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Influence of abscisic acid and other plant growth regulators on citrus defence mechanisms to salt stress

V. Arbona Mengual, M. L. Foó Serra, P. Escrig Marín, A. J. Marco Casanova, J. A. Jacas Miret and A. Gómez Cadenas*

Departament de Ciències Experimentals. Àrea de Producció Vegetal. Universitat Jaume I. Campus del Riu Sec. E-12071. Castelló. Spain

Abstract

Citrus yield and growth are deeply affected by salinity. In the present work we have studied the effectiveness of different plant growth regulators such as abscisic acid, jasmonic acid and 8'-methylene methyl abscissate in protecting citrus from salt-induced damage. Plants of Salustiana cultivar grafted onto Carrizo citrange were used for this purpose. Plants were watered with 100 mM NaCl and leaf abscission, ethylene production, chloride accumulation and net photosynthetic rate were measured. Non-treated plants showed a dramatic drop in photosynthetic activity in response to salinity, an increase in leaf ethylene production and a high abscission rate as a result of a massive leaf chloride accumulation. Plants treated with jasmonic acid or 8'-methylene methyl abscisate did not show any physiological change in response to salt stress. However, plants treated with abscisic acid showed a high reduction in the parameters considered. These results suggest that abscisic acid plays a role in modifying citrus physiological behaviour in response to salinity and could be helpful in their acclimation to saline conditions.

Key words: ABA, jasmonic acid, 8'-methylene methyl abscisate, Carrizo citrange, salinity, leaf abscission, photosynthetic rate.

Resumen

Influencia del ácido abscísico y otros fitorreguladores en los mecanismos de defensa de cítricos frente al estrés salino

El estrés salino afecta notablemente el crecimiento y la producción del cultivo de cítricos. En el presente trabajo se ha evaluado la efectividad de distintos tratamientos con fitorreguladores: ácido abscísico, ácido jasmónico y 8'-metilén abscisato de metilo, como paliativos de los efectos nocivos producidos por una concentración alta de sales. Para ello se cultivaron plantas de la variedad Salustiana, injertadas sobre el patrón citrange Carrizo, y se sometieron a tratamientos con NaCl (100 mM). Como parámetros de su comportamiento fisiológico se midieron abscisión, producción de etileno, acumulación de iones cloruro y tasa fotosintética neta en órganos foliares. Los resultados mostraron que, en plantas no tratadas con fitorreguladores, el estrés salino ocasionó una bajada drástica de la actividad fotosintética y un aumento de la producción de etileno como resultado de la acumulación masiva de iones cloruro y, por tanto, una abscisión foliar generalizada. Los tratamientos con ácido jasmónico u 8'-metilén abscisato de metilo no alteraron el patrón de cambios fisiológicos inducidos por el estrés salino. Por el contrario, las plantas tratadas con ácido abscísico mostraron una clara reducción en estos parámetros. Por tanto, los datos indican que el ácido abscísico modifica el comportamiento fisiológico de las plantas de cítricos y puede ayudar en su aclimatación a condiciones de estrés salino.

Palabras clave: ABA, ácido jasmónico, 8'-metilén abscisato de metilo, citrange Carrizo, salinidad, abscisión foliar, tasa fotosintética.

Introduction

Citrus are among the most widespread fruit crops throughout the world, being their global production around 100 million of tonnes per year, of which Spain produces 5% of the total (Anonymous, 2001). Yield and growth of this crop are severely affected by salt stress (Chapman, 1968), although substantial differences in the tolerance to stress within species, scions and rootstocks have been described (see recent review by Storey and Walker, 1999). Under salt stress condi-

* Corresponding author: cadenas@exp.uji.es
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tions, citrus rapidly reduce their leaf water potential. However, turgor potential in leaves remains at similar levels as in non-salinized plants. The osmotic adjustment is achieved by accumulating Na^+ and Cl^- ions and compatible solutes (Lloyd *et al.*, 1987; Bañuls and Primo-Millo, 1992; Gómez-Cadenas *et al.*, 1998). The accumulation of these ions has long term toxic effects and results in well-documented damage and lesions (Romero-Aranda *et al.*, 1998; Gómez-Cadenas *et al.*, 1998).

