

Differential Responses in Growth, Physiological Processes and Peroxidase Activity of Young Mango (*Mangifera indica*) and Citrus (*Citrus sinensis* L) Plants to Water Deficit

¹MOHD RAZI ISMAIL, ²ABD GHANI MUHAMMAD & ¹ISMAIL IBERAHIM

¹Department of Crop Science

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

²MARDI Research Station Jeram Pasu, 16800 Pasir Putih, Kelantan

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ABSTRAK

Pengaruh tegasan air terhadap pertumbuhan, proses fisiologi, aktiviti prolina dan peroksida telah dikaji pada tanaman mangga (*Mangifera indica*) dan limau (*Citrus sinensis*) didalam rumah tanaman. Dalam keadaan pengurangan air secara berperingkat, pokok mangga dan limau menunjukkan perbezaan dalam potensi air, konduksi stomata, perkembangan daun dan aktiviti peroksida. Perubahan stomata tidak bergantung pada perubahan potensi air daun pada kedua-dua tanaman apabila tegasan air berlangsung. Pengurangan konduksi stomata pada pokok mangga adalah lebih tinggi dibandingkan dengan limau, menunjukkan bahawa limau berupaya mengawal kehilangan air terhadap perubahan kedapataan air. Aktiviti peroksida menunjukkan peningkatan bererti dalam keadaan pengurangan air tanah bagi tanaman limau. Terdapat peningkatan di antara 6 hingga 10 kali ganda kandungan prolina bagi tanaman mangga dan limau apabila pokok didedahkan kepada tegasan air. Peningkatan prolina yang ketara bagi tanaman limau berbanding mangga menunjukkan ketahanan tegasan air yang tinggi tanaman limau berbanding mangga. Bukti ini diperkukuhkan dengan pemulihan semula yang cepat selepas pemberian air kepada pokok limau dibandingkan pokok mangga melibatkan regenerasi pucuk baru yang cepat.

ABSTRACT

The effects of water deficit on growth, plant physiological processes and peroxidase activity were studied for young mango (*Mangifera indica*) and citrus (*Citrus sinensis* L) plants in the greenhouse. Under gradually decreasing soil moisture content, mango and citrus differed in their leaf water potential, stomatal conductance, leaf growth and peroxidase activity. Stomata of both plants responded independently to the changes in leaf water potential as soil drying progressed. The reduction in stomatal conductance in mango was greater than citrus suggesting that citrus was able to control water loss better than mango to the changing condition of water availability in the root zone. Peroxidase activity increased significantly in water stressed citrus plants. There was a 6-10 fold increase in proline content when both species were exposed to water stress. Citrus plants accumulated higher proline levels suggesting that they can tolerate water stress compared to mango. This was also evident by a faster recovery after rewatering in citrus compared to mango plants that involved regeneration of new shoots.

INTRODUCTION

Water deficit is one of the environmentally limiting factors for fruit tree establishment in the field from the nursery. Roots of young fruit trees are often not fully developed at transplanting so a small degree of water deficit can cause plant water stress and mortality. Although Malaysia is tropical and characterized by adequate sources of water, the incidence of

water shortage occurs from time to time in some of the agricultural production areas. The problem of plant mortality is often serious in conditions where cultivation is to be done in undulating areas where there are difficulties in installing a proper irrigation system.

Plants usually developed physiological responses as well as ecological strategies to cope with water stress. These responses allow them to

survive and to sustain some growth under adverse conditions. Plants' responses depend on the species differences, nature of water shortage inducing physiological responses to short term changes, acclimation to a certain level of water availability and adaptation to drought (Jones 1983; Ruiz-Sanchez *et al.* 2000). Plant adaptability to localized water deficit has been attributed mainly to maintenance of water status by utilizing available soil and by chemical signaling. Various metabolites and chemicals have been observed to accumulate in plant tissues during water and salt stress and contribute to osmotic adjustment. Some species that adapt to mild and/or moderate drought stress exhibit increases in activities of antioxidant enzymes such as super oxide dismutase, catalase and peroxidase (Fu and Huang 2001). Although the concentration of some amino acids increase during water stress, there is general agreement that proline concentration is profound (Kohl *et al.* 1991; Albernethy *et al.* 1998). The information gathered on drought resistance mechanism makes it easier to plan for crop zoning based on the water availability and to designed irrigation strategies for optimizing water used.

