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Corresponding Author: Sergio G Nebauer

Corresponding Author's Institution: Universitat Politècnica de València

First Author: Catarino Ávila

Order of Authors: Catarino Ávila; José Luis Guardiola, Prof; Sergio G Nebauer

**Abstract:** The responses of photosynthesis and growth to water stress and recovery were studied and compared in two Citrus species. Fino lemon and Owari satsuma trees were subjected, under greenhouse conditions, to moderate (-2 MPa at predawn) and severe (-3 MPa) water stress levels, and rewatered after 60 days. Vegetative growth was inhibited during the stress assays, and strong defoliation levels were reported, especially in Fino lemon. The leaf loss was mainly produced during the water deficit period, and only scarcely after dehydration. Bud sprouting was induced after rewatering in both species. Flowers and vegetative shoots were developed in Owari satsuma after a drought period, irrespective of the stress level. In Fino lemon, vegetative shoots and flowers were mainly formed, after moderate and severe stress, respectively. The photosynthetic rate and stomatal conductance were reduced by water stress, with a marked increase in water use efficiency at moderate water deficit. Nevertheless, photosynthetic apparatus was not damaged, since maximum quantum yield, photosynthetic pigments concentration and Rubisco amount and activity did not change. Furthermore, measured MDA and peroxidase activity indicated that oxidative stress was not specifically triggered by water stress. Thus, gas exchange, fluorescence and biochemical parameters suggested that diffusional limitations to photosynthesis predominated in both studied Citrus species, and explained the rapid recovery of photosynthetic parameters after rehydration. Net CO<sub>2</sub> fixation rate and stomatal conductance recovered within 24 h in Fino lemon, whereas 3 days were needed in Owari satsuma, suggesting some metabolic limitations in this species. Furthermore, the sensibility of defoliation rates, the accumulation of proline and stomatal behaviour in response to water stress, indicated a higher tolerance of Fino lemon to drought, according to its better acclimation to hot climates.

Suggested Reviewers: Hipolito Medrano Prof

hipolito.medrano@uib.es

Expert in studies about the effect of water stress on photosynthesis

Rafael Ribeiro

rafael@iac.sp.gov.br

Works on ecophysiology of citrus trees



**Effect of a water stress period and rehydration on photosynthesis, biochemical parameters and growth in *Citrus***

Catarino Ávila<sup>2</sup>, José Luis Guardiola<sup>1</sup> (†), Sergio G. Nebauer<sup>1\*</sup>

<sup>1</sup>Departamento de Producción vegetal. Área de Fisiología vegetal. Universitat Politècnica de València. Edificio 3K. Camino de Vera sn. 46022-Valencia (Spain).

<sup>2</sup>Colegio de Postgraduados. Campus Veracruz. Veracruz. México

† Deceased

\* Corresponding author: Tel: +34-963877410; Fax: +34-963877419. E-mail address: [sergonne@bvg.upv.es](mailto:sergonne@bvg.upv.es)

## **Abstract**

The responses of photosynthesis and growth to water stress and recovery were studied and compared in two *Citrus* species. Fino lemon and Owari satsuma trees were subjected, under greenhouse conditions, to moderate (-2 MPa at predawn) and severe (-3 MPa) water stress levels, and rewatered after 60 days. Vegetative growth was inhibited during the stress assays, and strong defoliation levels were reported, especially in Fino lemon. The leaf loss was mainly produced during the water deficit period, and only scarcely after dehydration. Bud sprouting was induced after rewatering in both species. Flowers and vegetative shoots were developed in Owari satsuma after a drought period, irrespective of the stress level. In Fino lemon, vegetative shoots and flowers were mainly formed, after moderate and severe stress, respectively. The photosynthetic rate and stomatal conductance were reduced by water stress, with a marked increase in water use efficiency at moderate water deficit. Nevertheless, photosynthetic apparatus was not damaged, since maximum quantum yield, photosynthetic pigments concentration and Rubisco amount and activity did not changed. Furthermore, measured MDA and peroxidase activity indicated that oxidative stress was not specifically triggered by water stress. Thus, gas exchange, fluorescence and biochemical parameters suggested that diffusional limitations to photosynthesis predominated in both studied *Citrus* species, and explained the rapid recovery of photosynthetic parameters after rehydration. Net CO<sub>2</sub> fixation rate and stomatal conductance recovered within 24 h in Fino lemon, whereas 3 days were needed in Owari satsuma, suggesting some metabolic limitations in this species. Furthermore, the sensibility of defoliation rates, the accumulation of proline and stomatal behaviour in response to water stress, indicated a higher tolerance of Fino lemon to drought, according to its better acclimation to hot climates.

**Key words:** drought, recovery, flower induction, Owari satsuma, Fino lemon

## **Introduction**

A period of water stress during the winter induces *Citrus* bud sprouting and flowering in hot tropical and subtropical habitats (Cassin et al. 1969; Davenport 1990). The induction degree is proportional to the severity and the duration of the stress (Southwick and Davenport 1986). Out of season flowering

can also be triggered in some citrus species by a water stress period followed by rainfall or irrigation, which has been exploited for commercial production of Verdelli lemons in Sicily through the withdrawal of irrigation during the dry summer season (Barbera et al. 1985).

Drought results in the inhibition of photosynthesis (Boyer 1976; Ephrath and Hesketh 1991), either by diffusion limitations or by metabolic impairment (Sharkey 1990; Lawlor 1995). Stomatal closure seems to be the earliest response to drought and both stomatal and mesophyll conductances are the dominant limitations to photosynthesis in mild to moderate drought stress (Galmés et al. 2007). Biochemical limitations, involving the impairment of Rubisco activity and the reduction of RuBP content, further decrease photosynthesis under severe drought conditions. The relative water content at which these biochemical mechanisms are impaired is species-dependent (Bota et al. 2004).

Water stress triggers adaptive responses (Zhu 2002), regulating key genes for osmolyte biosynthesis, detoxification enzymes, water channel proteins, several proteases, transcription factors and some gene expression regulators (Shinozaki 2003). Sometimes a moderate water stress can improve photosynthetic capacity, water use efficiency and regulate carbon allocation due to acclimation to the environmental stresses (Xu and Zhou 2007).

Photosynthesis recovery after a mild stress is usually rapid and complete. In contrast, after severe stress the recovery is progressive and slow, lasting from days to weeks, and sometimes incomplete (Flexas et al. 2006). The relative contribution of biochemical and diffusion limitations during the recovery have to be further studied (Ennhali and Earl 2005; Galmés et al. 2007).

