



## Drought stress effect on morphological and physiological characteristics of different varieties of annual verbena (*Verbena hybrida*)

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### Abstract

Water scarcity is emerging as a problematic issue day by day for plant growth and development. Regarding this issue use of drought resistant plant cultivars is one of the strategies to cope water deficit conditions. Drought defiant plants are able to endure water deficit conditions and their growth and development is least effected by water scarcity. Current study was designed to check the ability of survival under water stress conditions of verbena cultivars F1 "Obsession" and "Quartz". After the appearance of seedlings plants were transferred to glass house and subjected to the drought conditions by applying different intervals of drought to the verbena cultivars. Different intervals of drought applied were T<sub>0</sub> (1 day), T<sub>1</sub> (3 days), T<sub>2</sub> (5 days) and T<sub>3</sub> (7 days). Morphological attributes studied, were plant height, number of leaves/plant, number of flowers/plant, leaf firing percentage, shoot fresh and dry weights and root fresh and dry weights. Physiological parameters including photosynthesis (A), chlorophyll contents, stomatal conductance (g<sub>s</sub>), sub-stomatal conductance (C<sub>i</sub>), transpiration rate (E), water use efficiency (WUE) and leaf water potential (Ψ<sub>wpd</sub>) were determined. Drought exerted negative impacts on both varieties regarding different morphological and physiological parameters. Overall analysis of final results of different parameters revealed that F1 Obsession is more drought resistant as compared to F1 Quartz showing better results regarding different morphological and physiological parameters.

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## Introduction

Scarcity of water utilize for the purpose of irrigation is the major issue, resulting in the stress on the growth and development of crops (Ashraf, 2010). As demand of water use for the irrigation is increasing day by day but still the water which is available as whole is decreasing. Population is increasing rapidly and to fulfill the requirements of food most of the barren lands are converted into productive land which ultimately create stress on the available resources of water (Somerville and Briscoe, 2001).

Pakistan also appears in the list of countries which are facing scantiness of water. Besides, drifting up of ground water use for the purpose of irrigation is causing decrease in soil water table which is in turn another serious problem. Like other cultivated crops in the world ornamental plants also need optimum supply of irrigation water for their survival, growth and augmentation. Verbena (*Verbena hybrida*) is an important bedding plant used in urban landscape and also at other places i.e. in containers. Its availability in various seasons and color range makes it a favorite bedding and container plant among the gardening community. It includes about 250 species of usually hairy, erect or decumbent to prostrate, annual or perennial herbs or sub shrubs; most are native to tropical and subtropical North and South America (Griffiths, 1994).

It is experimentally proved by the scientists that both the water inadequacy and excess irrigation have adverse effect on plant growth and augmentation (Tezara *et al.*, 1999). Excess irrigation renders to water logged conditions which suffocate the plant root system and results in less water uptake and low rate of respiration aiding the anaerobic conditions (Taiz and Zeiger, 2002).

Likewise water scanting conditions affect the morphology and physiology of plants which ultimately reduce the growth and proliferation of plants. Water scarcity sometimes exert severe negative impact on the metabolism of plants effecting the major metabolic processes which are the chief source of energy for plants like glycolysis,

respiration and photosynthesis so putting pressure on the agriculture and anthropogenic values (Tezara *et al.*, 1999). In order to thrive under the water deficit conditions plants show certain modifications in physiology, growth and development which alter the life history of plants (Geber and Dawson, 1990; Ingram and Bartels, 1996; Maggio *et al.*, 2006). The ability of plants to survive under stress conditions varies among different plant species and populations having multiple biological processes studied at various molecular and organizational levels (Ingram and Bartels, 1996; Hausman *et al.*, 2005; Juenger *et al.*, 2005).

The world botanists struggled for decades to know about the defending mechanism of plants against abiotic stress in applied and basic sciences (Araus *et al.*, 2002; Chaves *et al.*, 2003; Maggio *et al.*, 2006). Multifarious techniques have been used by the agricultural scientists and gardeners to overcome the problem of drought stress comprising the use of water conserving methods like sprinkler and drip irrigation, growing substances having good water holding capacity like mulches and cultivation of those varieties having high drought resistance ability (Anjum *et al.*, 2011). Among all the techniques, use of drought resistance cultivars seems to be appeared an optimistic strategy to overcome this problem. Current study was designed for the evaluation of influence of water deficit conditions on the growth and development of annual verbena by evaluating different morphological and physiological attributes. It was also aimed to check the ability of different varieties of annual verbena to cope with the water deficit conditions and to sort out the more drought resistant cultivar among F1 "Obsession" and "Quartz".

## Materials and methods

### *Experimental design and growth conditions*

A pot experiment was conducted to ascertain the drought tolerance of two annual verbena (*Verbena X hybrida*) cultivars F1 "Obsession" and "Quartz" in a greenhouse, at Institute of Horticultural Sciences, University of Agriculture, Faisalabad.