Mango and citrus are two of the fifteen fruit species that had been identified for future development as stated in the Third National Agricultural Policy 1998-2010 (Ministry of Agriculture Malaysia 1999). The area of cultivation is to be expanded and these crops will be cultivated on a large commercial scale throughout Malaysia. The aim of this study was to determine the growth and physiological responses of young mango and citrus plants exposed to water deficit, as well as to improve understanding on the drought tolerance mechanism involved in the responses of these fruit trees to water deficit. The role of proline and peroxidase in drought tolerance mechanism in both species was determined. The response of plant on recovery to water stress was assessed by the ability of plants to rejuvenate their vegetative stage.

MATERIAL AND METHODS

Eighteen months mango (*Mangifera indica*) cultivar Chukanan and fourteen months citrus (*Citrus sinensis* L) cultivar Limau Madu were grown in plastic containers containing 40 kg of soil mixtures. The plants were grown in well-watered conditions for 6 weeks prior to water

stress treatments allowing roots to grow and become established in the bottom section of the containers. During this 42 -day period, plants were watered daily until water drained freely from the drainage holes at the bottom and fertilized weekly with full strength of Cooper Solution (Cooper 1973). The experiment was conducted at the Department of Crop Science, Universiti Putra Malaysia, Serdang, Selangor.

The experiment consisted of two soil moisture treatments; control (well watered) and water stress. In the well-watered control, plants were irrigated daily until water drained freely. In water stress treatments, the whole soil in the container was allowed to dry down by withholding irrigation. Each group of watering treatments consisted of twenty plants arranged in a completely randomized design with four replicates. At each harvest, 4 plants were sampled for destructive samplings of leaf water potential and peroxidase determination. Soil moisture content was measured with a time domain reflectometry technique (Topp *et al.* 1980). After the drying cycle was completed, plants grown under water stress condition were irrigated to observe the potential of recovery on both plants species.

Leaf length increment was determined on the leaves that were tagged before treatments began. The leaf length increments were calculated at each sampling date by measuring the differences between the length on the sampling date and the initial measurements expressed in rate of leaf increment per day. The differential recovery of mango and citrus were assessed by regeneration of new shoots from the plants.

The onset of responses of water deficit was observed by measuring leaf water potential at mid-day from one young fully expanded leaf per plant and four plant plants per treatment, using a pressure chamber, following the recommendation of Turner (1986). Leaf stomatal conductance and net photosynthetic rate were measured at mid-day for a similar number and type of leaves as for leaf water potential. Stomatal conductance was measured on the abaxial surface of leaf using a diffusive porometer (AP-4, Delta-T Devices Ltd, Cambridge, UK). The photosynthesis rate was determined using a LCA-3 portable infrared gas analyzer (LCA3, Analytical Development Co. Hoddesdon, UK).

Peroxidase activity in the leaf tissue was determined on the youngest fully-developed leaf

sampled from both plant species. 0.5 g of leaf tissue was homogenised with a mortar and pestle, using 1 ml cold 0.05M sodium acetate buffer (pH5.0). Five milligrams of polyvinylpyrrolidone (PVPP) was added to each sample in order to decrease interaction of phenol-proteins during extraction. The homogenate was centrifuged at 14000 Xg for 20 minutes at 4°C. The supernatant was used to determine the enzyme activities. Extraction of ionically bound peroxidase was performed by re-homogenizing the pellets from the above extraction with the same buffer containing 1M sodium chloride (NaCl). Samples were incubated at 4-5 °C for 24 h and centrifuged as described above. The peroxidase activities of both soluble and ionically bound supernatant were determined. A 20 ml sample of the supernatant was added to 3 ml of the assay mixture, which consisted of a solution of 0.1M sodium phosphate buffer (pH6.0), 1mM hydrogen peroxide (H₂O₂) and 0.1mM 4-methoxyphenol (guaiacol). The increase in absorbance density at 470nm was recorded with a spectrophotometer (Spectronoc 20 Genesys) and the enzyme was expressed as the change in absorbance per minute per gram fresh weight. Determination of free proline levels was based on the method described by Bates *et al.* (1973). Proline was extracted from liquid nitrogen – frozen tissue by homogenizing 5 g of the sampled leaves with 10 ml of 3% sulfosalicylic acid at 25 °C. The homogenate was filtered through Whatman No 2 filter paper. Two ml of the filtrate was reacted with two ml of glacial acetic acid and two-ml acid ninhydrin in a test tube for one hour in a water bath at 95 °C. The reaction mixture was then cooled in an ice bath. Following that, 4 ml of toluene was added to the reaction mixture and mixed rigorously with a test tube stirrer for 20 seconds. The toluene layer on the top, which has a pink-red color, was collected with a pipette. The absorbency of the toluene layer was read at 520 nm with a spectrophotometer using toluene as a blank. Standard curve was produced ranging 0 to 30 ug/ml of L-proline (Sigma chemical Co. St Louis, Mo.) dissolved in 3% sulfosalicylic acid. Proline standard curve was used to calculate proline concentration in sample on fresh weight basis.