Changes in photosynthesis, biochemical parameters and growth in response to water stress and rehydration were determined in two citrus species, Fino lemon and Owari Satsuma. The study was performed in a shaded greenhouse during the spring months, in order to separate the effect of water stress from the effect of high temperatures and excessive irradiation that usually accompany water stress periods in field experiments.

## **Materials and methods**

### Plant material and growth conditions

Four year old trees of Owari satsuma (*Citrus unshiu* (Mak.) Marc.) grafted on Carrizo citrange (*C. sinensis* [L.] Osb. x *Poncirus trifoliata* [L.] Raf.) and Fino 49 lemon (*C. limon* [L.] Burm. F.) grafted on *C. macrophylla* Wester were grown in 20 L pots filled with a mix of peat:sand:perlite (7:2:1 by volume) and kept in a greenhouse covered with a reflective aluminized net. Maximum light (PAR) in the greenhouse approximated  $700 \mu\text{mol} / \text{m}^2 \text{ s}$  and the temperature ranged between 20 °C (minimum) and 32 °C (maximum).

Until the beginning of the experiment, the trees were watered every two days to run off and fertilized weekly with Hoagland's n° 2 solution (Hoagland and Arnon 1950). Predawn water potential ( $\psi_w$ ) was in the range of -0,9 to -1 MPa.

### *Experimental design*

Trees of both species were subjected to moderate (-2 MPa) or severe (-3 MPa) drought stress for 60 days. Watered trees were used as controls. Water stress was induced by withholding irrigation until the predawn water potential ( $\psi_w$ ) of the leaves, as determined with a pressure chamber (PMS Instrument Co, Corvallis, Oregon USA), reached the predefined water deficit levels. These predefined water stress levels were reached 6 to 12 days after withholding irrigation, respectively. Afterwards, this level of stress was kept by adding daily the transpirational water calculated from the weight of the pots as described by Chaikiattiyos et al. (1994). This procedure allowed the maintenance of the desired water stress with a variation of  $\pm 15\%$ . Sixty days after the establishing of the stress, the irrigation was restarted. Nine trees were used for each stress level. Within treatments, trees were randomly distributed in three blocks of three trees.

### Gas-exchange and chlorophyll fluorescence measurements

The rate of net CO<sub>2</sub> assimilation ( $A_N$ ), transpiration (E), and stomatal conductance ( $g_s$ ) were measured during the experiment at natural PAR and temperature conditions, with a LCi Portable Photosynthesis System (ADC, Herst, UK). Measurements were performed from 9 am to 12 pm to avoid the midday drop in photosynthesis. Water use efficiency (WUE) was estimated by the ratio  $A_N/E$ . Chlorophyll

fluorescence parameters were measured in the same leaves using a portable pulse amplitude modulation fluorometer (MINIPAM, Walz, Effeltrich, Germany). The maximal photochemical efficiency of photosystem II (PSII) was estimated by the fluorescence ratio  $F_v/F_m$  of half hour dark-adapted leaves, calculated from  $F_0$  and  $F_m$  (Genty et al. 1989).

Measurements were performed once a week during the water stress conditions, and daily after rewatering until photosynthesis was recovered. One measurement per tree was performed on a fully expanded mature leaf (third or fourth leaf from the shoot apex).

#### Biochemical analyses

Three independent extracts, corresponding to the three blocks of trees, were obtained for each treatment in all analyses. Within each block of trees, three mature leaves per tree were sampled and pulled together for each extract. Leaves were rinsed once with 0.1 % Tween 20 and three times in distilled water.

Chlorophylls and carotenoids were quantified as described by Lichtenthaler and Buschmann (2001). Lipid peroxidation was determined by measuring the malondialdehyde (MDA) content as described by Heath and Packer (1968). Peroxidase activity was determined using the guaiacol oxidation method as described by Bajji et al. (1998). One hundred mg of fresh leaf tissue was used in each independent extraction. Proline was determined as described by Bates et al. (1973). The leaf samples were oven dried at 60 °C during 48 h, and a 0.2 g finely ground aliquots were used for each extraction.

Rubisco carboxylase activity was determined as described by Lorimer et al. (1977). Rubisco was extracted from 1 g of fresh leaf tissue as described in Peñarrubia and Moreno (1988). Briefly, the leaves were homogenized in 10 ml extraction buffer (100 mM Tris pH 8.0, 10 mM  $MgSO_4$ , 20 mM 2-mercaptoethanol) and filtrated through 2 layers of Miracloth. PVP was added (2%) and after 10 min stirring, samples were centrifuged at 8000 xg for 20 min. The supernatant was used for Rubisco assay. Rubisco (20  $\mu$ L) was activated in buffer A (100 mM Tris pH 8.2, 20 mM  $MgCl_2$ , 10 mM  $NaHCO_3$ , 1 mM 2-mercaptoethanol) at 30 °C for 10 min, and the carboxylation reaction was assayed with 110 mM  $NaH^{14}CO_3$  in buffer A for 1 min. The reaction was stopped by adding 50  $\mu$ L of 2M HCl. Non



incorporated  $^{14}\text{CO}_2$  was eliminated at vacuum at 60 °C for 16 h. Radioactivity was then measured in a liquid scintillation counter (1214 Rackbeta; LKB). The amounts of Rubisco were determined by immunoblotting using rabbit polyclonal antibodies against the purified large subunit of *Euglena* Rubisco. Soluble protein content in the extracts was determined as described by Bradford (1976).

#### Defoliation and bud sprouting

Defoliation was measured after 30 and 60 days of drought stress. Four branches were randomly selected and tagged in each tree. The number of leaves per branch was counted and the results were expressed as percentages of defoliation.

Bud sprouting and flowering induction by drought was measured 30 days after irrigation was restarted. The results were expressed as the number of new formed shoots (vegetative or inflorescences) per 100 nodes.

#### Statistical analyses

Results were subjected to an analysis of variance (SPSS for Windows version 12.0.1, Illinois, USA). Bud sprouting and flower formation data were transformed as the arc sin of the square root. Mean comparisons were performed with Tukey's test.

Bivariate and partial correlations between photosynthetic parameters were performed using SPSS.

## Results

#### Plant growth and defoliation

Well watered control plants exhibited vigorous growth and scarce defoliation (Fig. 1) during the experimental period. In the stressed plants, growth ceased due to the abscission of the apical meristems. Moreover, defoliation was high in both species, and mainly occurred during the first month of stress. Leaf abscission increased with the intensity of the stress (Fig. 1). Fino lemon was more sensitive to severe stress (-3 MPa) (Fig. 1A) than Owari satsuma (Fig. 1B), and after 60 days of stress showed a higher proportion of leaf abscission (63 vs 48%, respectively;  $p < 0.05$ ).