The seeds were grown in seed germination trays having peat moss as growth media for germination. Seeds were sown and irrigated till germination was completed. After 4 weeks of seed germination, healthy and vigorous seedling were transplanted into plastic pots (25cm diameter and 25 cm depth) containing sand, silt, farm yard manure and leaf compost (1,1,1,1) as growth medium. Media saturated paste was made for the measurement of field capacity, which was maintained with each watering applied to plant. The seedlings were permitted to set up for 15–16 days before the start of water deficit treatments. Four drought treatments with following water interval levels, T<sub>0</sub> (Control, 1 day interval), T<sub>1</sub> (3 days interval), T<sub>2</sub>(5 days interval), T<sub>3</sub> (7 days interval) were maintained throughout the experiment. The experiment was laid out in Completely Randomized Design (CRD) with four replications in two factor factorial arrangements. Information accumulation was begun following 20 days of drought application.

*Data collection*

Data pertaining to various morphological characters including plant height, fresh and dry weights of roots and shoots (Bush, 1995), leaf firing percentage, number of leaves and flowers (Carrow and Duncan, 2003) were measured every 15<sup>th</sup> day.

Physiological attributes *viz.* photosynthetic rate, stomatal conductance, sub-stomatal conductance (Mosaad *et al.*, 1995), water potential (Makela *et al.*, 1998), transpiration rate (Subrahmanyam *et al.*, 2006) and water use efficiency(WUE) (Rafiq *et al.*, 2005) were calculated each month by the use of infrared gas analyzer (IRGA). Chlorophyll contents (a, b and total) were analyzed by the use of spectrophotometer by following method given by Arnon (1995), Taiz and Zeiger (2002) every 15<sup>th</sup> day till harvesting.

*Statistical analysis*

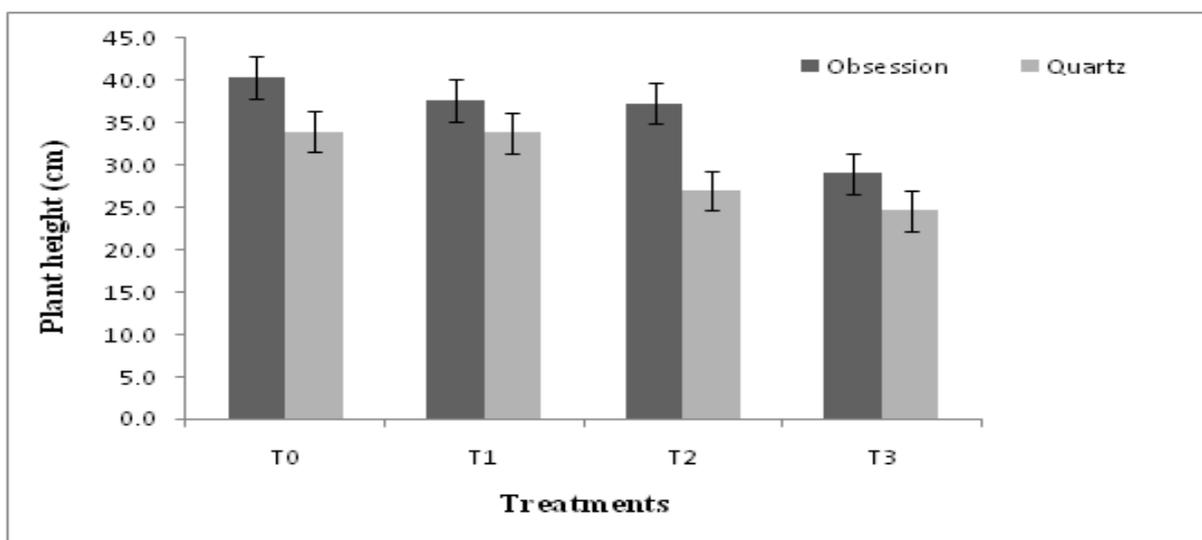
Data regarding to various morphological and physiological attributes were statistically analyzed following analysis of variance technique (ANOVA) using Statistix 8.1 software. The mean values were compared with least significance difference (LSD) test at 5% level of probability (Steel *et al.*, 1997).

**Results and discussion**

*Morphological characteristics*

*Plant height*

Maximum plant heights (40.3, 34.0 cm) were seen in plants, grown under control condition in both Obsession and Quartz cultivars, while minimum (29.0, 24.6 cm) at 7 days irrigation interval of both Obsession and Quartz cultivars, respectively.



**Fig. 1.** Response of two verbena varieties regarding plant height to drought stress conditions.

In obsession both at 3 and 5 days irrigation interval very little difference (37.7, 37.3 cm) was observed and sudden reduction was observed when irrigation interval was increased to 7 days. In case of Quartz at control and 3 days irrigation interval little difference (34.0, 33.8 cm) was observed and abrupt alleviation was seen as irrigation interval was increased up to 7 days.

