higher photosynthetic rate compared to those that have completed their development under low light (Jurik et al., 1979; Awang and Atherton, 1994). Optimum temperature for maximum photosynthesis varies between species and acclimation conditions. After maintenance at high temperature, plants of *F. chiloensis*, *F. virginiana* and *Fragaria* x *ananassa* have higher photosynthetic temperature optima. Furthermore, heat acclimated plants show a significantly lower reduction in net assimilation rate as temperature raises from 20 to 30 °C (Serce et al., 2000).

3. RESPONSE OF FRAGARIA SPECIES TO DROUGHT AND SALINITY STRESS

Salt sensitivity of the cultivated strawberry (*Fragaria* x *ananassa* Duch) has been reported since 1958 (Ehlig and Bernstein, 1958) and the reduction in yield usually depends on the plant's growth stage at the time it is exposed to salt stress. Salt injury in strawberry includes tip burning which advances progressively along the leaflet margins to the base forming concentric circles. Damage is mainly observed in the adult leaves while the younger ones remain curled (Ehlig and Bernstein, 1958; Martinez-Barroso and Alvarez, 1997).

Depression in fruit yield is correlated with a decline in inflorescence number. It has been proposed that the decrease in flowering could be minimized if salinization is delayed until sufficient vegetative development has taken place (Awang and Atherton, 1995). Early reports on the effect of salinity in cultivated strawberry indicated that the osmotic pressure of the nutrient solution was predominant factor determining growth in

sand culture (Ehlig and Bernstein, 1958) while leaf chloride content was directly correlated with the extend of leaf burn (Ehlig and Bernstein, 1958). Comparing leaf and root absorption of chloride and sodium by strawberry cultivars, using ground and foliar application of saline water, Ehlig, (1961) observed that more Cl^- is absorbed from saline root media than from saline sprays. Furthermore, sodium accumulation in leaves is much slower than chloride, thus it does not account for salt injury in the cultivars tested. Consistent with these conclusions is the finding that the combined effects of high EC and chloride in the irrigation water are the major factors causing salt injury in some cultivars, whereas the presence of Na_2SO_4 and $NaHCO_3$ do not cause phytotoxicity (Martinez-Barroso and Alvarez, 1997). However, Hoagland and Snyder, (1933) reported that the presence of sodium in the irrigation water may cause severe injury in sensitive cultivars.

Although cultivated strawberry is considered as salt sensitive (Maas, 1984), there is a large variation among different species of the genus *Fragaria*. According to Hancock and Bringhurst (1979), octoploid *Fragaria* species have undergone ecological differentiation with significant amounts of inter-populational variation in a number of polygenic and monogenic traits. Inter-population differences were found in the ability of the plant to survive under nutrient, salt, and shade stress but not in the ability of the plants to accumulate biomass under salt stress (Hancock and Bringhurst, 1979). These differences reflect the diversity of the environment the plants originated from. For example, some *F. chiloensis* ecotypes may tolerate up to 380 meq of NaCl, while many cultivars can tolerate only 40 meq or less (Hancock and Bringhurst, 1979). *Fragaria chiloensis*, native to the Pacific coasts of North and South America is reported to be drought tolerant, while *F. virginiana* originating in eastern North America is reported to

be drought susceptible (Zhang and Archbold, 1993b). McDonald and Archbold (1998), using an improved method to estimate tissue ionic conductance, found significant inter- and intra-specific variation in drought response of *F. chiloensis* and *F. virginiana* accessions

Morphological and physiological traits that may contribute to such variations include stomatal density and position (Darrow, 1966), cuticle thickness (Archbold, 1993), and osmotic adjustment through the accumulation of glucose and fructose that are the primary carbohydrates involved in osmoregulation (Zhang and Archbold, 1993a). Many *F. chiloensis* clones exhibit greater water use efficiency, lower leaf water potential, lower relative water content, greater membrane stability, and thicker cuticles (Archbold, 1993) than *F. virginiana*, although variability exists within both species (Zhang and Archbold, 1993a). Membrane stability is critical for drought tolerance (McDonald and Archbold, 1998), cold acclimation (O'Neil et al., 1981), and tolerance to the dehydration caused by extracellular ice formation (O'Neil et al., 1981).

In vitro evaluations of strawberry seedlings from different crosses have revealed that the use of *F. chiloensis* as a parent could provide the progeny with salt tolerance characteristics (Esensee et al., 1991). This could be useful for improving the horticultural characteristics of the cultivated *Fragaria* species (Tal, 1985).

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CHAPTER 1

Characterization of the photosynthetic performance of three strawberry selections grown in the greenhouse during salinity stress and recovery

Abstract

Salinity stress influences many aspects of plant physiology including net assimilation rate. The objective of this work was to study the effect of salinity stress and subsequent recovery on the photosynthetic performance of strawberry selections that differ in sensitivity. In two experiments, *Fragaria chiloensis* (FRA 24) and *Fragaria virginiana* (MR 10 and NC 95-21-1) plants were grown in pots and were receiving different levels of NaCl in the irrigation water. At early stages of salt imposition reduction in stomatal conductance (g_s) limited net assimilation rate (A) in all three selections tested. Later, carboxylation efficiency was strongly affected in the *F. virginiana* plants, but not in *F. chiloensisis,* and the effect was not reversible after the stress was released. Toxicity symptoms were apparent in both *F. virginiana* selections, starting approximately the second week of the treatments and the severity was correlated to the amount of salt present in the irrigation water. No symptoms developed in FRA 24 until the end of the experiment. High levels of Na^+ and Cl^- were detected in leaves of MR 10 and NC 95-21-1 treated with 50 and 100 mM NaCl while in FRA 24 only Cl^- was increased in the 100 mM treatment. Sodium appears to accumulate much slower than chloride in the leaf tissues. These data confirm the genetic variability in both photosynthetic rate and the accumulation of inorganic ions in the three selections tested.

Introduction

Reports on the effect of salinity in cultivated strawberry indicated that the osmotic pressure of the nutrient solution is the main factor determining growth, but leaf chloride content is directly correlated with the extend of leaf burn (Ehlig and Bernstein, 1958). Combined effects of high electrical conductivity (EC) and chloride in the irrigation water have been shown to cause salt injury in some cultivars, whereas it was suggested that the presence of Na^+ does not cause phytotoxicity (Martinez Barroso and Alvarez, 1997). However, Hoagland and Snyder, (1933) reported that the presence of sodium in the irrigation water may cause severe injury in sensitive cultivars. In fact, when talking about salinity stress, osmotic stress and ion excess are not alternative possibilities, but arise in sequence (Munns et al., 1995).

Although cultivated strawberry is considered as salt sensitive (Maas, 1984), there is a large variation among different species of the genus *Fragaria* in the amount of salinity the various native populations can tolerate, but not in the ability of the plants to accumulate biomass under salt stress. These differences reflect the diversity of the environments from which the plants originated (Hancock and Bringhurst, 1979). Understanding the physiological mechanisms that are involved in salinity tolerance is essential in determining traits useful in breeding programmes intended to develop cultivars suitable to saline conditions.

Fragaria chiloensis Duch. and *F. virginiana* Duch. that are the primary progenitor species of the cultivated strawberry (James et al., 1990) differ in drought and salinity tolerance with *F. chiloensis* being more tolerant than *F. virginiana* (Hancock and Bringhurst, 1979; Zhang and Archbold, 1993b). Anatomical characteristics of *Fragaria*

chiloensis including thicker cuticle and leaves (Archbold, 1993), sunken stomata, and reduced stomatal density per leaf area (Darrow, 1966) may play an essential role in regulating several aspects of strawberry physiology, including gas exchange, water status and accumulation of inorganic ions. Several reports exist that evaluate gas exchange characteristics of strawberry species and cultivars in relation to yield potential (Hancock et al., 1989b; Cameron and Hartley, 1990), method of propagation (Cameron et al., 1989), flowering and fruiting (Forney and Breen, 1985), fruit removal, (Schaffer et al., 1985), changing light regimes (Jurik et al., 1979; Awang and Atherton, 1994), leaf density, increasing air CO_2 concentration (Cameron, 1986), and high temperature (Serce et al., 2000), but there is a lack of information regarding the photosynthetic behavior of strawberry cultivars or wild species under salinity stress. Knowledge of the mechanisms involved in the regulation of the CO_2 assimilation rate in salinity stress conditions of the two progenitor species of the cultivated strawberry is essential, because they may serve as important resources for its genetic improvement.

The objective of this study was using strawberry plants that differ in salinity tolerance to evaluate their photosynthetic performance during salinity stress and subsequent recovery in order to determine how assimilation rate is affected by salinity.

Materials and methods

Experiment I

Plant material and growth conditions

Three strawberry selections, Montreal River 10 (MR 10) *(Fragaria virginiana),* native to Ontario, Canada), NC 95-21-1 *(Fragaria virginiana)*, native to Mississipi, and FRA 24 *(Fragaria chiloensis),* native to Colombia provided by Dr. James Hancock (Department of Horticulture, Michigan State University), were used for this study.

The experiment was conducted during summer 2000 (June, 18 - August, 3) in a research greenhouse in the Department of Horticulture at Michigan State University (East Lansing, MI). The glass was white washed to reduce the incident radiation and consequently the temperature during the summer months. Therefore, midday photosynthetic photon flux density on a sunny day varied from 550 to 850 μmol m^{-2} s^{-1}; average day/night temperature 30/22°C and relative humidity ranged between 60 and 75%.

Twenty plants of each type were potted in 2.5 L pots containing Baccto potting medium (Baccto, Michigan Peat Company, Houston,TX) (sphagnum peat 70-80%, pH 5.5-6.5). All plants were propagated by runners, and they were three months old when the experiment started. All flowers and runners were removed as soon as they appeared.

Mother plants, maintained in the same greenhouse throughout the experiments, were also grown in 2.5 L pots in Baccto medium and they were receiving 200 ppm soluble 20-20-20 Peters fertilizer weekly (Zhang and Archbold, 1993a; Zhang and Archbold, 1993b).

Salinity treatments

Preliminary experiments were conducted to determine the concentration of salt that could be used in our studies. In the trial experiment concentrations of 100, 200, and 400 mM were tested, but mortality occurred at 200 mM (especially the *F. virginiana* ecotypes) after one week of treatment and without any prior visible symptoms (data not shown).

Sodium chloride (J.T. Baker, Phillipsburg, NJ, USA) was dissolved in either tap water (known EC 1000-1200 μS/cm) or in 200 ppm fertilizer solution once a week. The treatments were stepped up in 25 mM increments every day until final treatment concentrations (0, 25, 50, 100 mM) (Everard et al., 1994). Plants were watered at least once a day with approximately 300 mL of solution, ensuring adequate leaching and preventing excess salinity. The leaching solution from each pot was collected every day in the beginning, and then twice a week in order to become aware of any significant salt built up over time. Electrical conductivity was measured in the lab using an ORION-150 conductivity meter, (Boston, MA, USA).

Analysis of photosynthetic performance

A. Gas exchange

Carbon assimilation (A), stomatal conductance (g_s), leaf intercellular CO_2 concentration (C_i), and transpiration (E) were determined using an open gas-exchange system (Layne and Flore, 1992). Gas exchange measurements were determined with a CIRAS-1 portable infrared gas analyzer (PP Systems, Haverhill, MA, USA) on a 7-days

interval, or when weather permitted between 10:00 am and 1:00 pm on the terminal leaflet of mature fully expanded leaves. Five plants per treatment and selection were used. Standard conditions during gas exchange were PPFD>500 μmol m^{-2} s^{-1}, VPD 14.5 ± 0.5 mbar, air CO_2 concentration 360 ± 10 μL/L, leaf temperature 24-28°C and air flow into the cuvette 200 ± 5 mL/min (3.3 mL/sec). Two leaves per plant were used. Each leaflet was inserted into the leaf chamber (2.5 cm^2) and one single measurement was collected, after a waiting period of 1-2 minutes for the atmosphere inside the chamber to equilibrate. Carbon assimilation (A), stomatal conductance (g_s), leaf intercellular CO_2 concentration (C_i) and transpiration (E) were the parameters calculated automatically by the instrument at each measurement.

B. Photosynthetic response curves to increasing light intensity

Light response curves were performed once during the experiment starting the 21st day after the initiation of the salt treatments. The photosynthetic response to light was carried out using the CIRAS-1 infrared gas analyzer in a walk-in growth chamber (PGV36 plant growth chamber with Conviron model 3023 control system, Controlled Environment Inc., Pembina, ND) set at 22°C, 75% RH. Three plants per treatment and selection were transferred to the lab for adaptation, the evening prior to the measurements. The terminal leaflet of a mature fully expanded leaf per plant was used for the response curves. The portion of the leaf blade enclosed in the assimilation chamber was maintained at a constant CO_2 supply of 360 μL/L by the CIRAS-1. The instrument was set to increase automatically stepwise the light intensity to the levels of PPF 0, 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 800, 1000, 1200, and 1400, μmol m^{-2} s^{-1}. The leaves were allowed to equilibrate for 3 minutes at each light level before the

measurement was taken automatically. In total, thirty six plants were used. Approximately one hour was necessary for each curve to be completed, so the curves were done within six days. In order to reduce the experiental error due to diurnal variation of gas exchange, all curves were carried out between 8:00 am and 3:00 pm, and adequate blocking was made to reduce the effect of additional of stress for the plants used the later days.

The calculated A was plotted versus the light intensity and nonlinear regression models were fitted to each response curve (Layne and Flore, 1992)

The monomolecular model:

$$y = a*[1-b*\exp(-c*x)]$$

was used to fit the response curves, where y (dependent variable) is the calculated A, x (independent variable) is the light intensity, and the parameters a, b, and c represent the A asymptotic value, the minimum value, and the rate constant, respectively (Layne and Flore, 1992). The best-fit curve was found with SigmaPlot software (version 8.02, Scientific Graphing Software, SPSS Inc. Chicago, IL). The light compensation point was extrapolated from each individual curve as the light intensity where the photosynthetic rate equals the rate of respiration (net photosynthesis equals zero). The maximum quantum yield of photosynthesis was calculated as the slope of the initial (linear) part of the response curve for each individual plant.

C. Photosynthetic response to increasing air CO_2 concentration

Photosynthetic response curves to increasing internal CO_2 were performed from day 28 to 32 of the experiment. The A/C_i curves were carried out using the CIRAS-1 infrared gas analyzer in a walk-in growth chamber (PGV36 plant growth chamber with Conviron model 3023 control system, Controlled Environment Inc., Pembina, ND) set at 22°C, 75% RH and PAR 400 μmol m^{-2} s^{-1}. Three plants per treatment and selection were transferred to the lab for adaptation, the evening prior to the measurements. The terminal leaflet of a mature fully expanded leaf per plant was used for the response curves. The portion of the leaf blade enclosed in the assimilation chamber was maintained at a constant PAR of 800 μmol m^{-2} s^{-1} by the CIRAS-1 external illumination supply unit. The instrument was set to increase automatically stepwise the CO_2 concentration of the air entering the leaf chamber to the levels of 0, 50, 100, 150, 250, 370, 500, 1000, 1500, and 2000 μL/L. The leaves were allowed to equilibrate for 5 minutes at each CO_2 concentration level before the measurement was taken automatically. In total twenty seven plants were used. Approximately ninety minutes were necessary for each curve to be completed, so the curves were done within 7 days. Similar to the light response curves in order to reduce the experimental error; all curves were obtained between 8:00 am and 3:00 pm each day. Adequate blocking was done to minimize the effect of additional stress for the plants used the later days.

The calculated A was plotted versus the internal CO_2 concentration (C_i) and nonlinear regression models were fitted to each response curve (Layne and Flore, 1992) The monomolecular model:

$$y = a*[1-b*\exp(-c*x)]$$

was used to fit the response curves, where y (dependent variable) is the calculated A, x (independent variable) is the intracellular CO_2 concentration (C_i), and the parameters a, b, and c represent the A asymptotic value, the minimum value, and the rate constant, respectively (Layne and Flore, 1992). The best-fit curve was found with SigmaPlot software (version 8.02, Scientific Graphing Software, SPSS Inc. Chicago, IL).

Data analysis for each individual A/C_i curve was performed as indicated by Farquhar and Sharkey (1982). CO_2 compensation point (Γ) was extrapolated as the value of internal CO_2 where A equals zero. The carboxylation efficiency (k) was calculated from the slope of the linear part of the $A\text{-}C_i$ curve (0-150 $\mu L/L$ CO_2). Stomatal limitations to CO_2 assimilation were calculated from individual A/C_i curves and expressed as percent of total resistances (Jones et al., 1985).

Leaf mineral composition analysis

Twenty one days after the beginning of the treatments (July 11, 2000) two to three mature leaves per plant were collected for analysis of Na^+, Cl^-, K^+ and Ca^{+2} composition. This was done before severe damage was evident in the most sensitive selection. Samples from each selection and treatment were pooled together and after drying they were analyzed. Each pooled sample was analyzed three times. Na^+ analysis was done using atomic absorption spectrophotometer, Cl^- was analyzed with Cl^- probes, while K^+ and Ca^{+2} were analyzed with a flame photometer.

Experiment II

Plant material and growth conditions

The experiment was conducted during summer 2001 (August, 10 – September, 16) in a research greenhouse in the Department of Horticulture at Michigan State University (East Lansing, MI). Midday photosynthetic photon flux density varied from 500 to 900 μmol m^{-2} s^{-1}; average day /night temperature 33/22°C and relative humidity ranged between 75 and 95%. Fifteen plants of each selection (*F .virginiana,* MR 10 and NC 95-21-1 and *F. chiloensis*, FRA 24) were potted in 2.5 L pots containing Baccto potting medium (Baccto, Michigan Peat Company, Houston,TX) (sphagnum peat 70-80%, pH 5.5-6.5). Plants were treated as described for the experiment I until the beginning of the experiment.

Salinity treatments

Sodium chloride was dissolved in either tap water or in 200 pm fertilizer solution once a week. The treatments were stepped up in 25 mM increments every day until final treatment concentration (100 mM) (Everard et al., 1994). Plants were watered at least once a day with approximately 300 mL solution, ensuring adequate leaching and preventing excess salinity. Electrical conductivity of the leaching solution was measured weekly. After twenty one days of salt treatment, a set of five plants from each selection was allowed to recover from the salt stress. These plants were receiving 300 mL of tap water daily following an initial washing of the soil with distilled water (Tattini et al., 1995).

Analysis of photosynthetic performance

A. Gas exchange

Carbon assimilation (A), stomatal conductance (g_s), leaf intracellular CO_2 concentration (C_i), transpiration (E) were determined as described for experiment I during the stress and recovery period. Briefly, gas exchange measurements were taken with a CIRAS-1 portable infrared gas analyzer on a 4-day interval, or when weather permitted between 10:00 am and 1:00 pm on the terminal leaflet of mature fully expanded leaves. Five plants per treatment and selection were used. Standard conditions during gas exchange were PPFD>500 μmol m^{-2} s^{-1}, VPD 15±1mbar, air CO_2 concentration 360 ± 10 μL/L, leaf temperature 24-32°C and air flow into the cuvette 200 ± 5 mL/min. Two leaves per plant were used. Each leaflet was inserted into the leaf chamber (2.5 cm^2) and one single measurement was collected, after a waiting period of 1-2 minutes for the atmosphere inside the chamber to equilibrate.

B. Photosynthetic responses to increasing air CO_2 concentration

Photosynthetic response curves to increasing air CO_2 concentration were performed twice during the experiment. Twenty one days after the initiation of the salt treatment, at the end of the stress period and fifteen days later at the end of the recovery period. The procedure was essentially the one used for experiment I, but eighteen plants were used instead (3 plants per selection and treatment).

C. Chlorophyll fluorescence

Determination of the light-induced fluorescence was done three times during the experiment, (on days 15, 28 and 36), using three leaves from three randomly selected plants of each treatment and selection, (total 9 measurements/treatment x selection). The leaves were tagged in the beginning of the measurements ensuring that the assessments were taken on the same leaf throughout the experiment

Measurements were carried out in the lab at room temperature, using attached leaves. Leaflets were covered with leaf clips for 30 min to ensure dark adaptation and monitoring of chlorophyll transient was done with a Plant Efficiency Analyzer, PEA (Hansatech, Norfolk, U.K) portable fluorometer. The light intensity at the leaf surface was approximately 2000 μmol m^{-2} s^{-1} supplied by an array of 6 high intensity LEDs, providing red radiation at a peak wavelength of 650 nm. Data recorded were, minimal fluorescence (F_o), maximal fluorescence (F_m), variable fluorescence (F_v), and the fluorescence efficiency (F_v/F_m).

Experimental design and statistical analysis

Both experiments were arranged in a completely randomized block design on a greenhouse bench to minimize the effect of temperature and light intensity gradient along the bench. Plants were selected for uniformity of vigor and leaf number and randomly divided into groups to be assigned to the different treatments. Statistical analysis was carried out using the PROC MIXED procedure in SAS/PC software (SAS Institute Inc., Cary, NC, USA). Repeated measures analysis was used for gas exchange, chlorophyll fluorescence and the parameters derived from A/C_i curves (In general, when the time was

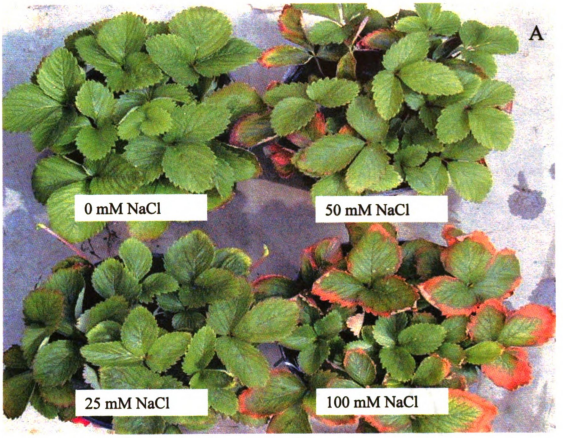
an additional factor in the experiments). Analysis of variance (ANOVA) and differences between means were determined by the Least-Squares Means test (LSMEANS). Probabilities less than or equal to 0.05 were regarded as significant within each date of measurement.

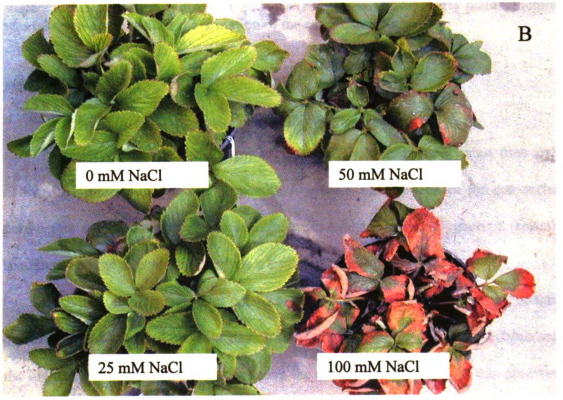
Results

Experiment I

Older leaves of NC 95-21-1 of plants receiving 100 mM salt showed necrosis in the margins as early as 10 days after the initiation of the treatments, while in plants receiving 50 mM salt visible symptoms developed 5 to 10 days later. MR 10 treated with 100 mM salt started developing marginal injury on day 15 while those receiving 50 mM treatment quite later (day 21). No damage was developed in plants of both ecotypes receiving 25 mM salt. Damage stared as tip burn which advanced progressively along the leaflet margins to the base of the leaf blade. No damage was evidence in petioles, young leaves or in emerging runners. Overall, NC 95-21-1 plants were more severely affected than MR 10 plants in all salt concentrations tested. In contrast, in FRA 24 no symptoms developed until the end of the experiment even in the highest salt concentration (Figures 1-2).

Figure 1. Effect of salinity stress on MR 10 (A) and NC 95-21-1 (B) (*F. virginiana*) strawberry plants. Pictures were taken five weeks after the initiation of the salt treatments.


A
0 mM NaCl
50 mM NaCl
25 mM NaCl
100 mM NaCl


B
0 mM NaCl
50 mM NaCl
25 mM NaCl
100 mM NaCl

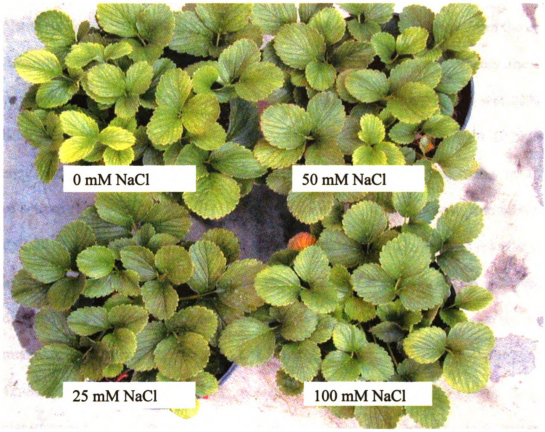


Figure 2. Effect of salinity stress on FRA 24 (*F. chiloensis*) strawberry plants. Picture was taken five weeks after the initiation of the salt treatments.

Analysis of photosynthetic performance

A. Gas exchange

Parameters derived from the gas exchange study were plotted versus time and are shown in Figures 3-6. Table 1, Appendix A gives a summary of all the gas exchange parameters during the experiment and the dates where effects of genotype (selection), treatment and the interaction genotype x treatment were significant.

An initial measurement was conducted just before the beginning of the treatments on June 18 and a second one four days later when 100 mM salt had been established for the highest treatment. Although a decline in the net assimilation rate (A) was observed 16

days after the beginning of the salt treatments (July, 6) in both MR 10 and NC 95-21-1 plants receiving 100 mM salt, the values were not significant different from the controls. A similar trend was observed on day 22 as well (July, 13) where only the highest treatment was significantly different from the rest. Net assimilation rate was 22% lower in MR 10, 50% lower in NC 95-21-1, and 30 % lower in FRA 24 ($P \leq 0.05$). No significant differences were observed in the 25 and 50 mM treatments in all three selections ($P \leq 0.05$). On day 30 (July 20), all treatments were different from each other at $P \leq 0.05$ in MR 10 with reductions 10, 30 and 50% respectively compared to the control. In NC 95-21-1 the 100 mM treatment showed a 65% reduction, but no difference was detected between control, 25, and 50 mM treatments. In FRA 24 also, only the 100 mM treatment showed a 30% reduction compared to the 25 and 50 mM treatments. On day 42, MR 10 and NC 95-21-1 plants receiving 100 mM salt were severely damaged so no measurements were taken. No significant difference was found between control and treated with 25 and 50 mM salt in MR 10, while all treatments were different from each other in NC 95-21-1 with reductions 20 and 30% respectively. FRA 24 plants receiving 100 mM salt were healthy with a minimum damage in some older leaves and photosynthetic rate 30% lower than the control. No difference was detected among the rest of the treatments throughout the experiment ($P \leq 0.05$) (Figure 3).

Stomatal conductance (g_s) was found to be significantly reduced in MR 10 in plants receiving 100 mM salt, with values 22, 28, and 36% lower than the control on day 16, 22, and 30 respectively. No difference was detected at $P \leq 0.05$ for the rest of the treatments during the experiment. A 50% reduction in stomatal conductance was observed on days 16 and 22 in NC 95-21-1 plants treated with 100 mM salt compared to the control.