## Photosynthesis

Net CO<sub>2</sub> fixation rate ( $A_N$ ) varied during the experimental period in well watered plants due to day to day changes in the light and temperature conditions of the greenhouse. Both in Owari satsuma and in Fino lemon,  $A_N$  was directly related both to PAR (in the range 70-700  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ;  $R^2 = 0.64$  and  $0.70$ , respectively) and to temperature (in the range 20-32 °C;  $R^2 = 0.35$  and  $0.40$ , respectively). In the unstressed plants, the average  $A_N$ ,  $g_s$  and  $E$  values during the experiment were higher in Owari satsuma than in Fino lemon (Fig. 2). Water stress reduced ( $p < 0.05$ ) the photosynthetic rate, stomatal conductance, transpiration and substomatal CO<sub>2</sub> concentration both in Fino lemon (Fig. 2A) and Owari satsuma (Fig. 2B). Compared to controls, a water potential of -2 Mpa at predawn resulted in a 20 % reduction in photosynthetic rate in Fino lemon and 30% in Owari satsuma. At a  $\Psi_w$  of -3Mpa, the reduction increased to 40 % and 50% in lemon and satsuma trees, respectively. This additional loss in  $A_N$  caused by a lower water potential, between moderate and severe stress, did not reached statistical significance. Overall photosynthesis and transpiration were correlated to stomatal conductance ( $R^2 = 0.70$  to  $0.76$ ;  $p < 0.05$ ). At intermediate  $g_s$  values, corresponding to the moderate stress, transpiration was more affected than photosynthesis by the closure of the stomata, leading to an increase of the water use efficiency both in Fino lemon (140% compared to controls) and in Owari satsuma (150%). Water potential had no effect in the maximum fluorescence yield (Fig. 2A and 2B).

## Biochemical changes

Water stress had no effect on the chlorophyll and carotenoid content in the leaves (Table 1) but in Owari satsuma, resulted in an increase ( $p < 0.05$ ) of the chlorophyll a/b ratio. The amount of total soluble protein, Rubisco content and activity did also not changed with the stress (Table 2 and Fig. 3). Water stress also had no effect on the activity of guaiacol peroxidase nor in MDA content (Table 3). Proline accumulated in the leaves in response to water stress (Table 3). In Fino lemon accumulation was the same at -2 and -3 MPa. In Owari satsuma the accumulation was observed at -3MPa, but not at -2 MPa (Table 3).

### Photosynthetic recovery after rewatering

The leaves of the stressed Fino lemon trees recovered the levels of  $A_N$  and  $g_s$  of the unstressed controls within 24 h upon rewatering (Fig. 4). In Owari satsuma, the recovery of the  $CO_2$  net exchange values also had occurred within 24 h upon rewatering, but the values of  $g_s$  only equaled those of the controls after 48 h (Fig. 4).

### Bud sprouting and flowering

Water stress induced bud sprouting in both species (Table 4). Bud sprouting and flower formation occurred between 15 and 30 days after rewatering the plants. In Fino lemon, drought intensity had no effect on flowering but in vegetative shoot formation ( $p < 0.05$ ; Table 4). Nevertheless, in Owari satsuma, mainly vegetative shoots were formed after moderate stress and inflorescences after severe stress.

## Discussion

Although the majority of commercially grown citrus is irrigated, most production areas in the world have climatic conditions leading to drought periods during the production cycle. Future climate changes may lead to increased intensity or duration of these periods (IPCC 2007; Sarris et al. 2007). Water deficit affects citrus vegetative growth as well as fruit yield and quality (Spiegel-Roy and Goldschmidt 1996). Bud sprouting and flowering is promoted by water deficit stress in several citrus species (Nir et al. 1972; Southwick and Davenport 1986; Chaykiattiyos et al. 1994). This positive effect has been reported as proportional to the duration of the drought period, and in some cases to the intensity of the stress. In this work, bud sprouting and flowering was increased by water stress. However, a different response was observed in both studied species, probably due to its origin and natural adaptation to the environment (Davies and Albrigo 1994). Flowering (Vu and Yelenosky 1988; Gómez-Cadenas et al. 1996; Pérez-Pérez et al. 2007) was induced in Owari satsuma by water stress, meanwhile in Fino lemon flowers were only developed after a severe stress period.

It has been described that leaves are injured in water stressed *Citrus* trees but do not abscise until water stress is released (Gómez-Cadenas et al. 1996). The transport of ACC and ABA from the roots to the shoots after rehydration, and subsequent synthesis of ethylene in leaves, would promote leaf abscission. We reported significant levels of defoliation in both studied species and mainly during the first 30 days of experiment. No additional leaf losses were reported when trees were rewatered after the drought periods, and thus the ABA from the roots would be not necessary for leaf abscission. The water stress-induced leaf abscission is one of the most important drought resistance mechanism developed by plants, by reducing the transpiring surface and prevents dehydration (Addicott 1982). Fino lemon showed higher defoliation at severe stress levels, probably indicating a better tolerance to extreme drought.

Fruit set and development in *Citrus* mainly depends on carbohydrate availability. After reserves have been depleted during bud sprouting and flowering, fruit development is supported by current photosynthesis (Bustan and Goldschmidt 1998). Although flower and vegetative shoot formation was induced by a drought period in both studied species, the effect of water stress on the photosynthetic integrity and recovery after rehydration has to be assessed in order to improve resource efficiency. It is well known that one of the primary physiological targets of drought is photosynthesis. The effects can be direct, as the decreased CO<sub>2</sub> availability caused by diffusion limitations, and alterations of photosynthetic metabolism, or secondary effects, namely oxidative stress (Chaves et al. 2009).

*Citrus* species showed a marked anisohydric behavior, with progressive decreases in leaf water potential as water stress intensified (Syvertsen and Lloyd 1994). The decrease in photosynthetic rate and stomatal conductance with drought intensity was higher in Owari satsuma than in Fino lemon. This increased tolerance could be related to the osmotic adjustment. Fino lemon leaves accumulated proline even at moderate stress levels. Compared to controls, stomatal conductance was more sensitive to water stress than photosynthesis, leading to an increase in water use efficiency. Improving water use efficiency is one of the main targets of crop research for Mediterranean environments (Reynolds and Tuberosa 2008). Regulated deficit strategies are potential ways to increase water savings in agriculture

by allowing crops to withstand water stress with no or only marginal decreases of yield and quality (Costa et al. 2007). The control of stomatal conductance under controlled irrigation may be a good alternative to improve water use efficiency, taking into account that the sensitivity of yield to water stress in *Citrus* is dependent on the phenological phase in which water stress is applied (Castel and Buj 1990; Domingo et al. 1996).