Salt stress affects a wide range of physiological parameters: reduces growth, especially in the aerial part (Syversten *et al.*, 1988; Lloyd and Howie, 1989); increases leaf succulence (Cerda *et al.*, 1977; Zekri, 1991); reduces photosynthetic rate and stomatic conductance (Bañuls and Primo-Millo, 1992; Bañuls *et al.*, 1997; Gómez-Cadenas *et al.*, 1998); decreases root hydraulic conductivity, affecting nutrient transport (Zekri and Parsons, 1989). Importantly, when the accumulation of chloride ions in leaves reaches toxic levels for the cellular metabolism, premature abscission occurs (Bañuls and Primo-Millo, 1995; Gómez-Cadenas *et al.*, 1998). Depending on the intensity, this defoliation can even result in plant death.

The physiological response to salt stress in citrus, as well as in many other species, is modulated by plant hormones, mainly abscisic acid (ABA) and ethylene (Gómez-Cadenas *et al.*, 1998). ABA is involved, apart from in many other physiological processes, in water use efficiency in plants under environmental stress. This hormone regulates the stomatal opening and, therefore, transpiration. One of the responses of citrus plants to water deficit is a rapid rise of the endogenous levels of ABA (Gómez-Cadenas *et al.*, 1996, 1998). Ethylene, on the other hand, regulates leaf abscission in citrus plants under water stress (Tudela and Primo-Millo, 1992; Gómez-Cadenas *et al.* 1996), or salt stress (Gómez-Cadenas *et al.*, 1998). The elevated levels of chloride ions in leaves of citrus under salt stress is linked to an increased concentration of 1-aminocyclopropano-1-carboxylic acid (ACC) followed by its oxidation to ethylene which, in turn, triggers leaf abscission (Gómez-Cadenas *et al.*, 1998).

Finally, evidence exists for the involvement of jasmonic acid (JA) in plant responses to stress, probably interacting with ABA and ethylene (Lehmann *et al.*, 1995; Wasternack and Parthier, 1997). However, data on the role of this hormone as a mediator of the responses of citrus to salt stress have not been reported.

Different combinations of phytohormones have been tested to alleviate salinization symptoms in cultivated plants. Pre-treatments with ABA effectively increased salt tolerance in crops such as tobacco and barley and forest species such as *Pinus banksiana* Lamb. (Larosa *et al.*, 1987; Popova *et al.*, 1995; Rajasekaran and Blake, 1999). In spite of this beneficial effect, ABA could have a limited effect since it could form conjugates and/or be degraded rapidly (Cutler and Krochko, 1999). JA has also been found to facilitate acclimation of crops such as barley and strawberry to saline conditions (Tsonev *et al.*, 1998).

In this article, the effects of ABA, JA and a synthetic derivative of ABA, 8'-methylene methyl abscissate (which chemical structure appears to delay its catabolism and, therefore, enhance its action), on the physiological behaviour of citrus grown under severe salt stress are studied.

Material and methods

Plant material

All the experiments were carried out in plants of the Salustiana cultivar [*Citrus sinensis* (L.) Osbeck] grafted onto Carrizo citrange (*Citrus sinensis* [L.] Osbeck × *Poncirus trifoliata* [L.] Raf) obtained from a commercial nursery. Plants were placed in 2-l plastic pots filled with inert sand as a substrate and kept in a greenhouse under the following conditions: day temperature, 24-28ºC, night temperature 18-20ºC; 16 h light/8 h dark photoperiod and relative humidity between 60 and 95%. All plants were watered twice a week with 500 ml of Hoagland solution modified for citrus (Bañuls *et al.,* 1997). Only intermediate leaves were used for the different determinations. Abscission was expressed as the percentage of leaves that shed with a gentle touch.

Chemical and salt treatments

Salt stress was imposed by adding sodium chloride to the irrigation solution to achieve a final concentration of 100 mM. The irrigation solution was supplemented with ABA, JA (both obtained from Sigma, Madrid, Spain) or 8-MAMe (Precision Biochemicals Inc., Vancouver, Canada) at a concentration of 10 µM each. Treatments with plant growth regulators were

initiated ten days before the beginning of salinization and maintained during the whole experimental period.

Leaf water potential

Leaf water potential was determined by using a pressure chamber (model 3000, Soilmoisture Equipment, Santa Barbara, CA), as described in Gómez-Cadenas *et al.* (1996).