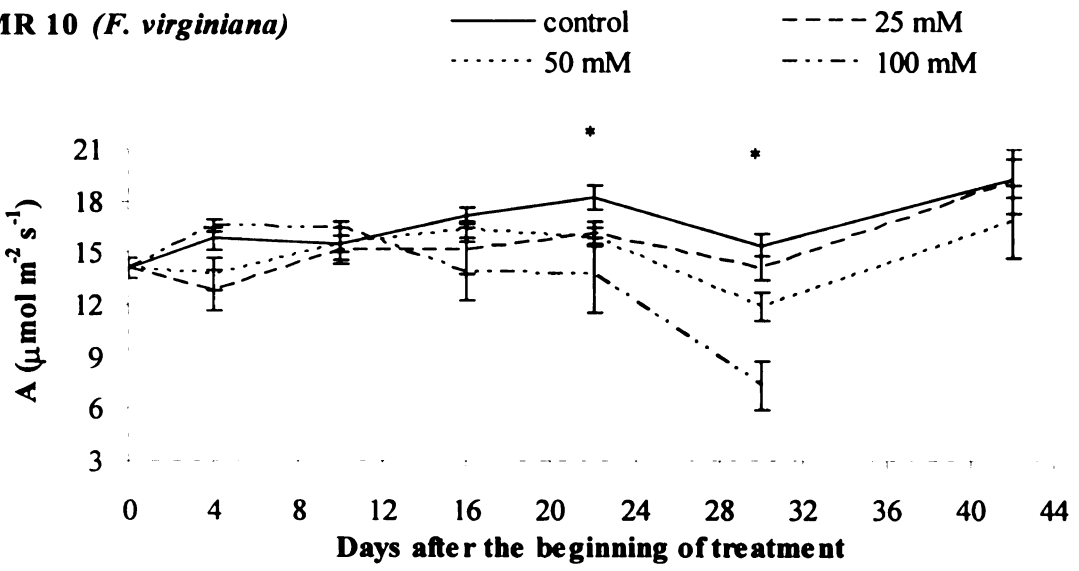
Similarly, plants receiving 100 mM salt had g_s 20% lower than the 25 mM and 5% lower than the 50 mM treatments. On day 30, plants treated with 100 mM exhibited g_s as low as 50% of controls. No differences were significant at $P \leq 0.05$ between control, 25, and 50 mM treatments. In FRA 24 no difference was detected between control and the treatment throughout the experiment ($P \leq 0.05$) with the exception the last day of the measurements where there was a 20% reduction in the 100 mM treatment compared to the control, 25, and 50 mM (Figure 4).

Transpiration rate (E) followed a pattern similar to the stomatal conductance (g_s) in all three selections tested, with only the 100 mM treatment showing significant difference from the rest throughout the experiment (Figure 5). Leaf intercellular concentration (C_i) maintained at the control's level in MR 10 until 30 days after the beginning of the salt treatments, where a slight increase (although significant at $P \leq 0.05$) was evident in 50 and 100 mM treatments compared to the control and 25 mM. In NC 95-21-1, a 30% reduction in C_i was observed in the 50 mM treatment compared to the rest on day 21, whereas a slight but significant increase was detected on days 30 and 42 in all treatments compared to the controls ($P \leq 0.05$). C_i maintained at control's values throughout the experiment in FRA 24 and in all treatments (Figure 6).

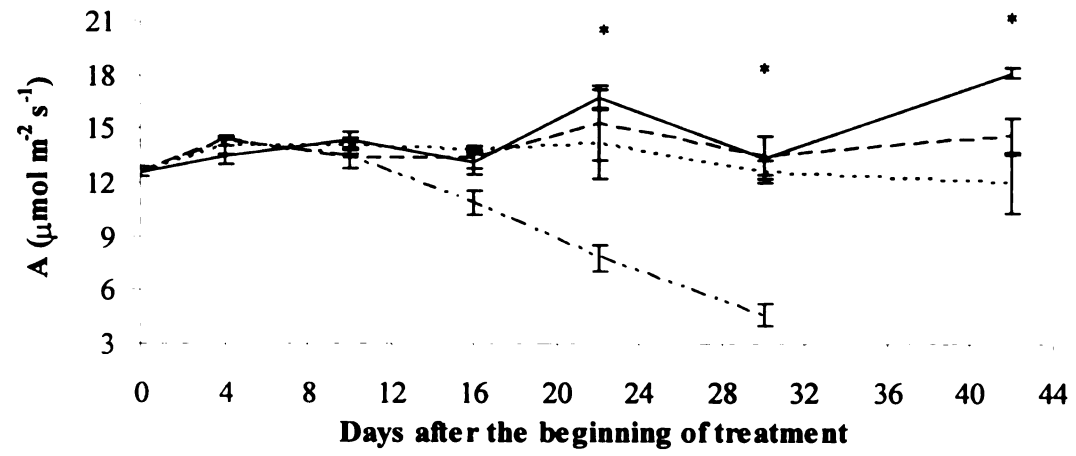
Comparison among selections within a treatment revealed a higher net assimilation rate (A) in MR 10 throughout the experiment, although not always different from the others ($P \leq 0.05$). In contrast there was not a clear trend for the stomatal conductance (g_s), transpiration rate (E) and leaf intercellular CO_2 (C_i).

Figure 3. Variation of leaf net assimilation rate (A) of strawberry plants during 42 days of salinity treatments. Each value represents the mean (±SE) of ten individual leaf measurements per selection and treatment. * indicates dates where statistically significant differences were observed between controls and stressed plants at $P \leq 0.05$ for each genotype (LSMEANS test) (see text for details).

MR 10 *(F. virginiana)*



NC 95-21-1 (*F. virginiana*)



FRA 24 (*F. chiloensis*)

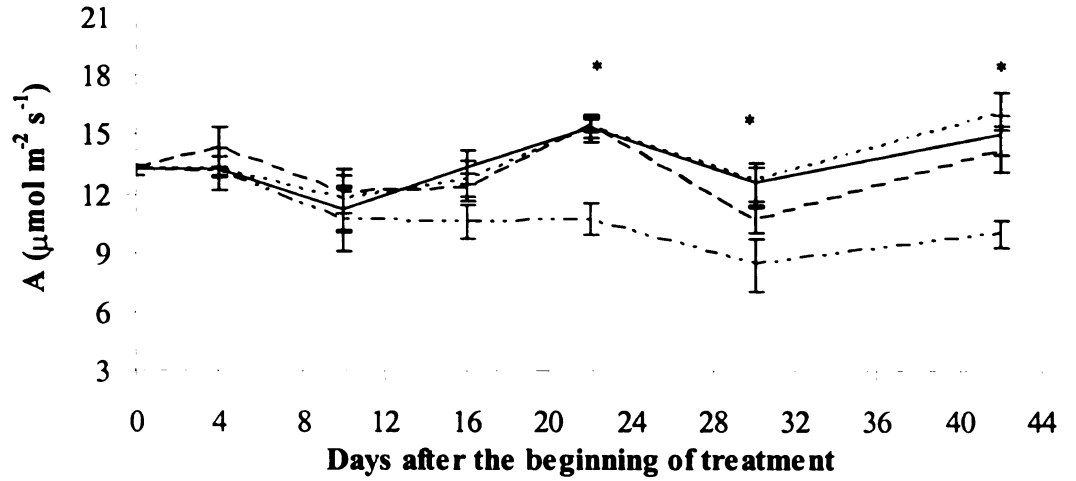
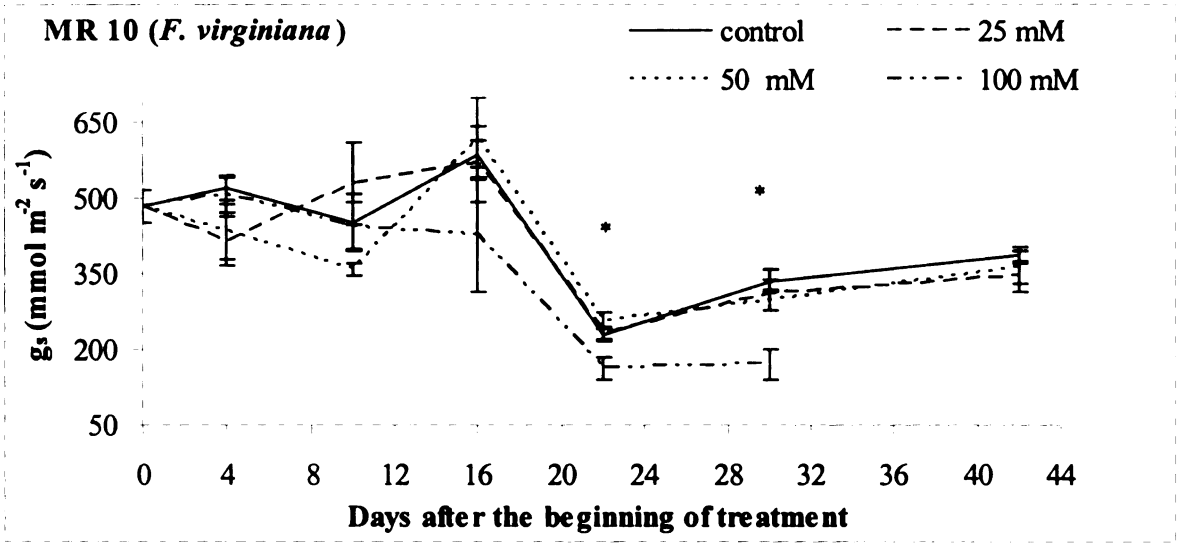
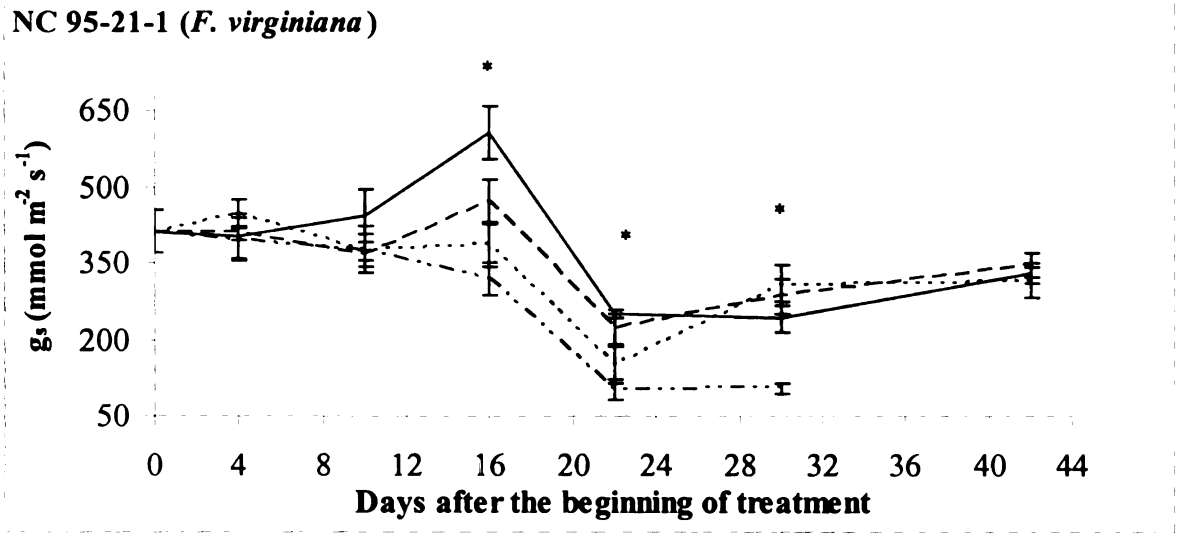


Figure 4. Variation of leaf stomatal conductance (g_s) of strawberry plants during 42 days of salinity treatments. Each value represents the mean (±SE) of ten individual leaf measurements per selection and treatment. * indicates dates where statistically significant differences were observed between controls and stressed plants at $P \leq 0.05$ for each genotype (LSMEANS test) (see text for details).


MR 10 (F. virginiana)
control
25 mM
50 mM
100 mM
650
500
350
200
50
gs (mmol m-2 s-1)
0
4
8
12
16
20
24
28
32
36
40
44
Days after the beginning of treatment


NC 95-21-1 (F. virginiana)
650
500
350
200
50
gs (mmol m-2 s-1)
0
4
8
12
16
20
24
28
32
36
40
44
Days after the beginning of treatment

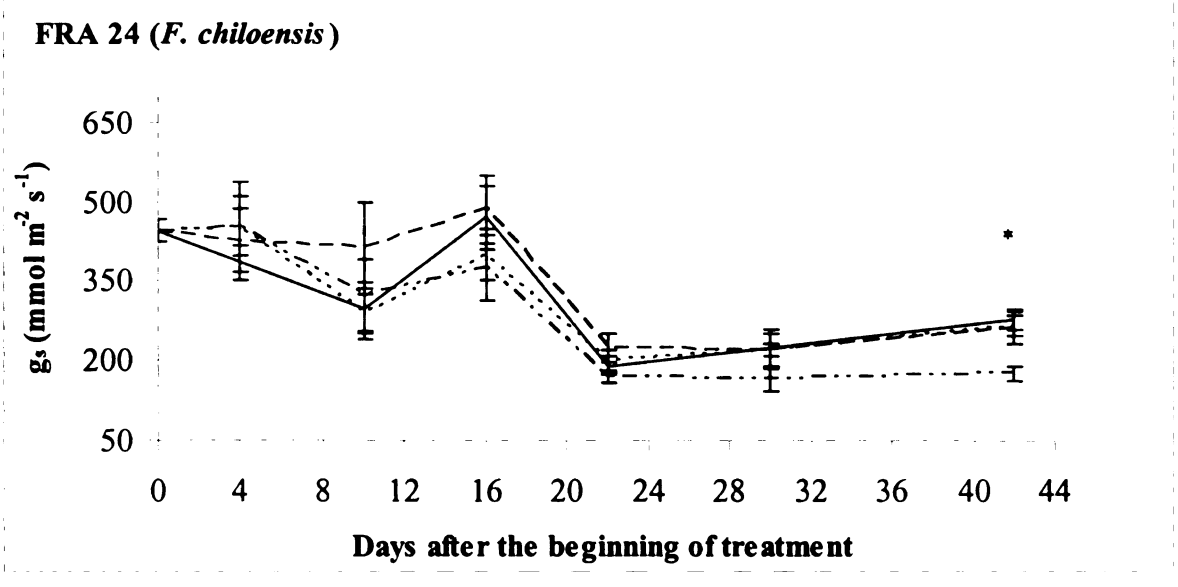
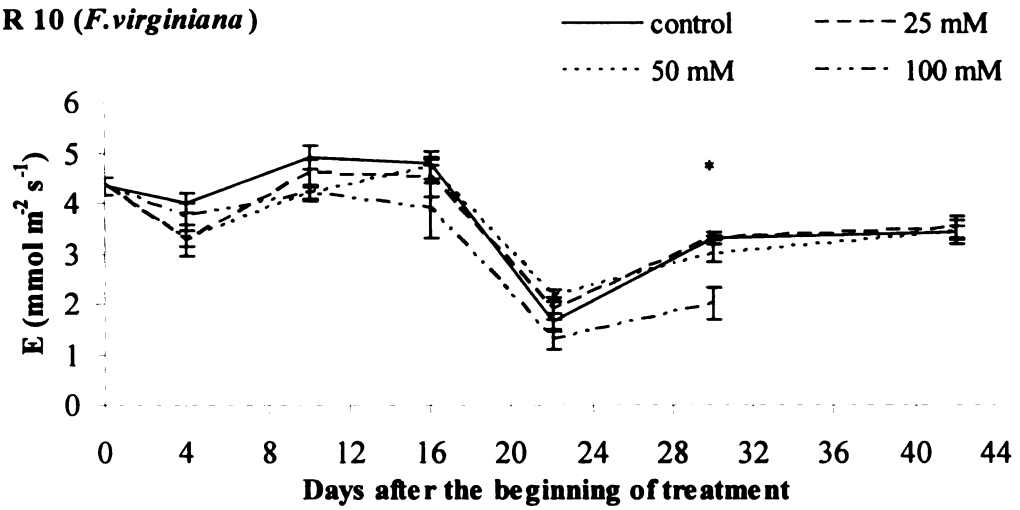
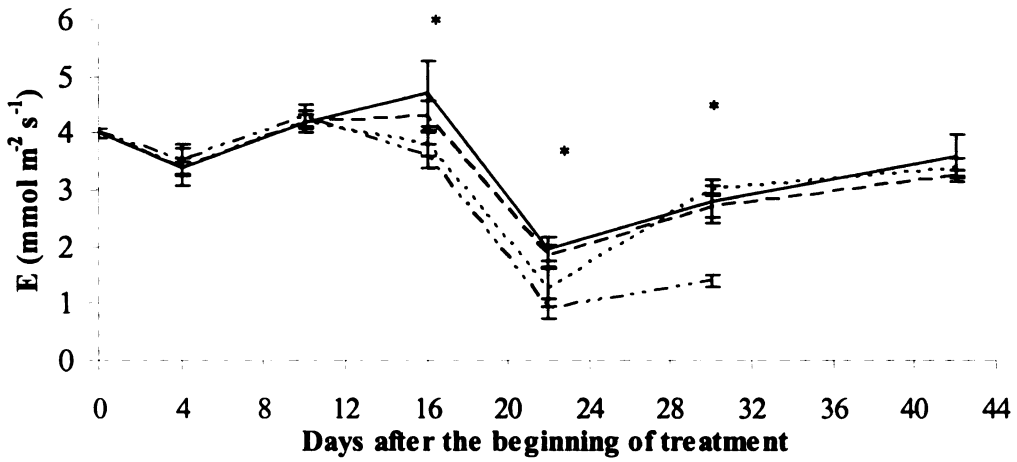

FRA 24 (F. chiloensis)
650
500
350
200
50
gs (mmol m-2 s-1)
0
4
8
12
16
20
24
28
32
36
40
44
Days after the beginning of treatment

Figure 5. Variation of leaf transpiration rate (E) of strawberry plants during 42 days of salinity treatments. Each value represents the mean (±SE) of ten individual leaf measurements per selection and treatment. * indicates dates where statistically significant differences were observed between controls and stressed plants at $P \leq 0.05$ for each genotype (LSMEANS test) (see text for details).

MR 10 (*F.virginiana*)



NC 95-21-1 (*F.virginiana*)



FRA 24 (*F. chiloensis*)

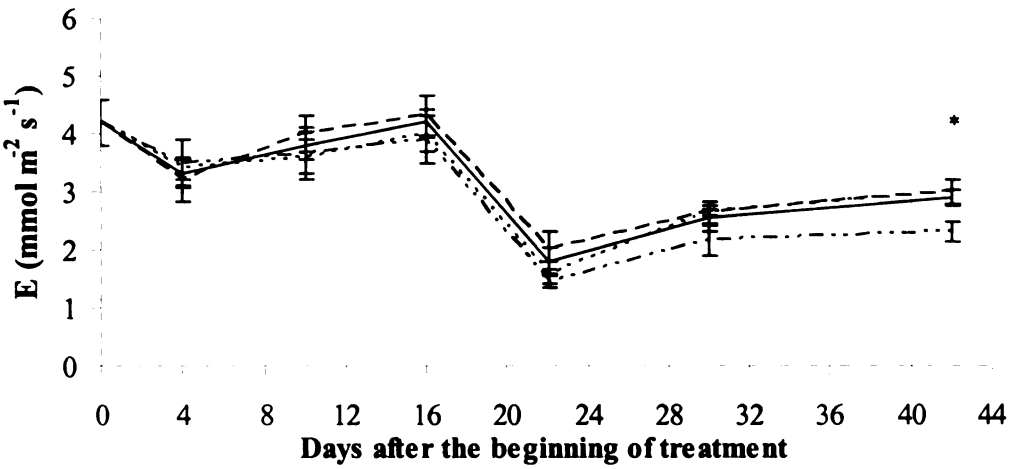
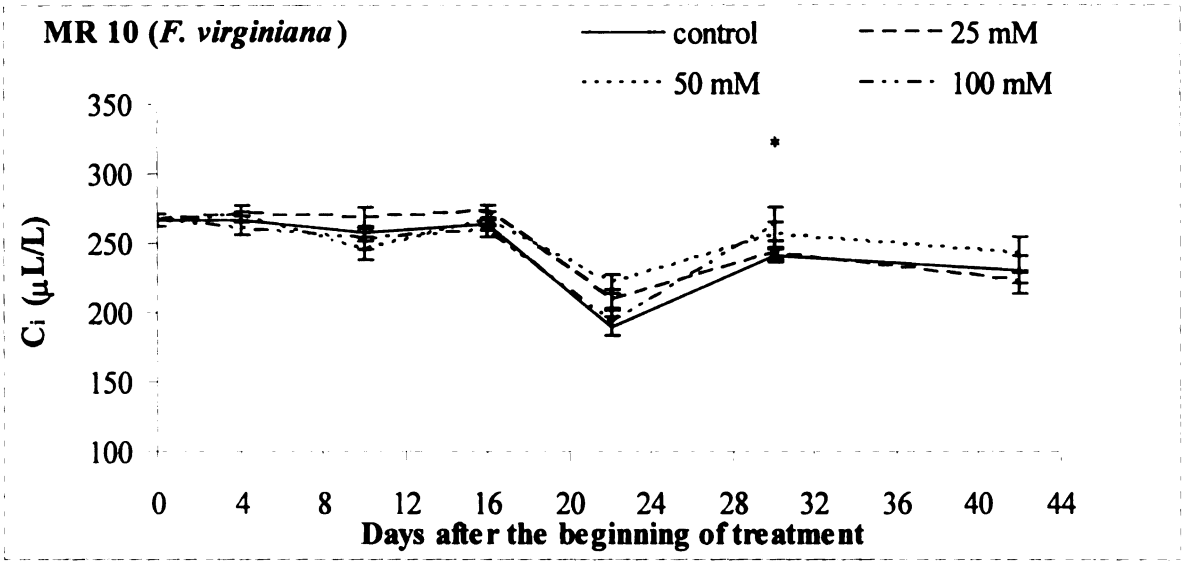
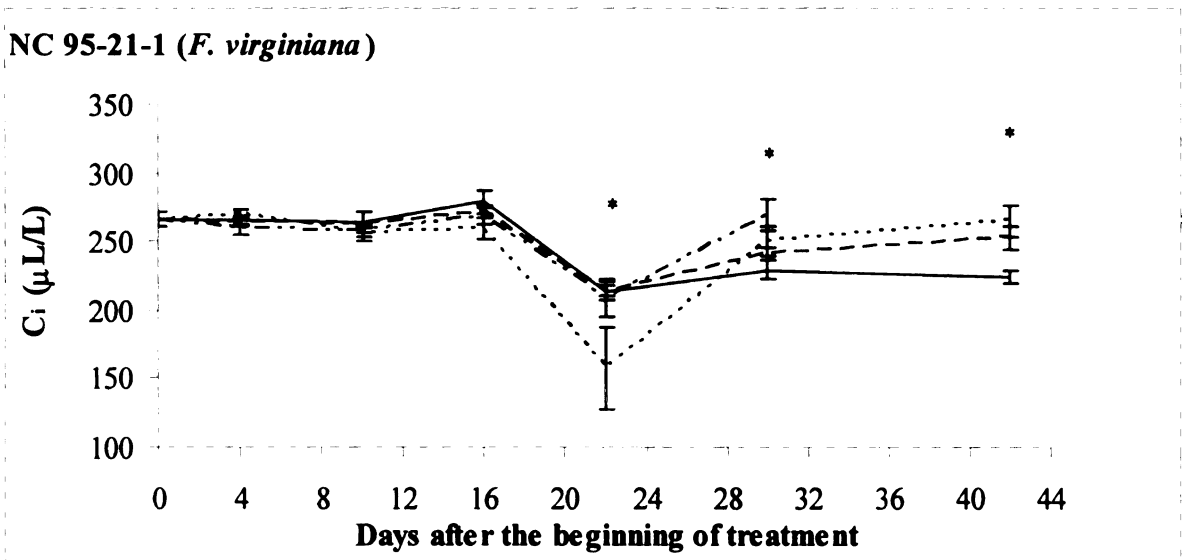
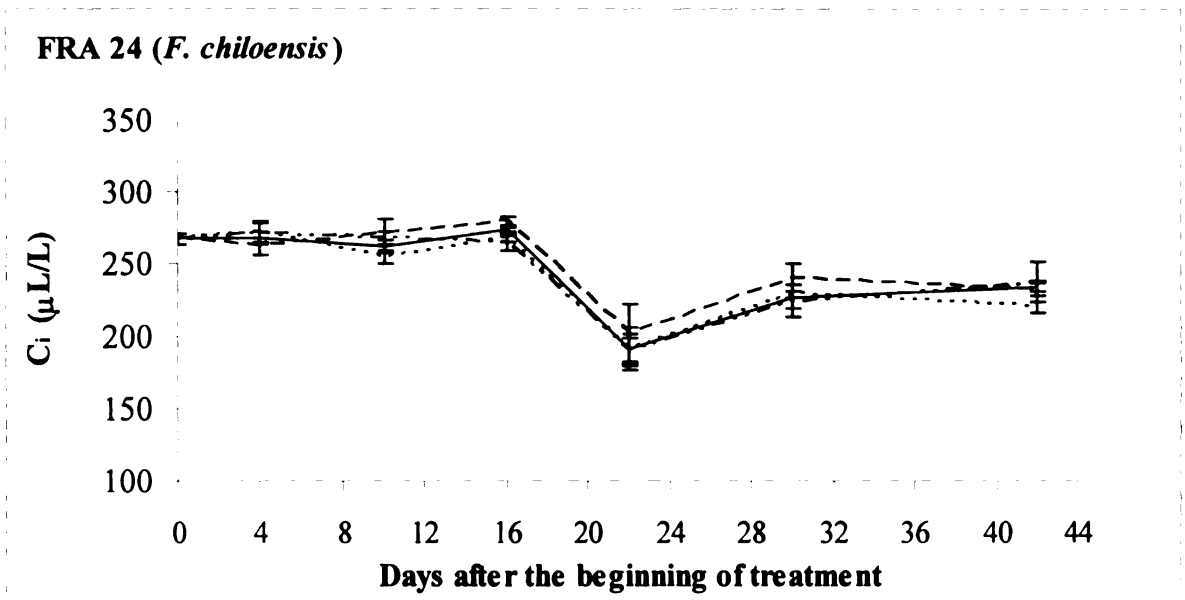


Figure 6. Variation of leaf intercellular concentration (C_i) of strawberry plants during 42 days of salinity treatments. Each value represents the mean (±SE) of ten individual leaf measurements per selection and treatment. * indicates dates where statistically significant differences were observed between controls and stressed plants at $P \leq 0.05$ for each genotype (LSMEANS test) (see text for details).


