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Mesophyll carbon intake concentrations exhibited high in citrus rootstocks grown on peat moss under water stress environment

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Abstract

The stomatal conductance (gs), photosynthesis rate (Pn), *intercellular* CO_2 *conc. (Ci)*, chlorophyll and *morphogeneic data* was collected from the 12 month old citrus rootstocks (Rough lemon, *Gada dehi*, *Flying dragon*, Brazilian *sour orange*, Citron *citrange*, *Carrizo citrange*, *Rubidoux trifoliate*, *Savage citrange*, *Yuma citrange and Citromelo*-1452) grown in peat moss under 100, 70, 40 and 10 %,s available water capacities in glass house. Pn, g_s, and *E* rates were decreased down with increase in water stress level. Rough lemon, Gada *dehi* and Savage *citrange* mesophyll *Ci* was not that much altered with change in water conc. However, Carrizo *citrange*, Citron *citrange*, Citromelo-1452 and Brazilian *sour orange* mesophyll carbon remained higher than that of Chlorophyll, Pn and gs ratios. C_i intake enhanced on 15 days and reached maximum in 45 days especially on 10% FC. The osmoprotactant cholorophyll conc. was opted as a drought resistant strategy (DRS) especially by trifoliate. Moreover, plant biomass of Keen *sour orange*, Rough lemon and *Gada dehi* was high comparatively even under stress.

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Introduction

Citrus belongs to family Rutaceae and it originated from South East Asia. Now it is commonly grown in favorable conditions with respect to soil and climate (Shah, 2004). The Pakistani citrus crop is under threat of biotic, abiotic stresses or their combinations. Among different stresses of citrus, drought is most important for citrus industry in Pakistan. In current research proposal this stress is chosen for crucial findings in field of plant physiology and horticulture. Drought in tropical and subtropical areas appear as predominant environmental stress but in arid and semiarid conditions, its affect is very devastating (Khan *et al*., 2003) due to improper drainage and substandard irrigation system (Binzel and Reuveni, 1994). Pakistan being subtropical country has arid and semi-arid climate. The irrigated land of Pakistan covers 16.795 million hectares while 15 million hectares is subjected to drought condition (Anonymous, 2007). Citrus seedling cultivars faced adverse problems and have become unthrifty in several countries of the world due to certain root fungi, nematodes, drought and salinity. To overcome such problems, now citrus is universally propagated by budding and grafting onto rootstocks. Davastating effects of these factors can be controlled by selecting favorable rootstocks (Lima *et al*., 1992). Local climatic and soil conditions are the first important factors to consider in rootstocks selection. Generally present knowledge of rootstock on the plant development under stress is limited and specifically in citrus it is very scarce. Less of the past research on its rootstocks has been focused on plant morphology, physiology, adaptability, development and quality parameters under different micro soil water environment. This study emphasized on the morpho-physiological characterization to assess the resilience in *Ci*trus rootstocks grown under water stressed conditions. This evaluation and characterization of *Ci*trus rootstocks will be helpful for researchers and farmers in selection of suitable rootstocks resistant.

Materials and methods

One and half year studies were conducted in Department of Horticulture PMAS-Arid Agri. University Rawalpindi. And experiment was conducted on 10 *Ci*trus rootstocks collected from Institute of Horticulture, University of Agriculture Faisalabad. These 10 rootstocks (Rough lemon, Gada dehi, Flying dragon, Brazilian Sour orange, Citron citrange, Carrizo citrange, Rubidoux trifoliate, Savage trifoliate, Yuma Citrange, Citromelo-145) having age nine month were evaluated in 3 liter pots against four available water capacities (100, 70, 40 and 10%,s) on peat moss growth medium. After 30 days of transplantation morpho-physiological data regarding height of plant, branches, leaves/plant, leaf area (LA), number of roots (NR), photosynthesis rate (Pn), stomatal conductance (gs), transpiration rate (E), and chlorophyll was taken.

The experiment was designed 4 treatments with 4 replicates. Prior to experimentation the peat moss material / mineral composition is noted from the factory packing material which was as below.