Measurement of photosynthetic rate

Net photosynthetic rate was measured with a LI-6200 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) equipped with a 250 ml cuvette. Determinations were made in the morning (9 a.m.). For the measurements, the inside of the cuvette was illuminated with a 150 W lamp (Philips EFR A1/232) and cooled with external fans to prevent the sample from heating. All measurements were made at a photosynthetic photon flux (PPF, 400-700 nm) of 900-1000 µmol m^{-2} s^{-1}, which exceeds saturating PPF for citrus. At least, ten mature leaves per treatment were used for the measurements.

Chloride analysis

Total chloride content in leaf tissue was determined by $AgNO_3$ titration (Chapman and Pratt, 1961). One gram of lyophilized leaf tissue was extracted in a basic medium achieved by adding 250 mg of CaO (PRS, Panreac, Barcelona) and some drops of water to the tissue to compact the powdered tissue. Samples were calcined in a muffle oven for 90 min at 550ºC. Ashes were then resuspended in 15 ml of ultra pure water at approximately 70ºC. This suspension was vacuum filtered using a Whatman paper nº 1. The filtered residue was washed five times with 10 ml of ultra pure hot water to obtain a final volume of 65 ml. The pH of the filtrate was adjusted to a value between 6.0 and 7.0 with diluted acetic acid. The titration was performed with 0.05 N $AgNO_3$ using 5% $KCrO_2$ as an indicator.

Ethylene production

Ethylene production was measured from intermediate leaves by gas chromatography. Leaves were separated from the plant by making an incision at the base of the petiole with a scalpel. Then, leaves were placed individually in 12-ml tubes with the petiole submerged in 100 µl of distilled water in the bottom of the tube to prevent dehydration. The tubes were aerated for 30 min and then hermetically closed with silicon stoppers. Following a 4 h incubation period, one ml of the enclosed atmosphere was injected into a gas chromatograph (AGILENT 4890D, Agilent Technologies, Inc., Wilmington, DE, USA) equipped with an activated alumina column and a flame ionization detector.

Results

Water potential

As a first approach to evaluate the effect of salinity on citrus plants, leaf water potential was measured (Fig. 1). Non-salinized plants showed leaf water potential values ranging between –8.42 and –12.50 bar throughout the period studied (data not shown). To facilitate the interpretation of data, a relative value of 100% was assigned to results obtained in control plants and those corresponding to the remaining treatments were expressed as a percentage of this value. All the plants exposed to salt stress showed a reduction in leaf water potential. After seven days of salinization,

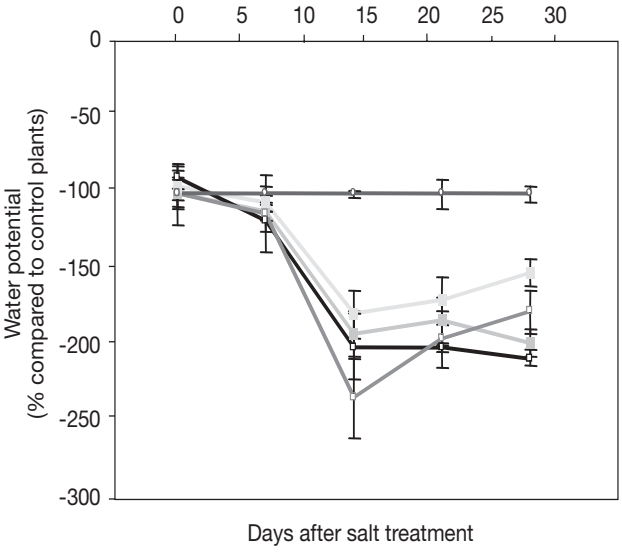

Figure 1. Leaf water potential. Control plants (——), salinized plants non-treated with phytohormones (——), salinized plants treated with: ABA (——), JA (——) and 8-MAMe (——). Each value is the mean of 3 measurements ± SE.

values began to drop and reached the lowest value on day 14 (–18.7 bar, 2.3 times smaller than the value for the non-salt treated control plants at that moment). After this, leaf water potential in plants under salt stress remained at low levels. Plants treated with plant growth regulators did not exhibit any differences in leaf water potential compared to non-treated salinized plants (Fig. 1).