RESULTS

Fig. 1 shows changes in soil moisture content and rate of leaf length increment as influenced

by withholding water for 15 days. Soil moisture declined from 0.26 to 0.12 g cm⁻³ by withholding water for 15 days in the containers. This reduction in soil moisture content had significantly reduced the rate of leaf length increment and plant physiological processes. Under well-watered conditions, leaf length increment was higher for mango compared to citrus plants (*Fig. 2*). The well-watered mango plants grew steadily for the first six days of plants in the treatments. Thereafter, leaf expansion declined or plateaued as the leaf reached its maximum growth. The rate of leaf increment of water-stressed plants declined progressively after day 3 of withholding water. The reduction in the rate of leaf length increment in water-stressed mango plants had resulted in a reduction of 8.84 cm in the final leaf length compared to well watered plants. In citrus, final leaf length was 2.1 cm shorter in water-stressed compared in the well watered plants. After rewatering, regeneration of shoots was faster in citrus than in mango plants (*Fig. 3*).

Fig. 4 shows changes in leaf water potential, stomatal conductance and photosynthetic rate of mango and citrus plants as influenced by water stress. Leaf water potential shows a significant difference between these two plant species. On the onset of water stress, leaf water potential of citrus declined rapidly compared to mango plants. Lower leaf water potential values in citrus were also observed for plants grown in well-watered conditions. The change in stomatal conductance during water stressed period followed quite a different pattern in comparison to the leaf water potential. This reduction in leaf water potential had caused similar reduction in stomatal conductance and leaf photosynthesis rate of plants exposed to water stress. The reduction in stomatal conductance was greater in mango than citrus plants. By day 6, stomatal conductance was reduced by 84% and 54 % in water stressed mango and citrus, respectively. Further exposure to water stress, both mango and citrus reached the same stomatal conductance values. The photosynthetic rate declined progressively with increased duration of water stress in both mango and citrus plants. There were significant reductions in the photosynthetic rates in both water-stressed mango and citrus plants when measurements were made on day 3 after withholding water. The results showed similar trends as observed in stomatal conductance where photosynthetic rate started to reach its

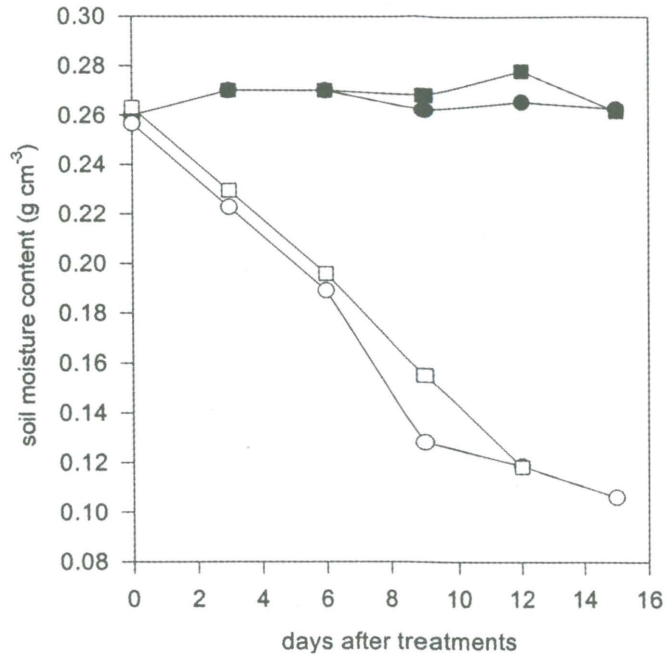


Fig. 1: Bulk soil water content in soil supporting plants under well-watered (closed symbol) and water stressed (open symbol) for mango (○) and citrus (□). Means of SE ± 3 replicates