Overall Obsession cultivar showed better results as compared to Quartz cultivar regarding plant height (Fig. 1). Similar consequences of the present study are in accordance with the discoveries of Sun and Dickinson (1995) who contended that diminishing stem stature was seen in the different types of the plants like *Eucalyptus camaldulensis* and *Casuarina cummehamiana* trees.

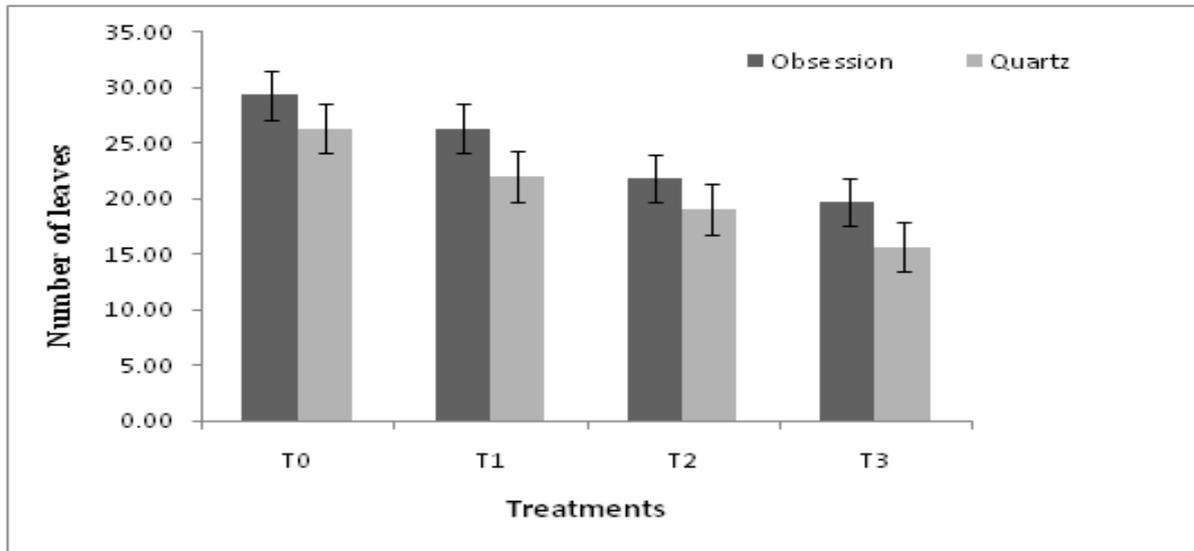


Fig. 2. Response of two verbena varieties regarding number of leaves to drought stress conditions.

Nautiyal *et al.* (2002), Schuppler *et al.* (1998) and Sundaravalli *et al.* (2005) likewise expressed that under water paucity trunk length was diminished in *Albizia* seedlings under water deficit conditions.

Decrease in plant height might be imparted to the lessening in the cell extension and more senescence of leaves in the plants under water deficit conditions (Manivannan *et al.*, 2007).

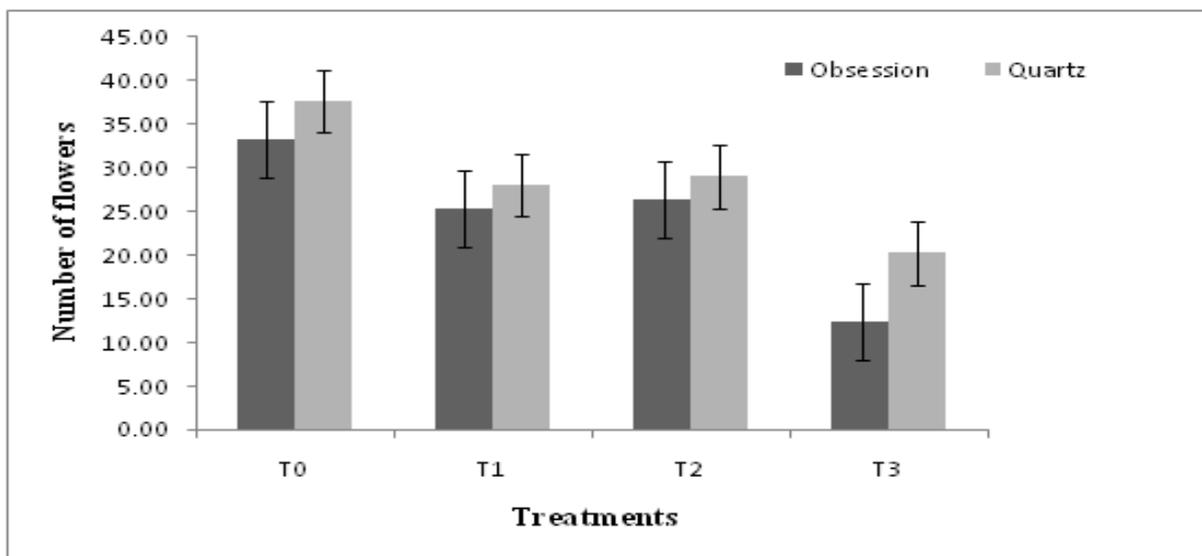


Fig. 3. Response of two verbena varieties regarding number of flowers to drought stress conditions.