Leaf abscission

Leaves in non-salinized plants did not abscise during the period studied (Fig. 2). In contrast, addition of 100 mM NaCl to the irrigation solution induced a massive drop of leaves. Therefore, after 20 days of saline treatment, leaf abscission was obvious and, in a short period of time (18 days), the salinized plants had lost nearly all their leaves. Treatment with 10 μM ABA resulted in an important reduction of the salt-induced abscission (56% abscission in plants treated with ABA compared to 98% in those not treated, 38 days after the onset of salinization). JA addition delayed leaf abscission induced by salt stress until day 30 although after this date leaf abscission rate increased and, 36 days after the beginning of salinization, 100% of leaves had abscised. Finally, 8-MAMe treatment did not delay the abscission process induced by salinity.

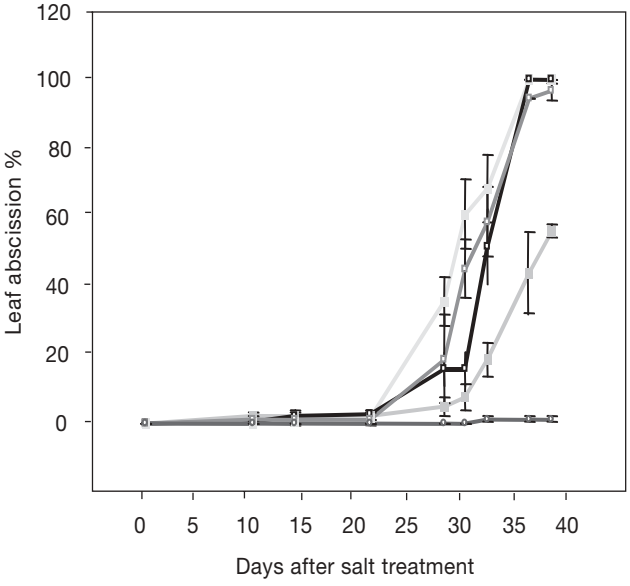

Figure 2. Leaf abscission. Control plants (——), salinized plants non-treated with phytohormones (——), salinized plants treated with: ABA (——), JA (——) and 8-MAMe (——). Each value is the mean abscission of at least 3 plants ± SE.

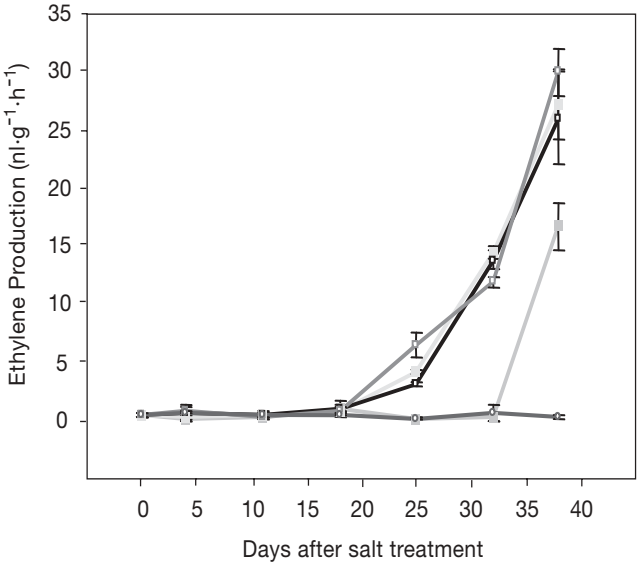

Figure 3. Leaf ethylene production. Control plants (——), salinized plants non-treated with phytohormones (——), salinized plants treated with: ABA (——), JA (——) and 8-MAMe (——). Each value is the mean of 3 independent measurements ± SE.

Leaf ethylene production

Leaves from non-salt treated control plants produced basal levels of ethylene, ranging between 0.30 and 0.68 nl g^{-1} h^{-1} (Fig. 3). Ethylene production in stressed plants remained low until day 18. However, after this date, leaves continuously increased the endogenous production of the plant hormone to reach 30 nl g^{-1} h^{-1} on day 38 (Fig. 3). Abscisic acid treatment suppressed ethylene production until 32 days after the onset of salt stress. Thereafter, leaf ethylene production in ABA-treated plants increased but levels on day 38 were still lower than those of non-treated plants.