MR 10 (F. virginiana)
control
25 mM
50 mM
100 mM
Ci (µL/L)
350
300
250
200
150
100
0 4 8 12 16 20 24 28 32 36 40 44
Days after the beginning of treatment


NC 95-21-1 (F. virginiana)
Ci (µL/L)
350
300
250
200
150
100
0 4 8 12 16 20 24 28 32 36 40 44
Days after the beginning of treatment


FRA 24 (F. chiloensis)
Ci (µL/L)
350
300
250
200
150
100
0 4 8 12 16 20 24 28 32 36 40 44
Days after the beginning of treatment

B. Photosynthetic response to increasing light intensity

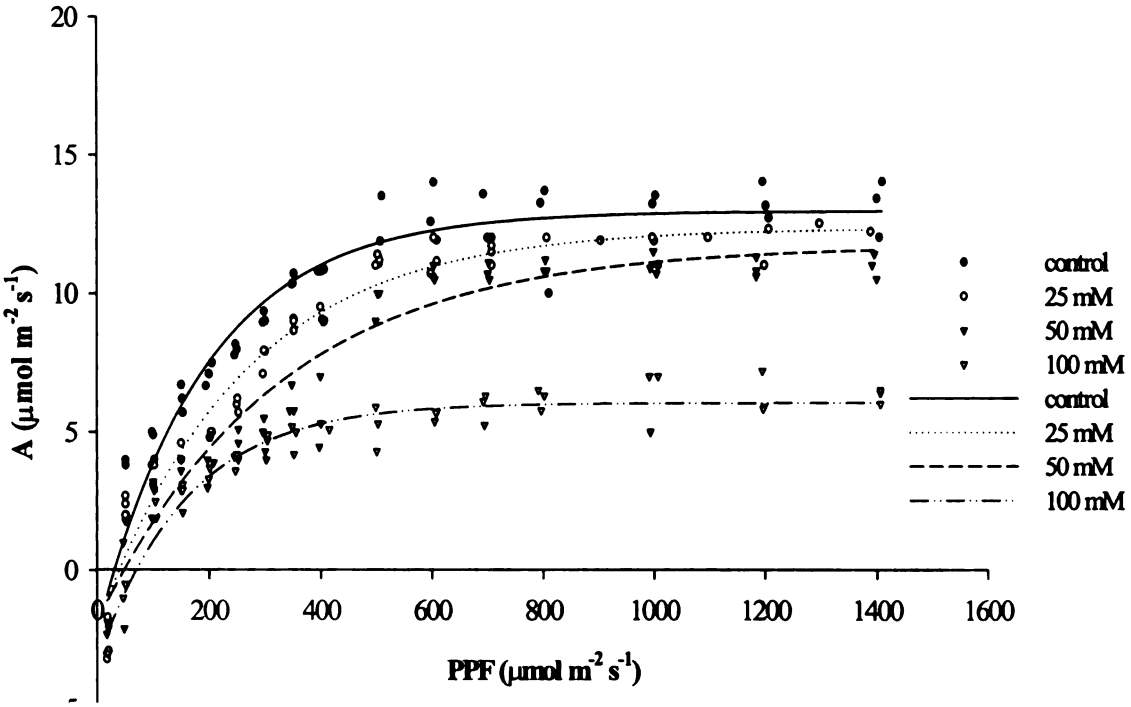
Light response curves were performed when a reduction in photosynthetic rate was evidence, during the fourth week of the experiment (days 21-26). The response of net assimilation rate (A) to increasing light intensity is shown in Figure 7. Curves were obtained after data were pooled from three plants per treatment. Equations of the fitted curves for all treatment x genotype combinations are shown in Table 2, Appendix A.

Gas exchange parameters derived from individual light response curves are summarized in Table 1. Detectable reduction in the maximum net assimilation rate (A_{max}) was observed in the 100 mM treatment in all selections. In addition, significant reduction was observed in MR 10 (*F. virginiana*) in the 50 mM treatment as well. A_{max} dropped from 14 to 6.9 μmol m^{-2} s^{-1} (50% reduction) in 100 mM treatment, whereas in 50 mM treatments the reduction was 15%. In NC 95-21-1, A_{max} dropped from 13.2 to 4.8 μmol m^{-2} s^{-1} (63% reduction) in the 100 mM treatment, while no significant difference was observed in the 25 and 50 mM treatments. In FRA 24, a reduction of 23% was evidence in the highest treatment whereas the slight decrease in 25 and 50 mM treatments was not significant ($P \leq 0.05$). Calculations of the maximum quantum yield efficiency revealed a statistically significant reduction in MR 10, from 0.052 in the control to 0.039 in the 25 mM, 0.036 in the 50 mM, and 0.03 in the 100 mM treatments. A quite different pattern was observed for NC 95-21-1 where no significant difference was detected ($P \leq 0.05$) between the control and the 25 and 50 mM treatments with values 0.053, 0.049 and 0.05. Significantly lower was the maximum quantum yield calculated for the 100 mM treatment. In FRA 24, the values were 0.056 in the control, 0.053 in the 25 mM, 0.049 in the 50 mM, and 0.043 in the 100 mM treatment.

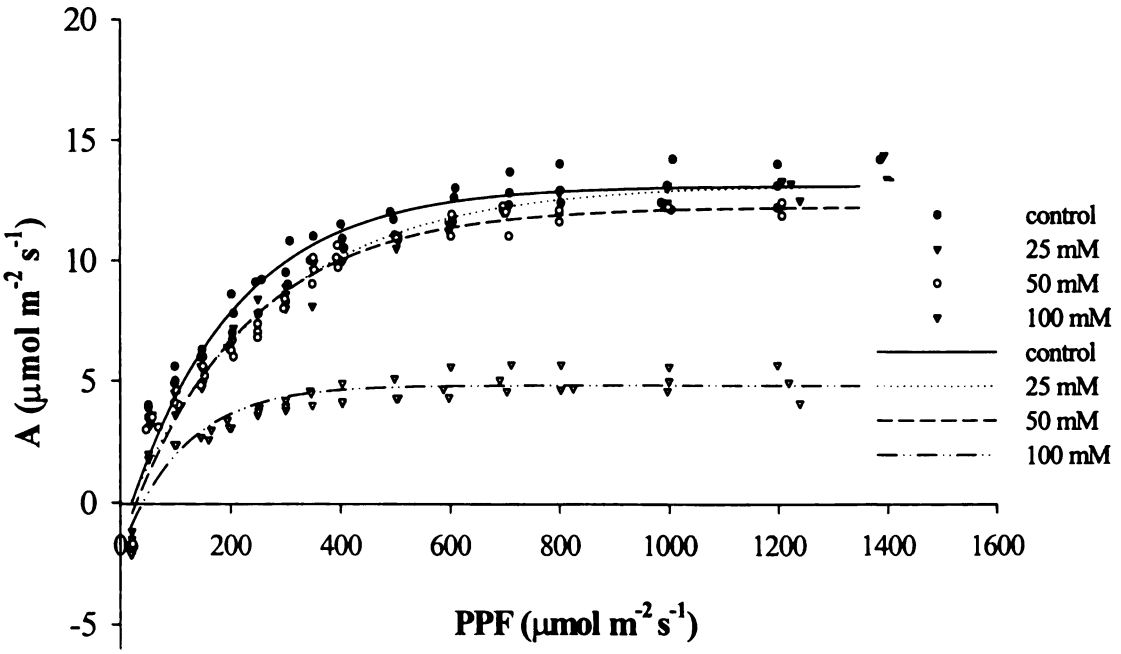
Salt treatment also affected the light compensation and saturation points in all three selections. The general trend was an increase in the compensation point and a decrease in the saturation point. More specifically, in MR 10, the light compensation points were 24, 26, 30 and 47 respectively, with only the highest treatment differing from the rest at $P \leq 0.05$. A similar pattern was observed in NC-95-21-1 as well, with values 18, 22, 29 and 39. Although there was an increase in the light compensation point in FRA 24, no difference was detected among treatments at $P \leq 0.05$. Light saturation point showed a decrease from the control to the 100 mM treatments in all three selections. More specifically, in MR 10 a statistically significant difference was observed between control and the rest of the treatments, whereas no difference was detected among the three salt treatments. In NC 95-21-1 there was no difference between control, 25, and 50 mM treatments while the 100 mM treatment was different from all the rest. In FRA 24 all three salt treatments were similar to each other but different from the control.

Figure 7. Variation of net assimilation rate in response to increasing light intensity (Light response curves). Data were collected on fully expanded strawberry leaves 21 to 26 days after the initiation of salt treatments. After photosynthetic parameters were calculated for each plant, data from three plants per treatment and selection were pooled and curves were fitted by nonlinear regression model for illustrative purposes
(A) MR 10 *(F. virginiana)* (B) NC 95-21-1 *(F. virginiana)* (C) FRA 24 (*F. chiloensis*)

MR 10 (*F. virginiana*) A



NC 95-21-1 (*F. virginiana*) B



FRA 24 (*F. chiloensis*) **C**

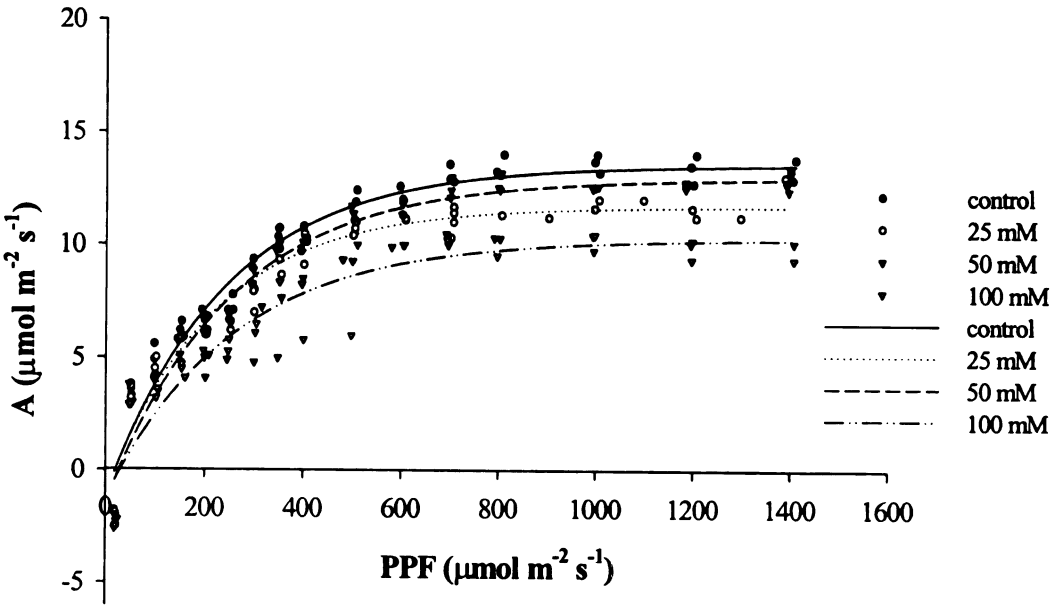


Table 1. Gas exchange parameters derived from light response curves of fully expanded leaves of MR 10 and NC 95-21-1 (*F. virginiana*) and FRA 24 (*F. chiloensis*) plants subjected to 21-26 days of salinity. Values represent means from three plants. Means followed by the same letter within a column do not differ significantly (LSMEANS, $P \leq 0.05$).

Genotype x treat		Max quantum yield (μmol CO_2 μmol^{-1} photons)	Light comp. point (μmol m^{-2} s^{-1})	Light sat. point (μmol m^{-2} s^{-1})	A_{max} (μmol m^{-2} s^{-1})
MR 10	control	0.052 *ab*	24 *cde*	765 *a*	14 *a*
	25 mM	0.039 *cd*	26 *cde*	633 *cde*	13.9 a
	50 mM	0.036 *d*	30 *cd*	633 *de*	11.8 *b*
	100 mM	0.030 *e*	47 *a*	560 *ef*	6.9 *c*
NC 95-21-1	control	0.053 *ab*	18 *e*	765 *ab*	13.2 *a*
	25 mM	0.049 *b*	22 *e*	733 *abc*	13.1 *a*
	50 mM	0.05 *ab*	29 *cde*	660 *bcde*	12.2 *a*
	100 mM	0.029 *e*	39 *b*	470 *f*	4.8 *d*
FRA 24	control	0.056 *a*	24 *cde*	730 *bcd*	13.5 *a*
	25 mM	0.053 *ab*	23 *cde*	565 *e*	12.2 *a*
	50 mM	0.049 *b*	28 *cde*	633 *cde*	12.8 *a*
	100 mM	0.043 *c*	34 *c*	590 *e*	10.3 *b*

C. Photosynthetic response to increasing air CO_2 concentration

Twenty eight days after the beginning of the salt treatments all MR 10 and NC 95-21-1 plants receiving 100 mM salt were severely damaged so that no CO_2 response curves were performed, but FRA 24 plants receiving the same amount of salt had no visible damage. Therefore, data from control, 25 mM, and 50 mM are presented for both ecotypes of *F. virginiana*, (Table 2) whereas data from control, 25 mM, and 100 mM are presented for FRA 24 (Table 3).

No major changes were observed in gas exchange parameters of MR 10 (*F. virginiana*) plants receiving 25 mM salt. In contrast, the maximum photosynthetic rate (A_{max}) and the carboxylation efficiency (k) of plants treated with 50 mM salt was significantly lower than the control. In NC 95-21-1 (*F. virginiana*), although differences in net assimilation rate at ambient CO_2 and A_{max} were not evident, there was a significant decrease in stomatal conductance and an increase in the CO_2 compensation point (Γ) as well as in limitations imposed by stomata in plants treated with 50 mM salt. No difference was detected in any of the other parameters.

In FRA 24 (*F. chiloensis*) treated with 100 mM salt there was a significant decrease in stomatal conductance (g_s), internal CO_2 concentration (C_i), and the ratio C_i/C_a whereas there was an increase in CO_2 compensation point (Γ) and stomatal limitation ($P \leq 0.05$).

Table 2. Gas exchange parameters derived from A/C_i curves of fully expanded leaves of MR 10 and NC 95-21-1 (*F. virginiana*) plants subjected to 30-35 days of salinity. Values represent means from three plants. Means followed by the same letter within a row do not differ significantly (LSMEANS, $P \leq 0.05$)

	MR 10		
Parameter	**Control**	**25 mM**	**50 mM**
A at 370 μL/L CO_2 (μmol m^{-2} s^{-1})	14.9 *a*	15.4 *a*	12.4 *b*
A_{max}	44.7 *a*	42.4 *ab*	37.2 *b*
k (μmol CO_2 $m^{-2}s^{-1}$ $\mu L^{-1}L$)	0.240 *a*	0.232 *a*	0.216 *b*
Γ (μL/L)	79	77	83
g_s (at 370 μL/L CO_2)	254	339	330
stomatal limit. (at 370 μL/L CO_2)	0.432	0.416	0.387
C_i (at 370 μL/L CO_2)	230	233	244
C_i/C_a (%)	62	63	66

	NC 95-21-1		
Parameter	**control**	**25 mM**	**50 mM**
A at 370 μL/L CO_2 (μmol m^{-2} s^{-1})	15.2	15.2	13.0
A_{max}	43.9	40.2	40.7
k (μmol CO_2 $m^{-2}s^{-1}$ μL^{-1} L)	0.225	0.214	0.21
Γ (μL/L)	78 *ab*	71 *a*	86 *b*
g_s (at 370 μL/L CO_2)	352 *a*	258 *ab*	216 *b*
stomatal limit. (at 370 μL/L CO_2)	0.354 *a*	0.384 *ab*	0.428 *b*
C_i (at 370 μL/L CO_2)	250	238	236
C_i/C_a (%)	68	64	64

Table 3. Gas exchange parameters derived from A/C_i curves of fully expanded leaves of FRA 24 (*F. chiloensis*) plants subjected to 30-35 days of salinity. Values represent means from three plants. Means followed by the same letter within a row do not differ significantly (LSMEANS, $P \leq 0.05$)

	FRA 24		
Parameter	**control**	**25 mM**	**100 mM**
A at 370 μL/L CO_2 (μmol m^{-2} s^{-1})	14.2	14.8	12.7
A_{max}	40.8 *a*	46.9 *b*	41.7 *ab*
k (μmol CO_2 $m^{-2}s^{-1}$ $\mu L^{-1}L$)	0.24	0.229	0.238
Γ (μL/L)	85 *a*	82 *a*	99 *b*
g_s (at 370 μL/L CO_2)	227 *a*	242 *a*	101 *b*
stomatal limit. (at 370 μL/L CO_2)	0.418 *a*	0.458 *a*	0.685 *b*
C_i (at 370 μL/L CO_2)	238 *a*	226 *a*	180 *b*
C_i/C_a (%)	64 *a*	61 *a*	49 *b*

Leaf mineral composition analysis

Analysis of leaf mineral content is presented in Table 4 for the two species. A fluctuation in leaf K^+ and Ca^{+2} content was observed in MR 10 and NC 95-21-1 with the highest value in the 100 mM treatment for both minerals. A quite different pattern was evidence in FRA 24 with the 100 mM treatment showing the highest value for K^+, while the rest did not differ from each other. In contrast, no difference was observed in Ca^{+2} content in all four treatments.

An increase in leaf sodium and chloride content was observed in all three selections with increasing salinity. The highest increase was detected in NC 95-21-1 where the amount of Na^+ found in leaves from plants receiving 25, 50, and 100 mM salt was respectively 1.5, 15, and 53 times more than the control. Chloride content increased 2.7, 9.5, and 20 times in the three treatments compared to the control. Significant differences were also detected between intermediate treatments ($P \leq 0.05$).

In MR 10, sodium content increased in all treatments compared to the control with values 1.2, 1.4, and 16 times higher in the three treatments respectively, while chloride content increased 2, 6, and 15.5 times respectively. In FRA 24 no difference was detected in sodium content for the 25 mM treatment, whereas an increase of 1.2, and 1.8 times was evidence for the 50 mM and 100 mM treatments compared to the control. The differences were not significant ($P \leq 0.05$). Chloride content increased 1.6 times in both 25 and 50 mM treatments and 4.5 times in the 100 mM treatment.

The ratio Na^+/K^+ was found higher 1.2, 1.8, 10 times in MR 10 plants receiving 25 mM, 50 mM and 100 mM salt compared to the control. Similarly, in NC 95-21-1 it was 1.2, 9 and 30 times higher respectively. In FRA 24 no increase was detected in the

Na^+/K^+ ratio for the 25 mM treatment, while it was 1.2 and 1.6 times higher for the 50 mM and the 100 mM treatments.

Table 4. Mineral composition of leaves from MR 10, NC 95-21-1 (*F.virginiana*) and FRA 24 (*F. chiloensis*) plants 21 days after the beginning of salt treatments. Values are means from three measurements. Means followed by the same letter within a column do not differ significantly, (LSMEANS, $P \leq 0.05$).

Genotype	x treat	K^+ (%DW)	Ca^{+2} (% DW)	Na^+ (%DW)	Cl^- (ppm)	Na^+/K^+
MR 10	control	0.77 *ab*	1.1 *ab*	0.05 *a*	2550 *a*	0.06 *a*
	25 mM	0.88 *bc*	0.9 *a*	0.06 *a*	5000 *c*	0.07 *a*
	50 mM	0.61 *a*	1.28 *b*	0.07 *a*	14700 *f*	0.11 *a*
	100 mM	1.33 *e*	2.61 *c*	0.8 *c*	40000 *h*	0.60 *b*
NC 95-21-1	control	0.77 *ab*	1.2 *ab*	0.04 *a*	2950 *a*	0.05 *a*
	25 mM	1.08 *cd*	1.1 *ab*	0.06 *a*	8200 *d*	0.06 *a*
	50 mM	1.31 *e*	1.31 *b*	0.58 *b*	28200 *g*	0.44 *b*
	100 mM	1.42 *e*	4.58 *d*	2.15 *d*	58800 *i*	1.51 *c*
FRA 24	control	1.02 *cd*	1.1 *ab*	0.05 *a*	2400 *a*	0.05 *a*
	25 mM	0.93 *bcd*	1.2 *ab*	0.05 *a*	4000 *b*	0.05 *a*
	50 mM	1.02 *cd*	1.15 *ab*	0.06 *a*	3900 *ab*	0.06 *a*
	100 mM	1.13 *e*	1.13 *ab*	0.09 *a*	11100 *e*	0.08 *a*

Experiment II

Analysis of photosynthetic performance

A. Gas exchange

Parameters derived from gas exchange studies were plotted versus time and are shown in Figures 8-11. Net assimilation rate (A) was affected by salt stress in all three selections tested. Stressed plants of MR 10 (*F. virginiana*) showed a reduction in photosynthetic rate as early as four days after the beginning of the salt treatment (Figure 8). Significant differences ($P \leq 0.05$) between the two treatments began on day 8, with stressed plants having 20% lower rates than the controls. Photosynthetic rate continued to decrease until day 28 where it was observed a 70% reduction compared to the control. In NC 95-21-1 (*F. virginiana*) plants treated with 10 mM salt a 20 % reduction in photosynthetic rate compared to control was observed on day 12 and it continued to decline until the end of the experiment where a 50% reduction was evident. In FRA 24 a 40% reduction was observed on day 8, and it remained significantly lower than the control until the end of the experiment. In general, reductions of 20 and 40% were observed in FRA 24 throughout the experiment.

Stomatal conductance (g_s) showed a 25% decrease in MR 10 on day 8 and it continued to decline until day 21 where the lowest value was recorded (50% lower than the control). In NC 95-21-1, a significant reduction in g_s was first evident on day 12 and it continued to decline until the end of the experiment where it was 65% lower than the control. In FRA 24, g_s was 55 and 65% lower than the control on days 8 and 12 respectively, while later, the difference became smaller because controls also exhibited a

reduction in g_s. After day 24 no significant difference was observed between stressed and control plants (Figure 9).

Transpiration rate (E) decreased significantly ($P \leq 0.05$) 12 days after the beginning of the treatment in MR 10 and it remained low with values ranging from 20 to 40% lower than the control until day 28, where no difference was observed. In NC 95-21-1, a significant reduction was evident on day 4, no difference was detected on day 8, whereas from day 12 until the end of the experiment, E remained 10 to 50% lower than the control. In FRA 24, E was found significantly lower than the control as early as 4 days after the initiation of the treatment and it remained 10 to 50% lower than the control until day 28 where no difference was detected (Figure 10).

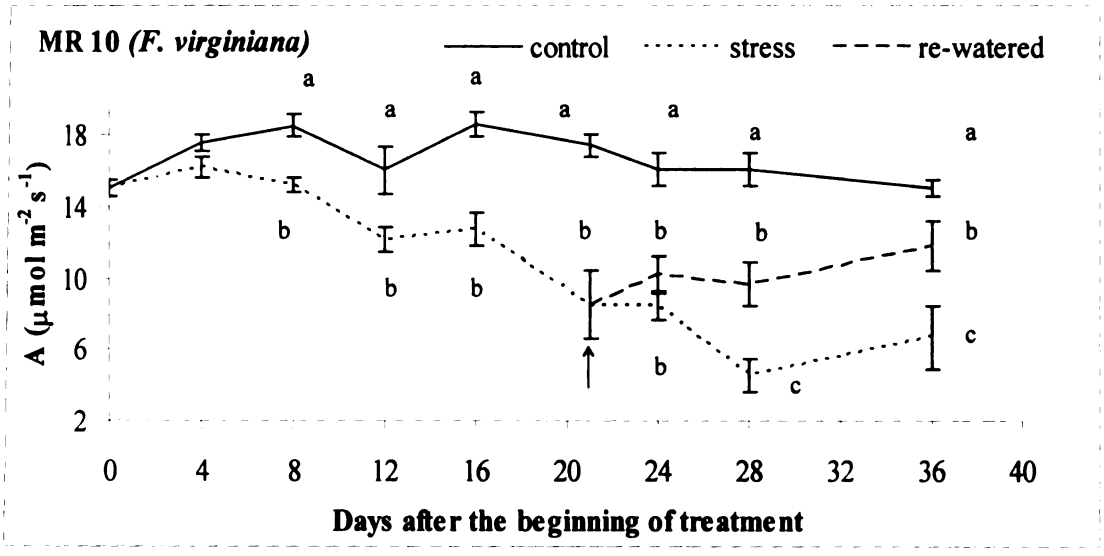
Leaf intercellular CO_2 (C_i) concentration showed an increase in MR 10 stressed plants on day 21 until the end of the experiment. In NC 95-21-1, it was lower than the control on days 16 and 21, but higher than the control on day 28 until the end of the experiment. In FRA 24, no differences were observed except on day 28 where stressed plants had higher C_i compared to the controls (Figure 11).

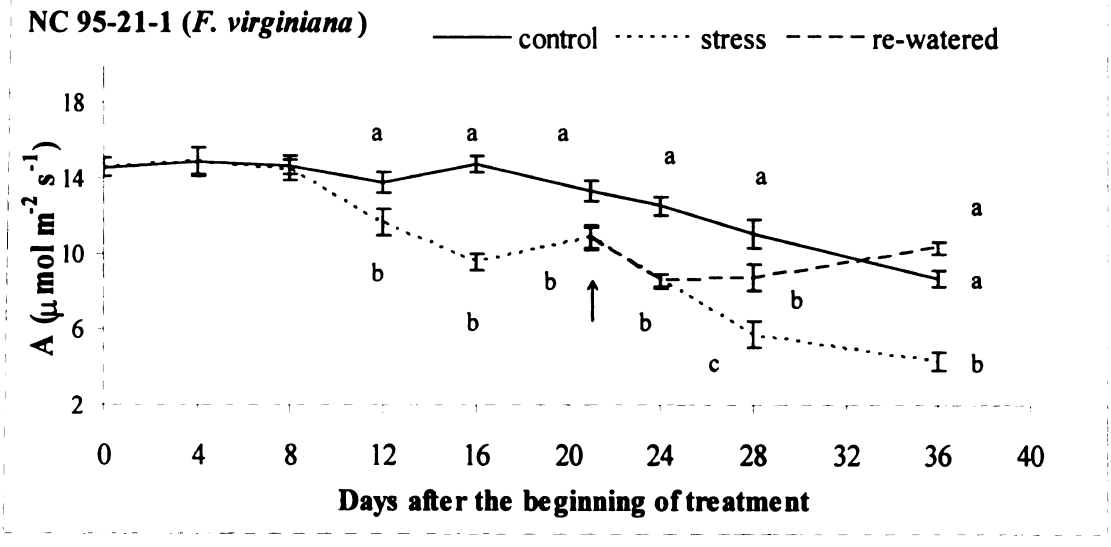
All plants allowed to recover from the stress showed an increase in the net assimilation rate (Figure 8). More specifically, in MR 10 there was a 50% recovery 8 days after the salt treatment had stopped. Although the value was significantly higher ($P \leq 0.05$) than that of the stressed plants, and it remained higher until the end of the experiment, it never reached the photosynthetic rate of the control. Both NC 95-21-1 and FRA 24 displayed a complete recovery of the photosynthetic rate on the last day of measurements ($P \leq 0.05$). Stomatal conductance increased in both MR 10 and NC 95-21-1 8 days after the beginning of the recovery period, and reached the control's values by the

end of the experiment ($P \leq 0.05$). In FRA 24 g_s was also found similar to control, but it is difficult to conclude if this was a treatment effect since control plants exhibited a low stomatal conductance as well (Figure 9).