Peat moss a Latvian product

It was used as growing medium for seeds of root stocks. Product brand name: pind strip plus peat substrate. Nutrition added in agent/m 3: Nitrate N 70g, Ammonium N50g, Phosphorus (P) 60g, Potassium (K) 200g, Magnesium (Mg)17g, Boron (B) 0.3g, Molybdenum (Mo) 2g, Cupper (Cu) 1.2g, Manganese (Mn)1.6, Zinc (Zn) 0.4g, Iron (Fe) 0.99g, pH5.6-6.4. Country of origin: Latvia, www. pindstrup.com

Peat moss a German product

It was used as growing medium for rootstocks seedlings. It was a mixture of more strongly decomposed peat (frozen black peat), slightly medium decomposed peat (white peat) and NPK fertilizers. pH ($CaCl_2$) 5.2-6.0, Salt Contents (H_2O)>1, 5g/l. Nutrients available for plants: 110-250mg/l Nitrogen (N), 60-140mg/l Phosphate (P_2O_5), 120-280mg/l Potassium (K_2O), Volume: 70 liters (EN 12580) Country of origin: Germany, Product Name: Substrate Semis Bouturage, Seedling substrate Manufacturer: Planta flor Humus Verkaufs-GmbH* Oldenburger Str. 4* D-49377 Vechta Storing: Cool and dry.

Planting methodology in glass house and plastic tunnel

15th October 2012; 10 *Ci*trus rootstocks fruit were taken and their seeds were extracted and grown in glass house in black color plastic trays having holes sizes 3 X 3 inch.Two types of peat mosses was used. Latvin origined was used for seed germination while for seedlings purpose German peat was used both were imported products.Prior to sowing seeds were treated with fungici*d*e radomil, uniform three to four leaves per stem were obtained during transplantation. After nine month the seedlings were transferred from the glass house to lath house in 1 liter clay pots for providing them closet natural condition. There were 10 genotypes (Rough lemon, Gada *dehi*, Flying *dragon*, Brazilian *sour orange*, *Ci*tron *citrange*, Carrizo *citrange*, Rubidoux *trifoliate*, Savage *trifoliate*, Yuma *citrange, Ci*tromelo-1452). Four water treatments were used on basis of available water capacity percentage (AWC) where AWC % was derived by subtracting wilting point from total field capacity(FC) on wieght basis.And following water treatments; T1=100 AWC %, T2=70 AWC %, T3=40 AWC % and T4=10AWC % were applied. Water application was started after replacing green sheet with plastic sheet as a safety from raining water, each treatment was applied with three days intervals and at the end of experiment (30th day), 100 AWC % was provided to each plant to minimize the water stress shock before taking the physio-chemical calculations. The agronomic traits were collected and were closely monitored throughout the experiment. A suite of measurements was conducted; the temperature of the green house and plastic tunnel were monitored by the installed instruments.

Morphological Data

The data regarding plant height (cm), Branch numbers, leave numbers ,leaf width were taken, and plant BM (g plant^{-1}) was measured by up rooting whole plant at the end of experiment from root starting point below soil. The cut plants were sun dried for three days and dry weight measured to the nearest 0.01g. On experiment completion plants were separated into roots and shoots. The lower portion was washed and weighed. Influence of aqua porins was avoided (Henzler *et al.,* 1999) by uprooting plant simultaneously b/w 10:00-11:00 am. Whole plant fractions were dried in forced-draft oven at 60ºC for 48 hours and re-weighed.