On the other hand, neither treatment with JA nor with 8-MAMe significantly modified leaf ethylene production in response to salt stress. It is interesting to note that the pattern of ethylene production in leaves mimicked that of leaf abscission.

Photosynthesis net rate

To demonstrate the effect of salt stress on the photosynthetic activity, CO_2 net assimilation rate was measured. The data obtained were normalized as indicated above. Non-salt treated control plants presented photosynthetic rates ranging from 5.7 to 7.9

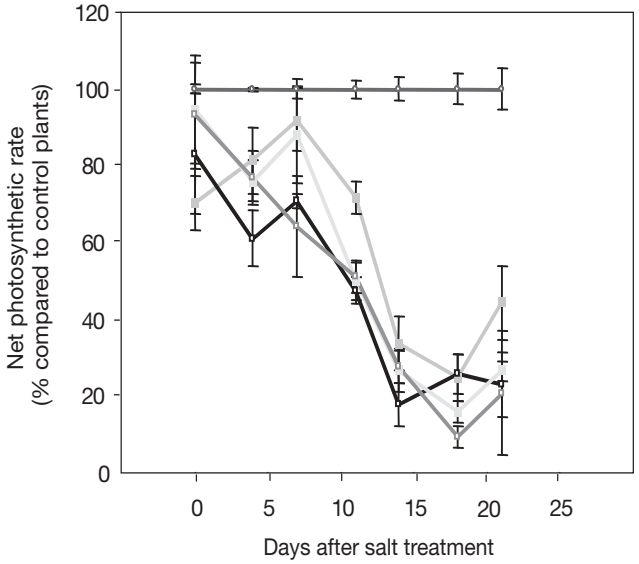

Figure 4. Net photosynthetic rate. Control plants (——), salinized plants non-treated with phytohormones (——), salinized plants treated with: ABA (——), JA (——) and 8-MAMe (——). Each point is the mean of 12 independent measurements ± SE.

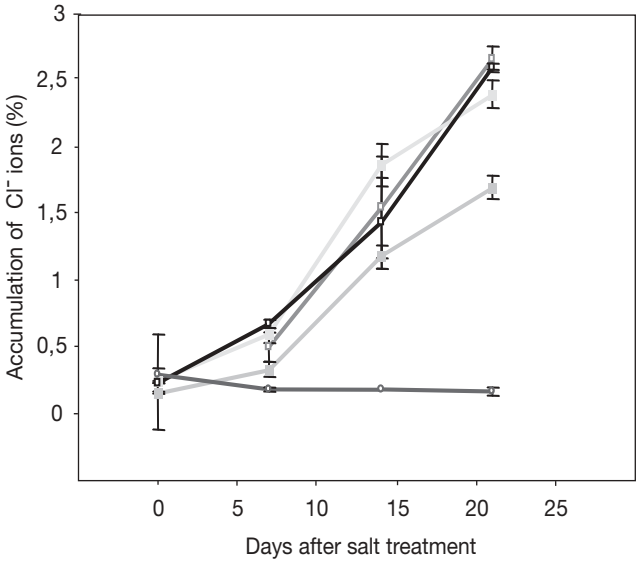

Figure 5. Accumulation of Cl^- ions in leaf tissue. Control plants (——), salinized plants non-treated with phytohormones (——), salinized plants treated with: ABA (——), JA (——) and 8-MAMe (——). Each value is the mean of three independent measurements ± SE.

µmol m^{-2} s^{-1} during the whole experimental period (data excluded). In salt-treated plants, a progressive inhibition of photosynthetic activity was observed obtaining, 18 days after the onset of the salinization, values 90.5% lower than those obtained in control plants (Fig. 4). ABA treatment caused a reduction in the initial photosynthetic rate (Fig. 4, day 0) but it remained constant after adding NaCl. After the fifth day, photosynthesis in ABA-treated plants substantially decreased although it still remained slightly higher that in non-treated plants. Treatments with JA or 8-MAMe did not have significant effects on the reduction of the photosynthetic rate induced by salt stress (Fig. 4).