Although transpiration rate reached the control's value in all three selections 8 days after the beginning of the recovery period, only in NC 95-21-1 it was significantly higher than the stress (Figure 10). Leaf intercellular CO_2 of plants allowed to recover was found significantly lower than stress and similar to the control in MR 10, similar to stress and higher than the control in NC 95-21-1, whereas no different from both stress and control in FRA 24 (Figure 11).

Figure 8. Variation of leaf net assimilation rate (A) of strawberry plants during 21 days of salinity treatment and 15 days of recovery. Each value represents the mean (±SE) of ten individual leaf measurements per selection and treatment. Letters indicate statistically significant differences between the treatments at $P \leq 0.05$ (LSMEANS test).
Arrow indicates the beginning of the recovery period.


MR 10 (F. virginiana)
control
stress
re-watered
A (µmol m-2 s-1)
Days after the beginning of treatment


NC 95-21-1 (F. virginiana)
control
stress
re-watered
A (µmol m-2 s-1)
Days after the beginning of treatment

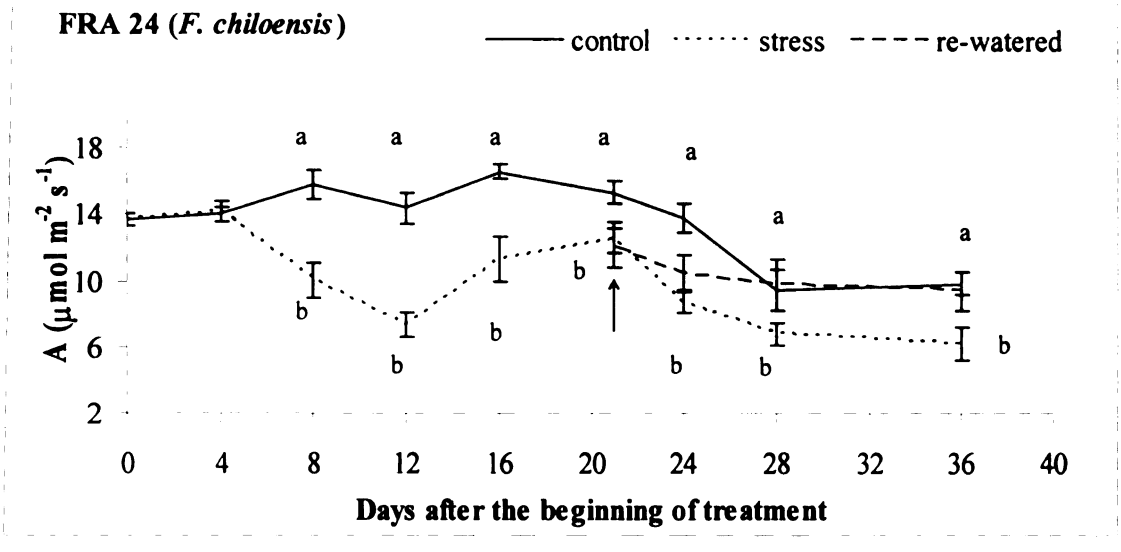
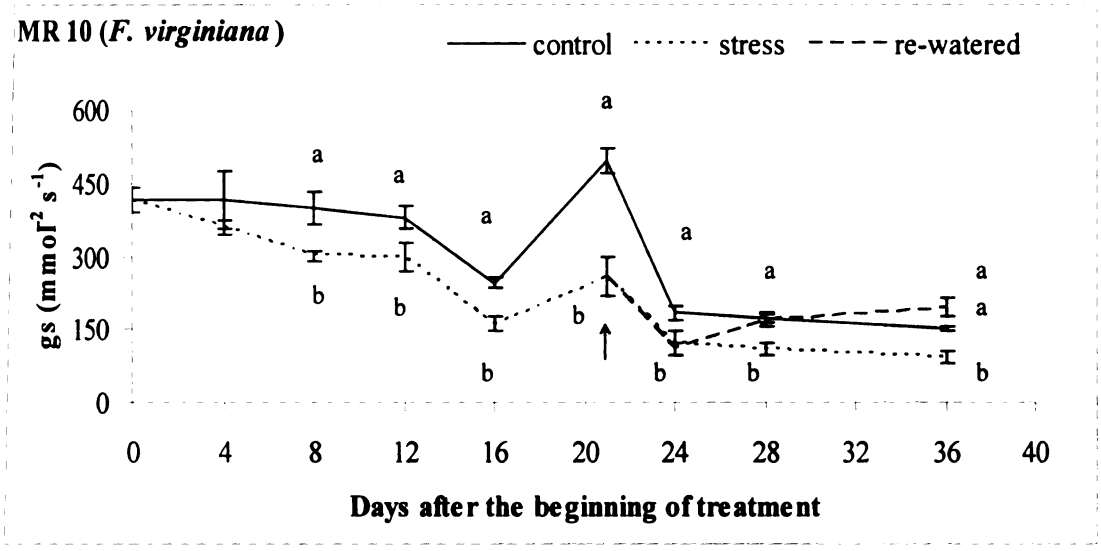
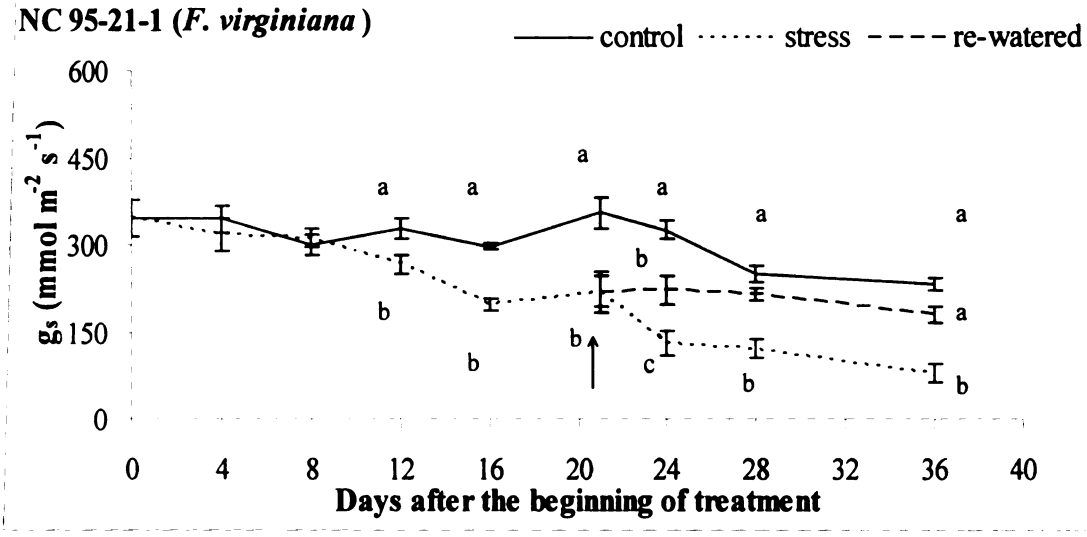

FRA 24 (F. chiloensis)
control
stress
re-watered
A (µmol m-2 s-1)
Days after the beginning of treatment

Figure 9. Variation of leaf stomatal conductance (g_s) of strawberry plants during 21 days of salinity treatment and 15 days of recovery. Each value represents the mean (±SE) of ten individual leaf measurements per selection and treatment. Letters indicate statistically significant differences between the treatments at $P \leq 0.05$ (LSMEANS test).
Arrow indicates the beginning of the recovery period.


MR 10 (F. virginiana)
control
stress
re-watered
gs (mmol m-2 s-1)
600
450
300
150
0
0 4 8 12 16 20 24 28 32 36 40
Days after the beginning of treatment


NC 95-21-1 (F. virginiana)
control
stress
re-watered
gs (mmol m-2 s-1)
600
450
300
150
0
0 4 8 12 16 20 24 28 32 36 40
Days after the beginning of treatment

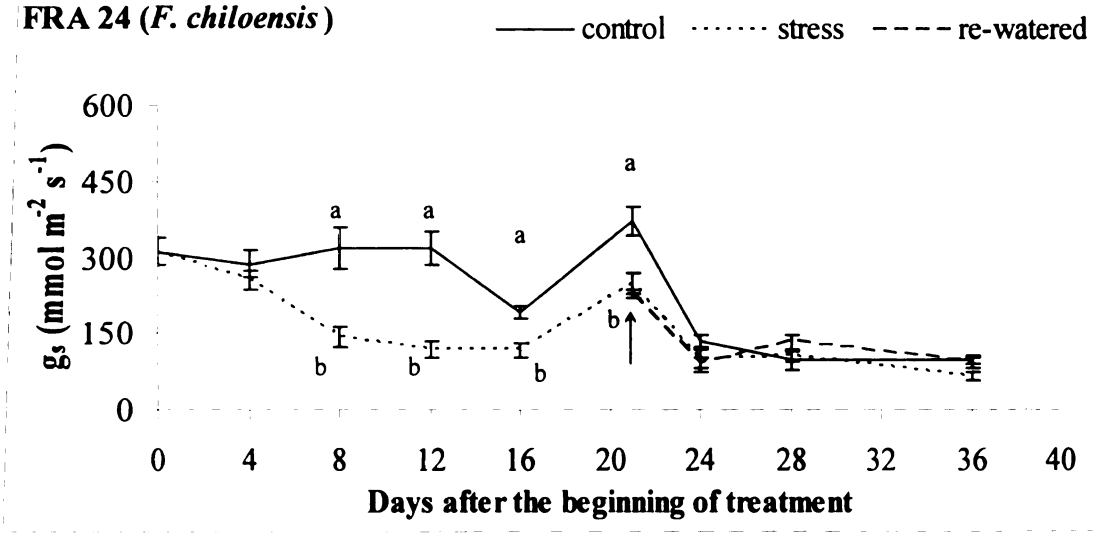
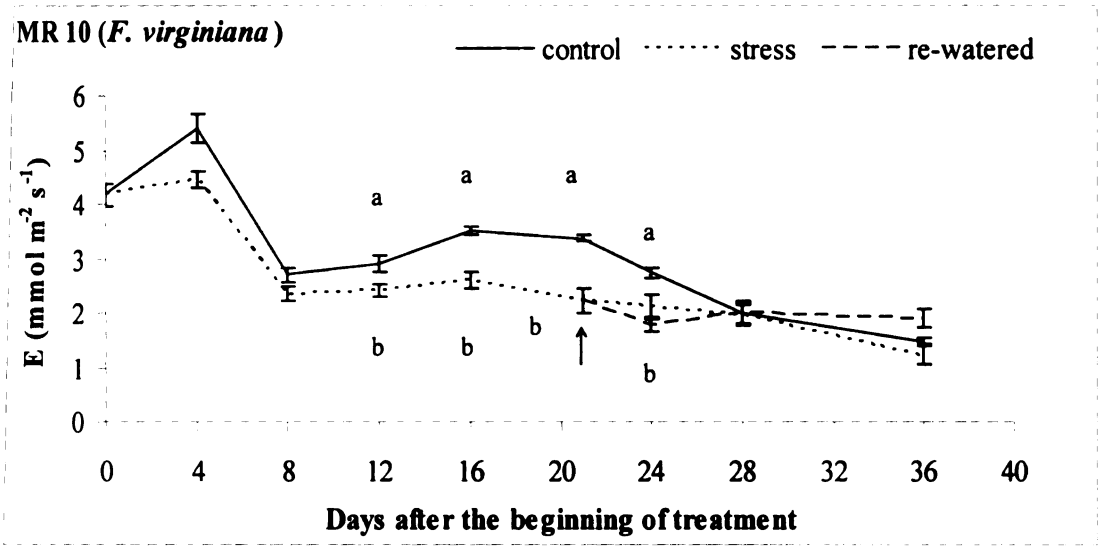
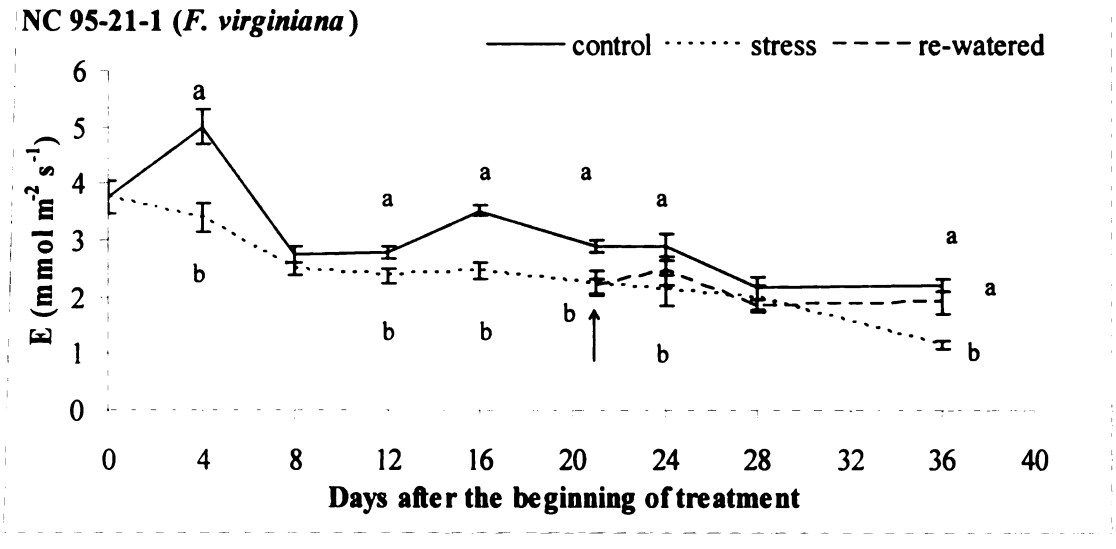

FRA 24 (F. chiloensis)
control
stress
re-watered
gs (mmol m-2 s-1)
600
450
300
150
0
0 4 8 12 16 20 24 28 32 36 40
Days after the beginning of treatment

Figure 10. Variation of leaf transpiration rate (E) of strawberry plants during 21 days of salinity treatment and 15 days of recovery. Each value represents the mean (±SE) of ten individual leaf measurements per selection and treatment. Letters indicate statistically significant differences between the treatments at $P \leq 0.05$ (LSMEANS test).
Arrow indicates the beginning of the recovery period.


MR 10 (F. virginiana)
control
stress
re-watered
E (mmol m-2 s-1)
Days after the beginning of treatment


NC 95-21-1 (F. virginiana)
control
stress
re-watered
E (mmol m-2 s-1)
Days after the beginning of treatment

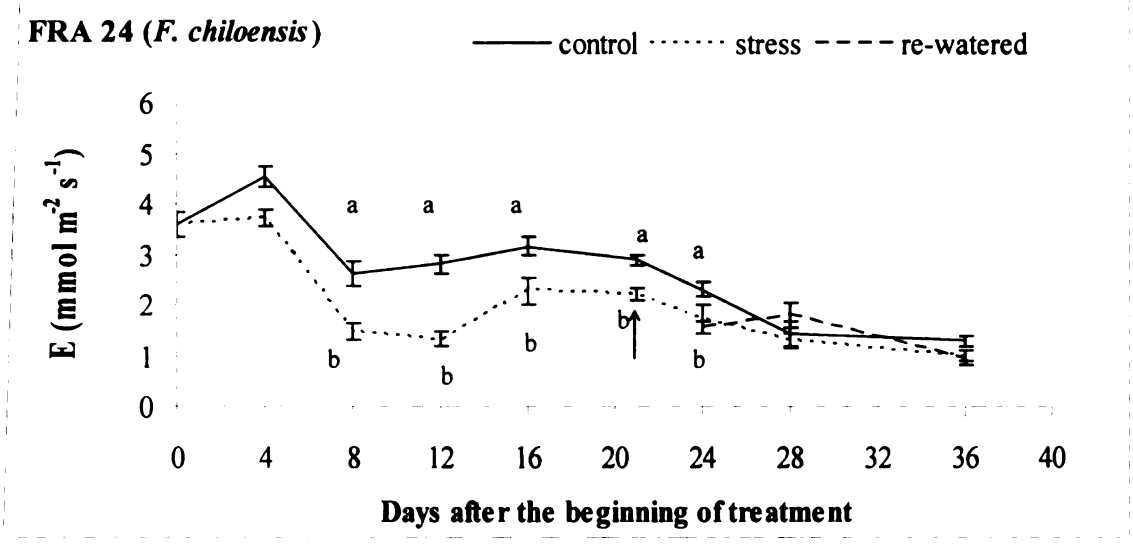
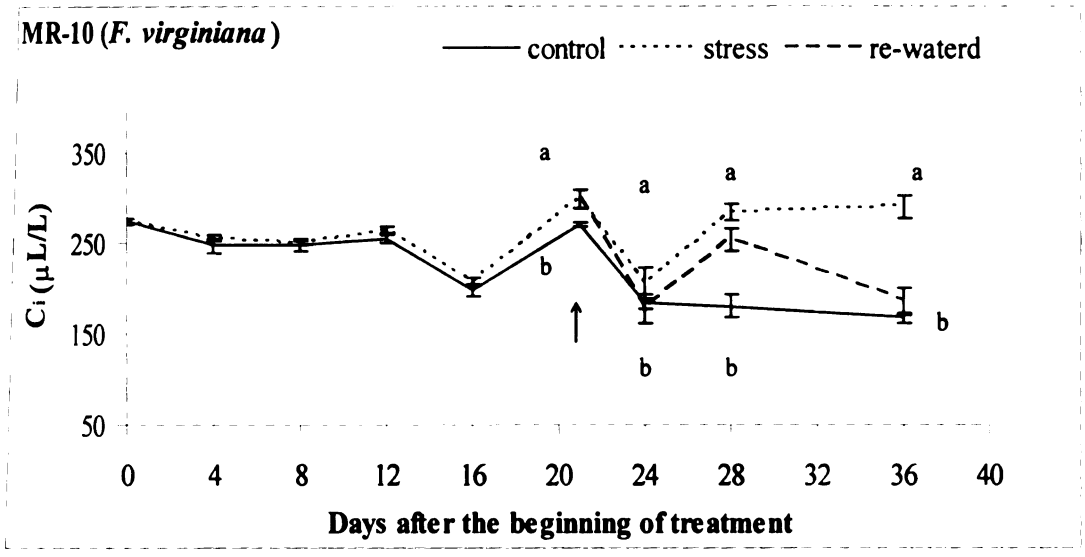
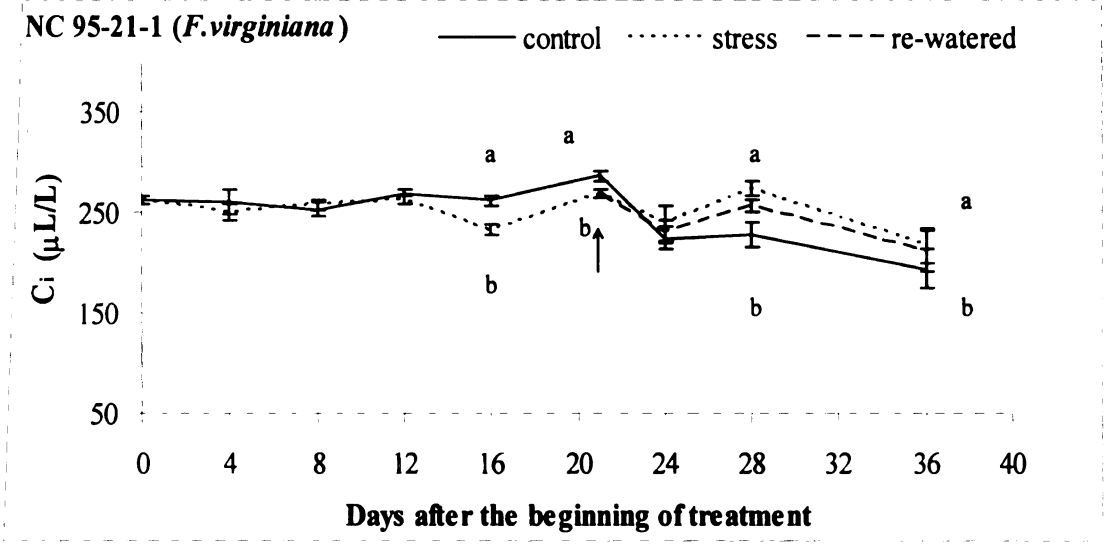
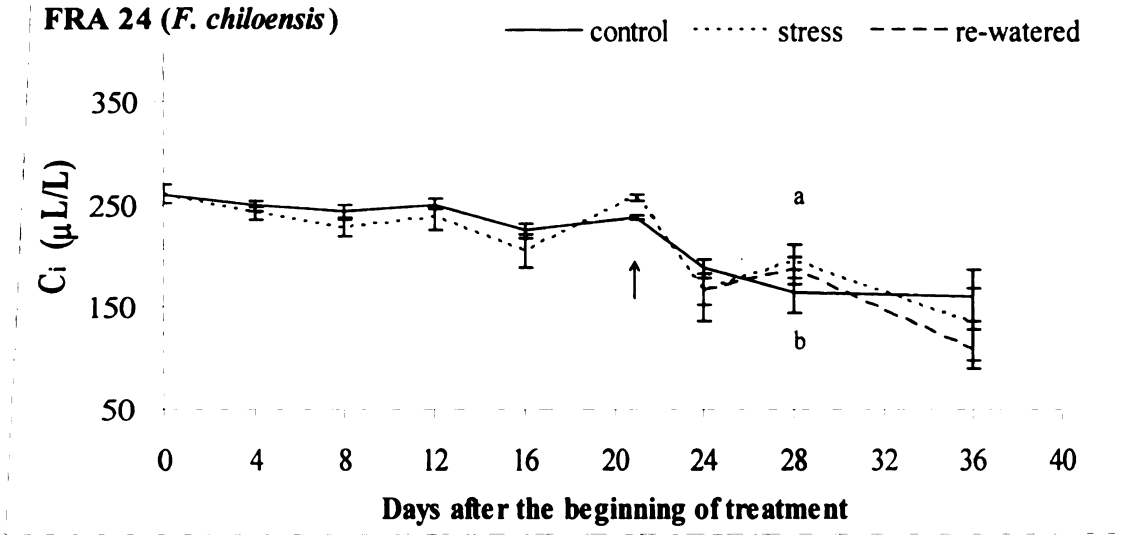

FRA 24 (F. chiloensis)
control
stress
re-watered
E (mmol m-2 s-1)
Days after the beginning of treatment

Figure 11. Variation of leaf intercellular CO_2 (C_i) of strawberry plants during 21 days of salinity treatment and 15 days of recovery. Each value represents the mean (±SE) of ten individual leaf measurements per selection and treatment. Letters indicate statistically significant differences between the treatments at $P \leq 0.05$ (LSMEANS test).
Arrow indicates the beginning of the recovery period.


MR-10 (F. virginiana)
control
stress
re-waterd
Ci (µL/L)
350
250
150
50
0
4
8
12
16
20
24
28
32
36
40
Days after the beginning of treatment


NC 95-21-1 (F.virginiana)
control
stress
re-watered
Ci (µL/L)
350
250
150
50
0
4
8
12
16
20
24
28
32
36
40
Days after the beginning of treatment


FRA 24 (F. chiloensis)
control
stress
re-watered
Ci (µL/L)
350
250
150
50
0
4
8
12
16
20
24
28
32
36
40
Days after the beginning of treatment

B. Photosynthetic response to increasing air CO_2 concentration.

Response of the net assimilation rate to increasing leaf intrercellular CO_2 concentration is shown in Figures 12-14 for each selection during stress and subsequent stress relief, (A/C_i curves). After photosynthetic parameters were calculated for each plant, data from three plants per treatment were pooled and curves were fitted by nonlinear regression model for illustrative purposes. Equations of the fitted curves for all treatment x selection combinations are shown in Table 3, Appendix A.

A reduction ($P \leq 0.05$) in the net photosynthetic rate (A_{amb}) at ambient CO_2 as well as in the A_{max} was observed in MR 10 (*F.virginiana*) plants treated with 100 mM salt at the end of the stress period, with values 40 and 20 % lower than the control respectively, while at the end of the recovery period a recover was evidence in A at ambient CO_2, but not in A_{max} (Table 5). Stomatal conductunce (g_s) was unaffected by the treatments, but leaf intercellular CO_2 concentration (C_i) was significantly higher in the stressed plants at the end of the stress period. After fifteen days of re-water with tap water, C_i in both treatments were similar. Slightly higher CO_2 compensation point (Γ) was observed in the stressed plants at the end of both periods but in neither case the values were different from the control ($P \leq 0.05$). Similarly, limitations imposed by stomata and the ratio C_i/C_a did not differ between the treatments ($P \leq 0.05$).

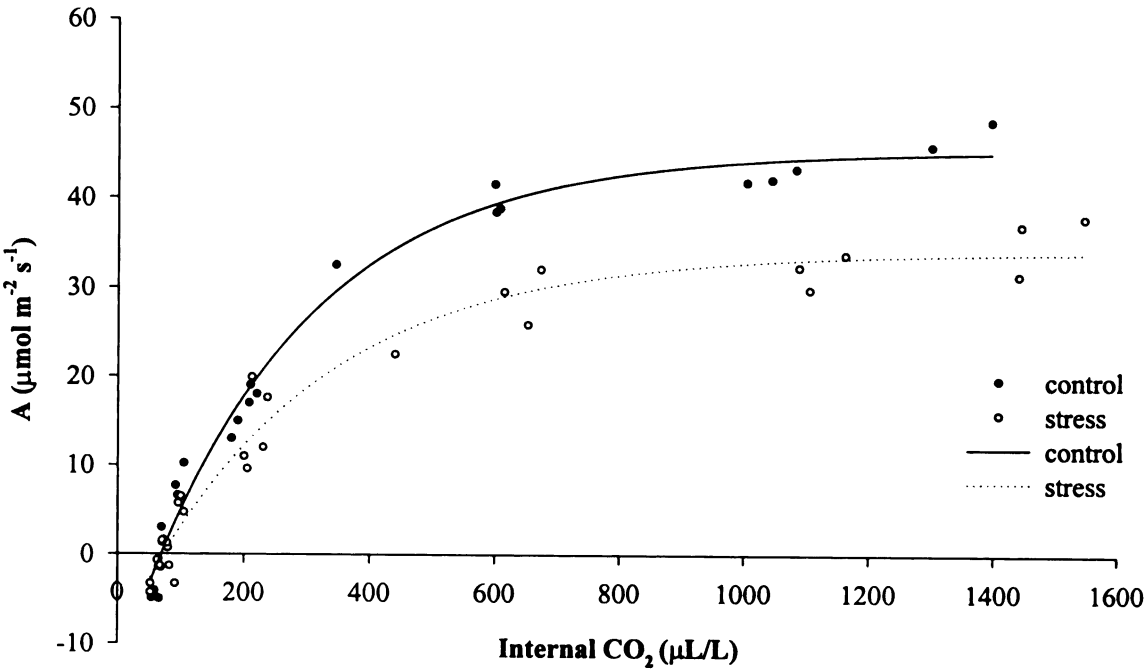
The initial slope of the A/C_i curve corresponds to the carboxylation efficiency (k) of the photosynthetic system. A significant decrease in k was found in stressed plants compared to the controls with values 0.20 and 0.32 respectively (36% lower than the control). After the re-watering no recovery was evidence ($P \leq 0.05$).

