Effect of saline conditions on the maturation process of Clementine Clemenules fruits on two different rootstocks

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Abstract

The production of mandarins is important in the Mediterranean area, where the continued use of saline water reduces fruit yield and modifies fruit quality. Grafted trees of Clemenules mandarin scion on Carrizo citrange and Cleopatra mandarin rootstocks, two of the most common citrus rootstocks employed in this area, were irrigated with two saline treatments (control and 30 mM NaCl). The fruit quality was studied through the last two months before the fruit harvest. Salinity reduced both the fruit number and the mean fruit weight on Carrizo trees whereas no fruit weight reduction was observed on Cleopatra. The decrease of fruit weight on Carrizo trees is probably due to the lower water content and consequently the lower juice percentage. Although the saline treatment produced significant differences in some fruit quality variables (shape and thickness indices) throughout the maturation process, they were minimal at the harvest time. Total soluble solids (TSS) were significantly higher in fruits from the saline treatments, probably due to a passive dehydration. It is also possible that *de novo* synthesis of sugars occurred, since fruits from Cleopatra trees receiving the saline treatment had similar water contents but higher TSS than control fruits. The external fruit colour indicated that the saline treatment accelerated the maturation process; however, the maturity index showed that the high acidity of these fruits delayed the internal maturation with respect to the control fruits.

Additional key words: acidity; Carrizo citrange; Cleopatra mandarin; fruit colour; maturity index; shape index; total soluble solids.

Resumen

Efecto del riego con agua salina en el proceso de maduración de los frutos de Clementina Clemenules

La producción de mandarinas es importante en el área mediterránea donde el uso continuado de agua salina reduce la producción y altera la calidad de los frutos. Árboles de mandarino Clemenules injertados en citrange Carrizo y mandarino Cleopatra, dos de los patrones de cítricos más empleados en la zona, se regaron con dos tratamientos salinos (control y NaCl 30 mM). Se estudió la calidad de los frutos en los dos últimos meses antes de la cosecha. La salinidad disminuyó tanto el número de frutos como el peso medio de los mismos en los árboles de Carrizo mientras que no redujo el peso en los frutos de Cleopatra. Dos meses antes de la cosecha del fruto, los árboles Carrizo de los tratamientos salinos tuvieron frutos con menor peso que los del control, probablemente debido al menor contenido de agua y por tanto al menor porcentaje de zumo. Aunque durante el proceso de maduración del fruto el tratamiento salino produjo diferencias significativas en algunas variables de calidad del fruto (índices de forma y de espesor de corteza), estas diferencias fueron mínimas en el momento de la recolección. Los sólidos solubles totales (TSS) fueron significativamente mayores en frutos de los tratamientos salinos, probablemente debido a una deshidratación pasiva. También pudo producirse una síntesis de azúcares *de novo*, ya que los frutos de los árboles de Cleopatra del tratamiento salino tuvieron igual contenido de agua pero mayor TSS que los frutos control. El color externo del fruto indicó que el tratamiento salino aceleró el proceso de maduración; sin embargo el índice de madurez mostró que la alta acidez de estos frutos retrasó la maduración interna respecto a los frutos control.

Palabras clave adicionales: acidez; citrange Carrizo; color del fruto; índice de forma; índice de madurez; mandarino Cleopatra; sólidos solubles totales.

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Introduction

The production of mandarins is economically important in south-eastern Spain, where the climate is semi-arid with dry, hot summers and a high evaporative demand. The scarcity of water resources in these semiarid regions forces to growers to use low-quality waters from aquifers containing excessive concentrations of soluble salts. Citrus species have been classified as salt-sensitive crops (Maas, 1993), and the continued use of saline water reduces citrus fruit yield and modifies the fruit quality. Salinity decreases the fruit size and modifies some fruit physical characteristics such as peel thickness and the percentages of juice, pulp and peel (Primo-Millo et al., 2000; Morinaga and Sykes, 2001; García-Sánchez et al., 2003, 2006). Some internal quality variables are also modified, such as total soluble solids (TSS), fruit acidity and the maturity index (Primo-Millo et al., 2000; Morinaga and Sykes, 2001; García-Sánchez et al., 2003, 2006).

