Effects of Irrigation Period on Biochemical Changes of some Citrus Species

Esmat ARABZADEH [1]
Reza FOTOUHI GHAZVINY [1]
Mahboubeh RAHMANI [1](✉)
Mohammad SAADATIAN [1, 2]

Summary

Water is the most important factor for plant growth, and deficiency of water is a reason for drought stress. In drought stress situations, antioxidant system and some biochemical properties are changed. To identify the effect of drought stress on some types of citrus species, we examined different irrigation periods on citrus cultivars. In this study, plants were irrigated every four, eight, twelve, and sixteen days. Antioxidant, superoxide dismutase (SOD), peroxidase (POD), chlorophyll content, relative water content (RWC), and proline were measured. Results showed that with increased irrigation period antioxidant capacity, SOD, POD, and proline were increased in all three citrus cultivars, but chlorophyll content and RWC were decreased. All cultivars expressed similar changing pattern and it means that with decrease of water availability antioxidant system activity increases to protect plants against drought stress

Key words

antioxidant system, environmental stress, citrus species, irrigation

[1] Department of Horticultural Sciences, Faculty of Agriculture, University of Guilan, Rasht, Iran
✉ e-mail: mh.rahmani21@gmail.com
[2] Soran University, Faculty of Education, Department of General Science, Soran, Kurdistan Regional Government, Iraq

Received: April 2, 2017 | Accepted: July 5, 2017

Introduction

Water demand is increasing on the global scale in view of fast population growth rates, improvement in the standard of living, irrigation schemes expansion and global warming (IPCC, 1996; UN Population Division, 1994). In the regions suffering from water scarcity like the Mediterranean basin water supplies are currently degraded or exposed to degradation processes, thereby exacerbating the water shortage (Chartzoulakis et al., 2001).

Whereas a high soil water potential during the growing season is essential for the maintenance of unimpaired crop growth and high economic yield, the stress imposition through longer irrigation intervals, higher moisture depletion or skipping irrigation during either the early vegetative stage or maturation can contribute to obtaining similar economic yields, along with saving irrigation water and ameliorating the water use efficiency (Attard et al., 1996). Higher plants are sessile and hence are continuously prone to various environmental stress factors like drought, salinity, heavy metals, nutritional disorders, radiation without any protection. The majority of these stresses have specific common impacts on plants, including induced oxidative stress by the reactive oxygen species (ROS) overproduction, as well as their own particular effects (Rao, 2006). Reactive Oxygen Species (ROS) are sometimes termed Active Oxygen Species (AOS), or Reactive Oxygen Intermediates (ROI), or Reactive Oxygen Derivatives (ROD) (Desikan et al., 2005).

ROS contains a miscellany of oxygen-radicals, for instance superoxide anion (O_2^{-}), hydroxyl radical ($OH^{\cdot}$), perhydroxyl radical ($HO_2^{\cdot}$) and hydrogen peroxide (H_2O_2). Singlet oxygen ($^1O_2$), another ROS type, can be created by the formation of the excited-chlorophyll in the photosystem II (PSII) reaction center and antenna systems. This is the principal formation mechanism of $^{1-}O_2$ in plant cells. Due to inadequate energy dissipations during the photosynthesis, the chlorophylls are excited, resulting in the chlorophyll (Chl) triplet state formation. The Chl triplet state reacts with $^3O_2$ to generate the highly reactive oxygen (Arora et al., 2002; Reddy and Raghavendra, 2006; Gill and Tuteja, 2010) as a metalloenzyme. The superoxide dismutase is the first enzyme in the detoxification processes to catalyze $O_2^{\cdot-}$ to H_2O_2 and O_2. SODs are divided into three groups in accordance with their metal cofactor: Fe-SOD (in chloroplasts), Mn-SOD (in mitochondria), and Cu/Zn-SOD (in chloroplasts, peroxisomes, and cytosol) (Gill and Tuteja, 2010).