Number of leaves

Results from different intervals of drought applied showed that water deficit conditions had highly significant effect on the number of leaves of both cultivars of *Verbena hybrida*. The results for number of leaves are shown in (Fig. 2) which presents that there was

alleviation in number of leaves by increasing irrigation intervals. Plants subjected to every day irrigation interval treatment (T<sub>0</sub>) revealed the maximum number of leaves (29.3, 26.3) and minimum number of leaves (19.7, 15.7) was observed in plants under 7 days irrigation interval treatment (T<sub>3</sub>) for Obsession and Quartz, respectively.

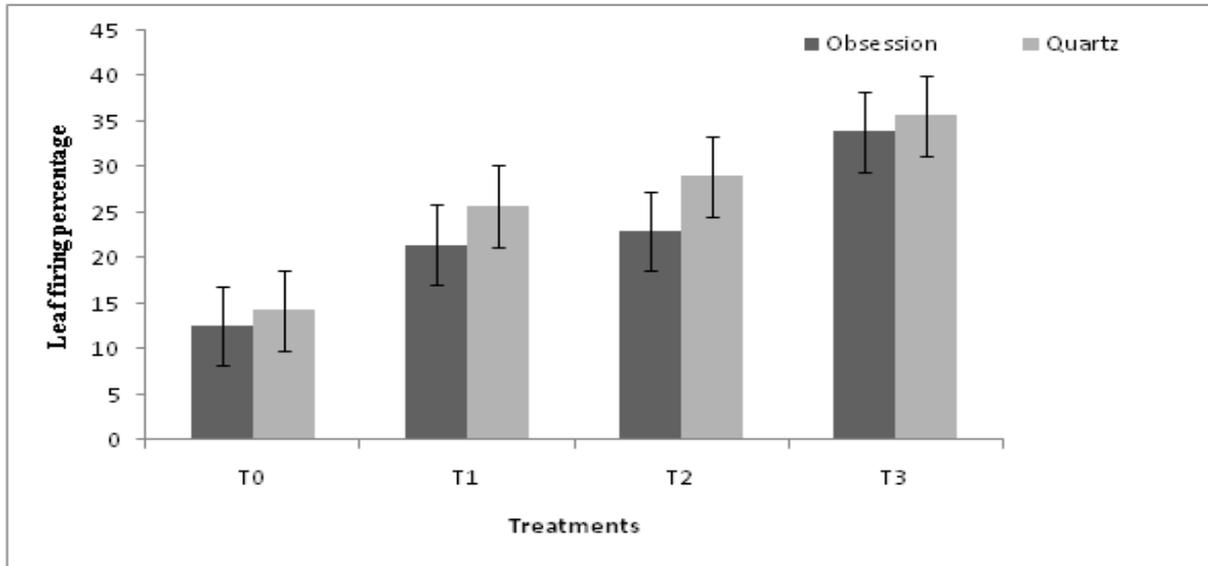


Fig. 4. Response of two verbena varieties regarding leaf firing percentage to drought stress conditions.

Both Obsession and Quartz showed gradual alleviation in number of leaves as irrigation intervals were increased. Overall Quartz showed more reduction in number of leaves as compared to Obsession as irrigation intervals were increased due

to its less drought tolerance capacity. Our outcomes are in line with the discoveries of El-Juhany and Ibrahim (2005) who reported diminishment in the number of leaves of *Gomphrena globosa* under dry spell stress.