A quite similar pattern was observed in NC 95-21-1 (*F. virginiana*). Net assimilation rate at ambient CO_2 (A_{amb}) and A_{max} of the stressed plants were 30% lower than the control at the end of the stress period. At the end of the recovery period there was a partial recovery in both values (Table 5). Stomatal conductance (g_s) showed a significant decrease during the stress period and a partial recover after the re-water treatment. No differences in both CO_2 compensation point (Γ), internal CO_2 concentration and the ratio C_i/C_a were observed. Stomatal limitations were higher in stressed plants at the end of the stress period, whereas they were similar to the control at the end of the recovery period. Carboxylation efficiency (k) was strongly reduced by the salt treatment with value 0.14 (40% lower than the control). A 10% increase observed in k after the recovery period was not significant.

FRA 24 (*F. chiloensis*) showed a different response, with photosynthetic rates (both at ambient CO_2 and A_{max}), CO_2 compensation point (Γ) and carboxylation efficiency (k) not affected by the treatment (Table 6). In contrast, a decrease in stomatal conductance (g_s), internal CO_2, the ratio C_i/C_a was evident at the end of the stress period and a partial recovery after the re-watering. Stomatal limitations were higher in plants receiving 100 mM salt at the end of the stress period compared to control, while these were partially reduced at the end of the recovery period.

Figure 12. Variation of net assimilation rate in response to increasing leaf intercellular concentration (A/C_i curves) for MR 10 (*F. virginiana*). Data were collected on fully expanded leaves at the end of the stress period (A) and at the end of the recovery period (B). After photosynthetic parameters were calculated for each plant, data from three plants per treatment were pooled and curves were fitted by nonlinear regression model for illustrative purposes.

MR 10 (*F. virginiana*) **A**



B

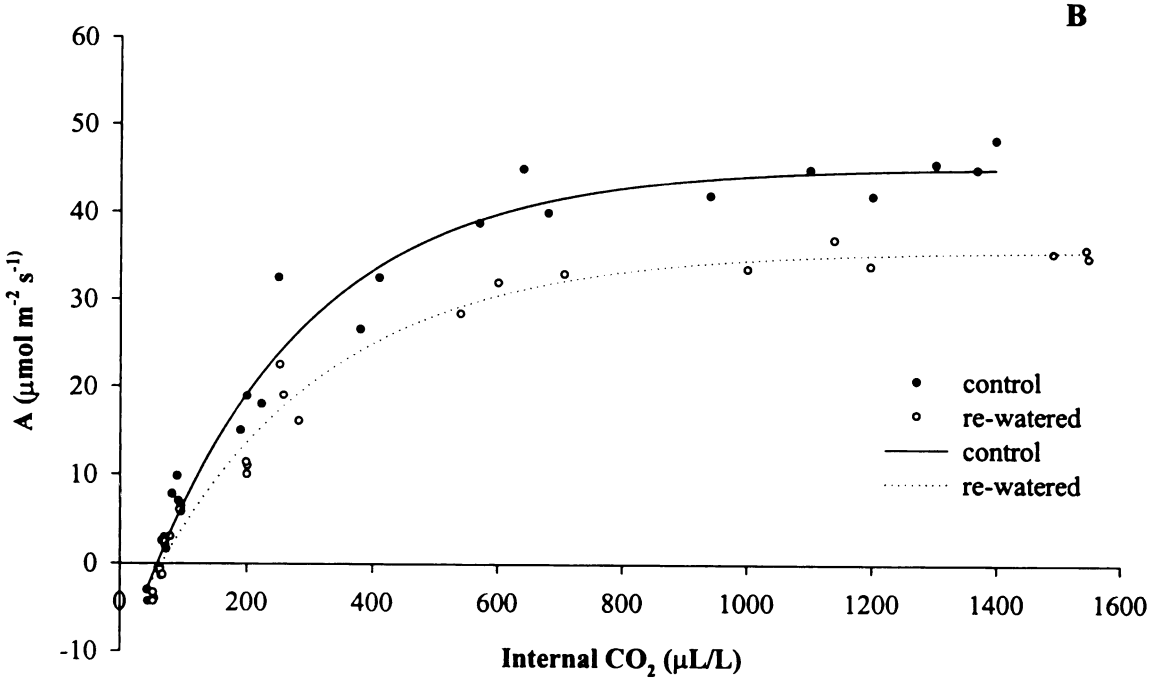
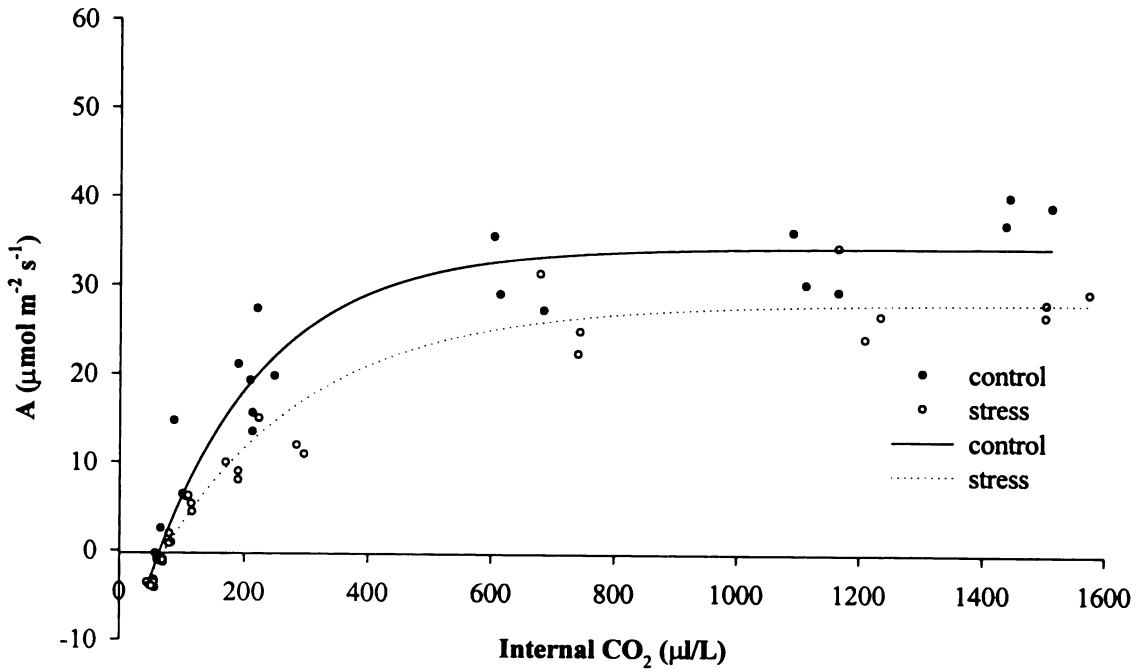


Figure 13. Variation of net assimilation rate in response to increasing leaf intercellular concentration (A/C_i curves) for NC 95-21-1 (*F. virginiana*). Data were collected on fully expanded leaves at the end of the stress period (A) and at the end of the recovery period (B). After photosynthetic parameters were calculated for each plant, data from three plants per treatment were pooled and curves were fitted by nonlinear regression model for illustrative purposes.

NC 95-21-1 (*F.virginiana*)

A



B

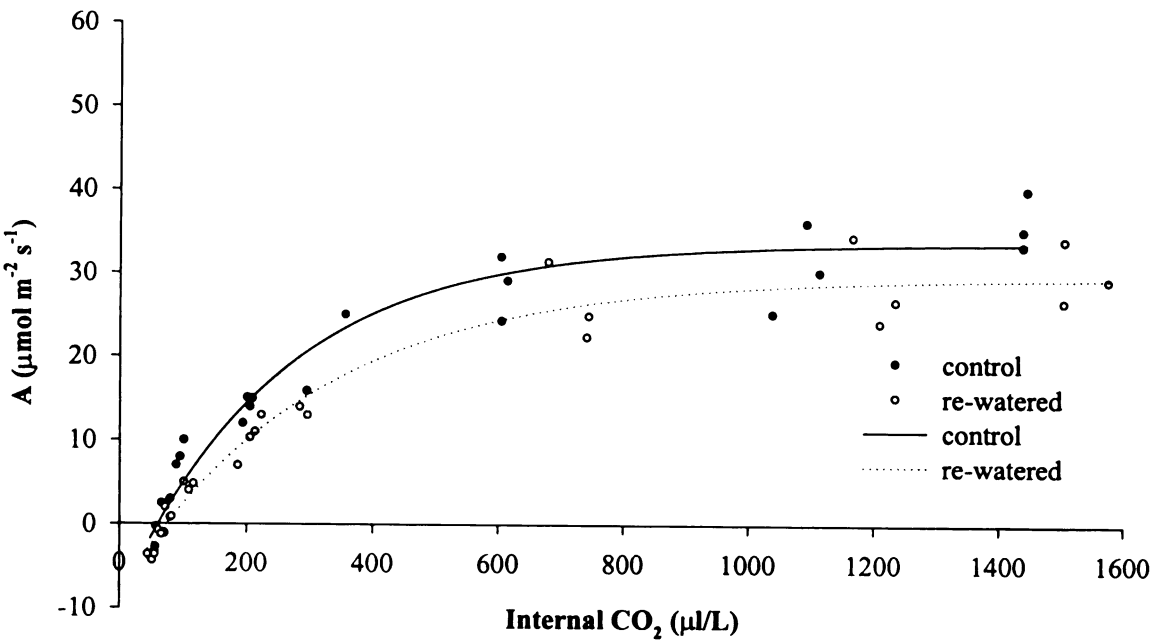
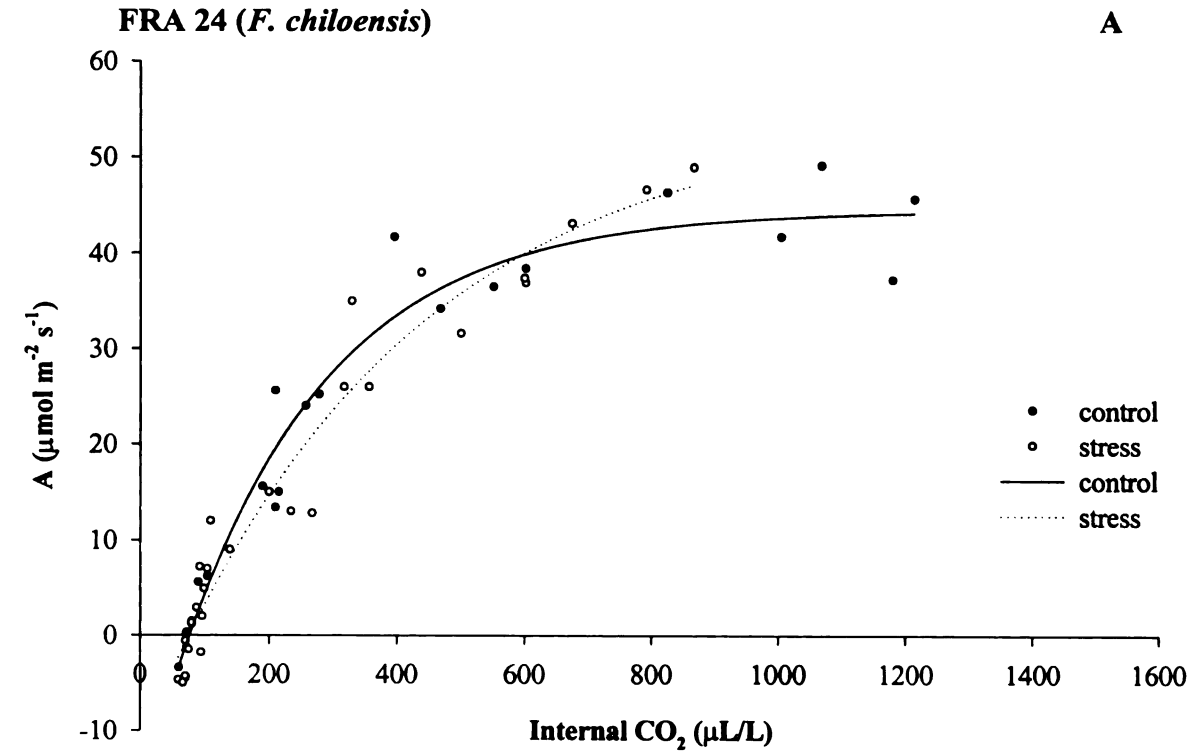


Figure 14. Variation of net assimilation rate in response to increasing leaf intercellular concentration (A/C_i curves) for FRA 24 (*F. chiloensis*). Data were collected on fully expanded leaves at the end of the stress period (A) and at the end of the recovery period (B). After photosynthetic parameters were calculated for each plant, data from three plants per treatment were pooled and curves were fitted by nonlinear regression model for illustrative purposes


FRA 24 (F. chiloensis)
A
A (µmol m-2 s-1)
Internal CO2 (µL/L)
control
stress
control
stress

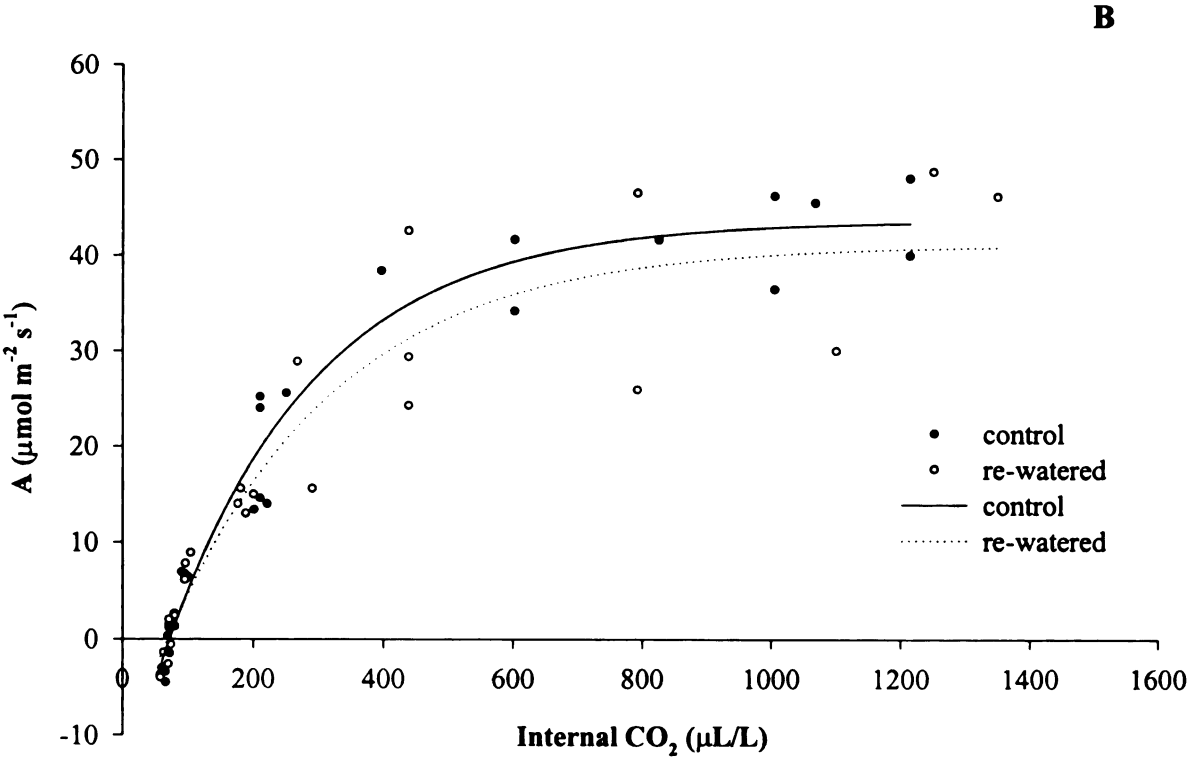

B
A (µmol m-2 s-1)
Internal CO2 (µL/L)
control
re-watered
control
re-watered

Table 5. Gas exchange parameters derived from A/C_i curves of fully expanded leaves of MR 10 and NC 95-21-1 (*F. virginiana*) plants subjected to twenty one days of salinity stress (stress period) and then fifteen days of stress relief (recovery period). Values represent means from three plants. Means followed by the same letter within a row do not differ significantly. Absence of letters within a row indicates no difference (LSMEANS, $P \leq 0.05$).

MR 10	**Stress period**		**Recovery period**	
Parameter	**control**	**stress**	**control**	**re-watered**
A at 370 μL/L CO_2 (μmol $m^{-2} s^{-1}$)	17.3 *a*	10.4 *b*	18.8 *a*	12.0 *b*
A_{max}	45.3 *a*	35.3 *b*	46.4 *a*	35.5 *b*
k (μmol CO_2 $m^{-2} s^{-1}$ μl^{-1} L)	0.32 *a*	0.20 *b*	0.29 *a*	0.24 *b*
Γ (μL/L)	70 *abc*	75 *a*	60 *c*	65 *abc*
g_s at 370 μL/L CO_2 (mmol H_2O $m^{-2} s^{-1}$)	203	190	220	202
stomatal limit. at 370 μL/L CO_2	0.51 *a*	0.48 *ab*	0.43 *b*	0.48 *ab*
C_i at 370 μL/L CO_2	183 *b*	201 *ab*	204 *a*	200 *ab*
C_i/C_a (%)	49	54	55	54

NC 95-21-1	**Stress period**		**Recovery period**	
Parameter	**control**	**stress**	**control**	**re-watered**
A at 370 μL/L CO_2 (μmol $m^{-2} s^{-1}$)	14.6 *a*	9.2 *c*	11.6 *b*	10.9 *b*
A_{max}	38.7 *a*	28.0 *b*	37.1 *ab*	30.0 *ab*
k (μmol CO_2 $m^{-2} s^{-1}$ μL^{-1} L)	0.24 *a*	0.14 *b*	0.23 *a*	0.15 *b*
Γ (μL/L)	69	72	66	71
g_s at 370 μL/L CO_2 (mmol H_2O $m^{-2} s^{-1}$)	196 *a*	138 *c*	192 *a*	170 *b*
stomatal limit. at 370 μL/L CO_2	0.43 *b*	0.54 *a*	0.45 *b*	0.46 *b*
C_i at 370 μL/L CO_2	205	183	200	201
C_i/C_a (%)	55	49	54	54

Table 6. Gas exchange parameters derived from A/C_i curves of fully expanded leaves of FRA 24 (*F. chiloensis*) plants subjected to twenty one days of salinity stress (stress period) and then fifteen days of stress relief (recovery period). Values represent means from three plants. Means followed by the same letter within a row, do not differ significantly. Absence of letters within a row indicates no difference (LSMEANS, $P \leq 0.05$).

FRA 24	**Stress period**		**Recovery period**	
Parameter	**control**	**stress**	**control**	**re-watered**
A at 370 μL/L CO_2 (μmol $m^{-2} s^{-1}$)	14.6	13.2	14.2	14
A_{max}	44	43	44.6	41.8
k (μmol CO_2 $m^{-2} s^{-1}$ μl^{-1} L)	0.30	0.29	0.29	0.28
Γ (μL/L)	76	78	68	70
g_s at 370 μL/L CO_2 (mmol H_2O $m^{-2} s^{-1}$)	200 *a*	156 *b*	193 *b*	179 *ab*
stomatal limit. at 370 μL/L CO_2	0.44 *b*	0.72 *a*	0.43 *b*	0.55 *b*
C_i at 370 μL/L CO_2	209 *a*	142 *c*	211 *a*	181 *b*
C_i/C_a (%)	58 *a*	38 *c*	57 *a*	48 *b*

C. Chlorophyll fluorescence

In MR 10 (*F. virginiana*), there was a significant decrease in chlorophyll efficiency (F_v/F_m) on day 15 which persisted until day 28. Values measured for plants allowed to recover, were significantly lower than the control, but they did not differ from the stress on day 28. However, at the end of the experiment, values from all three treatments were similar ($P \leq 0.05$) (Table 7). In NC 95-21-1 (*F. virginiana*) even though, there was an increase in the F_v/F_m at the end of the experiment the differences observed among treatments and time of measurement was not statistically significant at $P \leq 0.05$. Chlorophyll fluorescence measurements were also made on some symptomatic leaves

containing green sections that were large enough to be accommodated by the cuvette of the chlorophyll fluorescence system. No difference was detected between these and non-symptomatic leaves (data not included in table).

No differences were detected in chlorophyll efficiency between control, stress and re-watered plants of FRA 24 (*F.chiloensis*) throughout the experiment. However there was found a significant increase in the ratio at the end of the experiment with no difference among treatments.

No major differences were detected in F_o, F_m F_v values between control and stressed plants in both species (Table 4, Appendix A reports changes in F_m, F_o, F_v and F_m/F_o values during the experiment.)

Table 7. Chlorophyll efficiency (F_v/F_m) measured three times during the experiment. Values represent means from 9 measurements per treatment and selection. Means followed by the same letter within a column do not differ significantly. Absence of letters indicates no difference (LSMEANS, $P \leq 0.05$).

Time	Treatment	FRA 24	MR 10	NC 95-21-1
Day 15	control	0.813 *a*	0.843 *c*	0.840
	stress	0.793 *a*	0.814 *ab*	0.838
Day 28	control	0.812 *a*	0.820 *b*	0.831
	stress	0.824 *ab*	0.799 *a*	0.830
	re-watered	0.800 *a*	0.800 *a*	0.810
Day 36	control	0.827 *b*	0.822 *b*	0.844
	stress	0.834 *b*	0.830 *bc*	0.850
	re-watered	0.830 *b*	0.820 *b*	0.851

Discussion

In the present study, different salinity conditions in the irrigation water were used as a way to investigate the photosynthetic response of two *F. virginiana* and one *F. chiloensis* selections. The salinity treatments used were selected after preliminary experiments determined the salt concentration in the irrigation water that can be tolerated for a quite long period of time. Higher salt concentrations proved lethal within one week for the *F. virginiana* selections although without the development of visible damage.

Besides the environmental factors that affect maximum photosynthesis in strawberries [light intensity (Jurik et al., 1979), and temperature (Serce et al., 2000) etc.] genotype also determines the potential for high assimilation rate (Cameron and Hartley, 1990; Hancock, 1999). Compared to *F. virginiana,* higher photosynthetic rates have been reported in the literature for *F. chiloensis* selections but the two species have not been compared in a common environment (Larson, 1994; Hancock, 1999 and references therein).

Our study does not support this generalization, since in both years one of the *F. virginiana* selections used exhibited higher net assimilation rate than FRA 24. The values measured in FRA 24 were, however, well below those reported in the literature for greenhouse grown *F. chiloensis* clones (Cameron and Hartley, 1990). This may reflect the variability among different ecotypes of the same species (Hancock, 1999) or alternatively, conditions in the greenhouse may have differently affected the assimilation capacity of the selections tested.

At 25 and 50 mM NaCl, leaf net assimilation rate was little affected in all three strawberry selections until 30 days after the initiation of the salt treatments. Later, a

decrease was observed only in NC 95-21-1 but not in MR 10 or FRA 24. In contrast, higher salt concentrations resulted in a rapid decline in the photosynthetic rate in all three selections and in both years of experiments. Nonetheless, 100 mM salt resulted in severe marginal necrosis and rapid desiccation of older leaves in both NC 95-21-1 and MR 10, but not in FRA 24. This started approximately fifteen days after the initiation of the salt treatments in the experiment I but quite later in the experiment II. Senescence and shedding of older leaves has been considered as unavoidable result of continuous salt loading to transpiring tissues (Munns, 1993), but it may also act to eliminate excess salt accumulating in the shoot and leaf tissues (Albert, 1975). Although there was a difference between the two experiments in the timing of photosynthetic rate reduction as well as the development of leaf injury, clearly a similar pattern was evident in the two years and in all selections. Differences in relative humidity during the two experiments may partially explain these observations considering the impact that this has on stomatal conductance (g_s).

Low salt level (25 mM) caused some decrease in g_s and C_i in NC 95-21-1 but not in MR 10 (Table 2) or in FRA 24 (Table 3). There were negligible effects on A at ambient CO_2 and A_{max}. Intermediate level (50 mM) negatively affected carboxylation efficiency in MR 10 but not in the two others. Partial stomatal closure at 100 mM NaCl resulted in a lower C_i and C_i/C_a ratio in FRA 24, but not in NC 95-21-1 (Tables 5 & 6), indicating that at this salinity level stomatal constraints were a major factor determining photosynthesis in FRA 24, and to a lesser extent in NC 95-21-1. In FRA 24, only stomatal limitations seem to regulate photosynthesis at ambient CO_2 concentrations, since no significant reduction in carboxylation efficiency was observed. Furthermore, FRA 24

appears to be able to maintain considerable photosynthetic rate even at low internal CO_2 concentrations, as can be seen by the substantially lower C_i and C_i/C_a ratio compared to control. In NC 95-21-1, in addition to stomatal limitations, mesophyll limitations are also involved in restricting net assimilation rate. The similarity in C_i and C_i/C_a ratio between stress and control indicates that 100 mM salt decreased CO_2 assimilation and *k* to such an extent that even the lower CO_2 supply due to partial stomatal closure was adequate to satisfy the reduced demand. In contrast, in MR 10, decreased carboxylation efficiency was mostly responsible for the observed reduction in A. Actually, the lower stomatal limitations as component of total limitations and the higher C_i (although not different from the control) in stressed plants further supports the idea that at 100 mM salt, the reduction in photosynthesis in MR 10 is mainly due to mesophyll limitations.

A decrease in stomatal conductance in NC 95-21-1 and FRA 24 lowered also transpiration rate. Nevertheless we did not detect any significant increase in WUE (data not shown) in contrast to what it might have been expected (McCree and Richardson, 1987; Everard et al., 1994), primarily because a simultaneous decrease in photosynthetic rate had occurred due to non stomatal factors (eg. carboxylation efficiency) in NC 95-21-1. In FRA 24, there was a significant decrease in photosynthesis and transpiration of the controls as well, along with a severe reduction in g_s, during the second half of the experiment. The observed differences in net assimilation rate between the beginning and the end of the experiment in the controls may also have been affected by aging. Strawberry leaves have maximum photosynthetic rates 10-20 days after they become fully expanded (Jurik et al., 1979). In addition, control's transpiration rate maintained at low levels especially towards the end of the experiment perhaps due to low water vapor

pressure deficit from the leaf to the air, which decreased the driving force for transpiration (Cosgrove, 1998), as a result of the high humidity in the greenhouse.