It is well-known that fruit quality is also influenced by rootstock (Castle et al., 1993; Barry et al., 2004). Inherent rootstock differences affecting plant water relations are associated with differential sugar accumulation of citrus fruits, which are proposed as a primary cause of differences in quality among citrus rootstocks (Barry et al., 2004). The tree foliage supplies carbohydrates to the fruit but the rootstock determines the amount (Gardner, 1969). It is not clear how rootstocks exert their influence on the juice quality of Citrus species, but plant water relations are important factors in this (Castle, 1995; Navarro et al., 2010). Carrizo citrange and Cleopatra mandarin, the most-common rootstocks employed in Spain, have differing characteristics, resulting in different responses of quality (Romero et al., 2006; Pérez-Pérez et al., 2008). These genotypic differences could affect yield and fruit quality (Syvertsen et al., 2000; Barry et al., 2004; Koshita and Takahara, 2004).

Recently, we found that the response to drought stress of the fruit quality of Clemenules mandarin trees grafted on Cleopatra mandarin or Carrizo citrange differs (Navarro *et al.*, 2010). In order to evaluate the suitability of these rootstocks in a semi-arid environment, we examined the effect of irrigation with saline water on some important fruit quality variables during the last phases of the fruit maturation process for field-grown 'Clementine' citrus trees grafted on Cleopatra mandarin or Carrizo citrange, two rootstocks with differing sensitivities to salinity.

Material and methods

The study was carried out in Murcia (southern Spain), on 11-year-old citrus trees (Citrus reticulata Blanco) cv. 'Clemenules' grafted on two rootstocks, Cleopatra mandarin (Citrus reshni Hort. ex Tanaka) and Carrizo citrange (Citrus sinensis L., Osbeck × Poncirus trifoliata L.). The soil is an aridisol, with 27.9% clay, 33.5% loam and 38.6% sand. The soil had an organic matter content of 0.71% (dry soil), an EC₁₋₅ (electric conductivity) of 0.30 dS m⁻¹, 17.50% active CaCO₃ and a pH of 7.6. The weather is Mediterranean semi-arid, with a high mean daily solar radiation (>200 W m⁻²), >9 daily solar hours, a mean annual air temperature of around 17°C, scarce annual rainfall at the experimental site (283 mm) and a total annual reference evapotranspiration (ETo), calculated via the Penman-Monteith method (Allen et al., 1998).

The water applied came from the Tajo-Segura canal, with a pH of 8.99 and an electrical conductivity of 1.0 dS m⁻¹ and containing (mmol L⁻¹): Ca^{2+} 1.8, Mg^{2+} 3.7, K^{+} 0.2, Na^{+} 3.9, Cl^{-} 3.0, SO_{4}^{2-} 4.7 and HCO_{3}^{-} 1.8. A drip line was used in each tree row, with three self-compensating drippers (4 L h⁻¹) per tree, 0.75 m apart. The irrigation schedule was applied weekly using the crop coefficient (Kc) of 1, 0.7 and 0.6 during January and February, from March to June and from July to December, respectively, according to Amorós (1993) for mandarin trees in the Mediterranean area, and corrected by climatic data at the experimental site and neutron probe measurements. At the beginning of the experiment, the average of shaded area per tree was similar for Carrizo and Cleopatra, respectively; thus, the irrigation volume applied was the same. In 2004, the annual ETo was 1,255 mm and the rainfall was 434 mm, applying the total irrigation volume of 691 mm and 679 mm for control and saline treatments respectively, in both rootstocks.

The experiment was performed in trees having a fully-developed canopy, with tree-spacing at 3 × 4 m. The layout took the form of three completely-randomised, selected plots. Each treatment was applied to 9 trees (3 trees per treatment in each plot). The trial involved two saline treatments, 0 and 30 mM NaCl (0 and 3.3 dS m⁻¹, respectively), that were applied from March until the harvest of the fruits on the two rootstocks.