It is commonly accepted that diffusion limitations, mainly on stomatal and mesophyll conductances, predominate under most water stress situations (Flexas et al. 2004; Galmés et al. 2007). A general failure of metabolism only occurs when daily maximum stomatal conductance drops below  $0.1 \text{ mol H}_2\text{O} / \text{m}^2 \text{ s}$ . Pérez-Pérez et al. (2007) stated that the decrease in  $A_N$  in *Citrus* during drought stress is mainly due to non stomatal limitations, based on calculated  $C_i$  values. Nevertheless, and taking with caution the interpretations when using this parameter (Syvertsen and Lloyd 1994), we reported a significant decrease in  $C_i$  as net  $\text{CO}_2$  fixation decreased, suggesting that stomatal limitations are important in the response to water stress.

Metabolic impairments may also affect photosynthesis, especially under severe stress (Galle et al. 2009). Inhibition of Rubisco activity as response to water stress was reported in Valencia orange by losses in activation state and amount of the enzyme (Vu and Yelenosky 1988). Nevertheless, water stress did not changed total activated Rubisco activity and the amount of Rubisco in Fino lemon and Owari satsuma. In addition, no degradation of total soluble proteins was reported, and no changes in chlorophyll amounts were detected. However, Owari satsuma showed increased chlorophyll a/b ratio with drought, indicating changes in the distribution of chlorophylls to avoid photosynthetic apparatus damage. As reported in this study, small decreases in the chlorophyll fluorescence parameter  $F_v/F_m$  are described in several species in response to water stress (Valladares et al. 2005), indicating only a minor irreversible damage of the photosynthetic machinery during drought. When subjected to water stress the equilibrium in the reactive oxygen species production and removal can be broken, resulting in oxidative damage to proteins, nucleic acids and lipids. However, no MDA accumulation was produced and no changes in the activity of guaiacol peroxidase were observed in our study. Although other

antioxidant systems may be working, these results could indicate that marginal oxidative stress is occurring under water stress in *Citrus*, and in fact excessive light and temperature, usually associated with drought, are responsible for photoinhibition and photo-oxidation processes.

The photosynthetic recovery after a mild water stress, with  $g_s$  above  $0,15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , is usually rapid and almost complete (Vu and Yelenosky 1988; Gómez-Cadenas et al. 1996; Pérez-Pérez et al. 2007). In contrast, the recovery after severe stress is progressive, slow and sometimes incomplete (Miyashita et al. 2005; Flexas et al. 2006). A rapid recovery of net photosynthesis and leaf water potential was observed in our study even after severe water stress conditions. Stomatal conductance reached similar values than controls by 24 h in Fino lemon. Three days were needed in Owari satsuma, allowing the plants to limit water losses via transpiration. This may reflect different adaptation strategies to drought periods or sensitivity to the stress conditions.

### **Conclusions**

Both studied *Citrus* species showed similar photosynthetic responses to the water stress conditions. Water stress per se did not provoked important changes in the integrity of the photosynthetic apparatus as inferred from fluorescence and biochemical parameters. Furthermore, the rapid recovery of stomatal conductance and net  $\text{CO}_2$  fixation rates in Fino lemon suggested less biochemical limitations and an important role of stomatal closure under severe water stress. A marked abscission of the older leaves in order to reduce water loss by transpiration may be added to the tolerance responses to drought in this species. Based on the slower recovery of the stomatal conductance, Owari satsuma may present some metabolic limitations. In conclusion, a drought period induced vegetative sprouting and flowering in citrus, without damaging the photosynthetic apparatus, and thus allowing a rapid recovery of the plant and fruit growth after rewatering.

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## References

- Addicott FT (1982) Abscission. University of California Press, Berkeley
- Bajji M, Kinet JM, Lutts S (1998) Salt stress effects on roots and leaves of *Atriplex halimus* L. and their corresponding callus. *Plant Sci* 137: 131-142
- Barbera G, Fatta-del-Bosco G, Lo-Cascio B (1985) Effect of water stress on lemon summer Bloom: the Forzatura technique in the Sicilian citrus industry. *Acta Hort* 171: 391-397
- Bates, LS (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39, 205-207
- Bota J, Medrano H, Flexas J (2004) Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? *New Phytol* 162: 671-681
- Boyer JS (1976) Photosynthesis at low water potentials. *Phil Trans R Soc London B*273: 163-168
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254
- Bustan A, Goldschmidt EE (1998) Estimating the cost of flowering in a grapefruit tree. *Plant Cell Environ* 21: 217-224
- Cassin J, Bourdeaut A, Fougue V, Furon V, Gaillard JP, LeBourdelle J, Montagut G, Moreuil C (1969) The influence of climate upon blooming of *Citrus* in tropical areas. *Proc Int Soc Citr* 1: 315-323
- Castel JR, Buj A (1990) Response of Salustiana oranges to high frequency deficit irrigation. *Irrigation Sci* 11: 121-127
- Chaikiatitoyos S, Menzel CM, Rasmussen TS (1994) Floral induction in tropical fruit trees: Effects of temperature and water supply. *J Hort* 69: 397-415
- Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann Bot* 103: 551-560
- Costa JM, Ortuño MF, Chaves M (2007) Deficit irrigation as a strategy to save water: Physiology and potential application to horticulture. *J Integr Plant Biol* 49: 1421-1434