Peroxidase is an enzyme observed in miscellaneous organisms, ranging from bacteria and plants up to humans. Its function is to decompose hydrogen peroxide (H_2O_2) that is a toxin, created as a byproduct of utilizing oxygen for respiration. Chlorophyll content is one of the key factors influencing photosynthetic capacity. The decrease or constancy of the plants chlorophyll content under drought stress has been found in various plant species and its intensity is dependent upon the stress rate and duration (Jagtap et al., 1998). The leaf chlorophyll content is a plant tissues photosynthetic capability indicator (Nageswara et al., 2001). Schonfeld et al. (1988) revealed that wheat cultivars with high RWC show greater resistance to drought stress. Apparently, osmoregulation is one of the most crucial turger pressure preservation mechanisms in most plant species against water loss for the purpose of water absorption and metabolic activities retention (Gunasekera and Berkowiz, 1992). Proline Exogenous application induces the plants abiotic stress tolerance (Claussen, 2005). Much attention has been paid to the proline role in stress tolerance as a compatible osmolyte (Ali et al., 2007).

The purpose of the present study is to contribute to a better understanding of the physiological responses of the, plants to the irrigation time changes. Therefore, the influence of the amount of water and the period of irrigation on the chlorophyll content, relative water content (RWC), proline content, peroxidase (POD) content, antioxidant capacity, and superoxide dismutase (SOD) were investigated in 'Kinow' tangerine, 'Washington Navel' orange and 'Valencia' orange.

Material and methods

Plant Preparation:

The tests were done in approximately 80 days. The cultivars in the test were the 3-year-old trees of three citrus cultivars: 'Kinow' tangerine (A), 'Washington Navel' orange (N) and 'Valencia' orange (V). Four experiments (each in one treatment) were carried out. The samples were irrigated every four, eight, twelve and sixteen days. The leaves were tested using three replications and were randomly selected mature and immature leaves from the. The trees were in a greenhouse in the pots.

Peroxidase (POD) activity

The enzyme extracts were obtained by the homogenization of 0.5 g of leaves slices with 1 mL of 50 mM potassium phosphate buffer (pH 7), including 0.5 mM EDTA and 6% polyvinylpolypyrrolidone. The homogenate was centrifuged at 14000 rpm for 15 min at 4°C, and the supernatant was utili'zed for enzymes activity determination. The POD activity was assayed spectrophotometrically. Guaiacol was formed in l mL reaction mixture of 450 L 25 mM guaiacol, 450 L 225 mM H_2O_2 and 100 L crude enzyme. The activity was expressed as units per mg fresh weight, and each determination was implemented in triplicate.

Superoxide scavenging capacity

The superoxide scavenging capacity was evaluated by the method of Duh et al. (1999). The reaction mixture contained phosphate buffer, 200 µL of halophyte extracts, 200 µL of PMS solution, 200 µL of NADH, and 200 µL of NBT. The absorbance was read at 560 nm against blank after incubation at ambient temperature. The organ extract antioxidant activity evaluation was based upon IC50. The IC50 values were expressed as µg/ml. As for DPPH, the lower IC50 value pertains to a higher plant extract antioxidant activity. The superoxide anion generation inhibition proportion was calculated by the following formula:

$$\text{Superoxide quenching (\%)} = [(A0 - A1)/A0] \times 100$$

Where A0 and A1 have the same meaning as in Eq.

Antioxidant capacity

The antioxidant activity was assessed by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method based upon the procedure of Ghasemnezhad et al. (2011). In summary, 150 µL of the leaves extract was added to 850 µL of the DPPH radical, vortexed and placed at room temperature in darkness. The samples absorbance was assessed at 515 nm after 15 min via a UV/Vis spectrophotometer model PG Instrument +80, Leicester, United Kingdom. For each sample, three different determinations were made. The percentage of DPPH, which was scavenged (% DPPHsc), was calculated utilizing %DPPHsc: %DPPHsc = (Acont-Asam) ×100/Acont

Chlorophyll content

Determining the total chlorophyll content was based on Lemoine et al. (2009). Leaves were powdered in a refrigerated by liquid nitrogen mill and 0.4 g of the powder achieved was added to 5 ml of acetone and water at a ratio of 80:20, stirred and then centrifuged at 5000 rpm for 15 min. The obtained supernatant was used to determine the content of chlorophyll. The results were shown as mg chlorophyll /g FW.