The xylem water potential (Ψ_x) was measured fortnightly. One mature, fully-expanded leaf from the outer canopy, in the middle third of the tree, was taken from

6 trees per treatment. The leaves were enclosed within foil-covered plastic and aluminium envelopes at least 1 h before the midday measurement (McCutchan and Shackel, 1992). The midday Ψ_x was measured at noon (12:00-14:00), using a pressure chamber (model 3000; Soil Moisture Equipment Corp., Santa Barbara, California, USA) and following the recommendations of Turner (1988).

Fruits were harvested on the 15th of November, when they reached their commercial size. Individual tree yield was measured in the 9 trees per treatment. The number of fruits and the total fruit weight of each tree were measured. Some fruit sampling was carried out from the end of phase II and during all of phase III of fruit growth (60, 48, 20 and 10 days before the harvest). In all cases, a sample of 9 fruits per tree was collected, randomly, from the 9 trees per treatment, for analysis of fruit quality.

The external fruit colour was measured in the 9 fruits using a tri-stimulus colour difference meter (Minolta CR-300 colorimeter), on three locations around the equatorial plane of the fruit. The Hunter parameters a and b were used, and colour was expressed as the a/b Hunter colour ratio.

Fruit weight and the equatorial and longitudinal diameters were determined. The «Shape Index» was calculated as the ratio: longitudinal × (equatorial diameter)⁻¹. Fruits were cut in the equatorial area and the internal fruit colour was measured using the Minolta CR-300 colorimeter, at three locations of the equatorial plane, and expressed as the a/b Hunter colour ratio.

Peel thickness was measured at three points and the peel thickness index (PTI) was calculated as Domingo *et al.* (1996) equation:

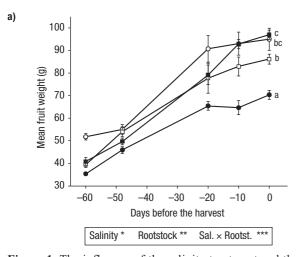
PTI = [peel thickness (mm)
$$\times$$
 200] \times [equatorial diameter (mm)]⁻¹

Fruits were squeezed and the juice filtered for measurements of the total soluble solids content (TSS) and titratable acidity (TA). All fruit fractions were separated, weighed and expressed as juice, peel and pulp percentages. The TSS of the juice was measured at 25°C with a digital refractometer (Atago, Palette PR100) and TA was determined by titration with 0.4 N NaOH and phenolphthalein indicator (results are expressed as percentage of citric acid in the juice). The maturity index (MI) was expressed as 10TSS/TA. For the fruit water content determination, four fruits per tree were harvested at each fruit sampling and they were weighed fresh and after drying.

The data were analysed using analysis of variance (ANOVA) procedures and means were separated by Duncan's multiple range test, using the SPSS software package (SPSS 7.5.1 for Windows, standard version, 1996).

Results and discussion

Salinity decreased fruit yield for both rootstocks due to both lower fruit number and lower mean fruit weight (Fig. 1a and Table 1). The fruit number reduction



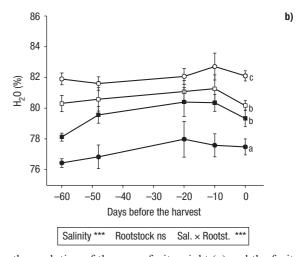


Figure 1. The influence of the salinity treatment and the rootstock on the evolution of the mean fruit weight (a) and the fruit water percentage (b) two months before the fruit harvest. Carrizo 0 mM NaCl, open circles; Cleopatra 0 mM NaCl, open square; Carrizo 30 mM NaCl, closed circles; Cleopatra 30 mM NaCl, closed square. The statistical analysis refers to harvested fruits. ns: no significant. *P < 0.05. **P < 0.01. ***P < 0.001. Separation by Duncan's multiple range test at the 95% confidence level.