- Davenport TL (1990) Citrus flowering. *Hortic Rev* 12: 249-408
- Davies FS, Albrigo LG (1994) Citrus. CAB International. Wallingford, UK, pp 126-134
- Domingo R, Ruiz-Sánchez MC, Sánchez-Blanco MJ, Torrecillas A (1996) Water relations, growth and yield of Fino lemon trees under regulated deficit irrigation. *Irrig Sci* 16: 115-123
- Ennhali S, Earl HJ (2005) Physiological limitations to photosynthetic carbon assimilation in cotton under water stress. *Crop Sci* 45: 2374-2382
- Ephrath JE, Hesketh JD (1991) The effects of drought stress on leaf elongation, photosynthetic and transpiration rates in maize (*Zea mays* L.) leaves. *Photosynthetica* 25: 607-619
- Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biol* 6: 269-279
- Flexas J, Bota J, Galmés J, Medrano H, Ribas-Carbó M (2006) Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiol Plantarum* 127: 343-352
- Galle A, Florez-Sarasa I, Tomas M, Pou A, Medrano H, Ribas-Carbó M, Flexas J (2009) The role of mesophyll conductance during water stress and recovery in tobacco (*Nicotiana sylvestris*): acclimation or limitation? *J Exp Bot* 60: 2379-2390
- Galmés J, Medrano H, Flexas J (2007) Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New Phytol* 175: 81-93
- Genty B, Briantais JM, Baker NR (1989) The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* 990: 87-92
- Gómez-Cadenas A, Tadeo FR, Talon 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
- Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125: 189-190
- Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil. *Calif Agric Exp Sta circular* 347



- IPCC (2001) Climate change 2001. In: Houghton JT (ed) The scientific basis. Cambridge University Press, Cambridge
- Lawlor DW (1995) The effects of water deficit on photosynthesis. In: Smirnov N (ed) Environment and plant metabolism. Bios Scientific Publishers, Oxford, pp 129-160
- Lichtenthaler HK, Buschmann C (2001) Current Protocols in Food analytical Chemistry, F4.2.1 and F4.3.1, John Wiley and Sons, Inc, NJ
- Lorimer GH, Badger MR, Andrews TJ (1977) D-Ribulose-1,5-bisphosphate carboxylase-oxygenase. Improved methods for activation and assay of catalytic activities. *Anal Biochem* 78: 66-75
- Miyashita K, Tanakamaru S, Maitani T, Kimura K (2005) Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Envir Exp Bot* 53: 205-214
- Nir I, Leshem B, Goren R (1972) Effects of water stress, gibberellic acid and 2-chloroethylammoniumchloride (CCC) on flower differentiation in Eureka lemon trees. *J Amer Soc Hortic Sci* 97: 774-778
- Peñarrubia L, Moreno J (1988) Ribulose 1,5-bisphosphate carboxylase oxygenase from citrus leaves. *Phytochem* 27: 1999-2004
- Pérez-Pérez JG, Syvertsen JP, Botía P, García-Sánchez F (2007) Leaf water relations and net gas exchange responses of salinized carrizo citrange seedlings during drought stress and recovery. *Ann Bot* 100: 335-345
- Reynolds M, Tuberosa R (2008) Translational research impacting on crop productivity in drought-prone environments. *Curr Opin Plant Biol* 11: 171-179
- Sarris D, Christodoulakis D, Körner C (2007) Recent decline in precipitation and tree growth in the eastern Mediterranean. *Glob Change Biol* 13: 1187-1200
- Sharkey TD (1990) Water-stress effects on photosynthesis. *Photosynthetica* 24: 651-651
- Shinozaki K (2003) Drought stress. In: Thomas B (ed) Encyclopedia of applied plant sciences. Elsevier Acad. Press, Oxford, UK, pp 1471
- Southwick SM, Davenport TL (1986) Characterization of water stress and low temperature effects on flower induction in *Citrus*. *Plant Physiol* 81: 26-29

- Spiegel-Roy P, Goldschmidt EE (1996) *Biology of Citrus*. Cambridge University Press, Cambridge, pp 131-136
- Syvetsen JP, Lloyd J (1994) Citrus. In: Schaffer BA, Andersen PC (eds) *Handbook of environmental physiology of fruit crops*. Vol II Subtropical and tropical crops,. CRC Press, Boca Raton, Florida, pp 65-99
- Valladares F, Arrieta S, Aranda I, Lorenzo D, Sánchez-Gómez D, Tena D, Suarez F, Pardos JA (2005) Shade tolerance, photoinhibition sensitivity and phenotypic plasticity of *Illex aquifolium* in continental Mediterranean sites. *Tree Physiol* 25: 1041-1052
- Vu JCV, Yelenosky G (1988) Solar irradiance and drought stress effects on the activity and concentration of ribulose biphosphate carboxylase in ‘Valencia’ orange leaves. *Isr J Bot* 37: 245-256
- Xu ZZ, Zhou GS (2007) Photosynthetic recovery of a perennial grass *Leymun chinensis* after different periods of soil drought. *Plant Prod Sci* 10: 277-285
- Zhu JK (2002) Salt and drought stress signal transduction in plants. *Ann Rev Plant Biol* 53: 247-273