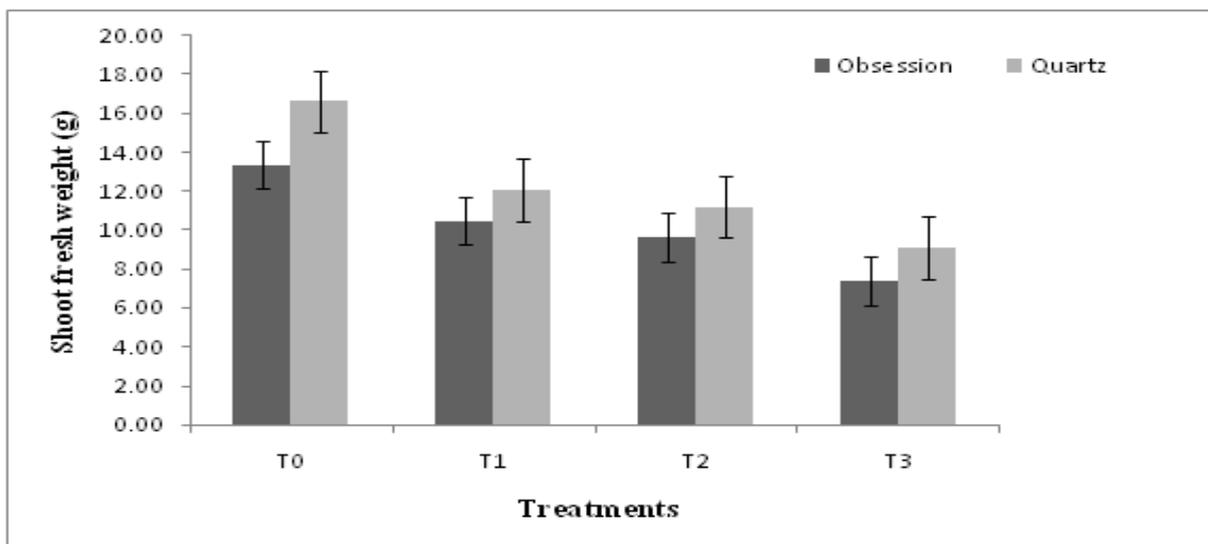
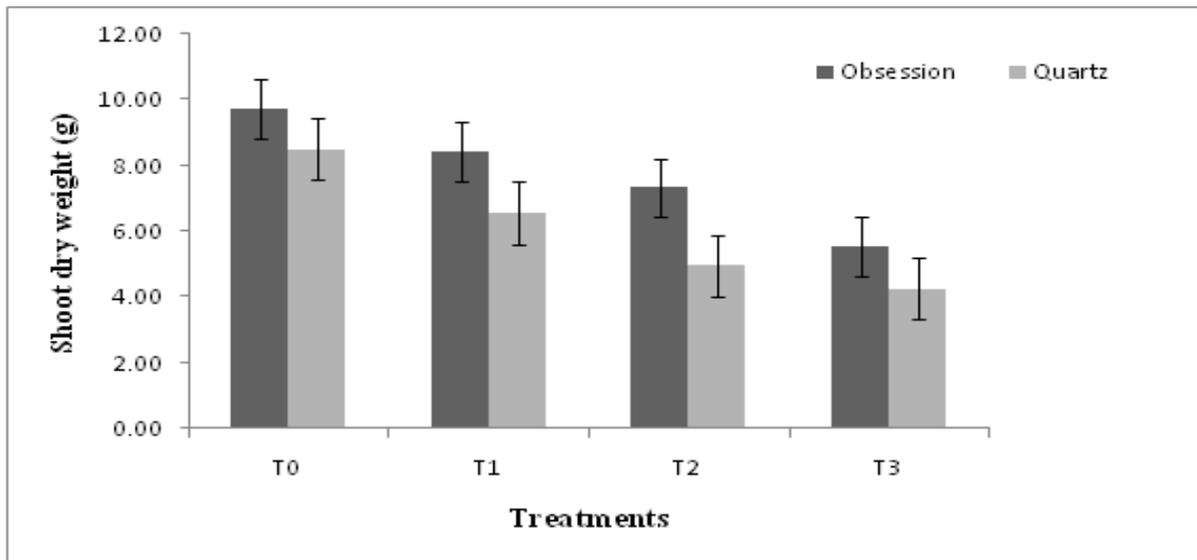


Fig. 5. Response of two verbena varieties regarding shoot fresh weight to drought stress conditions.

*Number of Flowers*

Data concerning number of flowers of two different cultivars of *Verbena hybrid* depicted that the effect of water deficit conditions on number of flowers was highly significant. Fig. 3 reveals that there was alleviation in number of flowers in T<sub>1</sub> and then

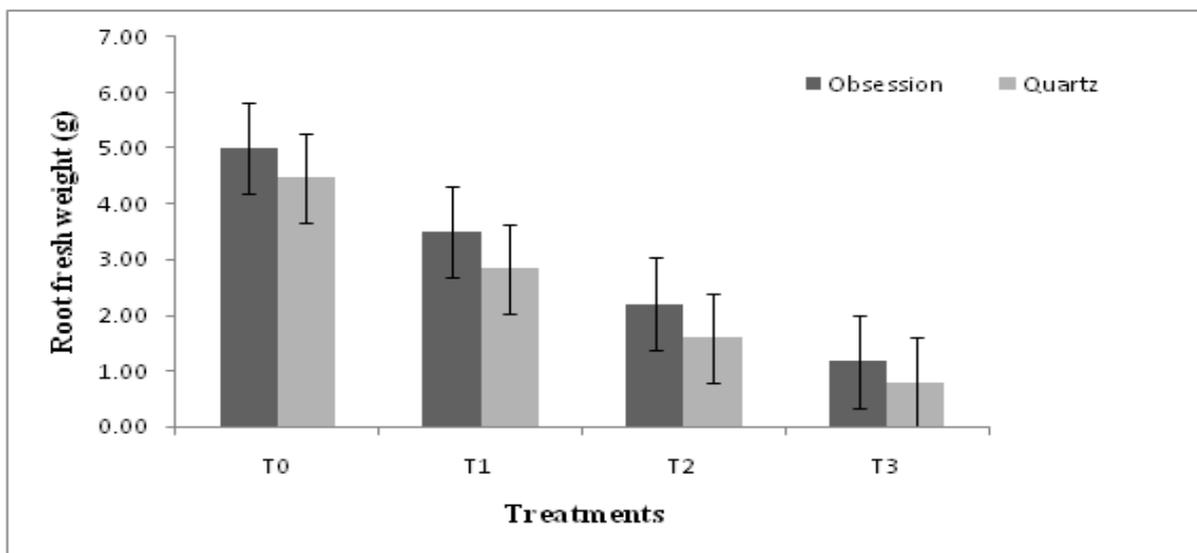
increase in T<sub>2</sub> and again reduction in T<sub>3</sub>. Plants subjected to every day irrigation interval treatment showed the maximum number of flowers (37.7, 33.3) and minimum number of flowers (20.2, 12.3) was seen in plants under 7 days irrigation interval treatment for Quartz and Obsession, respectively.