Table 1. The influence of the salinity treatment and the rootstock on Clemenules mandarin fruit yield variables at the end of the experimental period

Rootstock	Salinity	Yield (kg tree ⁻¹)	Fruit number
Carrizo	0 mM NaCl	97.2	1,321.9
	30 mM NaCl	62.0	1,203.1
Cleopatra	0 mM NaCl	72.2	986.0
	30 mM NaCl	56.3	728.5
Salinity (S)		***	*
Rootstock (R)		***	*
$S \times R$		ns	ns

ns: no significant. * P < 0.05. *** P < 0.001.

due to saline stress is well-described in mandarin trees (Morinaga and Sykes, 2001; García-Sánchez et al., 2006) and other citrus varieties and it has been attributed to an abnormal fruit abscission (Nieves et al., 1990, 1991; Dasberg et al., 1991; Primo-Millo et al., 2000; García-Sánchez et al., 2002). In other studies, the reduction of yield by salinity has been related with the lower fruit weight (Cerdá et al., 1986; Morinaga and Sykes, 2001; Boman, 2004). Salinity application decreased the fruit number for both rootstocks, but only for Carrizo rootstock were fruits of the salinity treatment smaller than control fruits (Fig. 1). Two months before the harvest, the salinity had already caused marked differences in the weight of fruits on Carrizo rootstock but not for those on Cleopatra. These differences increased until the harvest and the end of the experiment: the fruit weight from Carrizo decreased 26% with respect to the control whereas the weights of the fruits from Cleopatra trees were higher than their respective controls. These low fruit weights observed in fruits from Carrizo were due to both, the lower water content in the fruit (Fig. 1b) and the lower dry matter content since this effect was also observed in the fruit dry weight (data not shown). However, the water content of fruits on Cleopatra did not change with NaCl application.

Salinity treatment decreased the water potential in trees irrigated with saline treatments for both rootstocks (Fig. 2). The higher water stress achieved in Carrizo than in Cleopatra trees from the beginning of the experimental period could be the consequence of their different salinity tolerance. Moreover, Cleopatra is also better soil water extractor, maintaining a better tree water status than Carrizo (Romero *et al.*, 2006). Therefore, rootstock differences in root system morphology (higher root density or a deeper root system in Cleo-

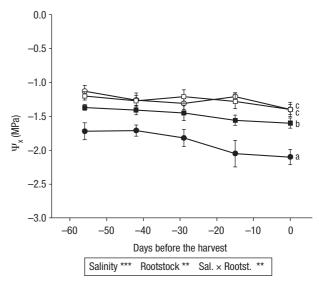


Figure 2. The influence of the salinity treatment and the rootstock on the xylem water potential (Ψ_x) two months before the fruit harvest. Carrizo 0 mM NaCl, open circles; Cleopatra 0 mM NaCl, open square; Carrizo 30 mM NaCl, closed circles; Cleopatra 30 mM NaCl, closed square. The statistical analysis refers to the last date. **P < 0.01. ***P < 0.001. Separation by Duncan's multiple range test at the 95% confidence level.

patra compared to Carrizo) could determine some shoot characteristics (water, nutrient, and carbon budgets, disease resistance, and plant growth regulators). These citrus rootstock characteristics impart some stress tolerances that are usually dominant in determining tree responses to environmental stresses (Syvertsen and Lloyd, 1994).

Shape index decreased with fruit maturation (Fig. 3a), regardless of treatment. Before the harvest, fruits from Cleopatra trees receiving the saline treatment had a more-rounded shape than control fruits. However, at harvest, no differences were found between treatments or rootstocks. For Cleopatra fruits, the PTI was lower with the saline treatment two months before the harvest; however, at the harvest time no differences were found between rootstocks or treatments (Fig. 3b). An increase of the PTI due to salinity has been found for Valencia orange grafted on Cleopatra rootstock (Francois and Clark, 1980), in Navelina orange and Clementina de Nules (Primo-Millo et al., 2000). Also, an increase of the peel thickness due to salinity has been found in Verna lemon and Star-Ruby grapefruit (Nieves et al., 1991; Porras et al., 2000).