Leaf Gas Exchange Data The portable instrument IRGA (infera red gas analyzer) CIRAS2 was used fow following below leaf gas exchange parameters. Prior to data CIRAS2 was set at a photosynthetically active radiation (PAR) level of 1000µ Mol photons m^{-2}sec^{-1} for performance. Method used for each parameter are as followed. *Photosynthesis Rate* (µmol CO_2 m^{-2}s^{-1}) of five plants was determined by taking their top most leaves (L1) and same process was repeated with next leaves (L2) after 15 days interval till physiological plant maturity with the help of Infrared Gas Analyzer Infrared (IRGA) in accordance with Long and Bernacchi, 2003 method. *Transpiration Rate* (mol H_2O m^{-2}s^{-1}) was calculated as same as photosynthesis methodology with IRGA. *Stomatal Conductance (gs)* Stomatal conductance (mol m^{-2}s^{-1}) was determined 5 randomly chosen citrus rootstocks as that of Pn rate with the help of IRGA. Intercellular CO_2 Conc. (µmol CO_2 m-²s-1) is very critical and play important role in plant cell metabolism so that it was observed from 5 randomly selected *Ci*trus rootstocks .The top leaves were selected for such parameter after 15 days interval during complete crop emergence and physiological maturity with Infrared Gas Analyzer (IRGA). The chlorophyll is measured by the SPAD (Special Products Analysis Division) which expressed chlorophyll index by utilizing the 650 and 940 nm wavelengths. From several locations measurements were done for the most representative spot. After ten days of transplantations 1st reading was taken. Then plants were subjected to water treatments and at the end of the experiment observations from all genotypes were taken and difference was noted.

Results and discussion

Morphogenetic Traits

Maximum plant height was expressed by Rubidoux *trif.,* and it is followed by *Ci*tron *citrange* and *Keen sour orange* (15cm).

Savage *citrange*, Rough lemon and Yuma *citrange* obtained mediocre position while Carrizo and Gada *dehi* acquired lowest plant heights (5cm) each. Branch means average placed Keen sour orange (3.75) on top ranking and was following with Gada *dehi* (2) and Rough lemon (2). Carrizo citrange came on bottom line by 0.7 branch generation. 100 and 70 AWC,s %'s yielded statistically at par digits 2.12 and 2.17 respectively. Likewise the 40 and 10 %'s produced 1.42 and 1.35 number of branches. The acquired data exhibited positive trend from lower to higher water levels but few rootstocks Carrizo citrange, B. *sour orange* and Rubidoux *trif.* behaved independently and could not compete well in changing water environment on peat moss and showed almost no significant results with respect to branch growth on all water levels especially on lowest 10% AWC which is new characteristic reflected by said rootstocks due to no availability of water that retarded growth (Pérez-Pérez *et al.*, 2010).

The plant biomass (Fig.3a,b) exhibited differently with respect to different *Ci*trus rootstock genotypes. Most of the rootstocks showed positive trend towards moisture level, However, rootstocks like Citromelo, Citron and Savage rootstocks expressed almost similar BM on 100,70 and 40% moisture levels.

On least water level rootstocks like Savage (9.5gm) and Citromelo-1452 (9.8gm) were statistically at par to each other and ranked 1[st] and was followed by all statically same BM of Gada dehi (8.2gm), Carrizo *cit.* (7.7gm), *Ci*tron (7.2gm), Keen sour orange (6.4gm) and Rough lemon (5.0gm). While Brazilian sour orange (2.0gm) was at lowest level and comparatively it could not compete well under all water regimes used in experiment. Keen sour orange, Rough lemon and Gada *dehi* maintained their performance in peat moss by taking part in metabolic process (Medina *et al.,* 1998).

The plant heights on peat were lower than that of heights on soil (Fochesato *et al.*, 2007) due to low water which retarded growth (Pérez-Pérez *et al.,* 2010). Citron Citrange, Brazilian sour orange and Yuma *citrange* showed more heights than that of Gada *dehi* and Savage *citrange* with assembling photosynthetic pigmentation and resulted into most BM (Fochesato *et al.,* 2007).

And findings are co-supported where *Ci*trus trees grafted on invigorating rootstocks (Rough lemon and Rangpur lime) expressed higher root hydraulic conductivity, gs, and E rates than trees on non-invigorating rootstocks such as Cleopatra mandarin and Brazilian sour orange (Romero *et al.,* 2006).

The carbon intake (Fig: 5) on 100 and 70%'s AWC's significantly differ within treatments. Both levels performed promising and significantly.