Accumulation of chloride ions in leaf tissue

To determine the level of intoxication, the amount of chloride ions in leaf tissue was measured (Fig. 5). Leaf Cl^- concentration in all plants under salt stress, treated or not with plant growth regulators, progressively increased over the time studied (Fig. 5). However, the final accumulation was clearly lower in ABA-treated plants than in those not treated with hormones. Treatments with JA or 8-MAMe did not cause any reduction in the accumulation of chloride ions in the leaf tissue of plants under salt stress (Fig. 5).

Discussion

The data presented here indicate that the deleterious effects of salt stress on citrus plants can be reduced by treatment with 10 µM ABA. However, neither JA nor 8-MAMe, applied in the same conditions, had any apparent effect on the physiological response of citrus to salt stress.

It was previously demonstrated that one of the first responses of Carrizo citrange to severe salt stress conditions (200 mM NaCl) was the massive abscission of leaves (Gómez-Cadenas *et al.*, 1998). This response has been directly related to the accumulation of Cl^- ions (Bañuls and Primo-Millo, 1995; Gómez-Cadenas *et al.*, 1998) as well as other damage and physiological alterations (Walker and Douglas, 1983; Bañuls *et al.*, 1997; Romero-Aranda *et al.*, 1998). It has also been confirmed that this response is mediated by ethylene, the hormone that triggers leaf and fruit abscission in citrus under salt, water and nutritional stress (Tudela and Primo-Millo, 1992; Gómez-Cadenas *et al.*, 1996, 1998, 2000). The data presented here reinforce these conclusions and extend the results to grafted citrus plants under less drastic conditions of salt stress. Hence, the massive and rapid leaf abscission observed 20 days after imposing the saline treatment (Fig. 2) was preceded by an important rise in leaf ethylene production (Fig. 3)

and was also correlated with an important accumulation of Cl^- ions in these organs (Fig. 5).

Considering that the accumulation of chloride ions is responsible for the overproduction of ethylene and the subsequent leaf abscission, the reduction in Cl^- concentration observed in plants treated with ABA (Fig. 5) can account for the inhibition of ethylene production (Fig. 3) and, therefore, in leaf abscission (Fig. 2). The mechanisms involved in Cl^- uptake and root to shoot transport are still unknown (Storey and Walker, 1999), although previous reports indicate that chloride uptake under salinization is primarily driven by passive forces, mainly water absorption (Moya *et al.*, 1999) which depends on transpiration and consequently on the stomatal aperture regulated by ABA. Thus, treatment with this phytohormone can induce stomatal closure and, in term, decrease transpiration, radicular absorption and the amount of chloride ions that pass from the substrate to the plant.

The data indicate that net photosynthetic rate is also affected by the salt stress imposed (Fig. 4). Previous studies have demonstrated the relationship between reduced photosynthetic rate and high levels of $Cl^-$and Na^+ (Walker *et al.*, 1982; Bañuls and Primo-Millo, 1992; García-Legaz *et al.*, 1993). Data shown in Fig. 4 are consistent with this proposal and show that the decrease in photosynthesis in salinized plants untreated with plant growth regulators was linear and coincident with the increase in leaf Cl^- ions (Fig. 5). On the other hand, treatment with ABA modifies the behaviour of CO_2 assimilation in saline conditions and appears to protect the photosynthetic machinery, as described in other species exposed to conditions of water deficit or high salt concentrations (Popova *et al.*, 1995; Rajasekaran and Blake, 1999).

It has also been proposed that ABA can act protecting membranes from the damage induced by reactive oxygen species (Rajasekaran and Blake, 1999), improving xylematic nutrient transport (Gadallah, 1996), regulating gene expression and inducing antioxidant enzymes (Gómez-Cadenas *et al.,* 1999; Bellaire *et al.,* 2000), etc. It is, therefore, likely that ABA works at different levels to reduce the deleterious effect of salinity on citrus.

On the other hand, Tsonev *et al*. (1998) showed that JA treatment in barley plants improved their resistance to high salinity. The data presented here, which indicate that treatment with JA does not modify the physiological responses of citrus to salt stress, do not agree with this results. This could be due to a different effect of the phytohormone in cereal and citrus plants or to the fact that the concentration of JA used was below the threshold required for its action. However, the application of JA 10 μM appears to have some effect on plant metabolism since, for example, a 17% reduction in the photosynthetic rate is observed on day 0 (Fig. 4). Moreover, in previous experiments it was shown that the addition of 100 μM JA produces toxic effects in citrus plants (data not shown). It, therefore, seems that although JA was absorbed by the plant, it had no significant effects on leaf abscission nor did it prevent the harmful effects of salt stress.