Relative water content (RWC)

To measure RWC, the leaves were sampled. They were laid in the polythene bags and transferred to the laboratory immediately, and the fresh weight (FW) was measured quickly. At the next step, the leaves were cut into 2 cm slices and floated on the distilled water for 4 h to measure the weight of the turgid leaves and obtain the turgid weight (TW). The samples were dried in an oven at 60°C for 24 h and then the dry weight (DW) was obtained. The RWC was estimated according to Barrs (1968): RWC (%) = [FW-DW]*100

Proline

Having passed the stress period, proline was extracted from fresh leaves and measured by the protocol given by Bates et al. (1973). Leaves were frozen by liquid nitrogen and separated 5 g was homogenized with 10 ml of 3% sulfosalicylic acid at 25C°. Filtering the homogenate was accomplished through the filter paper (Whatman NO_2). Two ml of the filtrate with two ml of glacial acetic acid and two ml acid ninhydrin were mixed in a tube in a water bath at 95°C for one hour. The reaction mixture was cooled in the ice bath. Following that, toluene in 4 ml was added to the mixture and then blended with a test tube stirrer for 20 seconds. The pink-red color toluene on the top of the mixture was extracted by a pipette. The absorbency of the toluene was read at 520 nm with a spectrophotometer. To calculate the proline concentration, the proline standard curve was used in a specimen on the fresh weight basis. The electrical conductivity was measured by a digital conductivity meter (JENWAY, Model 4070, and Essex, England).

Statistical analysis

The experiment was conducted using a completely randomized design with three replications. The recorded data were statistically analyzed (ANOVA analysis) using the software of SAS, sources of variation were different irrigation period and cultivars as the factors. Differences of least squared means were considered to be significant at P<0.01.

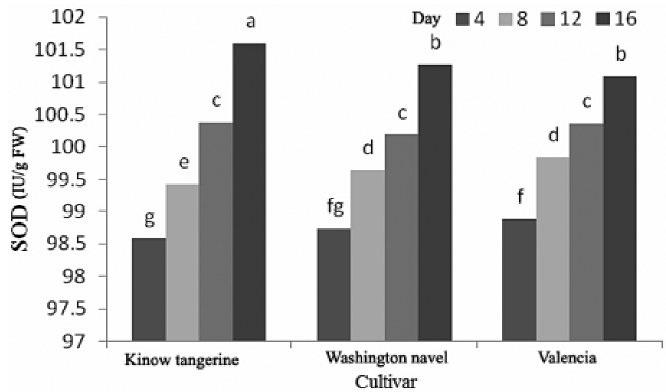

Figure 1. Effects of irrigation period on superoxide dismutase (SOD) activity in three cultivars of citrus

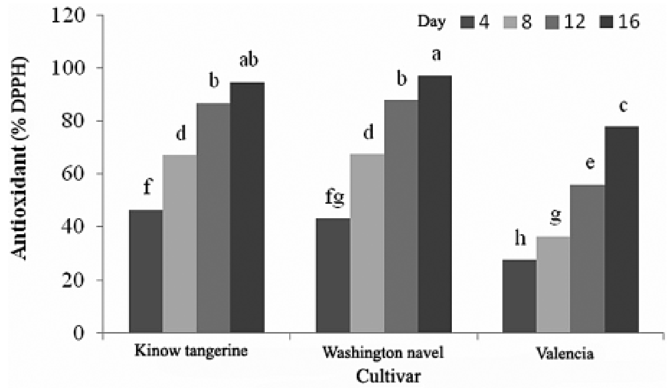

Figure 2. Effects of irrigation period on total antioxidant capacity in three cultivars of citrus

Result and discussion

SOD

All cultivars showed the same changing schema, indicating the minimum percentage of SOD activity and the amount of SOD enzymes in the samples that were irrigated every four days, that is an indication of the soil water stress. Actually, it was indicated that the content of SOD was greater in the leaves of three cultivars that were irrigated every sixteen days and was the lowest in the leaves that were irrigated every four days. 'Kinow' tangerine had stronger SOD activities at this level (101.5 U/mg /FW) than 'Valencia' orange (101 U/mg /FW) and 'Washington Navel' orange. There was no significant difference between orange cultivars (Fig. 1). Plants possess different evolutionary mechanisms to deal with the stresses produced by different environmental circumstances. SOD, which plants use to protect themselves against the damages of oxygen radicals, is considered as the first defensive line in antioxidant enzymes (Mittler, 2002). A rise for enzymes like SOD leads to increase of plant resistance to oxidative stresses (Sreenivasulu et al., 2000). The increased activities of antioxidant enzymes and the increased percentage of antioxidants under drought stress differ from each other not only among several plant species but also between two cultivars (Selote and Khanna-Chopra, 2004).