The external colour of citrus fruits is one of the main attributes of quality and a major variable for consumer acceptance. The fruit colour is expressed as a/b since this ratio has a high correlation with the visual appre-

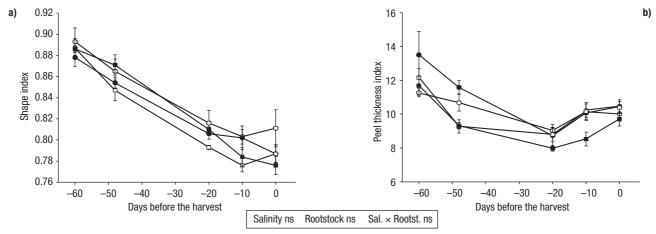


Figure 3. The influence of the salinity treatment and the rootstock on the evolution of the shape index (a) and the peel thickness index (b) two months before the fruit harvest. Carrizo 0 mM NaCl, open circles; Cleopatra 0 mM NaCl, open square; Carrizo 30 mM NaCl, closed circles; Cleopatra 30 mM NaCl, closed square. The statistical analysis refers to harvested fruits. ns: no significant.

ciation of the fruit colour (Stewart and Wheaton, 1971) and represents the colour variation from yellow to orange. The results show that the rootstocks had no effect on the external colour of the fruits (Fig. 4a). However, at the end of the maturation process, fruits from the saline treatment had a 25% higher a/b ratio than in the control fruits. During the maturation process the peel colour changes markedly, since chloroplasts (containing carotenoids and chlorophyll) are transformed into chromoplasts (having only carotenoids), taking place a strong modification of chlorophyll and carotenoid levels and a massive accumulation of carotenoid (Rodrigo et al., 2004). As the a/b ratio is negative for green fruits and positive for orange fruits, the

higher *a/b* ratio of saline fruits could show an apparent advance in their maturation process with respect to control fruits (Fig. 4a). With respect to the internal colour, only fruits from Carrizo trees subjected to the saline treatment had a higher *a/b* ratio than the others at the time of harvest (Fig. 4b). In other study, salinity increased the internal greenish colour of fruits from Clemenules mandarin trees although no differences were found on fruits from trees grafted on Carrizo or Cleopatra rootstocks (García-Sánchez *et al.*, 2006).

The rootstock, Carrizo or Cleopatra, did not modify the juice, pulp or peel percentages (Fig. 5). However, some studies have found a higher pulp percentage in orange fruits from Carrizo rootstock than in those from

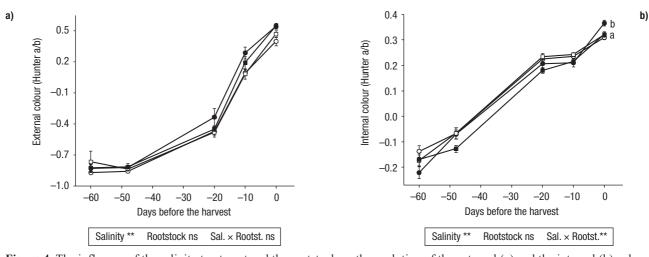


Figure 4. The influence of the salinity treatment and the rootstock on the evolution of the external (a) and the internal (b) colour of the fruits two months before the fruit harvest. Carrizo 0 mM NaCl, open circles; Cleopatra 0 mM NaCl, open square; Carrizo 30 mM NaCl, closed circles; Cleopatra 30 mM NaCl, closed square. The statistical analysis refers to harvested fruits. ns: no significant. ** P < 0.01. Separation by Duncan's multiple range test at the 95% confidence level.

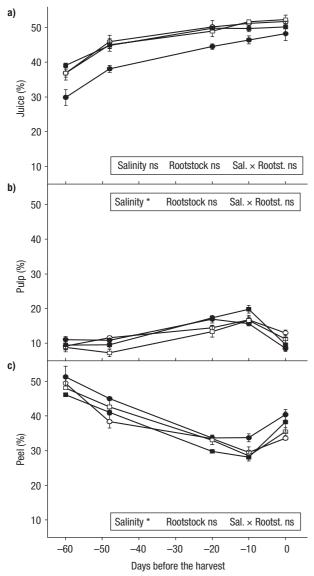


Figure 5. The influence of the salinity treatment and the rootstock on the evolution of the juice (a), pulp (b) and peel (c) percentages of the fruits two months before the fruit harvest. Carrizo 0 mM NaCl, open circles; Cleopatra 0 mM NaCl, open square; Carrizo 30 mM NaCl, closed circles; Cleopatra 30 mM NaCl, closed square. The statistical analysis refers to harvested fruits. ns: no significant. *P<0.05.