## Legends for figures

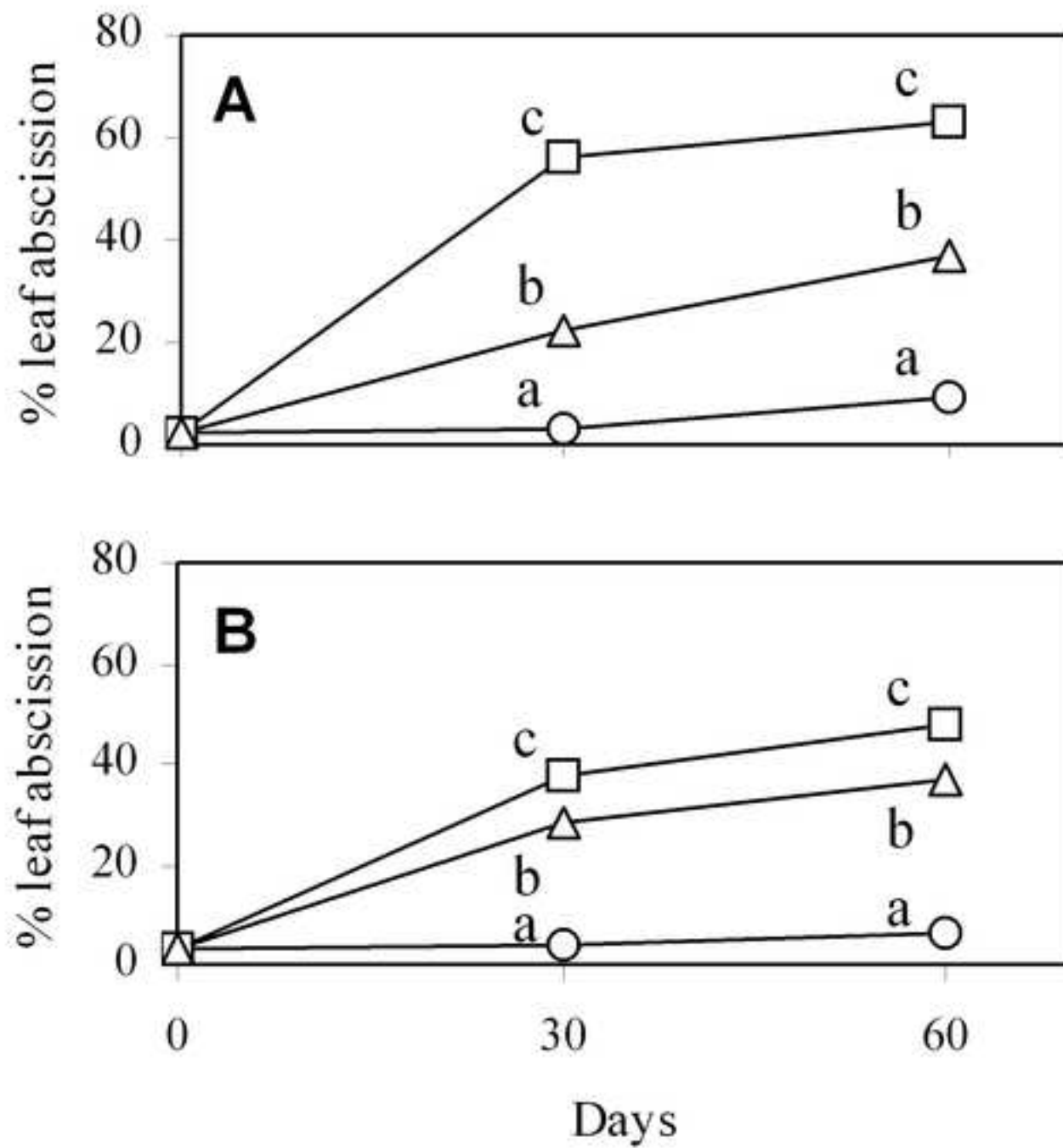
**Fig. 1** The effect of the duration (30 and 60 days) and intensity of water stress on defoliation in Fino lemon (A) and Owari satsuma (B). Each value is the mean of nine trees. In each date, different letters indicates statistical differences among treatments ( $p < 0.05$ ). Controls: ○; moderate stress (- 2 MPa): Δ; severe stress (-3MPa): □.

**Fig. 2** The effect of water stress on net CO<sub>2</sub> fixation ( $A_N$ ;  $\mu\text{mol} / \text{m}^2 \text{ s}$ ), stomatal conductance ( $g_s$ ;  $\text{mol} / \text{m}^2 \text{ s}$ ), transpiration ( $E$ ;  $\text{mmol} / \text{m}^2 \text{ s}$ ), substomatal CO<sub>2</sub> concentration ( $C_i$ ;  $\mu\text{mol} / \text{mol}$ ), WUE and maximum fluorescence yield ( $F_v/F_m$ ), in Fino lemon (A) and Owari satsuma (B) trees. Values are the averages of 8 measurements performed on 8 plants from day 5 to 55 (11 days of measurements). Results are presented as percentage of the values found in the non stressed controls (open bars). The absolute values for the controls are also shown. Different letters indicate significant differences ( $p < 0.05$ ). No letter indicates no differences.

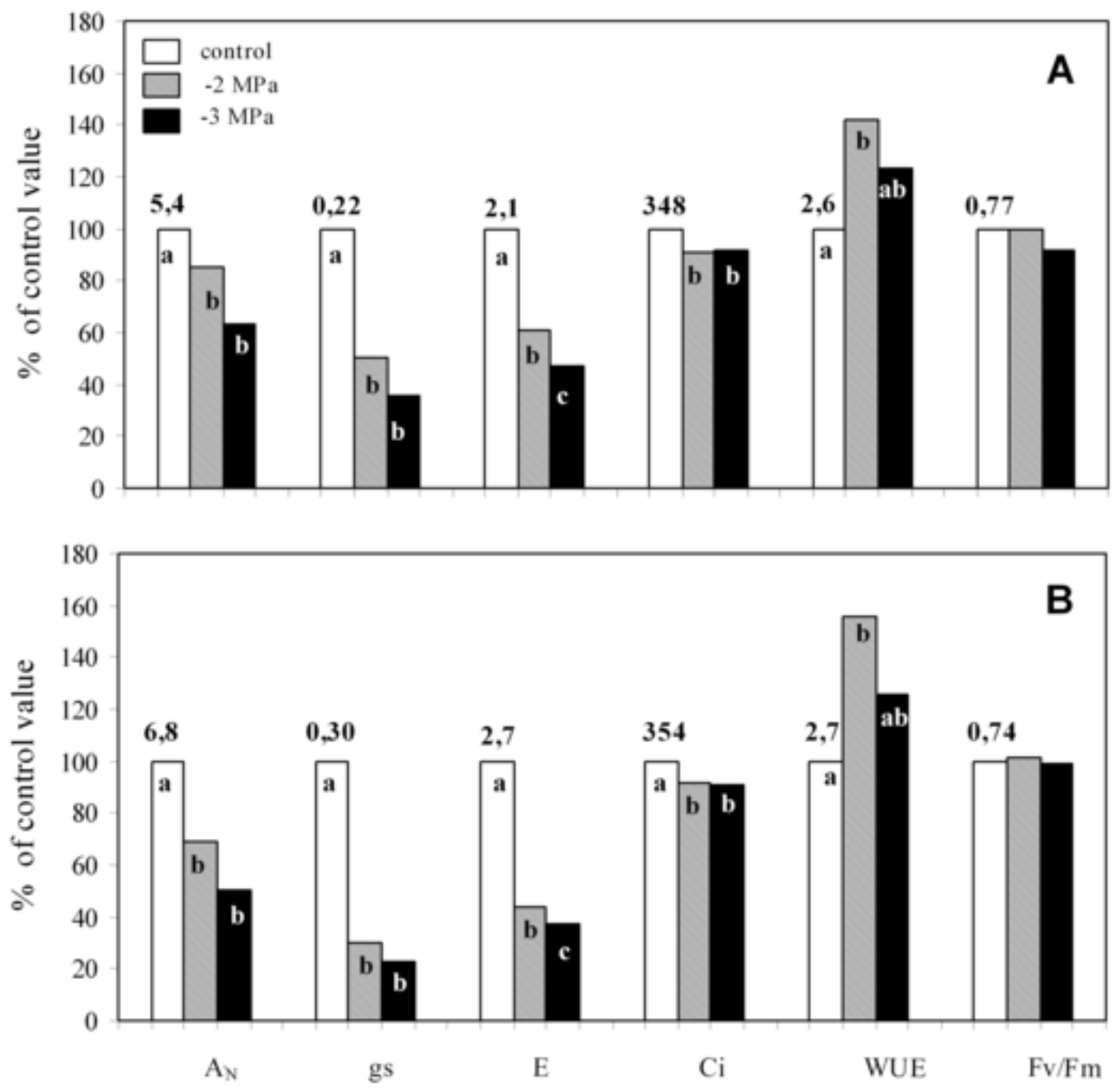
**Fig. 3** SDS-PAGE (A,B) and immunoblot (C;D) analysis of the Rubisco protein in water stressed Fino lemon and Owari satsuma trees. Lanes 1-3: controls; lanes 4-6: -2MPa; lanes 7-9: -3 MPa. rbsL and rbsS: Large and small subunits of Rubisco protein, respectively.