Changes in the maximum quantum yield (Table 1) observed in experiment I and in most of the treatments in combination with changes in maximum A, especially in the highest treatment, represent changes due to increased stomatal limitations, but also reflect decreases in carboxylation efficiency which may be due to significant alterations in the photosynthetic machinery and carbon metabolism (Everard et al., 1994.

By measuring the physiological parameters on the same leaves during stress and subsequent recovery, we attempted to determine whether inhibition of photosynthesis was reversible. The slight recovery in carboxylation efficiency observed in both *F. virginiana* ecotypes (although not significantly different from the stress) together with a partial increase in g_s in NC 95-21-1 was responsible for the measured recovery in A. This implies that A was partially reversible, but only where stomatal limitations were responsible. In FRA 24 the re-watering treatment was quite ineffective in increasing A, but at the same time we found a substantial decrease in the limitations imposed by stomata. Quite different results have been found in other studies where a full recovery in carboxylation efficiency occurred with release from salinization at least in the most salt tolerant olive cultivar (Tattini et al., 1997) or in spinach (Delfine et al., 1999). Apparently non stomatal (mesophyll) and stomatal limitations are responsible for the reduction in A in both *F. virginiana* ecotypes treated with 100 mM salt, although to a different extent for each one. These results are in agreement with reports from several other species (Banuls and Primo-Milo, 1992) (Bethke and Drew, 1992; Brugnoli and Bjorkman, 1992). Among the mesophyll limitations inhibiting A at this salinity level, was the reduction in *k*,

reflecting a decrease in the activity and /or content of the enzymes of the carbon reduction cycle (eg. RubisCO) or a decrease in the amount of the substrate, RuBP. At this point it is not clear which is most likely.

Effects of salinity on Chl fluorescence reported in the literature are contradictory, but they involve widely different plant species. For example, no salt effect was found on F_v/F_m cotton (Brugnoli and Bjorkman, 1992), barley (Sharma and Hall, 1991), or sorghum (Lu and Zhang, 1998), while Bongi and Loreto, (1989) reported a dramatic decrease in F_v/F_m in olive trees, but they did not find any change in F_o. Furthermore, (Everard et al., 1994) found a significant decrease in F_v/F_m along with an increase in F_o in celery plants treated with 300 mM salt. In contrast, we found a small but reversible decrease in the F_v/F_m ratio and only in one of the three strawberry selections tested (Table 7), implying that 100 mM salt did not impair the intrinsic capacity of strawberry leaves for electron transport. In addition we did not detect any changes in F_o which indicates structural integrity of the PSII and that all the reaction centers were open (oxidized) after the dark adaptation period (Demming-Adams et al., 1989; Krause and Weis, 1991).

In general, toxicity symptoms have been reported in cultivated strawberry with Na^+ greater than 0.1% and Cl- greater than 0.5% DW (Ulrich, 1980). The severity of injury in some cultivars has been associated with higher levels of chloride (Ehlig and Bernstein, 1958; Martinez Barroso and Alvarez, 1997) while in others, with higher level of sodium (Hoagland and Snyder, 1933). Leaves collected from FRA 24 plants were free of any visible symptom in all salinity treatments although chloride content (but not sodium) in leaves from plant treated with 100 mM NaCl was 11,100 ppm (1.1%) (Table 4), well above the minimum reported as a threshold for the induction of visible damage. In MR

10, leaves collected from the 50 and 100 mM treatments were symptomatic; while in NC 95-21-1 all leaves were symptomatic (except the controls) but to a different degree. Although it appears that in FRA 24 leaf chloride concentration does not determine the development of damage, data from MR 10 and NC 95-21-1 indicate that perhaps chloride plays the most significant role in developing leaf injury. Or alternatively, those two ions may act synergistically to induce greater damage (Martin and Koebner, 1995). In our study, sodium accumulation was much slower than chloride in all three selections, consistent with what is reported in the literature for strawberries (Hoagland and Snyder, 1933; Ehlig and Bernstein, 1958; Awang and Atherton, 1994). In addition, higher rates of delivery to the leaves for both ions were observed in NC 95-21-1, intermediate in MR 10 and much lower in FRA 24. Genotypic variability among the three selections was evident, as it has been reported for other species (Chartzoulakis et al., 2002).

Studies on leaf Na^+ and Cl^- content and photosynthetic rate from different crop species have shown that levels of both Na^+ and Cl^- in leaves were correlated to the degree of the reduction of photosynthesis in bell pepper (Bethke and Drew, 1992), However, chloride but not sodium was correlated with reductions in photosynthesis in peach (Ziska et al., 1990) and sweet orange (Banuls and Primo-Milo. 1992). In contrast, no correlation was found for either ion in olive trees (Tattini et al., 1995). Since leaf sampling for analysis was done only once during the experiment and photosynthetic measurements were taken only in non symptomatic leaves, no conclusion can be drawn for the relative importance of those two ions in the observed differences in photosynthetic rates between control and salt treated plants. Furthermore, the observation that photosynthetic rates recovered in the stress-relief experiment in all selections (although to a different degree)

probably indicates that osmotic effect outside the roots accounts for most of the observed depression in photosynthesis.

Salt tolerance in glycophytes is associated with the ability to limit uptake and /or to transport toxic ions (mainly Na^+ and Cl^-) from the root zone to the aerial parts (Greenway and Munns, 1980; Munns, 2002). Salts are delivered to shoots through the transpiration stream and deposited in leaves as water evaporates, so gradually salt builds up over time (Munns, 2002). No differences in the transpiration rate were observed among the three selections throughout both experiments which probably would have accounted for the differences in salt accumulation in the leaves. Alternatively, in FRA 24 salts must have been excluded from the transpiration stream either by mechanisms operating at the root cells (selectivity of uptake) or during loading to the xylem (Munns, 2002).

In summary, low salt concentrations did not affect photosynthesis in all selections tested, while higher levels negatively affected net assimilation rate in both sensitive and tolerant selections. Both stomatal and non-stomatal factors regulated photosynthesis in MR 10 and NC 95-21-1 whereas only a stomatal factor (an osmotic effect) was the key determinant of the photosynthetic rate in FRA 24. In addition, stress relief reversed this effect only to the extend where stomatal limitations are responsible.

The regulation of salt delivery to the leaves and the prevention of salt built up in the leaf tissues are key factors determining survival and tolerance in the studied selections.

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CHAPTER 2

Effect of salinity stress and recovery on photosynthesis, growth rate, soluble sugar and proline accumulation of two strawberry selections, MR 10 (*F. virginiana*) and FRA 24 (*F. chiloensis*)

Abstract

Salinity stress influences many physiological plant processes including photosynthetic rate, growth rate and the accumulation of certain metabolites to counteract the osmotic imbalance initially imposed by the increased salt concentration in the root medium. The effect of increased salinity and recovery on photosynthesis, growth and the accumulation of soluble sugars and proline were investigated in two strawberry selections that differ in salinity tolerance (*Fragaria virginiana*, MR 10 and *Fragaria chiloensis*, FRA 24). Increased salt level in the irrigation water brought about a reduction in photosynthesis, leaf area growth and runner production in both genotypes tested, while relief from stress resulted in full recovery of Pn in MR 10 and a partial recovery in FRA 24. Glucose and fructose accounted for 70% of the total soluble carbohydrates accumulated in leaves of both selections due to stress while no substantial amount of proline was detected during the stress or the recovery periods. Resumption of growth and leaf area production was evidence after the stress was released and it was accompanied by a decrease in total soluble carbohydrates in MR 10, but not in FRA 24.

Introduction

Salinity, whether natural or induced, is a widespread environmental stress that can limit growth and productivity of salt-sensitive species (Greenway and Munns, 1980). Understanding the physiological mechanisms that are involved in salinity tolerance is

essential in identifying traits useful in breeding programmes intended to develop cultivars adapted to saline environments. It is now accepted that plant growth is affected by salinity in two distinct phases. First, growth is reduced due to high ionic concentration outside the roots and this is essentially a water stress. While later (after days or weeks), growth reduction results from salts accumulating in transpiring tissues to excessive levels (Munns, 2002).

Reduction in growth due to salinity stress has been reported in the literature for many species of agricultural importance (Munns et al., 1982; Awang and Atherton, 1995; Tattini et al., 1995; Lutts et al., 1996; Tattini et al., 1997; Chartzoulakis et al., 2002). When studied, resumption of growth was also evidence after the stress was released (Tattini et al., 1995; Tattini et al., 1997; Pardossi et al., 1998).

Much of the physiological research into salinity has concentrated on photosynthesis and the accumulation of particular metabolites, e.g., reducing sugars (O'Neil, 1983; Zhang and Archbold, 1993b), fructans (Kerepesi et al., 1998), polyols (Wang and Stutte, 1992; Everard et al., 1994), and certain amino acids and proline in particular (Hanson et al., 1977; Lutts et al., 1996), assuming that these processes would affect growth and potentially tolerance to osmotic stresses (Munns et al., 1982; Ziska et al., 1990). Most of these studies have established patterns of responses in many crop species, but they have not elucidated the mechanisms at either the biochemical or the whole plant level (Munns, 1993). Some have argued that salinity reduces growth more than photosynthetic rate per se, causing a subsequent carbohydrate built up (either as starch and/or soluble sugars) due to the lack of utilization which in turn affects photosynthetic rate (Greenway and Munns, 1980; Munns et al., 1982). Data supporting

the opposite view comes from experiments with peach trees, (Ziska et al., 1990) where mean soluble and insoluble carbohydrate content decreased with increased salinity, indicating lack of feedback inhibition to CO_2 uptake.

Furthermore, active carbohydrate accumulation when present in plant tissues has been seen as a response mechanism through which the plant uses sugar components in a stress adaptive process. Alteration of carbon partitioning under salt stress has been considered an adaptive response rather than a consequence of the stress (Everard et al., 1994; Kerepesi et al., 1998; Kerepesi and Galiba, 2000). Although accumulation of non-structural carbon compounds which facilitates osmotic adjustment and turgor maintenance does not result in loss of carbon from the plant, (e.g., respiration) it may remove it from the pool available for immediate metabolism or growth (Cheeseman, 1988).

Accumulation of proline has been implicated in osmotic stress tolerance by some authors, but it remains a matter of debate whether or not it actually contributes to tolerance or if it is a consequence of other stress-induced changes in metabolism. Genotypic variation exists in the amount of proline accumulated due to stress that affects cellular water status in rice (Lutts et al., 1996), barley (Hanson et al., 1977), citrus (Nolte and Hanson, 1997), and many other species. However salt–induced proline synthesis is not always rapid and begins only when cell injury is evident (Hanson et al., 1977). Also it has been pointed out by Lutts et al., (1996) that lower proline accumulation observed in salt-resistant rice cultivars could indicate a lower perturbation of cellular metabolism. The absence of a positive correlation between proline accumulation and tolerance to

osmotic stress in some species may simply reflect the predominance of other tolerance mechanisms in those species (Delauney and Verma, 1993).

Cultivated strawberry (*Fragaria* x *ananassa*) is considered a salt sensitive crop (Maas, 1984), but there is a large variation among different species of the *Fragaria* genus in the amount of salinity that can be tolerated. In contrast, there is not much variability in some selections in their capacity to accumulate biomass under salt stress (Hancock and Bringhurst, 1979). Reduction in growth and yield in cultivated strawberry has been described due to salt stress (Awang and Atherton, 1994) but sensitivity to salt stress varies at different developmental stages (Awang and Atherton, 1995). Depression of yield is closely correlated with a reduction in inflorescence number and this could be minimized if sufficient vegetative development takes place before the initiation of the salt stress (Awang and Atherton, 1995). *Fragaria chiloensis* Duch. and *F. virginiana* Duch., the primary progenitor species of the cultivated strawberry (James et al., 1990); differ in drought and salinity tolerance with *F. chiloensis* being more tolerant than *F. virginiana* (Hancock and Bringhurst, 1979; Zhang and Archbold, 1993b). Osmotic adjustment in *Fragaria* species occurs through the accumulation of carbohydrates (O'Neil, 1983; Zhang and Archbold, 1993b). In water-stressed *F. chiloensis*, total soluble carbohydrate concentration increased 1.4 to 2.4 –fold during wilting cycles. Glucose and fructose were the primary carbohydrates involved in osmoregulation accounting for more than 50% of the total osmotic potential (Zhang and Archbold, 1993b).

Salinity stress affects photosynthetic rate in strawberry plants, and this is controlled by both stomatal and non–stomatal mechanisms depending on the species as well as the duration and the severity of the stress (see Chapter 1). The present study was

carried out to determine if the rate of photosynthesis in stressed plants limits growth rate and the carbohydrate supply in the growing tissues, or alternatively, if reduction in growth rate is partly responsible for the observed photosynthetic depression. Furthermore, we wanted to establish a possible correlation between tolerance to salt stress and accumulation of specific organic compounds that potentially contribute to the observed better performance of *F. chiloensis* selection compared to *F. virginiana.* Identification of such mechanisms would be of interest in breeding programmes where salinity tolerance of the cultivated strawberry is a desirable trait.

Materials and methods

The experiment was conducted during summer 2002 (June, 15-July, 18) in a research greenhouse in the Department of Horticulture at Michigan State University (East Lansing, MI). The glass was white washed to reduce the incident radiation and consequently the temperature during the summer months. Therefore, midday photosynthetic photon flux density on a sunny day varied from 550 to 900 $\mu mol\ m^{-2}\ s^{-1}$; average day/night temperature 35/22°C and relative humidity ranged between 65 and 80%.

Twenty plants of each genotype, *Fragaria chiloensis*: FRA 24 and *Fragaria virginiana*: Montreal River (MR 10), were potted in 2.5 L pots containing Baccto potting medium (Baccto, Michigan Peat Company, Houston,TX) (sphagnum peat 70-80%, pH 5.5-6.5). All plants were propagated by runners, and they were three months old when the experiment started.

Salinity treatment

Sodium chloride (J.T. Baker, Phillipsburg, NJ, USA) was dissolved in either tap water (known EC 1000-1200 μS/cm) or in 200 ppm fertilizer solution once a week. The treatments were stepped up in 25 mM increments every day until final treatment concentration (100 mM) (Everard et al., 1994). Plants were watered at least once a day with approximately 300 mL solution, ensuring adequate leaching and preventing excess salinity. The leaching solution from each pot was collected every day in the beginning, and then twice a week to guarantee that there was not any significant salt built up over time. Electrical conductivity was measured in the lab using an ORION-150 conductivity meter, (Boston, MA, USA).

After fifteen days of salt treatment, stressed plants were allowed to recover from the stress. These plants were receiving 300 ml of tap water daily after an initial washing of the soil with distilled water (Tattini et al., 1995). Within two days the EC of the leaching solution of the treated plants was similar to the controls.

Evaluation of plant growth

Leaf area estimation

Before the beginning of the experiment, ten plants per genotype were used to develop regression equations for the leaf area estimation. Equations were developed independently for each genotype. Briefly, leaves were separated in mature, fully expanded (85-90 leaves) and young, still growing (60-65 leaves) and the length and width of each single leaflet were measured and recorded individually. Then the area of each leaflet was measured using a portable leaf area meter (LI-3000 LAMBDA Instruments

Corporation). Facing the upper (abaxial) side of the trifoliate, the leaflet on the left was designated as number one, the one in the middle as number two, and the one on the right as number three. Regression equations for each leaflet were developed using the PROC REG procedure in SAS/PC software (SAS Institute Inc., Cary, NC, USA). Only equations developed for young, growing leaves were used in the experiment (table 1).

Table 1. Equations used for the leaf area calculation. W=width, L =length

genotype	leaflet	equation	degrees of freedom	r^2
MR 10	1	4.3W+1.92L-11.76	63	0.87
	2	3.22W+2.86L-12.9	65	0.93
	3	4.54W+1.77L-11.8	63	0.91
FRA 24	1	3.63W+0.64L-5.22	61	0.91
	2	4.24W+0.91L-8.06	61	0.92
	3	1.47W+2.27L-5.29	61	0.94

Average size of leaves at full maturity and days to mature

After leaf area calculation for each leaf, leaves from stressed plants were divided into two groups and analyzed separately for size at full maturity and days to complete development:

Group I including those leaves emerged and reaching maturity during stress

Group II including those emerged and reaching maturity during the recovery period

Leaf expansion rate

At the onset of the experiment, all the young unfolded leaves from seven plants per treatment and genotype were tagged and the length and width for each individual leaflet were recorded every day for the first ten days and then at two day intervals until they reached full maturity and stopped growing. All new leaves emerged during the experiment were also measured on these plants. The obtained values of leaf area were used to estimate the total leaf area produced during the experiment and the mean relative expansion rate (RLER) which was calculated using the formula (Beadle, 1993):

$$RLER = \frac{\ln(LA_2)-\ln(LA_1)}{t_2-t_1}$$

Where t_1 and t_2 indicate the initial and final time, respectively, of a discrete time interval of measurements, LA_1 and LA_2 are the values of the leaf area measured at t_1 and t_2 and ln indicates the natural logarithm.

Leaf and runner emergence rate

The number of runners was counted twice a week and they were removed as soon as they appeared. The number of young, just emerging leaves was recorded every day and at the end of the experiment the number of healthy leaves was counted for each plant.

Photosynthetic measurements

Carbon assimilation (A), stomatal conductance (g_s), leaf intercellular CO_2 concentration (C_i), and transpiration (E) were determined using an open gas-exchange

system (Layne and Flore, 1992). Gas exchange measurements were taken with a CIRAS-1 portable infrared gas analyzer (PP Systems, Haverhill, MA, USA) on a 2-day intervals in the beginning and then every 5-7 days, or when weather permitted between 10:00 am and 1:00 pm on the terminal leaflet of a mature fully expanded leaf. Five plants per treatment and genotype were used. Standard conditions during gas exchange were PPFD>500 μmol m^{-2} s^{-1}, VPD 12.5 ± 0.5 mbar, air CO_2 concentration 370 ± 10 ppm, leaf temperature 25-34°C and air flow into the cuvette 200 ± 5 mL/min (3.3 ml/sec). Two to three leaves per plant were used. Each leaflet was inserted into the leaf chamber (2.5 cm^2) and one single measurement was collected, after a waiting period of 1-2 minutes for the atmosphere inside the chamber to equilibrate. Carbon assimilation (A), stomatal conductance (g_s), leaf intracellular CO_2 concentration (C_i), and transpiration (E) were the parameters calculated automatically by the instrument at each measurement.

Carbohydrate and proline determination

Sample collection and preparation

Leaf samples were collected two times during the experiment. One at the end of the stress period (July, 5) and a second at the end of the recovery period (July, 21). Leaves were separated in mature, fully expanded with no visible symptoms of salt damage and young, still growing and analyzed separately for carbohydrate content. For proline determination, only mature leaves were used. Three plants per treatment and genotype were used for leaf sampling. Samples were collected in the midday and they stored in liquid nitrogen until they were transferred to the lab. Samples were freeze-dried, ground with a mortar and pestle to pass through a 40 mesh screen and then stored in a

desiccator over anhydrous calcium sulfate® (Drierite) (W.A. Hammond Drierite Company Ltd., Xenia, OH, USA) until they were used for carbohydrate and proline extraction.

Carbohydrate extraction and quantification

For carbohydrate extraction, the protocol described by (Everard et al., 1994) was followed with some modifications. Approximately 0.1 g of dried, ground tissue was extracted in 3.5 mL 80% ethanol (v/v with H_2O) and transferred to a test tube. After 15 min at room temperature (vortex mixing every 5 min), extracts were centrifuged for 5 min at 3000 rpm on a Sorvall RC-5B centrifuge. The supernatants were decanted; the pellets were re-extracted twice with 3.5 mL ethanol and the resulting supernatants were pooled with the initial extract. Five milliliters of H_2O were added to the pooled supernatants followed by partitioning with 5 mL of chloroform to remove the chlorophyll from the sample extract. The aqueous phase was dried at 60°C and subsequently stored over Drierite at –20°C. Dried carbohydrate samples were dissolved in 2 mL H_2O and passed through C_{18} Sep-Pack® cartridges (Waters Corporation, Milford, MA, USA) to remove any residual lipophilic compounds. Then they were filtered through 0.2μm pore size cellulose acetate membrane (Life Science Products Inc., Frederick, CO, USA) and injected onto the HPLC column. HPLC analysis was performed at 90°C using a Sugar-PACK I column (300 X 6.5 mm) (Waters Corporation, Milford, MA, USA) eluted with water (flow of 0.5 mL/min) supplemented with 50 mg/L calcium EDTA. Identity and quantity of the carbohydrates present in the samples were confirmed by comparing the retention times with those of authentic standards of sucrose, glucose and fructose

Proline extraction and quantification

The acid-ninhydrine method described by Bates et al., (1973) with some modifications was used for proline extraction and quantification. Purified proline (Sigma-Aldrich, St Louis, MO, USA) was used to standardize the procedure and quantify the samples.

Reagents. Acid-ninhydrine was prepared by warming 1.25 g ninhydrin (Sigma-Aldrich, St Louis, Mo, USA) in 30 mL glacial acetic acid and 20 mL phosphoric acid, with agitation, until dissolved.

Procedure. Approximately 0.1 g dry tissue was homogenized with 3 mL distilled water and heated at 80°C for 15 min. The homogenate was then shaken for 1 hour. 200 µL of the homogenate were extracted with 200 µL acid ninhydrine and 200 µL phosphoric acid in water bath at 100°C for 1 hour. The reaction was terminated in anice bath. The cool extracts were partitioned in 1 mL toluene and the absorbance was read at 515 nm using toluene as blank in a spectrophotometer. The proline concentration was determined from a standard curve and calculated on a dry weight basis as follows:

[(µg proline / mL x mL toluene) * 3] / g sample.

Experimental design and statistical analysis

The experiment was arranged in a completely randomized block design on a greenhouse bench to minimize the effect of temperature and light intensity gradient along the bench. Plants were selected for uniformity of vigor and leaf number and randomly divided into groups to be assigned to the different treatments. Statistical analysis was carried out using the PROC MIXED procedure in SAS/PC software (SAS Institute Inc.,

Cary, NC, USA). Repeated measures analysis was used for gas exchange and leaf carbohydrate concentration. Analysis of variance (ANOVA) and differences between means were determined by the Least-Squares Means test (LSMEANS). Probabilities less than or equal to 0.05 were regarded as significant within each date of measurement.

Results

Evaluation of plant growth

Average size of leaves at full maturity and days to mature

Control leaves from MR 10 attained an average final size of 66 cm^2 and reached full maturity in approximately 15 days while those from FRA 24 were 45.4 cm^2 and reached full maturity in 13.1 days. Group I leaves from MR 10 reached an average size of 45.1 cm^2 (31% reduction compared to the control) and they stopped growing after 9.6 days while those from FRA 24 reached the size of 33.5 cm^2 (26% smaller than the control) within 10 days. Group II leaves from MR 10 reached an average size of 49 cm^2 and completed their development in 11.7 days whereas those from FRA 24 had an average size of 35.1 cm^2 and they reached full expansion in 13.6 days (Table 2). Group I and II leaves from both genotypes were smaller when they appeared and in general it took longer until they reached a measurable size, compared to their corresponding controls.

In MR 10 differences between control and group I and II leaves, ($P \leq 0.05$) were observed in the size at full expansion and the number of days that the leaves were actively growing. Although leaves emerged and developed during the recovery period

(group II) had slightly bigger final size and they grew for more days compared to group I they did not differ statistically from group I.

In FRA 24 differences between control and group I leaves, ($P \leq 0.05$) were also observed in both the final size and the number of days that the leaves were actively growing. Furthermore, leaves emerged and developed during the recovery period (group II) were actively growing for more days than group I and similar to the control. However their final size was lower than the control and comparable to group I ($P \leq 0.05$).

Comparison between the two genotypes showed that leaves from MR 10 developed to a greater size compared to FRA 24 under any given condition and they grew for more days under control conditions but not during stress (Table 2).

Table 2. Average leaf size at maturity and days to complete expansion for strawberry leaves from MR 10 (*F. virginiana*) and FRA 24 (*F. chiloensis*) plants during the experiment

Group I: leaves emerged and reached maturity during stress

Group II: leaves emerged and reached maturity during the recovery period

Values are the means (±SE) of 3-8 leaves per plant from seven plants for each genotype and treatment combination. Means followed by the same letter within a column did not differ significantly (LSMEANS test, $P \leq 0.05$)

genotype	group	area (cm^2)	days
MR 10	control	66.09± 2.4 *a*	14.6±0.6 *a*
	I	45.1± 1.9 *b*	9.6±0.8 *c*
	II	49± 1.5 *b*	11.7±0.7 *bc*
FRA 24	control	45.4± 1.7 *b*	13.1± 0.5 *b*
	I	33.5±1.4 *c*	10±0.8 *c*
	II	35.1±1.2 *c*	13.6±0.6 *b*

Leaf and runner emergence rate

Although there was a decrease ($P \leq 0.05$) in the rate of leaf emergence in both control and stress treatments towards the middle of the experiment, with the lowest values observed on days 10 and 15, no difference was observed between stress and control in MR 10 plants throughout the experiment for any given day. Values ranged between 0.16 and 0.47 (leaves /day) in the controls and 0.21 and 0.33 (leaves /day) in the stress (Figure 1). In contrast, in FRA 24 the rate of leaf emergence in stressed plants was always statistically lower than the controls, with the exception on day 15 when no difference was observed ($P \leq 0.05$) (Figure 1). Values for control plants ranged between 0.29 and 0.45 leaves/per day whereas for stressed 0.14 to 0.33 leaves/per day.

A lower rate of runner emergence was observed in stressed MR 10 plants compared to the controls throughout the experiment ($P \leq 0.05$). On the other hand, the rate was not affected in FRA 24 during the first fifteen days of the experiment (stress period), whereas a significantly lower rate was observed in the stressed plants during the recovery period ($P \leq 0.05$) (Figure 2).

Consequently, no difference was detected in the total number of leaves produced per plant throughout the experiment in MR 10 stressed plants compared to the control (8.8 vs. 10.4), whereas significantly less leaves were produced in FRA 24 stressed plants (7.4 vs.11.7) (Figure 3A). Overall, there was a 40% reduction in the total number of runners in both genotypes throughout the experiment with values 12.4 vs 7.7 for control and stress respectively MR 10 and 9 vs. 5 for FRA 24 (Figure 3B).

Comparison between the two genotypes (LSMEANS test, $P \leq 0.05$) showed that control MR 10 and FRA 24 plants differed in the rate of leaf emergence only on day 10

(between 5-10 days after the beginning of the experiment) (Table 3), with FRA 24 having higher rate, while stressed plants did not differ at any given time throughout the experiment (Table 4). In addition, control MR 10 plants had higher rate of runner emergence only in the beginning of the experiment (day 5) compared to FRA 24 ($P \leq 0.05$), (Table 3). On the other hand, stressed MR 10 plants had higher runner emergence rate ($P \leq 0.05$), on day 5 as well as during the recovery period (day 31) (Table 4).