Cleopatra (Forner-Giner et al., 2003; Pérez-Zamora, 2004). Also, for fruits from Carrizo, a higher juice percentage and a lower peel percentage than for fruits from Cleopatra have been found (García-Sánchez et al., 2003, 2006). Before the harvest, the juice percentage of fruits from Carrizo irrigated with saline water was significantly lower than in control fruits (Fig. 5a), probably due to the lower water content of these fruits relative to the control (Fig. 1b). However, at the harvest time,

salinity did not modify significantly the juice percentage of these fruits (Fig. 5a). As was observed for the water content, fruits from Cleopatra trees subject to salinity did not show differences in the juice percentage with respect to control fruits (Fig. 5a). Salinity application did not produce clear responses of the pulp percentage during the experiment; however, at the harvest time, fruits from trees irrigated with saline water had a lower pulp percentage (Fig. 5b). By contrast, at the end of the experimental period, the peel percentage was increased significantly for fruits of trees irrigated with salinity (Fig. 5c). Similar results have been found at the harvest time in Clemenules mandarin fruits of trees irrigated with saline water (García-Sánchez, 2006). However, other studies have found a higher juice percentage and lower peel percentage in fruits of varieties Navelina and Clementina de Nules due to salinity (Primo-Millo et al., 2000).

One of the most-important quality variables of citrus juice is the TSS (sugars and citric acid being the most abundant compounds), which plays an important role in determining the taste of citrus fruits. From the end of phase II and throughout phase III of fruit growth, TSS increased (Fig. 6a). Rootstocks may influence the TSS through the degree of dilution, since invigorating rootstocks are better extractors of soil water (Castle and Krezdorn, 1977). Since Carrizo is a more-invigorating rootstock than Cleopatra, the water percentage of control fruits was higher than in control fruits from Cleopatra and the dilution effect was higher, resulting in lower TSS values through the experiment (Fig. 6a). Salinity significantly increased TSS during the experiment, probably due to a concentration effect since these fruits had lower water content (Fig. 1b). It is also possible that de novo synthesis of sugars occurred, to achieve osmotic adjustment in the fruit, since TSS increased in fruits from Cleopatra due to salinity but their water content was similar to that of control fruits (Fig. 1b). With similar salinity levels, García-Sánchez et al. (2006) only found an increase of TSS in trees on Carrizo related with the reduction in juice percentage and a possible osmotic adjustment.

The TA of the citrus juice is also an important quality factor and it is determinant for establishing the optimal harvest time (Harding *et al.*, 1940). The total acids in fruits increase in the first stages of maturation, but later remain constant. As juice percentage increases, acidity decreases with maturation due to a dilution effect. Two months before the harvest, fruits from the saline treatment showed acidity levels higher than

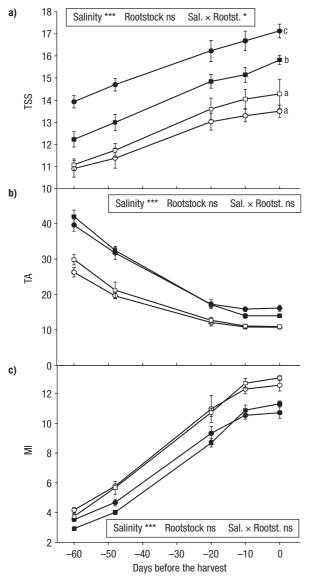


Figure 6. The influence of the salinity treatment and the rootstock on the evolution of the total soluble solids, TSS, (a), titratable acidity, TA, (b) and maturity index, MI, (c) of the juice fruit two months before the fruit harvest. Carrizo 0 mM NaCl, open circles; Cleopatra 0 mM NaCl, open square; Carrizo 30 mM NaCl, closed circles; Cleopatra 30 mM NaCl, closed square. The statistical analysis refers to harvested fruits. ns: no significant. *P<0.05. ***P<0.001. Separation by Duncan's multiple range test at the 95% confidence level.