Finally, treatment with 8-MAMe, the chemically modified abscisic acid methyl ester, did not have the desired effects on leaf abscission, ethylene production or chloride ion accumulation. Although this compound had not been tested previously on citrus, it could be hypothesized that the chemical modification delayed its breakdown, favouring the action of this compound similar to abscisic acid. However, 8-MAMe failed to modify the behaviour of citrus plants in the parameters studied. This failure could be due to the presence of the methylic ester or to the fact that the chemical modification in the 8' position affects its physiological activity.

As a conclusion, abscisic acid, regularly added to the irrigation solution can reduce the damaging effects of high NaCl concentrations on young citrus plants. Treatment with ABA reduces defoliation triggered by ethylene by reducing the amount of chloride ions in the leaf tissue as well as protecting the photosynthetic machinery under saline conditions. This work opens up new possibilities for future studies on the management of citrus crops grown in areas with salinized irrigation water.

Acknowledgments

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References

ANONYMOUS, 2001. Food and agricultural organization of the United Nations (FAO), Statistical data bases. Published in internet, available in http://apps.fao.org/default.htm.

BAÑULS J., PRIMO-MILLO E., 1992. Effects of chloride and sodium on gas exchange parameters and water relations of *Citrus* plants. Physiol. Plant. 86, 115-123.

BAÑULS J., PRIMO-MILLO E., 1995. Effects of salinity on some *Citrus* scion-rootstock combinations. Ann. Bot. 76, 97-102.

BAÑULS J., SERNA M.D., LEGAZ M., PRIMO-MILLO E., 1997. Growth and gas exchange parameters of *Citrus* plants stressed with different salts. J. Plant. Physiol. 150, 194-199.

BELLAIRE B.A., CARMODY J., BRAUD J., GOSSETT D.R., BANKS S.W., LUCAS M.C., FOWLER T.E., 2000. Involvement of abscisic acid-dependent and –independent pathways in the upregulation of antioxidant enzyme activity during NaCl stress in cotton callus tissue. Free. Radic. Res. 33, 531-545.

CERDÁ A., FERNÁNDEZ F.G., CARO M., 1977. The effects of sodium chloride in the irrigation water on the succulence of leaves of citrus rootstocks. An. Edafol. Agrobiol. 36, 393-398.

CHAPMAN H.D., 1968. The mineral nutrition of *Citrus*. In: Reuther W, Batchelor LD, Webber HJ, editors. The citrus Industry II. University of California Press, Berkeley, pp. 127-289.

CHAPMAN H.D., PRATT P.F., 1961. Methods of analysis for soils, plants and waters. University of California Press, Berkeley, 309 pp.

CUTLER A.J., KROCHKO J.E., 1999. Formation and breakdown of ABA. Trends Plant Sci. 4 (12), 472-478.

GADALLAH M.A.A., 1996. Abscisic acid, temperature and salinity interactions on growth and some mineral elements in *Carthamus* plants. Plant Growth Regul. 20, 225-236.

GARCÍA-LEGAZ M.F., ORTIZ J.M., GARCÍA-LIDON A.G., CERDÁ A., 1993. Effect of salinity on growth. Ion content and CO_2 assimilation rate in lemon varieties on different rootstocks. Physiol. Plant. 89, 427-432.

GÓMEZ-CADENAS A., MEHOUACHI J., TADEO F.R., PRIMO-MILLO E., TALÓN M., 2000. Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. Planta 210, 636-643.

GÓMEZ-CADENAS A., TADEO F.R., PRIMO-MILLO E., TALÓN M., 1998. Involvement of abscisic acid and ethylene in the response of citrus seedlings to salt shock. Physiol. Plant. 103,475-484.

GÓMEZ-CADENAS A., TADEO F.R., TALÓN M., PRIMO-MILLO E., 1996. Leaf abscission induced by ethylene in water stressed intact seedlings of Cleopatra mandarin requires previous abscisic acid accumulation in roots. Plant. Physiol. 112, 401-408.