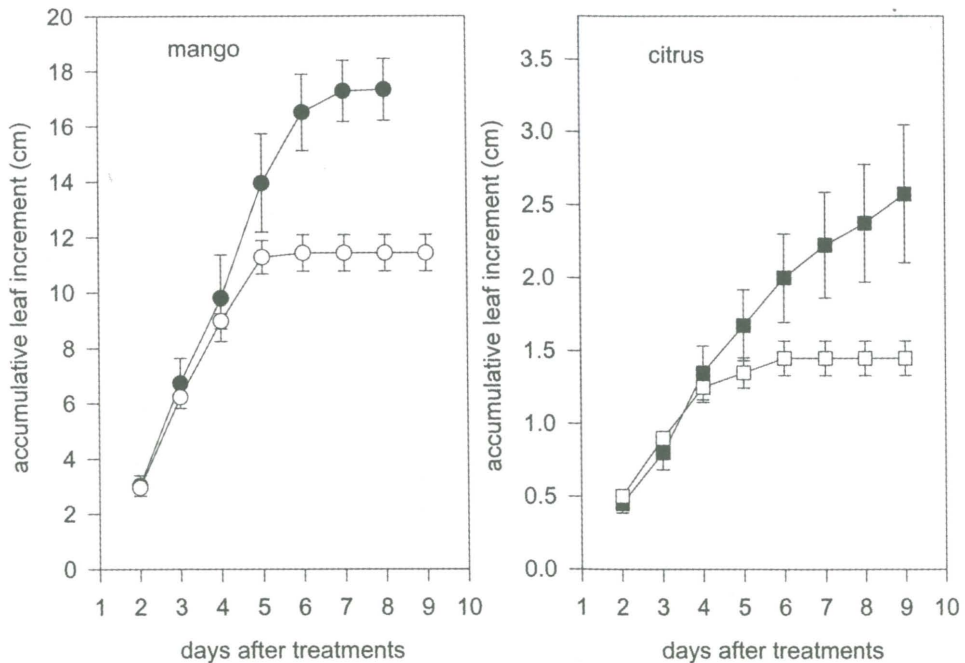


Fig. 2: Accumulative leaf length increment of mango (○) and citrus leaves (□) which were either well-watered (closed symbol) or water-stressed (open symbol). Bars represent ±SE. of five replicates

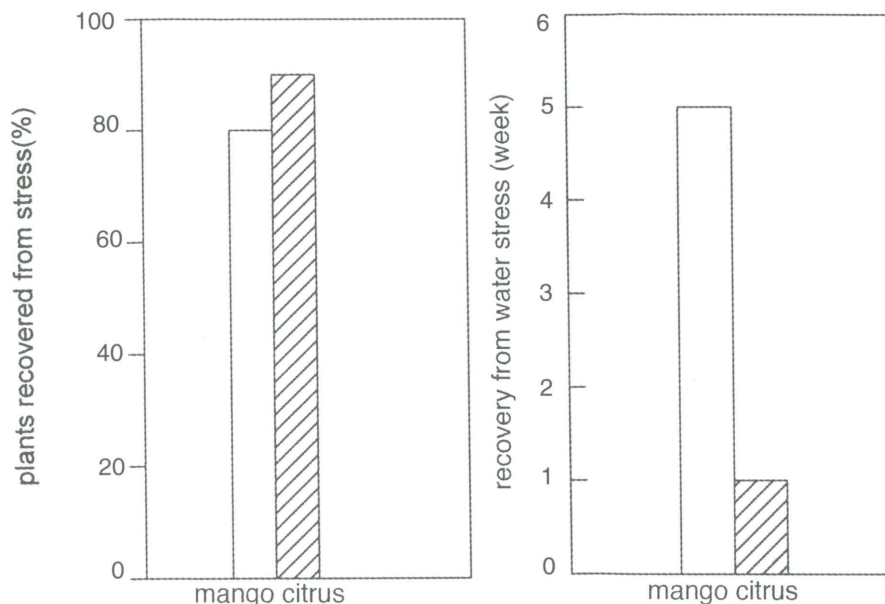


Fig. 3: Effects of re-watering of plants on percentage and duration for recovery in mango and citrus plants. Data was based on observation of plant numbers recovered from pre-stress treatment and no SE of mean presented

minimum values after 6 days of withholding water. Thereafter, the photosynthetic rate remained lower and reached the lowest values after prolonged exposure to water stress in both mango and citrus plants.