**Fig. 6.** Response of two verbena varieties regarding shoot dry weight to drought stress conditions.

Both cultivars showed sudden increase in number of flowers as irrigation interval was increased from 3 to 5 days and then abrupt alleviation was observed in number of flowers as irrigation interval was increased to 7 days revealing reduction in plant flowering with the increase in drought stress. Overall, Quartz showed

maximum number of flowers as compared to Obsession due to its more drought tolerance capacity. Water paucity diminishes the development of the plants by creating the decrease in aggregate leaf range and pushing leaf senescence (Kafiand Damghani 2001).

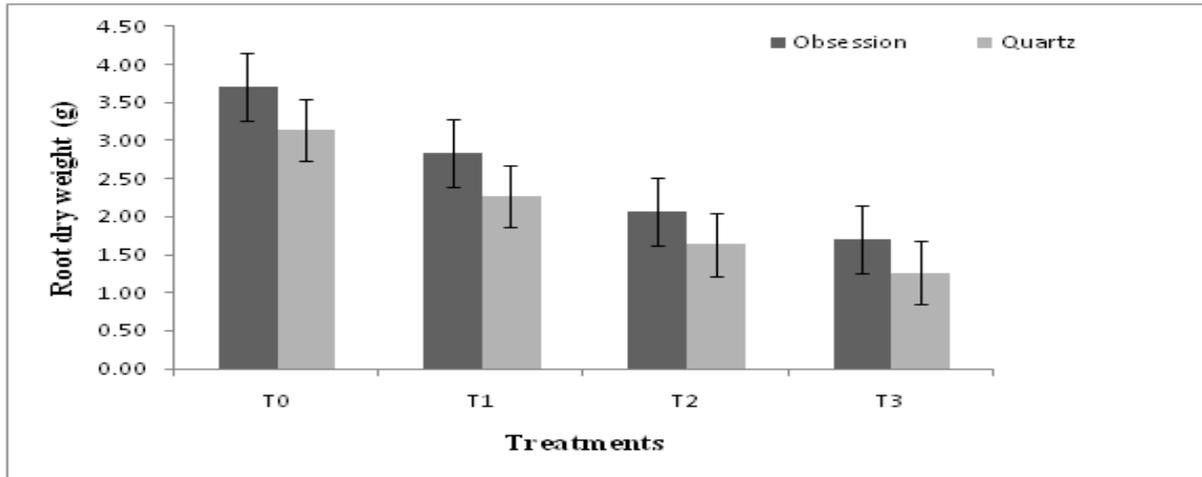


**Fig. 7.** Response of two verbena varieties regarding root fresh weight to drought stress conditions.

*Leaf firing percentage*

The impact of water stress on leaf firing percentage was highly significant on both cultivars Obsession and Quartz. Leaf firing percentage increases as the drought intervals are increased from daily to 7 days (Fig 4). Minimum leaf firing percentage (14.7%, 11.7%) was seen when both the cultivars were watered daily and maximum leaf firing percentage (82.3%,

68.3%) was found when plants were irrigated after 7 days irrigation interval of both Quartz and Obsession, respectively. Overall Obsession showed better results by less firing of leaves as compared to Quartz. The reason for increase in leaf firing might be high evapotranspiration rate and smaller root biomass and density during water stress (Sifers and Beard, 2000).