Relative leaf expansion rate

Even though there was a significant reduction in the RLER throughout the experiment in both genotypes ($P \leq 0.05$), no difference was detected between stress and control plants within a genotype at any given time (Figure 4). Moreover, no difference was detected between the controls of the two genotypes throughout the experiment (Table 3) while between stressed plants, higher RLER was observed in FRA 24 compared to MR 10 during the first period of the experiment (day 5)($P \leq 0.05$). Later, no difference was noticed between the genotypes (Table 4).

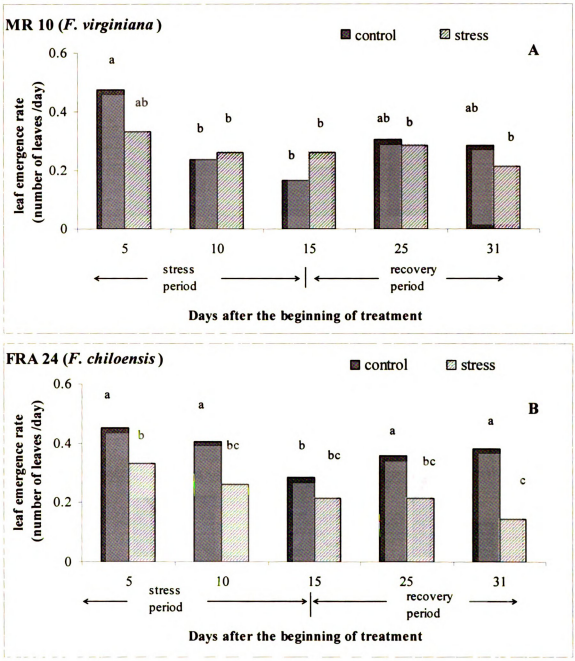


Figure 1. Leaf emergence rate (number of leaves/day) calculated throughout the experiment for MR 10 (A) and FRA 24 (B). Each bar represents the average of seven plants. Different letters indicate significant differences between the treatments within a day as well as differences within a treatment during time (LSMEANS test, $P \leq 0.05$).

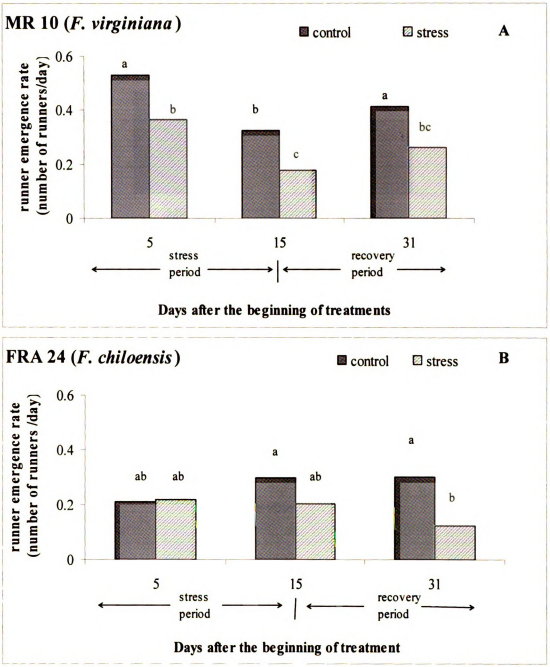


Figure 2. Runner emergence rate (number of runners/day) calculated throughout the experiment for MR 10 (A) and FRA 24 (B). Each bar represents the average of seven plants. Different letters indicate significant differences between the treatments within a day as well as differences within a treatment during time (LSMEANS test, $P \leq 0.05$).

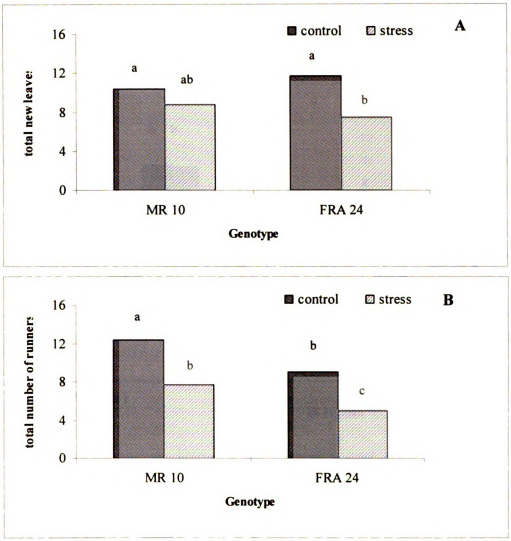
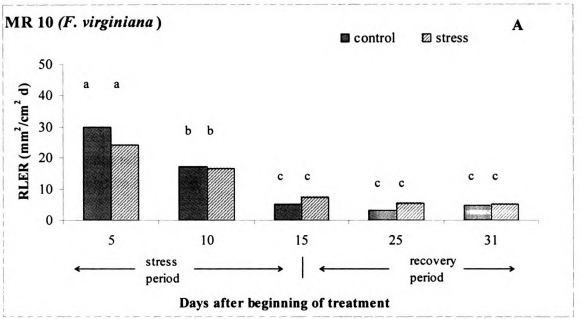


Figure 3 Total number of leaves (A) and runners (B) produced from MR 10 and FRA 24 plants throughout the experiment. Bars represent the average of seven plants. Different letters indicate significant differences between treatments and genotypes (LSMEANS test, $P \leq 0.05$)



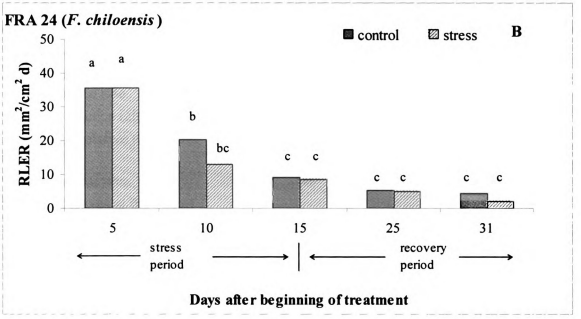


Figure 4. Relative Leaf Expansion Rate of MR 10 (A) and FRA 24 (B) plants, throughout the experiment. Each bar represents the average of seven plants. Different letters indicate significant differences between the treatments within a day as well as differences within a treatment during time (LSMEANS test, $P \leq 0.05$)

Table 3. Time (days) where statistically significant differences were observed in different growth parameters between control plants of MR 10 and FRA 24, * & ** indicate significance at P≤0.05 and 0.01, respectively (LSMEANS test).

	days after the initiation of the experiment				
parameter	5	10	15	25	31
leaf emergence rate	ns	*	ns	ns	ns
runner emergence rate	**		ns		ns
relative leaf relative expansion rate (RLER)	ns	ns	ns	ns	ns
cumulative leaf area	ns	ns	ns	ns	ns
number of leaves at the end of the experiment					ns

Table 4. Time (days) where statistically significant differences were observed in different growth parameters between stressed plants of MR 10 and FRA 24, * & ** indicate significance at P≤0.05 and 0.01, respectively (LSMEANS test).

	days after the initiation of the experiment					
	stress period			recovery period		
parameter	5	10	15	25	28	31
leaf emergence rate	ns	ns	ns	ns		ns
runner emergence rate	*		ns			*
relative leaf relative expansion rate (RLER)	**	ns	ns	ns		ns
cumulative leaf area	ns	ns	ns	ns	*	*
number of leaves at the end of the experiment						*

Cumulative leaf area

Stressed MR 10 plants produced an average of 306.5 cm^2 per plant while controls produced 425 cm^2 during the experiment. A significant difference between stress and control in MR 10 plants was observed on day 10 with 67% reduction compared to the control ($P \leq 0.05$). After day 15, when the recovery period started, this difference started to decrease until the end of the experiment, where the total area produced by the stressed plants was 28% lower than the control (Figure 5). Stressed FRA 24 plants produced an average of 208 cm^2, while controls 461 cm^2 through the experiment. A significant difference between stress and control was evidence on day 12 with a 50% reduction in the stress compared to the control. A further 5% reduction was detected at the end of the experiment where the total area produced by the stressed plants was 55% lower than that of the control. No recovery was detected when the stress was removed (Figure 5). A significant difference was detected in the average number of viable leaves per plant at the end of the experiment between stress and control in MR 10 (12.3 vs. 7.1) ($P \leq 0.05$), whereas no difference was evidence for FRA 24 plants, with 15 leaves per plant in the stressed and 17.8 in the control ($P \leq 0.05$) (Figure 6).

Comparison between the two genotypes showed that there was no significant difference ($P \leq 0.05$) in the total leaf area produced by the controls throughout the experiment (Table 3), whereas a statistically significant difference was observed at the end of the experiment between the stressed plants (on days 28 and 31) with MR 10 plants having produced more leaf area (Table 4). In addition, no difference was found between the controls in the number of viable leaves, while stressed, FRA 24 plants retained significantly higher number of leaves at the end of the experiment (Figure 6).

Both genotypes showed injury symptoms at the end of the experiment, although in MR 10, these were developed about two weeks after the initiation of the treatment, while in FRA 24 these developed only during the last week of the experiment. Overall, MR 10 was more severely affected than FRA 24.

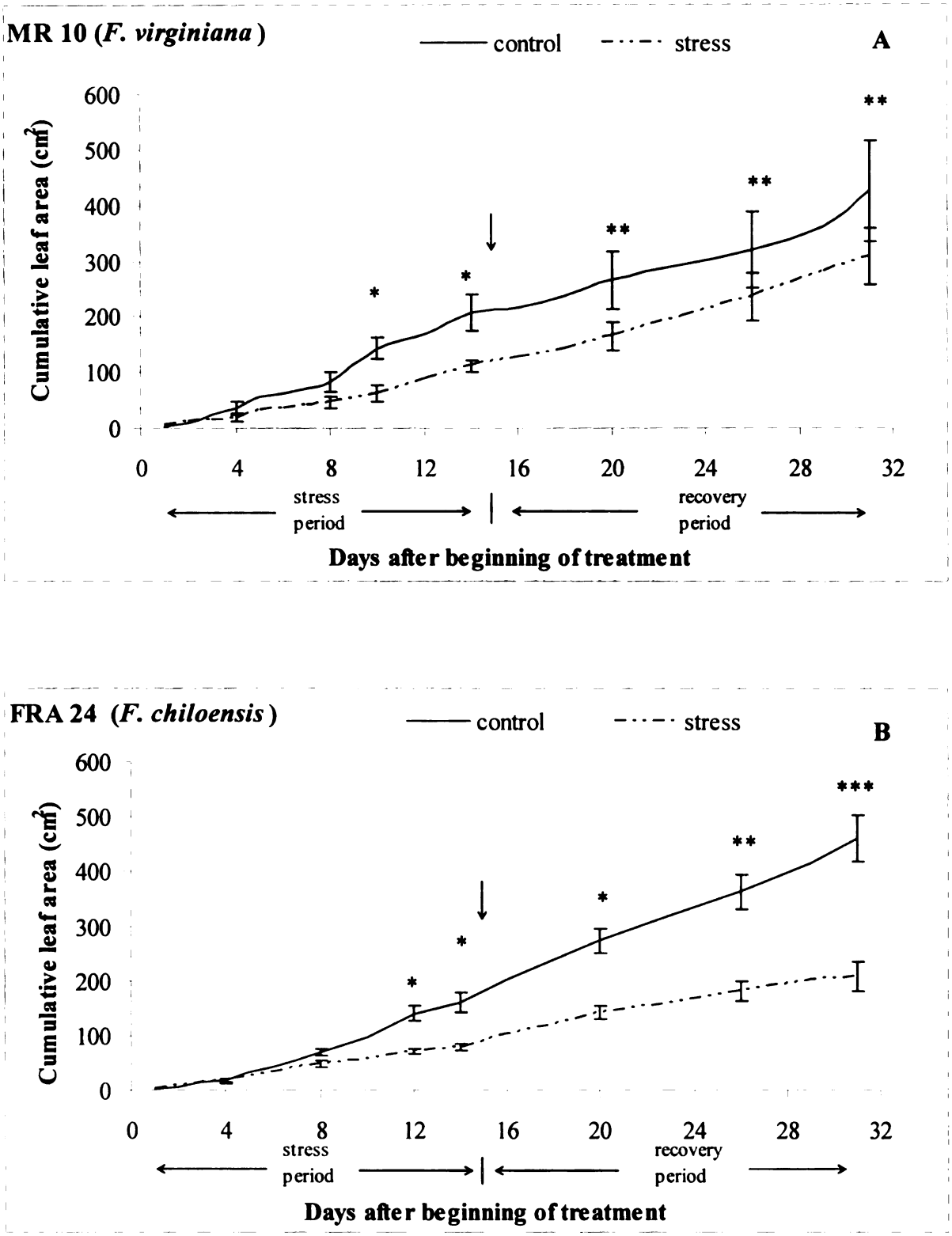


Figure 5. Total leaf area produced by MR 10 (A) and FRA 24 (B) plants throughout the experiment. Arrows indicates the beginning of the recovery period. Each data point is the mean of seven plants *, **, *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001 respectively (LSMEANS test).

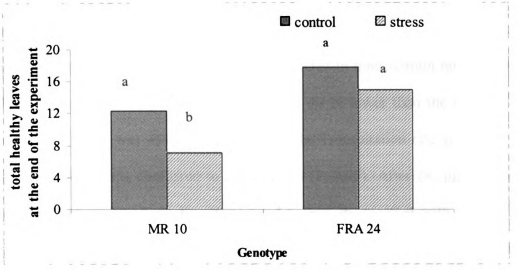


Figure 6. Total number of viable leaves of MR 10 and FRA 24 plants at the end of the experiment. Bars represent the average of seven plants. Different letters indicate significant difference between treatments and genotypes (LSMEANS test, $P \leq 0.05$).

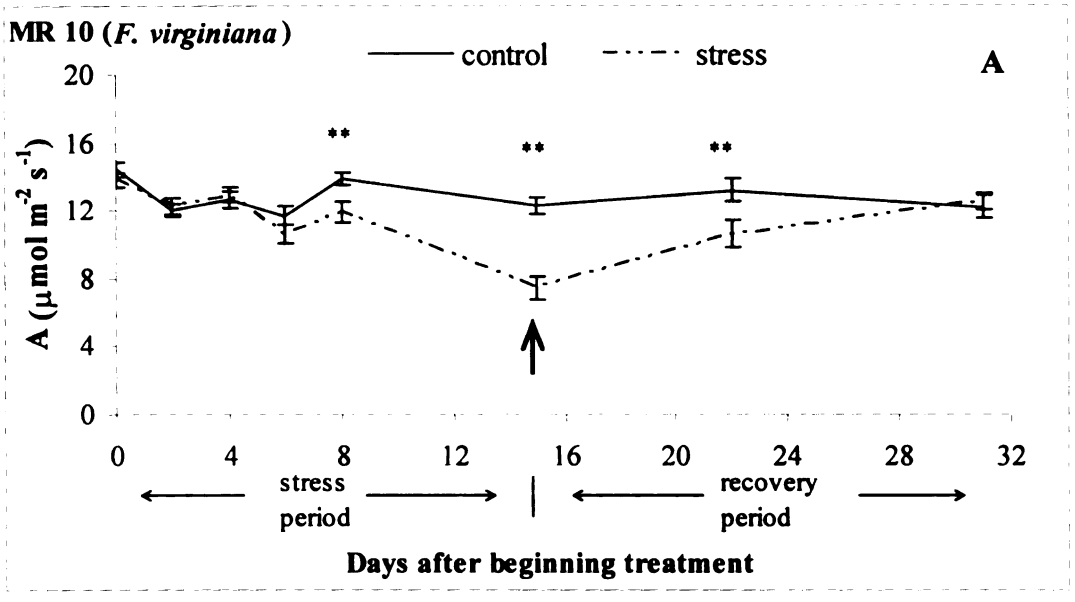
Photosynthetic performance

A decrease in the net photosynthetic rate (A) along with a decrease in stomatal condactunce (g_s) was first evident in MR 10 stressed plants on day 8 with reductions of 15 and 24% respectively. No differences were detected in transpiration rate (E). On day 15, photosynthetic rate of the stressed plants was 40 % lower than the controls while stomatal conductance was 45% lower than controls. Transpiration rate was also lower (28%) in stressed plants compared to the controls ($P \leq 0.05$). After the initiation of the recovery treatment, photosynthetic rate showed a recovery together with an increase in stomatal conductance (g_s) and transpiration rate (E). On day 22, although A in stressed plants was lower, the value was only 20% less than the control, while stomatal conductance was 23% lower and transpiration rate did not differ from the control ($P \leq 0.05$). At the end of the experiment (day 31) all gas exchange paramenters had reached the control's values (Figure 7).

In stressed FRA 24 plants net assimilation rate (A) was reduced 20% on day 4, along with a 30% reduction in stomatal conductance (g_s) and 20% reduction in transpiration rate (E) ($P \leq 0.05$). Photosynthetic rates were 20 % lower than controls until day 8 and 30% lower on day 15. Stomatal conductance and transpiration continued to decrease until day 15 where they were 40 and 30% lower, respectively. During recovery, all gas exchange parameters increased but they were always lower than the control ($P \leq 0.05$). More specifically, on day 31, the net assimilation rate, stomatal condactunce and transpiration rates of the stressed plants were 18, 40, and 30% lower than the control respectively (Figure 8).

Comparison between the two genotypes for gas exchange paramenters is presented in Table 1, Appendix B. It shows the time (days) of occurrence of statistically significant differences between control (A) and stressed (B) plants. Higher net assimilation rates were observed in MR 10 compared to FRA 24 controls on days 6 and 8, while no difference was detected on any other day ($P \leq 0.05$). Stomatal conductance was higher in MR 10 plants on days 2, 6, 8 and 15 whereas transpiration rate was higher only on day 6 ($P \leq 0.05$). In stressed plants, differences between the two genotypes were observed during the stress period starting on day 2 with MR 10 having always higher values for all parameters ($P \leq 0.05$). In the beginning of the recovery period no differences were detected while at the end of the experiment, net assimilation rate, stomatal conductance and transpiration rates of the stressed MR 10 plants were higher than FRA 24 ($P \leq 0.05$).

Figure 7. Variation of leaf net assimilation rate (A), transpiration rate (B) and stomatal conductance (C) of MR 10 (*F. virginiana*) plants during 15 days of salinity treatment and 15 days of recovery. Each value represents the mean (±SE) of ten to fifteen individual leaf measurements. ** indicates dates wher significant differences were observed between controls and stressed plants at $P \leq 0.05$ (LSMEANS test). Arrow indicates the beginning of the recovery period.


MR 10 (F. virginiana)
control
stress
A
20
16
12
8
4
0
A (μmol m-2 s-1)
**
**
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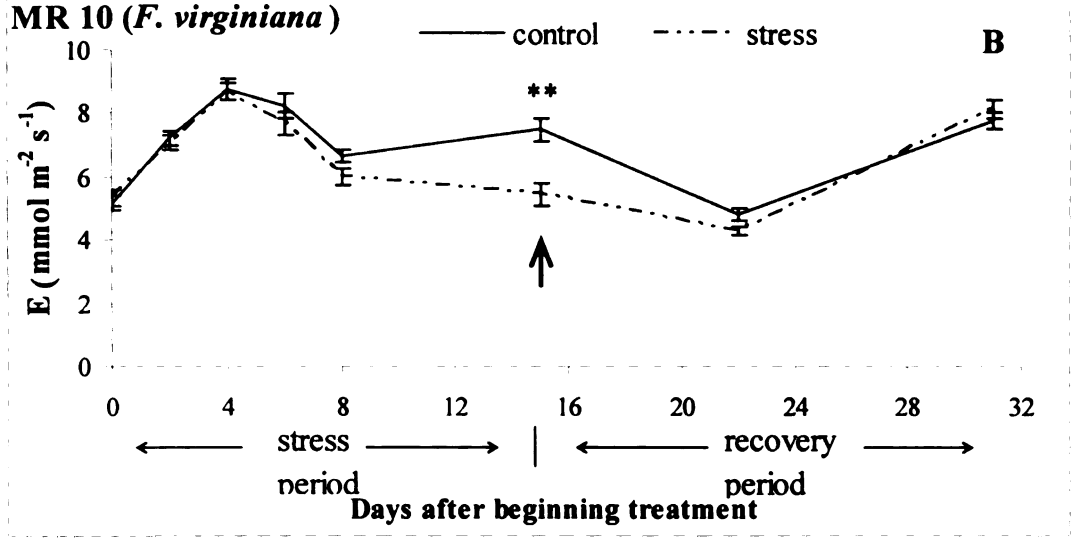
28
32
stress period
recovery period
Days after beginning treatment


MR 10 (F. virginiana)
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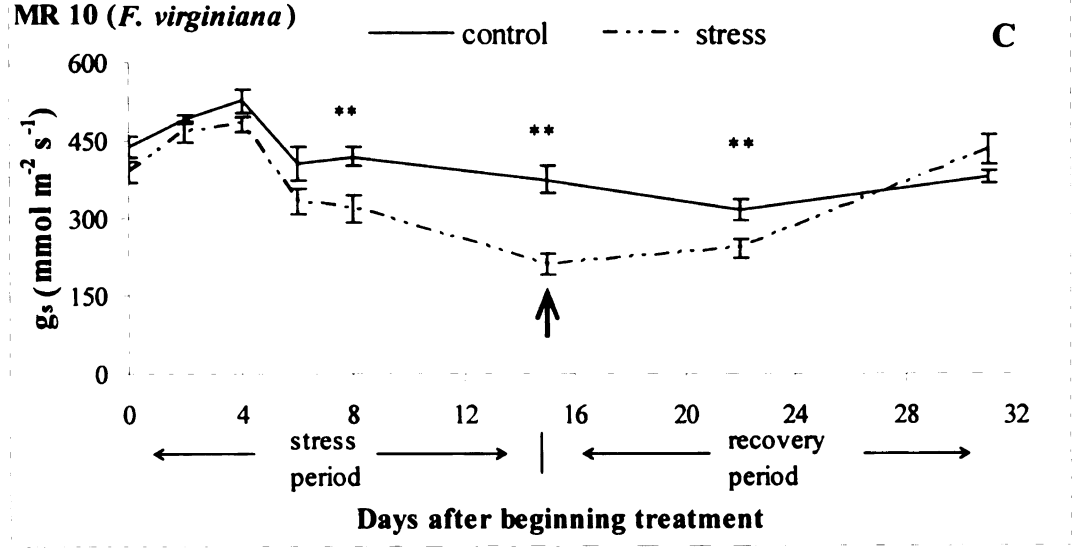
stress period
recovery period
Days after beginning treatment


MR 10 (F. virginiana)
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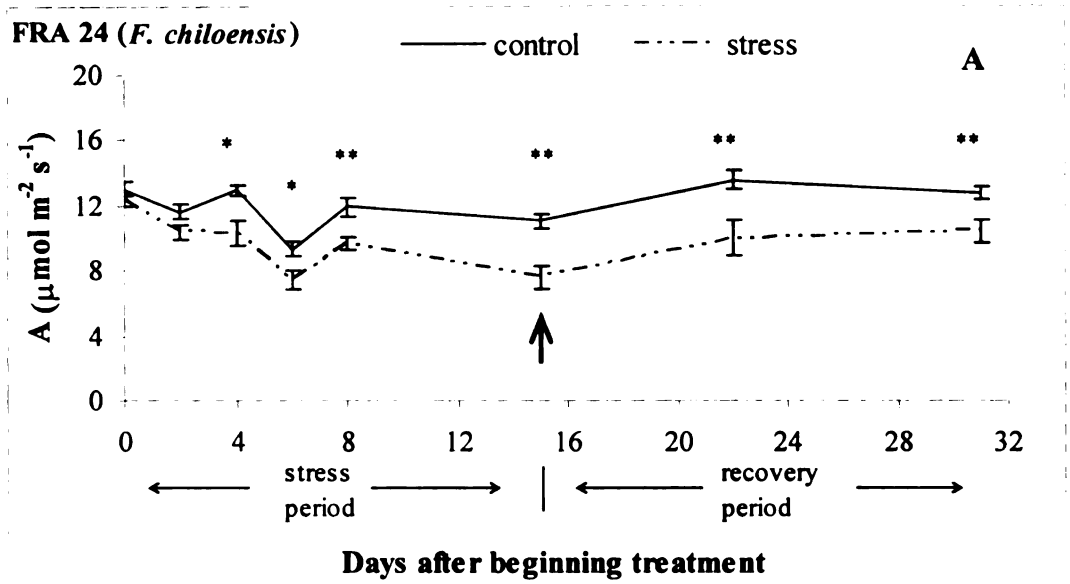
stress period
recovery period
Days after beginning treatment

Figure 8. Variation of leaf net assimilation rate (A), transpiration rate (B) and stomatal condactunce (C) of FRA 24 (*F. chiloensis*) plants during 15 days of salinity treatment and 15 days of recovery. Each value represents the mean (±SE) of ten to fifteen individual leaf measurements. * & ** indicate dates where significant differences were observed between controls and stressed plants at $P \leq 0.05$ & 0.01 respectively (LSMEANS test). Arrow indicates the beginning of the recovery period.