Antioxidant

It was demonstrated that the lowest antioxidant capacity was in 'Valencia' orange irrigated every four days. The results also indicated that there was no significant difference between 'Kinow' tangerine and 'Washington Navel' orange that were irrigated every eight and every twelve days. 'Kinow' tangerine and 'Washington Navel' orange showed the highest antioxidant activities in all four types of irrigation and 'Valencia' orange had the lowest activity (Fig 2). These results revealed that the genotype related studies exhibit genetic changes concerning adaptation and reaction in water stress. Misra and Gupta (2006) stated that the amount of antioxidant enzymes varies noticeably, highly depending on the cultivar, the stress regimes, the growth phases, and the environmental stresses. Antioxidants play a crucial part in protecting plants in inappropriate conditions (Lohrmann et al., 2004).

POD

In this study, POD was considerably active in the 'Kinow' tangerine irrigated every sixteen days, not demonstrating any significant difference in comparison with the 'Valencia' orange. However, it showed the lowest activity in the samples irrigated every four days. Then a re-increase in POD was observed in the irrigated 'Kinow' tangerine and 'Valencia' orange irrigated every eight days (Fig 3). The cultivars under water deficit presented higher POD activity than normal irrigation management, which can be a defense mechanism against free radical formation resulting from water deficit. Strong plants, such as salt-tolerant tomatoes tend to have a greater proportion of POD than the weak plants under stress (Shalata and Tal, 1998). The POD activity can be a useful tool in the selection of the cultivars resistant to environmental stress such as the water deficit. Xiong et al. (2002) reported that in plants under water deficit there is an increase in peroxidase activity that is correlated with the increase of water deficit tolerance. In this study, this increase in peroxidase activity under water deficit was evident indicating greater tolerance of these cultivars to water deficit.

Chlorophyll

The highest chlorophyll content was observed in all three cultivars irrigated every four days. The results also indicated that all cultivars had the lowest chlorophyll content when they were irrigated every eight and twelve days, and there was no significant difference between 'Valencia' orange and 'Washington Navel' orange (Fig 4). These results suggested that there was no considerable difference among cultivars.

RWC

Based on the results, RWC was decreased in all of the cultivars in irrigation period (Fig. 5). Drought stress did not lead to a significant reduction in relative water content (RWC) when plants were treated by different irrigation regimes in all cultivars. Similar results were observed in the maize when moderate stress did not significantly change the relative water content. Alizadeh et al. (2011) found a decrease in water relations in four apple dwarf rootstocks under drought stress but it differed between rootstocks. In the study of two cultivars of citrus, leaf RWC was smaller in the drought stress samples than in the controlled plants and the genotype that showed less reduction was supposed to be more tolerant than another genotype because of its water status (Garcia-Sanchez et al., 2007). RWC has also been considered as a main water index, as compared to other water potential factors in drought stress (Lugojan and Ciulca, 2011). A decline in the relative water content blocks stomata, and after the obstruction of stomata the photosynthesis level decreases. Also, RWC is closely associated with the cell content and may vividly show the equilibrium between the leaf water supply and the amount of transpiration (Schonfeld et al., 1988). This enables the plant to be released from stress, affecting the yield and the yield persistence (Lilley and Ludlow, 1996).

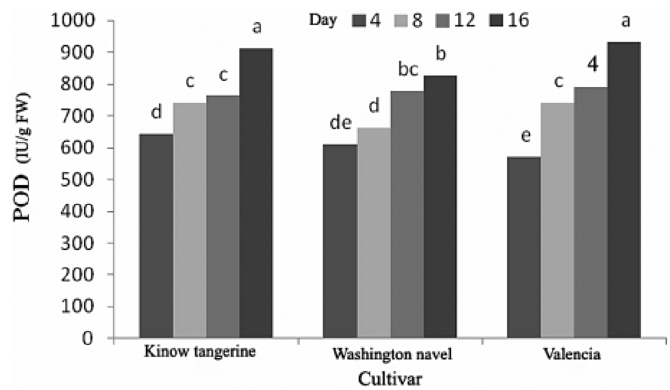

Figure 3. Effects of irrigation period on peroxidase (POD) activity in three cultivars of citrus