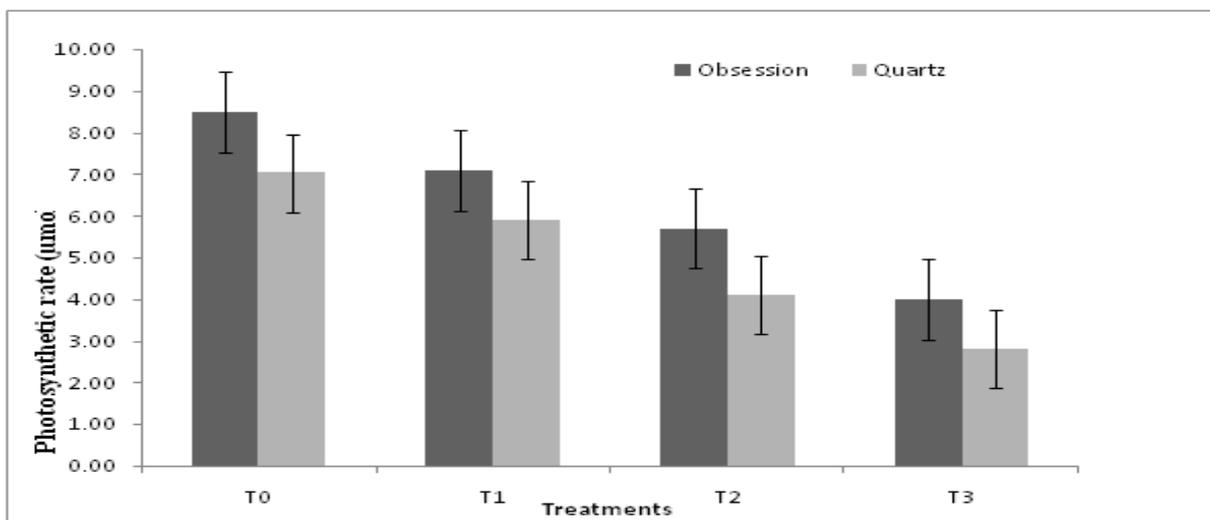


**Fig. 8.** Response of two verbena varieties regarding root dry weight to drought stress conditions.

*Shoot fresh and dry weights*

Regarding shoot fresh and dry weights the effect of drought was highly significant. Both cultivars were also found significantly varied between themselves for shoot fresh and dry weights. Figures (5 and 6) present the alleviation in shoot fresh and dry weights by accessing water deficit period up to 7 days irrigation

intervals. Obsession and Quartz had maximum shoot fresh (13.4, 16.6g) and dry (8.6, 7.4 g) weights at one day irrigation interval (T<sub>0</sub>) while minimum shoot fresh (7.4, 9.1g) and dry (3.8, 2.7 g) weights were recorded at seven days irrigation interval (T<sub>3</sub>), respectively.



**Fig. 9.** Response of two verbena varieties regarding photosynthetic rate to drought stress conditions.

Overall Quartz showed better results as compared to Obsession regarding shoot fresh and dry weights. Results of the present study are in agreement with the experiments of Pattangual and Madore (1999).

Durable dry spell had intense and exceptional consequences for plant wellbeing (Ashraf and Oleary, 1996; Tahir and Mehdi, 2001).

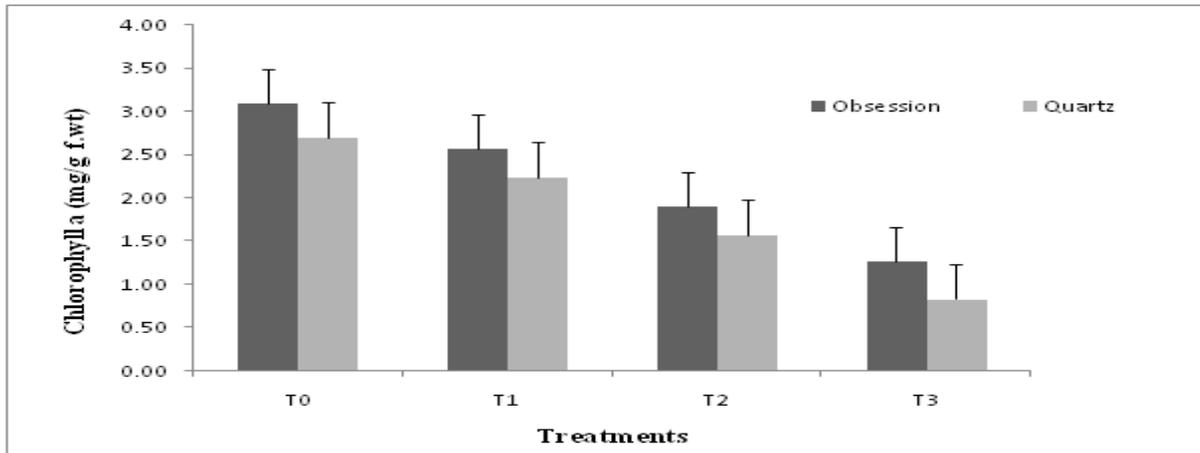


Fig. 10. Response of two verbena varieties regarding chlorophyll “a” contents to drought stress conditions.

*Root fresh and dry weights*

Root fresh and dry weights were affected significantly under water deficit conditions. Also the influence of drought conditions on fresh and dry weights of roots regarding cultivars was significant. Figures (7 and 8) show that paramount root fresh (5.0, 4.5 g) and dry

(3.7, 3.1g) weights were obtained when plants were acquiring water daily and least fresh (1.2, 0.8g) and dry (1.7, 1.3g) weights were observed in plants under 7 days irrigation interval of Obsession and Quartz, respectively. Overall, Obsession revealed better results as compared to Quartz.