those of control fruits (Fig. 6b). Although these differences became less as fruits ripened, at the harvest time the acidity of fruits from the saline treatment was still higher (by 28%) than in control fruits. No differences were found between rootstocks. Similar to TSS, the increase of the acidity due to salinity could have been due to a concentration effect, since these fruits had lower

water content, but also to a *de novo* synthesis of organic acids in the osmotic-adjustment process that occurred in response to salinity (García-Sánchez *et al.*, 2002). The increase of TSS and the acidity of the fruits with salinity has been observed also by Dasberg *et al.* (1991), in Shamouti orange, by Cerdá *et al.* (1986) and Nieves *et al.* (1991), with lemon Verna on Cleopatra rootstock, and by Bielorai *et al.* (1978), for Marsh grapefruit grafted on sour orange. However, other authors have found an increase of TSS but a decrease in the acidity due to salinity (Primo-Millo *et al.*, 2000; García-Sánchez *et al.*, 2003).

In the most important citrus-growing areas, the balance between the TSS and the sourness produced by acidity is a sure, useful and reliable method for evaluating fruit quality and for determining the fruit maturation process, and it is also the best criterion in correlating fruit quality with consumer acceptance (Harding and Fisher, 1945; Erickson, 1968; Davies and Albrigo, 1994). The standard suitable for early harvesting is an 8:1 ratio, but most people prefer a sweeter-tasting mandarin; a pleasant taste is achieved when this ratio is greater than 10. In this experiment, fruits were harvested at the same time, so the MI shows the state of the maturation process of the fruit in each treatment. Unlike other studies (Conesa, 1999; Wagner et al., 2002; Forner-Giner et al., 2003; García-Sánchez et al., 2003, 2006), and as we have previously found (Navarro et al., 2010), no differences were found in MI between the two rootstocks (Fig. 6b). Salinity decreased the MI of fruits by 16% with respect to control fruits, since the increase of the acidity due to salinity was higher than that of the TSS (Fig. 6). Francois and Clark (1980) also found a delay in the maturation of fruits grown under saline conditions. However, in previous studies, similar increases of TSS and acidity due to salinity have been found, resulting in MI values similar to those of control fruits (Metochis, 1989; Dasberg et al., 1991; García-Sánchez et al., 2003), and other authors have even observed an increase in the MI with salinity (Primo-Millo et al., 2000; Morinaga and Sykes, 2001; García-Sánchez et al., 2006).

Although the effect of salinity on the quality of citrus fruits has been evaluated in several studies, conflicting results frequently can be found because of the multiple influences usually present. First, different levels of salinity are used in these studies, and they are usually applied during different periods of time. Second, the characteristics of the fruits vary enormously in different citrus varieties, even among cultivars. Fruit quality is

also influenced by the rootstock; inherent rootstock differences affecting plant water relations are associated with differential sugar accumulation of citrus fruits, which is proposed as a primary cause of differences in quality among citrus rootstocks (Barry et al., 2004). Moreover, the responses of citrus to salinity could be complex since they depend not only on the rootstock and the variety but also on the rootstockscion interactions. Even for the same rootstock-scion combination, comparing field performance under saline conditions is difficult because of the enormous potential for interactions with other environmental factors (soil characteristics, climatic conditions, mineral nutrition, agromanagement techniques, plant age, size of the root system, etc.) that could affect the plant response to salinity and hence fruit quality. Although we could not find studies with similar salinity levels, the same rootstock-scion combination and the same experimental conditions, such studies help us to draw general conclusions for a better understanding of the mechanisms involved in plant responses to salinity.

Conclusions

Irrigation with 30 mM NaCl affected the fruit yield and quality of Clemenules mandarin, particularly for fruit from trees grafted on Carrizo —where the fruit water content, and therefore juice percentage, was reduced.

Although salinity produced important differences in most of the variables of fruit quality during the maturation process, these decreased until the fruit harvest.

The fruit colour indicated an apparent advance of fruit maturation with the saline treatment; however, the maturity index showed that the high acidity of these fruits delayed their maturation with respect to control fruits.

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