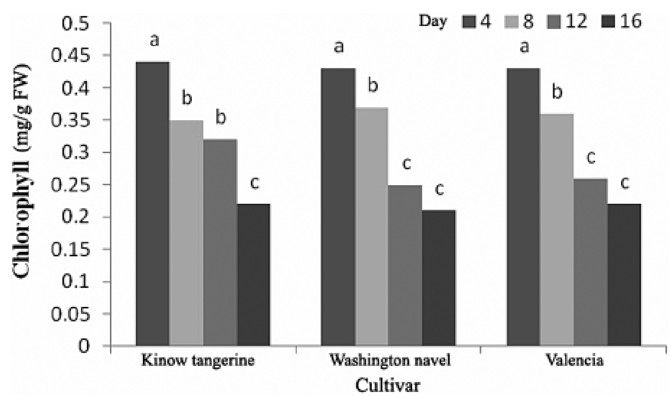

Figure 4. Effects of irrigation period on chlorophyll content in three cultivars of citrus

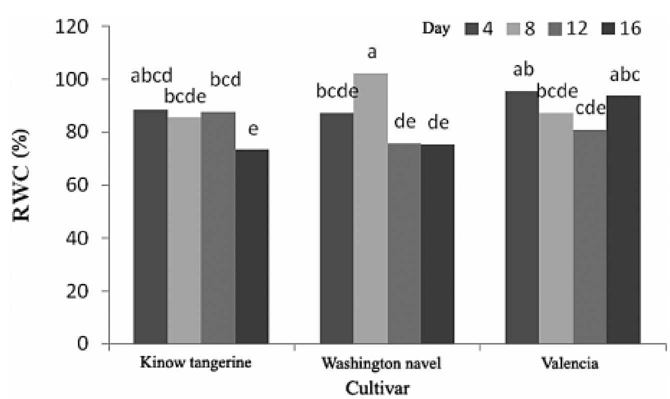

Figure 5. Effects of irrigation period on relative water content (RWC) in three cultivars of citrus

The cultivars, which are able to keep more water in their leaves, show more resistance to drought stress (Garcia-Sanchez et al., 2007). It has been indicated that high relative water content is resistant to drought and that high relative water content is the product of regulating more osmotic or lower tissue cell wall elasticity (Ritchie et al., 1990). Hence, slight changes in RWC of the drought plants during sixteen days proved well the osmotic

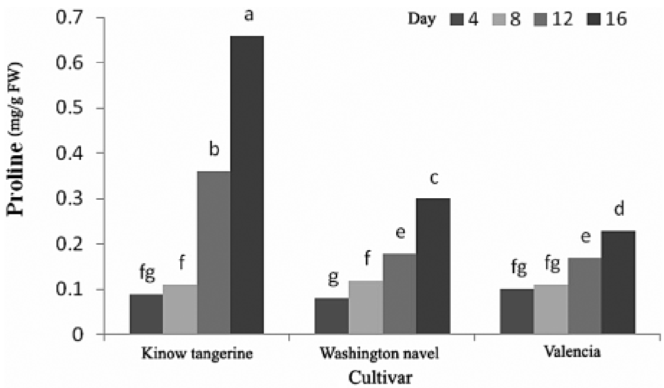

Figure 6. Effects of irrigation period on proline activity in three cultivars of citrus

adjustment, and the subsequent increase of the drought tolerance of our studied plants revealed that the above-mentioned points would be taken into account.

Proline

Overall, content of proline in all three cultivars was increased by water deficit. 'Kinow' tangerine tended to accumulate more proline than other two cultivars when they were irrigated every sixteen an twelve days. However, it is observed that there was no significant difference among cultivars when plants were exposed to four and eight days shortages of water. The highest amount of proline was obtained when plants were exposed to fourteen days of water deficit in cultivar 'Kinow' tangerine (Fig. 6).