**Fig. 4** Photosynthetic rate ( $A_N$ ,  $\mu\text{mol} / \text{m}^2 \text{ s}$ ) (A,B) and stomatal conductance ( $g_s$ ,  $\text{mol} / \text{m}^2 \text{ s}$ ) (C,D) recovery in water stressed plants of Fino lemon and Owari satsuma after fully rewatering. Values are the mean of eight to ten determinations. Controls: ○; moderate stress (-2 MPa): Δ; severe stress (-3 MPa): □.

**Figure 1**  
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**Figure 2**  
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**Figure 3**  
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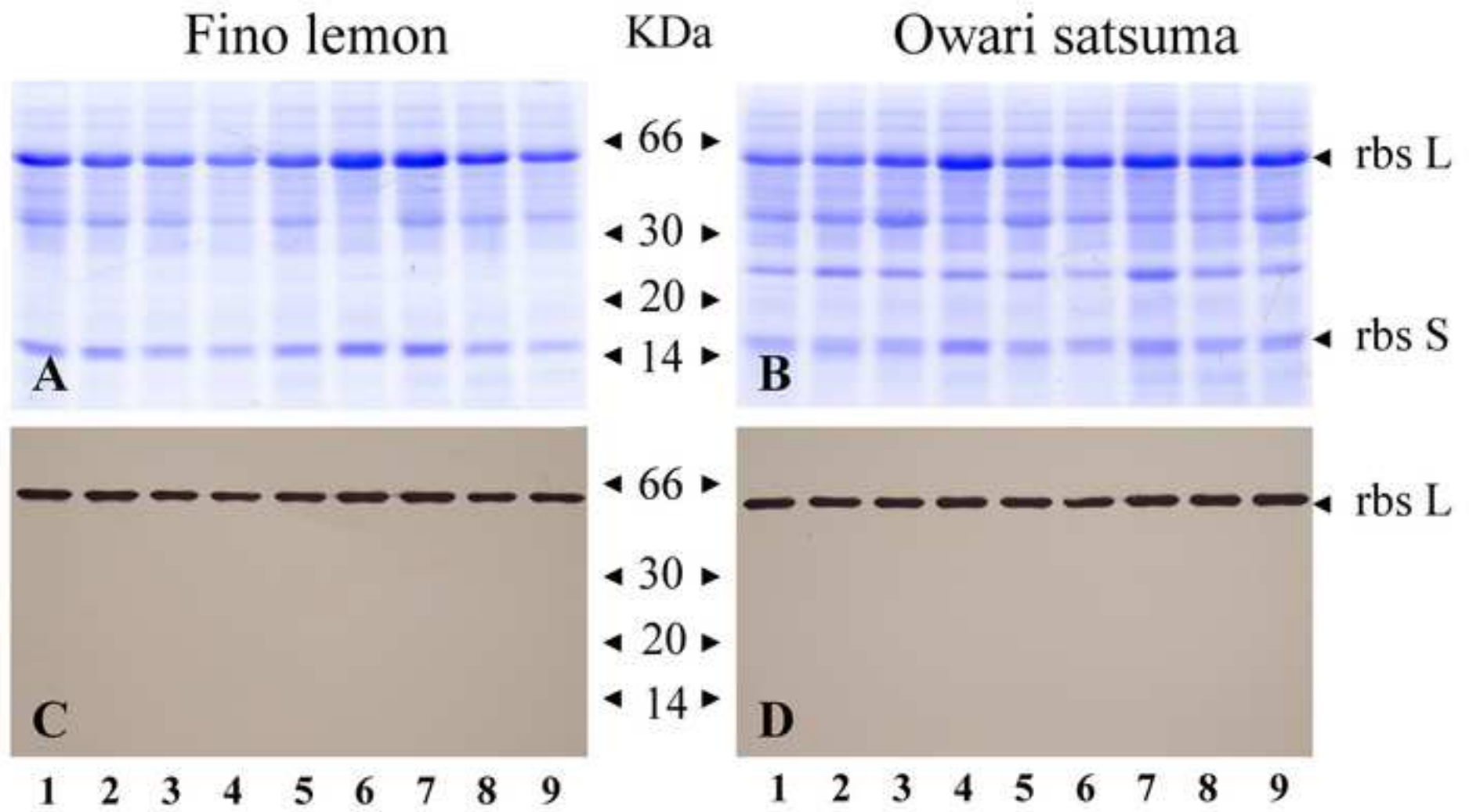
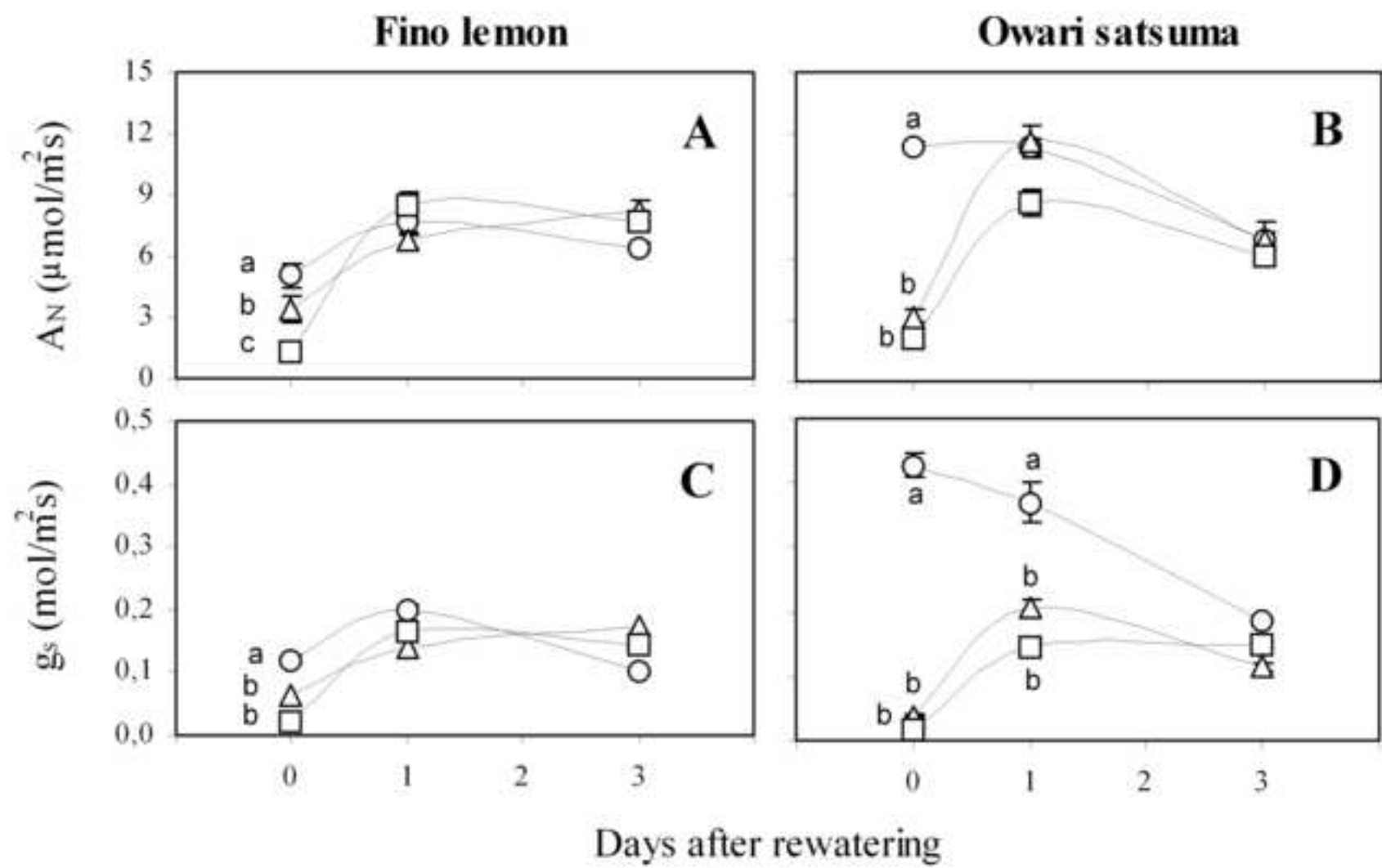