FRA 24 (F. chiloensis)
control
stress
A
A (μmol m-2 s-1)
20
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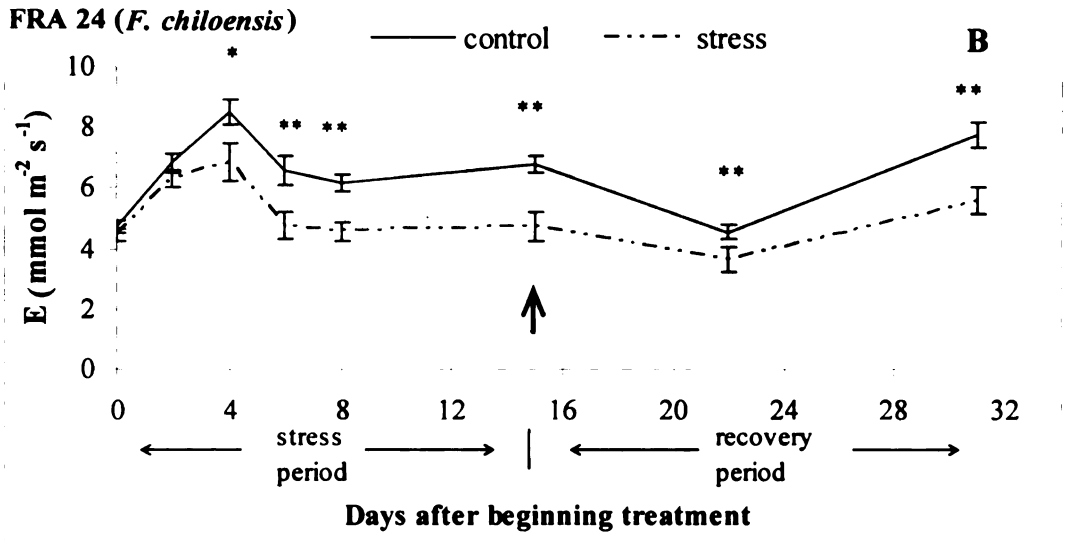
32
stress period
recovery period
Days after beginning treatment


FRA 24 (F. chiloensis)
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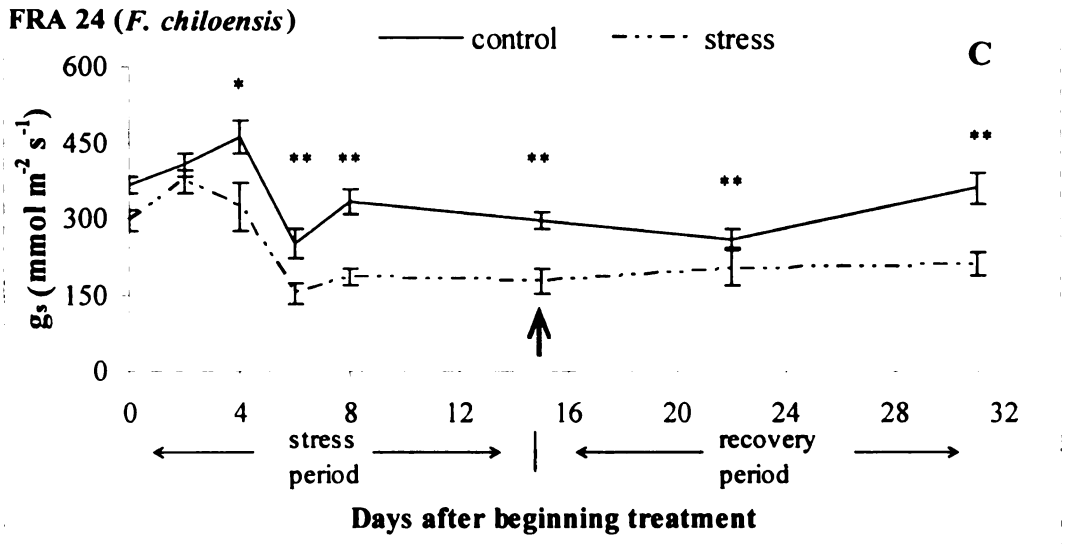
stress period
recovery period
Days after beginning treatment


FRA 24 (F. chiloensis)
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gs (mmol m-2 s-1)
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stress period
recovery period
Days after beginning treatment

Carbohydrate and proline accumulation

Total soluble carbohydrate concentration. TSC concentration of the young leaves of MR 10 (*F. virginiana*) stressed plants was not statistically different from those of the controls at the end of both the stress and the recovery periods. In mature leaves, there was a 1.6-fold increase in the amount of TSC at the end of the stress period (statistically significant at $P \leq 0.05$), while at the end of the recovery period the foliar TSC was reduced to control levels (Table 5).

The TSC of stressed young leaves of FRA 24 *(F. chiloensis*), although higher, did not differ from controls ($P \leq 0.05$) at the end of the stress period. In contrast, significant differences were found at the end of the recovery period with stressed leaves having 2-fold higher TSC than the controls. A quite different pattern was observed in mature leaves, where differences between stress and control were found during both stress and recovery periods, ($P \leq 0.05$). TSC of the stressed plants was 2.2-fold that of controls at the end of the stress period, whereas at the end of the recovery period they were 1.7–fold higher than the controls (Table 5).

Comparison between the two genotypes (Table 5 and 6) showed a higher amount of TSC in mature leaves of MR 10 in both control and stressed plants at the end of the stress period while no difference was observed in young leaves ($P \leq 0.05$). At the end of the recovery period, no difference was detected between the two genotypes in control plants whereas in stressed plants higher TSC were found in both young and mature leaves of FRA 24 compared to MR 10 ($P \leq 0.05$).

Individual carbohydrates. In MR 10, sucrose level was higher in mature leaves compared to the young ones throughout the experiment ($P \leq 0.05$) with values ranging

from 101.4 to 124.3 μmol/g leaf DW and from 13.3 to 33.3 μmol/g respectively. No difference was detected between control and stressed plants during the stress or the recovery periods (Table 5). Glucose and fructose concentrations were also not different ($P \leq 0.05$) between stress and control in the young leaves at the end of both stress and recovery period. In contrast, in mature leaves both sugars were higher in the stressed plants at the end of the stress period with values 3 and 2.3 times higher than the control, respectively. At the end of the recovery period, glucose concentration was 2-fold that of the control, while fructose was reduced to the control level (Table 5). However, when individual carbohydrates were expressed as a percentage of the total, sucrose was the major fraction (53%) with glucose and fructose consisting the rest (47%) in mature control leaves, while under stress conditions glucose and fructose represented the 70% of the total carbohydrates present. At the end of the recovery period sucrose percentage increased to the control levels. No difference was evidence in the relative carbohydrate concentrations between stress and control young leaves (Table 2, Appendix B).

In FRA 24, no difference was detected between stress and control young leaves in all three sugars throughout the experiment, while in mature leaves a 4-fold increase in glucose and 2.8-fold increase in fructose were evident at the end of the stress period with no difference in sucrose concentration. At the end of the recovery period, sucrose was lower in stressed plants (62.8 vs. 113.3 μmol/g leaf DW) while glucose was 4-fold and fructose 2-fold higher than the controls ($P \leq 0.05$) (Table 5). When individual carbohydrates were expressed as percentage of the total a pattern similar to MR 10 was observed, with sucrose being the major fraction in mature control leaves (54%), while

under stress sucrose was reduced to 30% of the total. At the end of the recovery period, sucrose remained at low levels i,e. 17% of TSC (Table 2, Appendix B).

Comparison between the two genotypes showed that there was no difference in the concentration of individual sugars at the end of the stress period with the exception of sucrose in mature leaves, with MR 10 higher compared to FRA 24. On the other hand, at the end of the recovery period, significant differences were detected. More specifically, mature leaves of MR 10 had higher sucrose concentration (101.4 vs. 61.8 μmol/g leaf DW) while they had lower glucose and fructose concentrations with values 69.9 vs. 138.4 μmol/g leaf DW and 85.8 vs. 172.7 μmol/g leaf DW respectively (Table 5 and 6).

Proline concentration. Only trace amount of proline was detected (~1μmol/g DW) in both genotypes. No difference in concentration was evident between stress and control plants or between the two genotypes during the two sampling periods (Table 7).

Table 5. Carbohydrate concentration (μmol/g leaf DW) of young and mature leaves of MR 10 (*F. virginiana*) and FRA 24 (*F. chiloensis*) plants subjected to two weeks of salinity stress (stress period) and then two weeks of stress relief (recovery period). Values represent means from three plants per treatment and genotype. Means followed by the same letter within a column for each genotype, do not differ significantly (LSMEANS, $P \leq 0.05$)

MR 10	treat	age	Suc	Glu	Fru	Total
Stress period	control	young	33.31 *a*	46.33 *ab*	80.43 *a*	160.07 *ab*
	stress	young	27.82 *a*	58.75 *abc*	68.58 *a*	155.14 *ab*
	control	mature	124.26 *b*	39.02 *ab*	69.71 *a*	233.00 *b*
	stress	mature	113.47 *b*	116.75 *d*	159.99 *b*	390.20 c
Recovery period	control	young	13.35 *a*	59.73 *bc*	39.00 *a*	112.08 *a*
	stress	young	14.64 *a*	77.50 *cd*	85.42 *a*	172.68 *ab*
	control	mature	108.55 *b*	31.2 *a*	63.34 *a*	203.1*b*
	stress	mature	101.45 *b*	69.91 *c*	85.76 *a*	257.11 *b*

FRA 24	treat	age	Suc	Glu	Fru	Total
Stress period	control	young	14.10 *a*	97.57 *bc*	51.07 *a*	162.75 *a*
	stress	young	21.80 *ab*	102.33 *bc*	93.56 *ab*	217.69 *ab*
	control	mature	80.77 *cd*	25.08 *a*	45.67 *a*	151.52 *a*
	stress	mature	93.35 *cd*	109.63 *bc*	128.04 *bc*	331.02 *bc*
Recovery period	control	young	15.50 *a*	76.60 *ab*	45.60 *a*	137.70 *a*
	stress	young	23.87 *ab*	129.68 *bc*	128.17 *bc*	281.71 *c*
	control	mature	113.32 *d*	29.39 *a*	70.11 *ab*	212.82*ab*
	stress	mature	62.82 *bc*	138.43 *c*	172.7 *c*	373.94 *c*

Table 6. Comparison between the two genotypes for leaf carbohydrate concentration throughout the experiment (LSMEANS test, $P \leq 0.05$)

	treatment	age	suc	glu	fru	total
stress period	control	young	ns	ns	ns	ns
		mature	**	ns	ns	**
	stress	young	ns	ns	ns	ns
		mature	ns	ns	ns	**
recovery period	control	young	ns	ns	ns	ns
		mature	ns	ns	ns	ns
	stress	young	ns	**	**	**
		mature	**	**	**	**

Table 7. Foliar proline concentration (μmol/g leaf DW) of MR 10 (*F. virginiana*) and FRA 24 (*F. chiloensis*) plants subjected to two weeks of salinity stress (stress period) and then two weeks of stress relief (recovery period). Values represent means from three plants per treatment and genotype. Means do not differ significantly (LSMEANS, $P \leq 0.05$)

	treatment	MR 10	FRA 24
stress period	control	0.95±0.04	1.06 ±0.06
	stress	1.08±0.1	1.1 ±0.1
recovery period	control	1.01±0.07	0.96±0.1
	stress	1.05±0.06	1.02± 0.8

Discussion

Salinity stress affects photosynthetic rate in strawberry plants and this is controlled by both stomatal and non–stomatal mechanisms depending on the species as well as the duration and the severity of the stress (see Chapter 1). In an attempt to determine if the rate of photosynthesis in stressed plants limits growth rate and the carbohydrate supply in the growing tissues we determined the leaf growth rate, photosynthetic performance and the amount of soluble carbohydrates in young (expanding) and mature (expanded) leaves. Furthermore, proline was determined in leaf tissues in order to examine any positive correlation between salinity tolerance and the accumulation of proline in the two selections tested.

Leaf size is a function of cell number and cell size within lamina tissues. Cell division dictates potential size, while cell enlargement is responsible for achieving that potential (Kriedemann, 1986). Meristematic activity in the terminal apices and subsequent emergence of successive leaves has been proposed to be relative insensitive to drought and salinity, but these do appear limited by photoassimilate supply (Kriedemann, 1986). In our study, leaves from control plants and both genotypes completed their expansion within 13-15 days, consistent with what is reported for strawberry species growing under high light (Jurik et al., 1979), while leaves from stressed plants stopped growing much earlier in both genotypes tested. Consequently, in contrast to what was previously found for water stressed strawberry selections in regards to leaf area (Archbold and Zhang, 1991) our results showed that there was a considerable reduction in area per leaf due to stress in both genotypes.

Total leaf area is a function of both the number of leaves as well as the area of the individual leaf. In MR 10 (*F.virginiana*), the decrease in leaf area observed during the stress period derived from the smaller sizes attained by the individual leaves and not from any effect on leaf number. Although the relative leaf expansion rate (mm^2/cm^2 d) was not affected by the treatment, smaller leaves were produced during the stress period. This was due to shorter time in which those leaves were growing and it may reflect a lower cell number per leaf area or smaller size of those cells (Kriedemann, 1986; Munns, 2002). On the other hand, in FRA 24 (*F. chiloensis*) the decrease in leaf area observed is related to the shorter time in which the leaves were actively growing as well as to a decreased rate of leaf emergence. Inhibitory effect of osmotic stress in leaf emergence and initiation rate have been described in *F. virginiana* plants (O'Neil, 1983), while contrasting results have been found by Archbold and Zhang, (1991) where the number of leaves was not affected by water stress in a *F. virginiana* selection, but it was reduced in *F. chiloensis*.

In this study, we observed a strong reduction in the rate of leaf emergence in FRA 24 *(F. chiloensis)* stressed plants throughout the experiment, but no effect was evidence for MR 10 (*F. virginiana*) plants. Decreases in leaf initiation and emergence rate due to salinity stress have been also reported in celery (Everard et al., 1994; Pardossi et al., 1998). Runner production was affected in both genotypes, although in MR 10 much earlier than in FRA 24.

MR 10 plants seemed to be able recover growth after the stress was released and this was evident in that there was an enhancement in the leaf area produced by the previously stressed plants, while in FRA 24 no such recovery was observed. Resumption in growth once salinization relieved has been reported for species such as celery

(Pardossi et al., 1998), and olive (Tattini et al., 1995). (Kriedemann, 1986) pointed out that the extent rather than the activity of the photosynthetic surface is the key determinant of plant productivity. Loss of only a few leaves would not be of great importance for the growth of a large plant, but if the rate of death exceeds the rate of new leaf production, then there would be a substantial drop in the supply of assimilates to the growing leaves and eventually growth would be reduced (Munns et al., 1995).

Although fewer leaves were retained by stressed MR 10 plants at the end of the experiment, indicating a higher rate of senescence and abscission, the supply of assimilates to the growing tissues seems to have met the requirements to support the increased growth rate observed after the release of stress. In addition, the decrease in the total carbohydrates at the end of the recovery period may have been the result of the increased utilization of the photosynthates due to the recovery in growth rate.

A quite different situation emerged from FRA 24 where, although there was a significant recovery in photosynthetic rate after the stress released, we did not observe any positive effect in the growth rate. In addition, the increased amount of soluble carbohydrates present at the end of the stress period was also evident at the end of the recovery period, perhaps indicating lack of utilization.

Concentration of total soluble sugars and other organic compounds has been shown to increase in response to water and/or salinity stress in several species, including apple (Wang and Stutte, 1992; Wang et al., 1995), strawberry (O'Neil, 1983; Zhang and Archbold, 1993b). Furthermore, osmotic and salt stress induced alteration in soluble carbohydrate content in wheat (Kerepesi et al., 1998; Kerepesi and Galiba, 2000), and celery (Everard et al., 1994). In water-stressed *F. chiloensis*, total soluble carbohydrate

concentration has been found to increase 1.4 to 2.4–fold during wilting cycles. Glucose and fructose were the primary carbohydrates involved in osmoregulation accounting for more than 50% of the total osmotic potential. In the present study, sucrose was the major carbohydrate present in control mature leaves of both genotypes, while much less sucrose was present in young leaves, consistent with the notion that sucrose concentration increases as leaf matures (Davis et al., 1988). At the end of the stress period 70% of the TSC present in mature leaves in both MR 10 and FRA 24 were glucose and fructose. Although there was a significant carbohydrate accumulation in mature leaves and in both genotypes due to salt stress, it is not clear whether this accumulation was due to active osmotic adjustment or if it was a consequence of the reduced growth. Taken together, the timing of the first observed reduction in photosynthesis and the timing when a significant reduction in leaf growth was observed, it is obvious that reduction in leaf photosytnthetic rate precedes the reduction in growth. This could support the idea that photosynthetic rate limits growth under these experimental conditions. On the other hand, the fact that growing leaves in both genotypes had carbohydrate contents similar to the controls, does not favor this interpretation, since a reduced carbohydrate accumulation would have been expected if there was a decreased rate of delivery in the growing tissues.

The method we used for proline analysis has been questioned because other amino acids (e.g., glutamine) may increase the color yield of proline leading in inaccurate readings. Under stress conditions, the increase of these amino acids should be negligible in relation to many-fold proline increase (Bates, 1973). In addition our results from proline determination showed only trace amounts in both control and stressed plants throughout the experiment, indicating that strawberry does not accumulate proline in

response to salt stress, as it has been found for water-stressed plants as well (Zhang and Archobold, 1993b). Nolte and Hanson, (1997) have proposed a threshold of 4 μmol/g DW, to distinguish between proline accumulators and non-accumulators. In addition, if proline accumulates after severe cellular damage has occurred (Hanson et al., 1977; Lutts et al., 1996), we would not expect any significant accumulation in our plants since in both sampling times only healthy leaves were collected. In summary, our results indicate that salinity stress affected leaf growth in both genotypes but in quite different manner, in the sense that MR 10 plants were able to resume growth soon after the stress was released while FRA 24 are not. Salinity stress reduced photosynthetic rate and caused carbohydrate accumulation in mature leaves, while it did not limit carbohydrate supply to the young, still growing leaves. Proline accumulation did not appear play any role in controlling salinity tolerance in the strawberry selections tested. Further research is needed to evaluate the relative importance of glucose and fructose in active osmotic adjustment in those two genotypes.

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CONCLUSIONS AND FURTHER RESEARCH

The goals of the present study included the evaluation of the photosynthetic performance and the accumulation of inorganic ions in strawberry plants during salinity stress and subsequent recovery in order to identify possible mechanisms that are involved in salinity tolerance in wild species of the genus *Fragaria*. The second goal was to determine if the rate of photosynthesis in stressed plants limits growth rate and the carbohydrate supply in the growing tissues, or alternatively, if reduction in growth rate is partly responsible for the observed photosynthetic depression. Furthermore, we wanted to establish a possible correlation between tolerance to salt stress and accumulation of specific organic compounds that potentially contribute to the observed better performance of *F. chiloensis* selection compared to *F. virginiana.*

In order to accomplish the first objective we evaluated gas exchange parameters and the accumulation of specific inorganic ions in leaf tissues of two selections of *F. virginiana* and *F. chiloensis* under salinity stress conditions and subsequent recovery. Exposure of strawberry plants to low salinity in the irrigation water did not result in a significant reduction in net assimilation rate, while higher salt concentration caused photosynthetic depression in both sensitive and tolerant selections. Depression in photosynthetic rate was correlated with a reduction in stomatal conductance along with significant decrease in carboxylation efficiency in *F. virginiana* selections, whereas in *F. chiloensis,* only stomatal limitations were responsible for the observed reduction. Lack of substantial accumulation of Na^+ and Cl^- in leaf tissues, together with the absence of significant leaf injury in *F. chiloensis* indicates that mechanisms operate in this selection to control the absorption and/or transport of these ions from the root zone to the aerial

parts. Based on these results further research is needed to determine when salt delivery to the leaves is restricted in the tolerant selection. In addition, from a breeder's point of view, it would be interesting to determine the heritability of this trait in order to be used in breeding programmes aimed to develop strawberry cultivars able to tolerate salinity stress.

To pursue the second goal one selection of each species was chosen to study the effect of salinity on photosynthetic rate, leaf emergence and expansion rates and carbohydrate supply to the growing leaf tissues. Salinity stress resulted in reduced leaf growth in both genotypes but in a quite different manner, in the sense that MR 10 plants were able to resume growth soon after the stress was released while FRA 24 were not. Salinity stress reduced photosynthetic rate and caused carbohydrate built up in mature leaves, while it did not limit carbohydrate supply to the young still growing leaves. Proline accumulation did not appear to play any role in affecting salinity tolerance in the strawberry selections tested. Further research is necessary to evaluate the importance of glucose and fructose in active osmotic adjustment in those species.

APPENDIX A

Table 1. Summary of statistics showing the effects of genotype, treatment and the interaction genotype x treatments on photosynthetic parameters during the experiment (LSMEANS test). * indicates significance at $P \leq 0.05$, ** indicates significance at $P \leq 0.01$, ns indicates no significance.

Effect	Genotype				Treatment				Genotype x treatment			
day	A	E	g_s	C_i	A	E	g_s	C_i	A	E	g_s	C_i
0	ns	ns	ns	ns								
4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
10	**	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
16	**	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns
22	*	ns	*	ns	**	*	**	ns	**	ns	*	ns
30	ns	*	*	*	**	**	**	ns	**	ns	ns	ns
42	**	*	**	*	**	**	**	ns	**	ns	ns	*

Table 2. Regression equations and r^2 for the light response fitted curves

Genotype	Treatment		
MR 10	Control	y = 12.9*(1-1.14*exp(-0.005*x))	$r^2 = 0.94$
	25 mM	y = 12.3*(1-1.12*exp(-0.008*x))	$r^2 = 0.95$
	50 mM	y = 11.7*(1-1.1*exp(-0.003*x))	$r^2 = 0.94$
	100 mM	y = 6.1*(1-1.26*exp(-0.005*x))	$r^2 = 0.95$
NC 95-21-1	Control	y = 13.09*(1-1.11*exp(-0.005*x))	$r^2 = 0.95$
	25 mM	y = 13.1*(1-1.07*exp(-0.004*x))	$r^2 = 0.97$
	50 mM	y = 12.2*(1-1.14*exp(-0.004*x))	$r^2 = 0.96$
	100 mM	y = 4.8*(1-1.43*exp(-0.008*x))	$r^2 = 0.87$
FRA 24	Control	y = 13.5*(1-1.08*exp(-0.004*x))	$r^2 = 0.95$
	25 mM	y = 11.7*(1-1.09*exp(-0.005*x))	$r^2 = 0.93$
	50 mM	y = 12.9*(1-1.11*exp(-0.04*x))	$r^2 = 0.95$
	100 mM	y = 10.2*(1-1.11*exp(-0.004*x))	$r^2 = 0.89$

Table 3. Regression equations and r^2 for the A/C_i fitted curves.

Genotype	Treatment		
Stress period			
MR 10	control	y = 42.86*(1- 1.66*exp(-0.007*x))	r^2=0.97
	stress	y = 32.7*(1- 1.45*exp(-0.005*x))	r^2=0.97
NC 95-21-1	control	y = 34.2*(1- 1.5*exp(-0.006*x))	r^2=0.95
	stress	y = 27.6*(1- 1.5*exp(-0.006*x))	r^2=0.94
FRA 24	control	y = 43.7*(1- 1.4*exp(-0.004*x))	r^2=0.97
	stress	y = 48.5*(1- 1.2*exp(-0.003*x))	r^2=0.89
Recovery period			
MR 10	control	y = 44.1*(1- 1.3*exp(-0.005*x))	r^2=0.97
	re-watered	y = 35.1*(1- 1.3*exp(-0.004*x))	r^2=0.98
NC 95-21-1	control	y = 32.3*(1- 1.5*exp(-0.006*x))	r^2=0.92
	re-watered	y = 28.4 *(1- 1.5*exp(-0.006*x))	r^2=0.94
FRA 24	control	y = 43*(1- 1.4*exp(-0.005*x))	r^2=0.92
	re-watered	y = 40.3*(1- 1.3*exp(-0.04*x))	r^2=0.92

Table 4. Chlorophyll fluorescence parameters, minimal fluorescence (F_o), variable fluorescence (F_v), maximal fluorescence (F_m) and F_m/F_o measured three times during the experiment. Values represent means from 9 measurements per treatment and ecotype. Means followed by the same letter within a column do not differ significantly (LSMEANS test, $P \leq 0.05$)

Day 15	genotype	F_o	F_v	F_m	F_m/F_o
control	FRA 24	602 abc	2619 ab	3221 a	5.35
stress	FRA 24	624 c	2420 a	3051 a	4.89
control	MR 10	524 abc	2755 b	3268 a	6.24
stress	MR 10	595 abc	2645 ab	3250 a	5.46
control	NC 95-21-1	497 ab	2652 ab	3157 a	6.35
stress	NC 95-21-1	450 a	2627 ab	3134 a	6.96

Day 28	genotype	F_o	F_v	F_m	F_m/F_o
control	FRA 24	632 ab	2737 b	3369 b	5.33
stress	FRA 24	581 ab	2720 b	3302 ab	4.68
Re-watered	FRA 24	698 b	2565 ab	3264 ab	5.68
control	MR 10	599 ab	2731 b	3330 ab	5.56
stress	MR 10	658 ab	2616 ab	3274 ab	5.07
Re-watered	MR 10	642 ab	2615 ab	3257 ab	4.98
control	NC 95-21-1	530 a	2615 ab	3145 ab	5.93
stress	NC 95-21-1	541 a	2635 ab	3176 ab	4.69
Re-watered	NC 95-21-1	645 ab	2479 a	3024 a	5.87

Day 36	genotype	F_o	F_v	F_m	F_m/F_o
control	FRA 24	566 b	2710 a	3277 b	5.79
stress	FRA 24	547 ab	2754 a	3302 b	5.93
Re-watered	FRA 24	571 b	2816 b	3387 b	6.04
control	MR 10	555 ab	2557 a	3112 b	5.61
stress	MR 10	559 ab	2720 a	3279 b	5.29
Re-watered	MR 10	531 ab	2613 a	2811 a	5.87
control	NC 95-21-1	479 a	2599 a	3078 b	6.43
stress	NC 95-21-1	483 a	2746 a	3229 b	6.75
Re-watered	NC 95-21-1	463 a	2660 a	3123 b	6.69

APPENDIX B

Table 1. Time (days) of occurrence of statistical differences between genotypes for gas exchange parameters, measured for control plants (A) and stressed plants (B) throughout the experiment. * & ** indicate significance at $P \leq 0.05$ & 0.01 respectively (LSMEANS test)

Gas exchange parameter	days after the initiation of the experiment						
	stress period				recovery period		
A . control	2	4	6	8	15	22	30
A	ns	ns	**	**	ns	ns	ns
E	ns	ns	**	ns	ns	ns	ns
g_s	**	ns	**	**	**	ns	ns
B. stress							
A	*	**	**	**	ns	ns	**
E	ns	**	**	**	ns	ns	**
g_s	**	**	**	**	ns	ns	**

Table 2. Sucrose, glucose and fructose levels as percentage of the total soluble carbohydrates in young and mature leaves of MR 10 (*F. virginiana*) and FRA 24 (*F. chiloensis*) plants subjected to two weeks of salinity stress (stress period) and then two weeks of stress relief (recovery period). Values represent means from three plants per treatment and genotype. Means followed by the same letter within a column for each genotype, do not differ significantly (LSMEANS, $P \leq 0.05$)

MR 10	treat	age	Suc	Glu	Fru
Stress period	control	young	19.0 *ab*	29.8 *b*	51.2 *c*
	stress	young	15.4 *ab*	41.5 *bc*	43.1 *ab*
	control	mature	53.1 *d*	16.5 *ab*	30.4 *a*
	stress	mature	29.0 *bc*	30.0 *b*	41.0 *ab*
Recovery period	control	young	11.8 *a*	53.3 *c*	34.9 *a*
	stress	young	7.9 *a*	47.0 *c*	45.1 *b*
	control	mature	53.4 *d*	15.4 *a*	31.2 *a*
	stress	mature	41.0 *cd*	27.0 *ab*	32.0 *a*

FRA 24	treat	age	Suc	Glu	Fru
Stress period	control	young	8.7 *a*	59.9 *e*	31.4 *ab*
	stress	young	10.8 *a*	47.5 *de*	41.7 *ab*
	control	mature	54 *c*	18.3 *ab*	27.7 *a*
	stress	mature	29.8 *b*	31.8 *bc*	38.4 *ab*
Recovery period	control	young	11.5 *a*	55.5 *e*	33.0 *ab*
	stress	young	8.8 *a*	48.2 *de*	43.0 *ab*
	control	mature	53.2 *c*	13.8 *a*	33.0 *ab*
	stress	mature	16.7 *ab*	37.1 *cd*	46.2 *b*