The results of this study are in agreement with the findings on drought tolerance of citrus and mango plants when there was a 6-10 fold increase in proline content (Mohd Razi et al., 2004). Because of osmoregulatory role of proline in plants, cultivar that can accumulate higher proline levels during water stress can be considered more tolerant to water stress (Mohd Razi et al., 2004). In faba bean, it has been proved that cultivars represent higher yield durability in case of water deficiency (Karamanos et al., 1995) and the greatest potential for the amount of proline. Thus, the proportion of proline may be considered as a practical instrument for the estimating of the drought resistance of the crop cultivars. Our data suggests that 'Kinow' tangerine accumulated higher proline and tended to survive water stress more readily comparing to other cultivars. The influence of cultivar on high accumulation is obvious and may originate from their natural growth. It has become evident that the larger the growth rate in a given phase is, the greater the potential for accumulation will be. The biological period may also be another significant parameter, with the recent cultivars often showing higher concentration than the earliest ones (Karamanos, 1995).

Conclusion

In this research, all of the citrus cultivars had similar respond to the irrigation periods. It seems that enhanced antioxidant system activity affected was by drought stress. Increase in proline content in this research is a way of plant adaptation against drought stress because proline is a chemical that have an osmoregulatory role in plants. With enhanced environmental stress in plant chlorophyll degradation is increased. With long period between irrigation, it is possible that photosynthesis rate decreases because of decrease in chlorophyll content.

References

Ali Q., Ashraf M. and Athar H.R. (2007): Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. Pak. J. Bot. 39(4): 1133-1144

Alizadeh A., Alizade V., Nassery L., Eivazi A. (2011): Effect of drought stress on apple dwarf rootstocks. *Technical Journal of Engineering and Applied Sciences.* 1(3):86-94

Arora A., Sairam R.K., Srivastava G.C. (2002): Oxidative stress and antioxidative system in plants, Curr. Scie.. 82(10): 1227-1237

Attard D.J., Axiak V., Borg S., Cachia J., de Bono G., Lanfranco E., Micallef R.E., Mifsud J. (1996): Implications of expected climatic changes for Malta. In: Jeftic L, Keckes S, Pernetta J.(eds.), Climate Change and the Mediterranean, vol. 2. Arnold, UK, pp 322–430

Barrs H. D. (1968): Determination of water deficits in plant tissues. In: Water Deficits and Plant Growth, Kozolvski I TT (ed.) Academic Press, New Delhi, pp 235–368

Bates L. S, Waldren R. P., Teare I. D. (1973): Rapid determination of free proline for water-stress studies. *Plant Soil.* 39:205–207

Chartzoulakis K.S., Paranychianakis N.V., Angelakis, A.N. (2001): Water resources anagement in the island of Crete, Greece with emphasis on the agricultural use. Water Policy 3, 193–205

Desikan R., Hancock J., Neill S. (2005): Reactive oxygen species as signaling molecules, In: Smirnoff N (ed) Antioxidants and Reactive Oxygen Species in Plants, Blackwell Pub, Ltd, Pp 69-196

Duh P.D, Yen G.C. (1999) Antioxidant activity of water extract of Harng Jyur (Chrysanthemum morifolium Ramat.) varieties in soybean oil emulsion, Food. Chem. 66: 471–476

Garcia-Sanchez F., Syvertsen J. P., Gimeno V., Botia P., Perez-Perez J.G. (2007): Responses to flooding and drought stress by two critrus rootstock seedlings with different water-use efficiency. Physiologia Plantarum 130: 532-542

Ghasemnezhad M., Sherafati M., Payvast A. (2011): Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (Capsicum annum) fruits at two different harvest times. Journal of Functional Foods 3: 44–49

Gill S.S., and Tuteja N. (2010): Reactive oxygen species and antioxidant machinery in biotic stress tolerance in crop plants, Plant Physiol Biochem 48: 909-930

Gunasekera D, Berkowitz G.A. (1992): Evaluation of contrasting cellular-level acclimation responses to leaf water deficits in three wheat genotypes. Plant. Sci. 86: 1-12

Jagtap V., Bhargava S., Sterb P., Feierabend J. (1998): Comparative effect of water, heat and light stresses on photosynthetic reactions in Sorghum bicolor (L.) Moench. J. Exp. Bot. 49: 1715-1721

Claussen W. (2005): Proline as a measure of stress in tomato plants. Plant Sci. 168:241-246

IPCC. (1996). Houghton J.T., Meira Filho L.B., Callander B.A.,Harris N., Kattenberg A., Maskell K. (eds.). Climate Change (1995): The Science of Climate Change. Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge

Karamanos A. J, Peppa A., Avgoulas C. E. (1995): A comparative study of the water relations and productivity of contrasting faba bean(*Vicia faba* L.) cultivars. In: Improving Production and Utilisation of Grain Legumes (proc. 2nd Eropean Confrence on Grain Legumes). AEP-Copenhagen. pp.124-125.