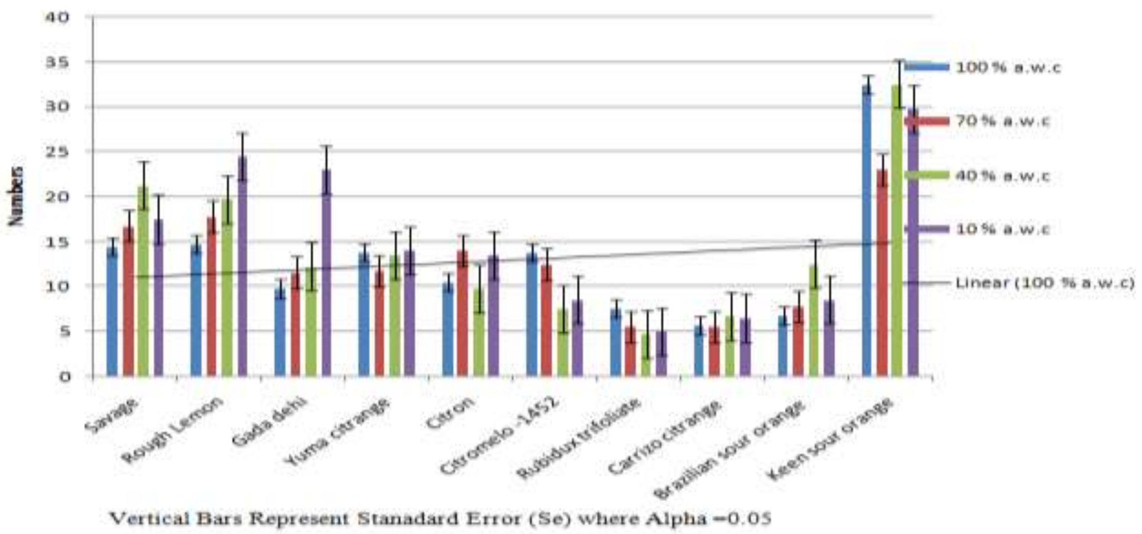

Fig. 1. Effect on the numbers of leaves of *Citrus* rootstocks with change in water regimes in peat moss.

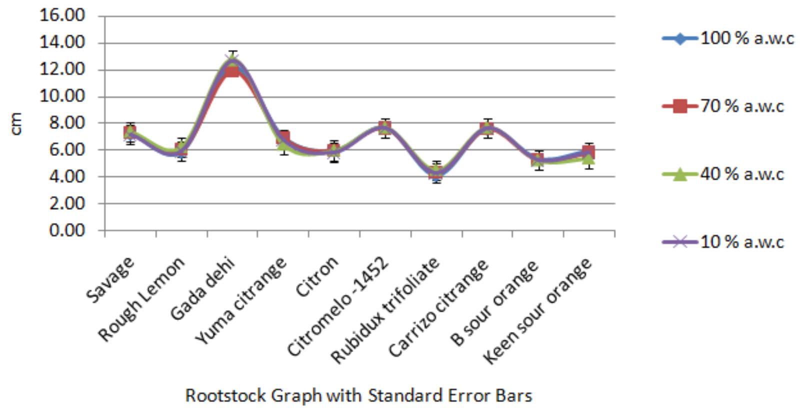

Fig. 2. Root growth pattern of *Citrus* rootstocks against four available water capa*ci*ties in peat moss.