Measurements on peroxidase activity show significant higher values observed in water-stressed citrus compared to mango plants (Fig. 5). In both species, proline content increased with exposure to water stress. There were marked differences in proline content between citrus and mango in both well-watered and water-stressed conditions. Higher concentration of proline was observed in citrus compared to mango plants. There was a 6-10 fold increase in proline content when both species were subjected to water stressed conditions.

DISCUSSION

Leaf growth was very sensitive to the reduction in soil water availability. Rapid reduction in leaf increment suggests that available water is one of the major factors that determines growth of mango plants at establishment in the field. Nonami and Boyer (1989) suggest that leaf growth could be inhibited at low water potential despite complete maintenance of turgor in the growing region. However, the explanation for this situation of growth inhibition might be

metabolically regulated through osmotic adjustment or cell wall associated enzymes possibly serving an adequate role by restricting the development of transpiring surface areas. Nevertheless, leaf growth of these plants species is sensitive to soil drying. In retrospect, leaf growth can be influenced through the mechanism of non-hydraulic factor synthesis from the roots (Bacon *et al.* 1998) or inhibition of leaf growth as a result of decrease in cell wall extensibility (Lu and Newman 1998). The threshold value of around -1.20MPa and -0.95MPa can be observed below which leaf increment was almost zero in citrus and mango, respectively.

In this study, leaf water potential was used as an indicator for water stress in both plant species. The decline in water availability by withholding water had significantly reduced leaf water potential and stomatal conductance on both plant species. In the water-stressed plants, minimum leaf water potential at midday was around -2.5MPa and -2.0MPa for citrus plants, respectively. The values of leaf water potential measured here for stressed plants were comparable with those usually reported for other citrus species under severe stress in different climates (Kaufman and Levy 1976; Levy 1983). As shown in Fig. 4, stomatal conductance was higher in mango than citrus plants in well-