Figure 4  
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**Table 1** The effect of water stress on the concentration of photosynthetic pigments (mg/g FW) in Fino lemon and Owari satsuma leaves. Leaves were sampled after 45 days of water stress at -2 or -3 MPa at predawn. Each value is the mean of three determinations. C<sub>a</sub>: chlorophyll a; C<sub>b</sub>: chlorophyll b; C<sub>a+b</sub>: total chlorophylls; a/b: C<sub>a</sub>/C<sub>b</sub>; C<sub>x+c</sub>: carotenoids (xanthophylls + carotenes).

Treatment	Fino lemon					Owari satsuma				
	C <sub>a</sub>	C <sub>b</sub>	C <sub>a+b</sub>	a/b	C <sub>x+c</sub>	C <sub>a</sub>	C <sub>b</sub>	C <sub>a+b</sub>	a/b	C <sub>x+c</sub>
Control	1.5	0.6	2.1	2.4	0.9	1.4	0.7	2.1	2.0 a	0.7
-2 MPa	1.5	0.6	2.1	2.5	0.8	1.1	0.5	1.6	2.3 b	0.7
-3 MPa	1.2	0.5	1.8	2.4	0.7	1.2	0.5	1.7	2.6 c	0.6
SE	0.2	0.1	0.3	0.1	0.1	0.2	0.1	0.3	0.1	0.1

Values within a column with different letters are statistically different ( $P < 0.05$ ). No letter indicates no differences.



**Table 2** The effect of water stress on soluble protein content (SP, mg/ g FW), Rubisco protein content (mg/ g FW) and Rubisco activity ( $\mu\text{mol CO}_2/\text{min mg}$  Rubisco) in Fino lemon and Owari satsuma leaves. Leaves were sampled after 45 days of stress. Each vale is the mean of three determinations.

Treatment	Fino lemon			Owari satsuma		
	SP	Rubisco content	Rubisco activity	SP	Rubisco content	Rubisco activity
Control	14.5	12.3	0.20	20.3	16.7	0.16
-2 MPa	15.7	14.0	0.20	23.9	16.1	0.17
-3 MPa	18.7	16.2	0.15	21.3	17.4	0.17
SE	1.9	1.9	0.02	1.9	0.5	0.01
Significance (F test)	0.40	0.46	0.10	0.30	0.61	0.43

Values within a column with different letters are statistically different ( $P < 0.05$ ).

No letter indicates no differences.

**Table 3** The effect of water stress on malonyl dialdehyde (MDA, nmol/g FW) and proline content (mg/ g DW), and guaiacol peroxidase activity (GUPX,  $\mu\text{mol}/\text{min g FW}$ ) in Fino lemon and Owari satsuma leaves. Leaves were sampled after 50 days of stress. Each vale is the mean of three determinations.

Treatment	Fino lemon			Owari satsuma		
	MDA	GUPX	Proline	MDA	GUPX	Proline
Control	61	30	9 a	78	17	15 a
-2 MPa	55	32	18 b	68	20	16 a
-3 MPa	66	28	19 b	78	21	30 b
SE	7	4	2	6	5	2
Significance ( F test)	0.54	0.78	0.01	0.10	0.83	0.000

Values within a column with different letters are statistically different ( $P < 0.05$ ). No letter indicates no differences.

**Table 4** The effect of water stress on bud sprouting in Fino lemon and Owari satsuma. The number of formed shoots (vegetative, inflorescences and total shoots) per 100 nodes is presented. Each value is the mean of seven trees.

Treatment	Fino lemon			Owari satsuma		
	Vegetative	Inflorescences	Total	Vegetative	Inflorescences	Total
Control	1 a	0 a	1 a	2 a	0 a	2 a
-2 MPa	16 b	6 b	22 b	23 b	27 b	50 b
-3 MPa	6 c	20 c	26 b	36 b	23 b	58 b

Values within a column with different letters are statistically different ( $P < 0.05$ ).