Bioexchange Traits

Where 100% AWC in Carrizo *ci*trange yielded highest carbon exchange 545.75µmol CO_2 m^{-2} s^{-1} and was followed by *Ci*tromelo-1452 and Savage *citrange*. While low water conc., Savage, Yuma *citrange* and *Ci*tron expressed statistically at par with 400.02µmol CO_2 m^{-2} s^{-1}. Carrizo citrange took highest carbon intake (*Ci)* due to its vigorous nature even on 100% water level while Savage *citrange* behaved same. Over all *Ci* intake scenario (Fig. 5) showed positive effect on the rootstocks growth after participation in carboxylation reactions, in result osmolytes or osmoprotactants were boosted. Chlorophyll conc. was affected by the atmospheric *Ci* intake in Savage, Rough lemon, *Ci*tron, Rubidoux and Gada dehi that combated stress even under 10% AWC while on highest water level the rootstocks found favorable conditions to flourish and exhibited more *Ci* intake. The stomatal conductance (E) of all rootstocks remained lower than (2.00m mol m^2s^{-1}) except Carrizo *ci*trange which differ due to high root-shoot water translocation. The stomatal conductance scenario (Fig. 6) of the Carrizo *ci*trange repeated same on 100,70 and 10 AWC's percentages while Rough lemon exhibited 60 m mol m^2s^{-1} on all water levels, such attribute showed strong drought resistant trait. Savage and Gada dehi performed allike Rough lemon and reflected drought or moisture resistant traits on peat moss. The findings are co-related to the E and gs rates as well. Brazilian sour orange with low Pn and gs rate expressed water sensitive rootstock. In addition results were in accordance to findings of which revealed that high decrease in gs and temperature with minor reduction in Pn under water stress caused much photosynthetic WUE in the resistant cultivars. An increase in mesophyll conductance (MC) enhanced *Ci* due to drought stress of the susceptible cultivars that exhibited the increased sensitivity of the photosynthetic apparatus to water stress (Flexas *et al.,* 2008) which appeared in shape of chlorophyll loss (Fig. 4. c,d). Rubidoux trif and Brazilian sour orange could not take more *Ci* intake due to mesophyll carbon production that retarded atmospheric *Ci* intake. Thus, beyond stomata, a considerable body of evidence from above findings expressed that leaf mesophyll conductance to CO_2 and also responded changes in environmental conditions such as drought and temperature. In consequence Rough lemon, Savage, Gada dehi and *Ci*tron relied on mesophll CO_2 other than atmospheric CO_2 intake (Caemmerer, 2000) where mesophyll and stomatal elements characterized the photosynthesis diffusion component, and determined the carbon availability for the carboxylation reaction. Intercellular leaf spaces CO_2 diffusion from the atmosphere occurred (Flexas *et al.,* 2008) which was a conservative response under conditions of water scar*ci*ty (Flexas & Medrano 2002) shown by current drought resistance rootstocks. Rootstocks under three water levels did not show noticeable *Ci* while same rootstocks on lowest water level a countable reduction expressed and moderate stress under 70 and 40% AWC were overcome by same rootstocks while *Ci* was compromised (Lisar *et al*., 2012).

The physiology under stress was highly complex and was involved in adaptive or deleterious alterations. This complexity comprises on multifactor like variety, specie, stress duration and soil dynamics other than water demand from air (Zhao *et al.*, 2006). Thus, *Ci*trus rootstocks; Savage, *Ci*tron and Gada dehi and Rough lemon plants under said strategies (tolerance & avoidance) coped well with drought (Szabados and Savoure, 2009). Chlorophyll as osmoprotectant in *Ci*tromelo-1452, Savage and Rough lemon did not exhibited salient difference in mentioned rootstocks. Such trait hindered due to mesophyll CO_2 production, stomatal closure and plant internal respiration process, under such condition ABA might produced in roots and transferred into shoots that caused stomatal closure and leave senescence occurred as part of drought combating strategy (Garcea-Sanchez *et al.*, 2006). This limitations might be due to the non-stomatal mesophyll carbon assimilation (A_{CO_2}) presence. As in *Ci*trus, calculated *Ci* was not considered an important limiting factor however the mesophyll CO_2 diffusion in cells or chloroplast counted much.

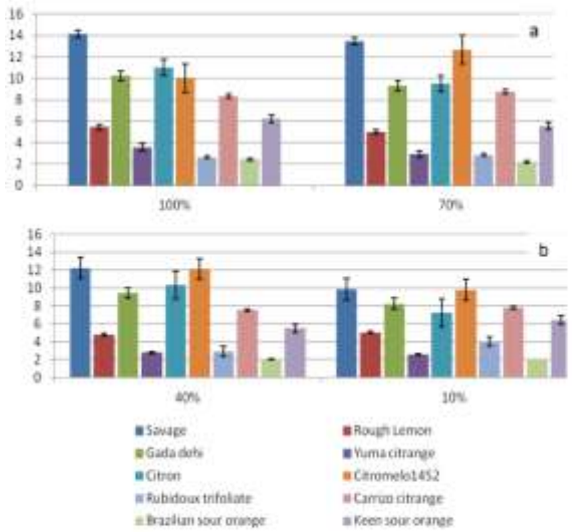

Fig.s 3 a,b: The effect of four water regimes (100.70,40 and 10 AWC,s %) on the plant biomass (gms) of *Ci*trus rootstocks grown on peat moss medium. The trend depicts that the *Ci*trus rootstocks did not show water stress on 100 and 70% water percentages and least biomass was observed on 10% AWC. Vertical Bars Represent ±SE.