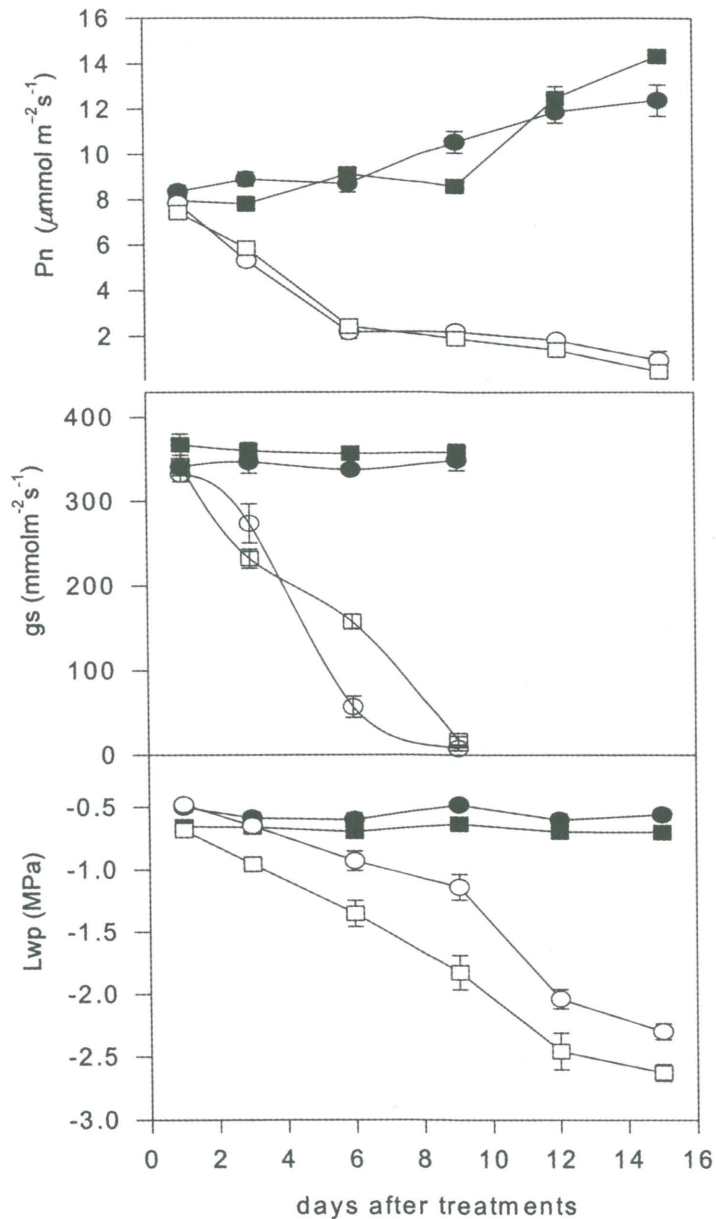


Fig. 4: Changes in leaf water potential (Lwp), stomatal conductance (gs) and photosynthesis rate (pn) of mango (o) and citrus (\square) which were either well watered (closed symbol) and water stress (open symbol). Bars represent \pm SE of 4-5 replicates

watered plants. Both plant species showed no significant differences in stomatal conductance between stressed and control plants at 1 d after withholding water. Imposition of water stress had resulted in a further reduction in stomatal conductance. The differences between both plant species were obvious on days 6 and 9 of imposing stress with a higher stomatal conductance recorded on citrus compared to mango plants.

This could explain findings from the present study that show a greater drought tolerance and recovery at a faster rate after rewatering in citrus compared to mango plants. The results agreed with the findings on drought tolerance of *Eucalyptus* clones that show that plants that have strong stomatal control of water loss enables rapid responses to changing conditions of water availability and possessed characteristics of

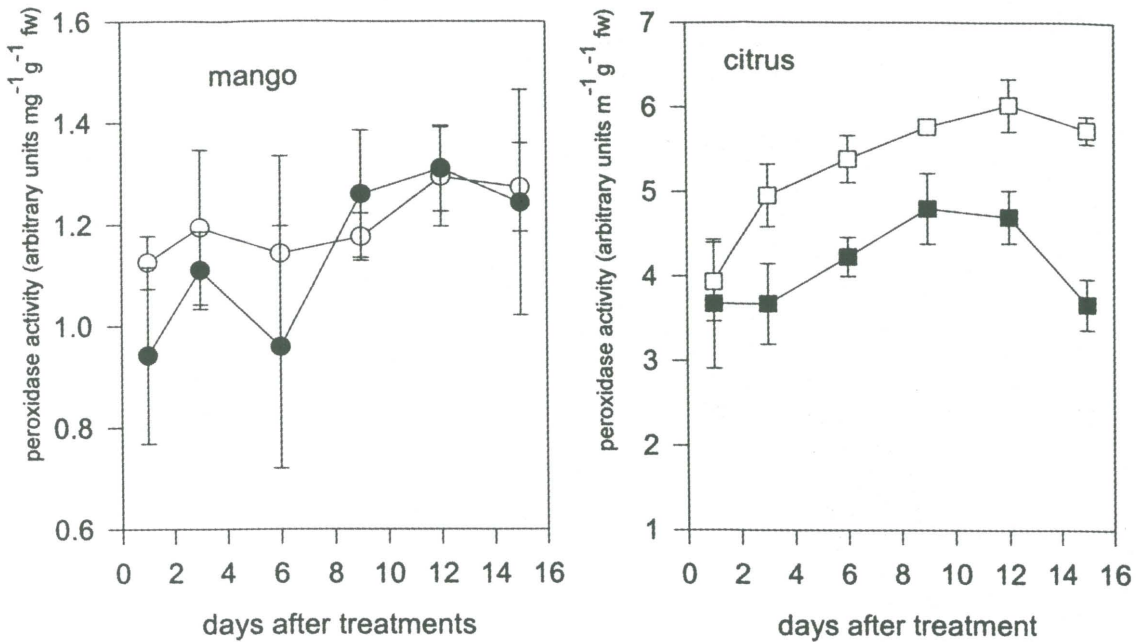


Fig. 5: Peroxidase activity in mango (o) and (□) citrus which were either well watered (closed symbol) and water stressed (open symbol). Bars represent \pm SE of four replicates