GÓMEZ-CADENAS A., VERHEY S.D., HOLAPPA L.D., SHEN Q., HO T-H.D., WALKER-SIMMONS M.K., 1999. An abscisic acid-induce protein kinase, PKABA1, mediates abscisic acid-suppressed gene expression in barley aleurone layers. Proc. Natl. Acad. Sci. USA 96, 1767-1772.

LAROSA P.C., HASEGAWA P.M., RHODES D., CLITHERO J.M., WATAD A.E.A., BRESSAN R.A., 1987. Abscisic acid stimulated osmotic adjustment and its involvement in adaptation of tobacco cells to NaCl. Plant Physiol. 85, 174-181.

LEHMANN J., ATZORN R., BRUCKNER C., 1995. Accumulation of jasmonate, abscisic acid, specific transcripts and proteins in osmotically stressed barley leaf segments. Planta 197 (1), 156-162.

LLOYD J., HOWIE H., 1989. Salinity, stomatal responses and whole-tree hydraulic conductivity of orchard Washington navel orange, *Citrus sinensis* (L.) Osbeck. Aust. J. Plant Physiol. 16, 169-179.

LLOYD J., KRIEDEMANN P.E., SYVERSTEN J.P., 1987. Gas exchange, water relations and ion concentrations of leaves on salt-stressed Valencia orange *Citrus sinensis* (L.) Osbeck. Aust. J. Plant Physiol. 14, 387-396.

MOYA J.L., PRIMO-MILLO E., TALÓN M., 1999. Morphological factors determining salt tolerance in citrus seedlings: the shoot to root ration modulates passive root uptake of chloride ions and their accumulation in leaves. Plant Cell Envir. 22, 1425-1433.

POPOVA L.P., STOINOVA Z.G., MASLENKOVA L.T., 1995. Involvement of abscisic acid in photosynthetic process in *Hordeum vulgare* L. during salinity stress. J. Plant Growth Regul. 14, 211-218.

RAJASEKARAN L.R., BLAKE T.J., 1999. New plant growth regulators protect photosynthesis and enhance growth under drought of jack pine seedlings. J. Plant Growth Regul. 18, 175-181.

ROMERO-ARANDA R., MOYA J.L., TADEO F.R., LEGAZ F., PRIMO-MILLO E., TALÓN M., 1998. Physiological and anatomical disturbances induced by chloride salts in sensitive and tolerant citrus: beneficial and detrimental effects of cations. Plant Cell Envir. 21, 1243-1253.

STOREY R., WALKER R.R., 1999. Citrus and salinity. Sci. Hort. 78, 39-81.

SYVERTSEN J.P., LLOYD J., KRIEDEMANN P.E., 1988. Salinity and drought stress effects on foliar ion concentration, water relations and photosynthetic characteristics of orchard citrus. Aust. J. Agric. Res. 39, 619-627.

TSONEV T.D., LAZOVA G.N., STOINOVA Z.G., POPOVA L.P., 1998. A possible role for jasmonic acid in adaptation of barley seedlings to salinity stress. J. Plant Growth Regul. 17, 153-159.

TUDELA D., PRIMO-MILLO E., 1992. 1-aminocyclopropane-1-carboxylicic acid transported from roots to shoots promotes leaf abscission in Cleopatra mandarin (*Citrus reshni* Hort. ex Tan.) seedlings rehydrated after water stress. Plant Physiol. 100, 131-137.

WALKER R.R., DOUGLAS T.J., 1983. Effect of salinity level on uptake and distribution of chloride, sodium and potassium ions in citrus plants. Aust. J. Agric. Res. 34, 145-153.

WALKER R.R., TÖRÖKFALVY E., DOWNTON W.J.S., 1982. Photosynthetic responses of the *Citrus* varieties Rangpur lime and Etrog citron to salt treatment. Aust. J. Plant Physiol. 9, 783-790.

WASTERNACK C., PARTHIER B., 1997. Jasmonate signalled plant gene expression. Trends Plant Sci. 2 (8), 302-307

ZEKRI M., 1991. Effects of NaCl on growth and physiology of sour orange and Cleopatra mandarin seedlings. Sci. Hort. 47, 305-315.

ZEKRI M., PARSONS L.R., 1989. Growth and root hydraulic conductivity of several citrus rootstocks under salt and polyethylene glycol stresses. Physiol. Plant. 77, 99-106.