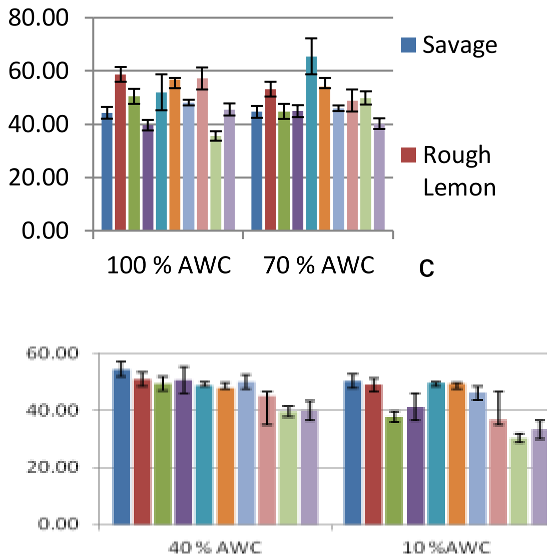

Fig.s. 4 c, d. The effect of four water regimes (100.70, 40 and 10 AWC, s %) on the plant chlorophyll conc. (SPAD) of *Ci*trus rootstocks grown on peat moss medium. The trend depicts that the *Ci*trus rootstocks did not show water stress on 100 and 70% water percentages and least biomass was observed on 10% AWC. Vertical Bars Represent ±SE.

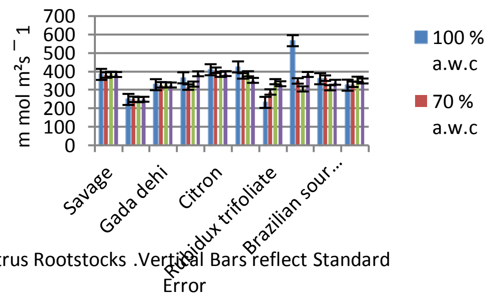

Fig. 5. Mesophyll Carbon intake (*Ci*) with in leaves by *Citrus* rootstocks in four available water capa*ci*ties on peatmoss.

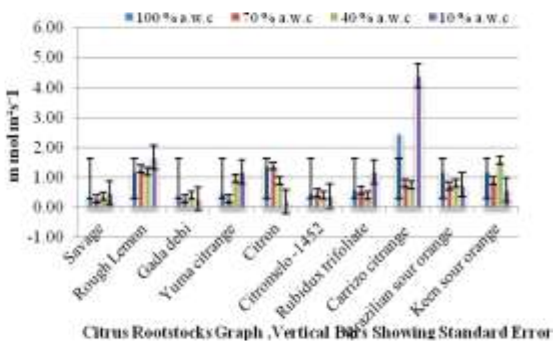

Fig. 6. Stomatal conductance of ten *Ci*trus rootstocks under four available water capa*ci*ties in peat moss in Rawalpindi.

A_{CO_2} limiting factor (Flexas *et al.*, 2004), so that mesophyll CO_2 could not be ruled out in water stress in *Ci*trus. This factor reduces production and quality of fruit but its intensity is dependent on the severity of stress. The chlorophyll formation on least water level was expressed almost same by Savage, Gada dehi, Citromelo-1452, and Carrizo *citrange* (Fig. 5a,b) which exhibited increased chlorophyll contents on least water level due to active root-shoot water pull (Perez-Perez *et al.*, 2008a). Bio-exchange parameters like gs, Pn and E $_{leaf}$ regulated plant growth even under stress environment (Syvertsen and Levy, 2005), Rubidoux trifoliate, Flying dragon and Cleopatra mandarin had showed better plant water status under minimized water % (Perez-Perez *et al.*, 2008).