tolerance to environmental stresses (Farrell *et al.* 1996). The more drought tolerant species control stomatal function to allow some carbon fixation at stress, thus improving water use efficiency or open stomata rapidly when water deficit is relieved (Yordanov *et al.* 2000). There was a similar trend of reduction in photosynthetic rate as in stomatal conductance with exposure of plants to water deficits. The results clearly indicate that stomatal limitation had contributed to a decrease in the photosynthesis rate. This is in agreement with Farquhar *et al.* (1989) who indicated that stomatal factors are more important than non-stomatal factors in affecting photosynthesis rate under water deficit, mainly because of leaf stomatal heterogeneity.

Water deficit can result in an increased production of reactive oxygen species and therefore requires elevated levels of antioxidants for stress compensation. The ability of plants to overcome the effects of different stresses and to sustain their productivity may be related to increased enzymes such as super oxide dismutase, peroxidases and reductases (Polle and Rennenberg 1994; Awad *et al.* 2000). The response of increase peroxidase activity with water stress was clearly evident in citrus plants. In contrast, the present study shows that water deficit did not cause a significant increase in

peroxidase activity in mango plants. Our results on the insignificant effects of water stress to caused elevation of peroxidase activities agreed with other findings (Zhang and Kirkham 1996; Brown *et al.* 1995; Fu and Huang 2001). Both citrus and mango plants were found to accumulate proline when exposed to increasing water stress (Fig. 6). Proline is considered to be involved in the adaptation mechanism in drought stress. The accumulation of proline in plants under identical stress condition is species-specific (Heuer 1999). In *Citrus macrophylla* seedlings, proline accumulation was proportional to the severity of water stress and not to leaf water potential (Levy 1983). As proline plays an osmoregulatory role in plants, species that can accumulate higher proline levels during water stress can be considered more tolerant to water stress. Our data suggest that citrus accumulated higher proline and tended to survive water stress more readily and regenerate shoots rapidly following stress relief compared to mango plants.

The present study shows the differential plant responses to water deficit for both mango and citrus plants. This is important in optimizing water resources for cultivation of these plants in the field conditions. A proper water management needs to be adopted in establishing mango plants to avoid plant desiccation and mortality. The

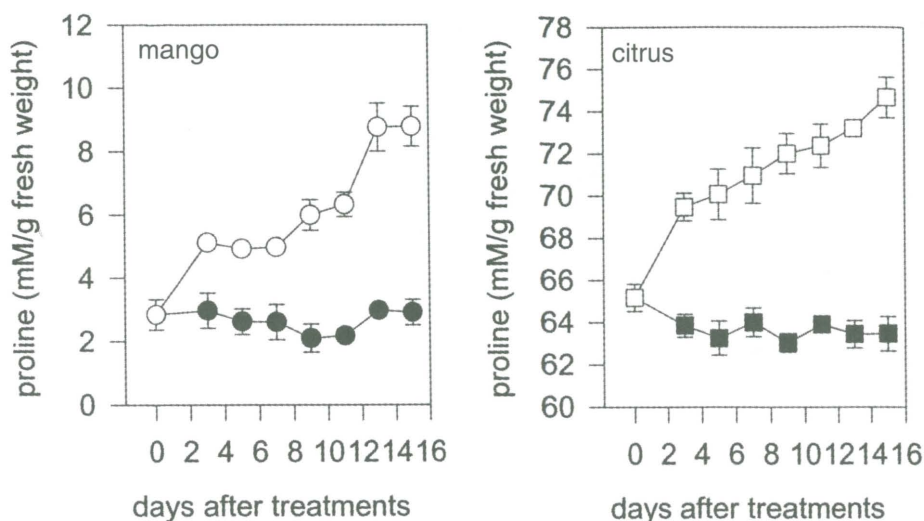


Fig. 6: Proline in mango (mango (o) and citrus (□) which was either well watered (closed symbol) and water-stressed (open symbol). Bars represent ± SE of 4 replicates

capability for adaptation to localized drought stress can be related to a sustained stomatal conductance, higher proline and peroxidase activity in citrus than mango plants. Further studies need to be conducted to identify the possible candidate that controls leaf expansion and stomatal conductance for improvement of water use efficiency in mango and citrus plants during water deficit condition.

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