Karamanos A. J. (1995): The involvement of proline and some metabolites in water stress and their importance as drought resistance indicators. *Bulg. Journal of Plant Physiology.* 21(2-3):98-110.

Lemoine M.L., Civello P., Chaves A., Martıinez G.A. (2009): Hot air treatment delays senescence and maintains quality of fresh-cut broccoli florets during refrigerated storage. Food Science and Technology 42: 1076–1081

Lilley J. M., Ludlow M. M. (1996): Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. *Field Crop Research.* 48: 185–197

Lohrmann N. L, Logan B. A., Johnson A.S. (2004): Seasonal acclimatization of antioxidants and photosynthesis in Chondrus crispus and Mastocarpus stellatus, two co-occurring red algae with differing stress tolerances. *The Biological Bulletin.* 207:225–232

Lugojan C., Ciulca S. (2011): Evaluation of relative water content in winter wheat, Journal of Horticulture. *Foresty and Biotechnology.* 15(2):173-177

Misra N., Gupta A. K. (2006): Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in catharantus roseus seedlings. *Journal of Plant Physiology.* 163: 11-18

Mittler R. (2002): Oxidative stress, antioxidants and stress tolerance. *Trends Plant Science.* 7:405–410

Mohd R.I, Abd-Ghani M., Ismail I. (2004): Differential responses in growth, physiogical processes and peroxidise activity of young mango (*mangifera indica* L.) and Citrus (Citrus sinensis L.) plants to water deficit. *Pertanika Journal of Tropical Agricultural Science.* 27(1):47-55

Nageswara R.R.C, Talwar H.S., Wright G.C. (2001): Rapid assessment of specific leaf area and leaf nitrogen in peanut (Arachis hypogaea L.) using chlorophyll meter. J. Agron. Crop Sci. 189: 175-182

Ramachandra-Reddy A., Viswanatha Chaitanya K., Vivekanandan M. (2004): Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physioogyl.*161: 1189–1202.

Rao K.V.M. (2006): Introduction. In: Rao KVM, Raghavendra AS, Reddy KJ (eds) Physiology and Molecular Biology of Stress Tolerance in Plants. Springer-Netherlands, pp 1-14.

Reddy A.R. and Raghavendr A.S. (2006): Photooxidative stress. In: Rao KVM, Raghavendra AS, Reddy, KJ (eds) Physiology and Molecular Biology of Stress Tolerance in Plants, Springer-Netherlands, pp 157-186.

Ritchie S. W., Nguyan H. T., Holaday A. S. (1990): Leaf Water content and gas exchange parameters of two wheat genotypes differing in drought resistance. Crop science. 30:105-111.

Schonfeld M. A, Johnson R. C., Carver B. F., Mornhigweg D. W. (1988): Water relations in winter wheat as drought resistance indicators. *Crop Science.* 28: 526–531.

Selote D.S., Khanna-Chopra R. (2004): Drought-induced spikelet sterility is associated with an inefficient antioxidant defense in rice panicles. *Plant Physioogyl.* 121: 462–471.

Shalata A., Tal M. (1998): The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*, *Plant Physioogyl.* 104: 169–174.

Sreenivasulu, N, Grimm B., Wobns U., Weschke W. (2000): Different response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensetive seedling of foxtail millet. *Physiology Plantarum.* 109: 435-442.

UN Population Division. (1994): World population prospects (sustaining water: an update). The 1994 Revision. The UN, New York

Xiong L., Schumaker K. S., Zhu J. K. (2002): Cell signaling during cold, drought and salt stress. The Plant Cell, Rockville, v. 14, Supplement. pp 165-183

---

acs82_06