Conclusion

The current experiment explored lower carbon assimilation from atmosphere than mesophyll carbon intake. However, Gada dehi, Rough lemon, Keen sour oranges and Citron overall explored salient drought resistant traits as compared to sensitive rootstock Brazilian sour orange. Flying dragon could not gain height due to low carbon intake from atmosphere. The biomass of vigorous rootstocks Carrizo citrange, Keen sour orange exhibited high under all water levels due to their root to shoot water translocations that caused early growth media dryness. The use of Gada dehi, Keen sour orange and Citron citrange has tendency to combat water scarcity. Rough lemon genetic makeup still has water resistant traits which might be in interest of breeders in future.

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References

Anonymous. 2007. Agricultural Statistics of Pakistan. MINFAL, Islamabad.

Binzel ML, Reuveni M. 1994. Cellular Mechanism of salt tolerance in plants. Horticultural Review **38**, 33-69.

Flexas J, Medrano H. 2002. Drought inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. Annals of Botany **89**, 183-189.

Flexas J, Ribas-Carbo M, Diaz-Espejo A, Galmes J, Medrano H. 2008. Mesophyll conductance to CO_2: current knowledge and future prospects. Plant, Cell and Environment **31**, 602-621.

Flexas J, Barbour MM, Cabrera HM. 2004. Mesophyll diffusion conductance to CO_2: an unappreciated central player in photosynthesis. Plant Science, in Press.

Garcia-Sanchez F, Perez-Perez JG, Botia P, Martinez V. 2006. The response of young mandarin trees grown under saline conditions depends on the rootstock. European Journal of Agronomy **24**, 129-139.

Khan AA, Rao SA, McNeilly T. 2003. Assessment of salinity tolerance based upon seedling root growth response functions in maize (*Zea mays* L.). Euphytica **131**, 81-89.

Lima M, Furlani PR, Miranda JB. 1992. Divergent selection for aluminium tolerance in maize (*Zea mays* L,) population. Maydica **37**, 123-132.

Lisar SYS, Motafakkerazad R, Hossain MM, Rahman IMM. 2012. Water Stress in Plants: Causes, Effects and Responses, in Water Stress; Prof. Ismail Md. Mofizur Rahman, (ed.), in Tech: New York, USA.

Medina C L,Machado EC, Pinto JM. 1998. Fotossíntese de laranjeira Valência enxertada sobre quatro porta-enxertose submetidas a deficiência hídrica. Bragantia **57**, 1-14.

Pérez-Pérez JG, García J, Robles JM and Botía P. 2010. Economic analysis of navel orange V.‘ Lane Late’ grown on two different drought-tolerant rootstock under deficit irrigation in south-eastern Spain. Agricultural Water Management **97**, 157-164.

Pérez-Pérez JG, Romero PJM, Navarro, Botía P. 2008. Response of sweet range cv 'Lane late' to deficit irrigation in two rootstocks. I: water relations, leaf gas exchange and vegetative growth. Irrigation Science **26,** 415-425.

Romero P, Navarro JM, Perez-Perez J, Garcia-Sanchez F, Gomez-Gomez G, Porras I, Martinez V, Botia P. 2006. Deficit irrigation and rootstock: their effects on water relations, vegetative development, yield, fruit quality and mineral nutrition of *Clemenules mandarin*. Tree Physiology **26,** 1537-1548.

Shah MA. 2004. Citrus Cultivation in N.W.F.P. Proceedings of the 1st International conference on citri culture, University of Agriculture Faisalabad p. 36-39.

Syvertsen JP, Levy L. 2005. Salinity interactions with other abiotic and biotic stresses in citrus. Horticultural Technology 15, 100-103.

Szabados L, Savourea A. 2009. Proline: A Multifunctional Amino Acid. Trends in Plant Science **15,** 89-97.

Zhao TJ, Sun S,Liu Y, Liu JM, Liu Q, Yan YBN, Zhou HM. 2006. Regulating the drought-responsive element (DRE) mediate signaling pathway by synergic functions transactive and trans inactive DRE binding factors in *Brassica napus*, Journal of Biological and. Chemical **281,** 10752-107.