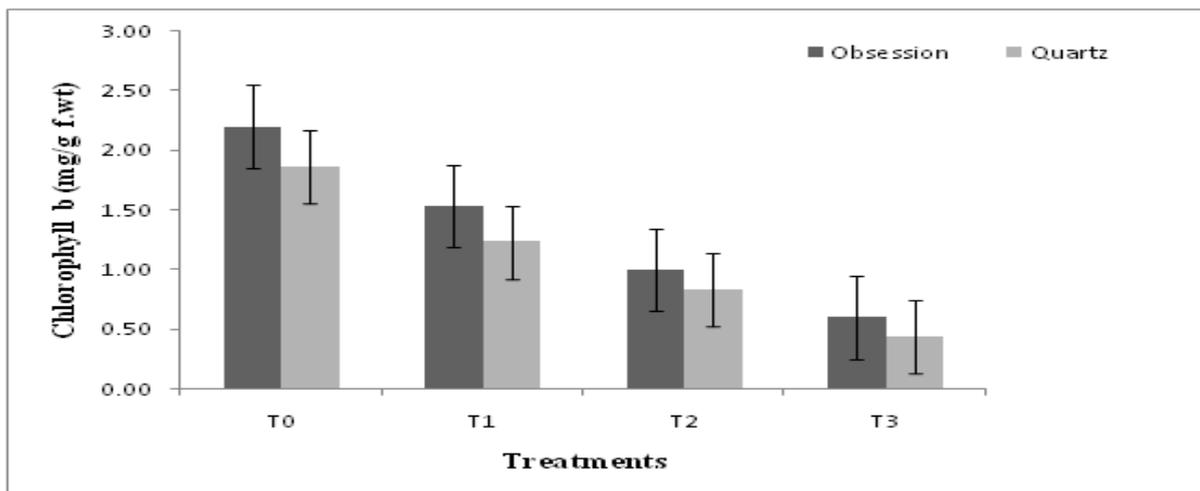


Fig. 11. Response of two Verbena varieties regarding chlorophyll “b” contents to drought stress conditions.

Similarly, Willekens *et al.* (1997) observed diminished development of roots in *Populus cathayami* under water deficit conditions. Riaz *et al.* (2013) reported that root fresh and dry weights were antagonistically influenced by the dry spell.

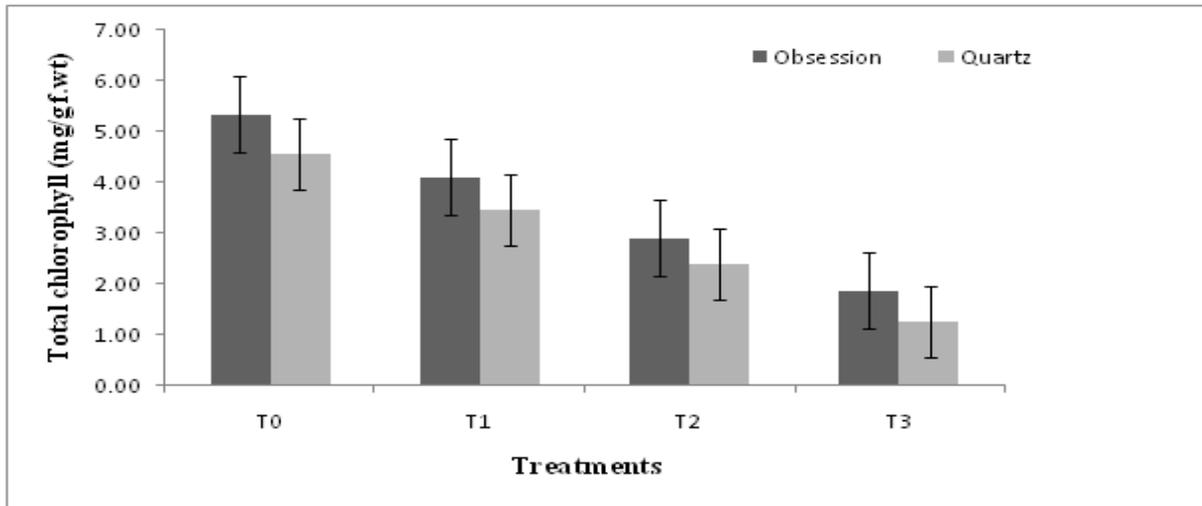
Compelling alleviation was found in fresh and dry weights of roots under water deficit conditions in cotton (Yin *et al.*, 2004). Our results are relevant to the observations concluded on various plant species by Morgan and Condon (2002) and Chaves and Oliveria (2004).

*Physiological characteristics*

*Photosynthesis (A)*

Photosynthesis assumes a fundamental part in building up the structural as well as non-structural components vital for appropriate plant growth. A progressive reduction in photosynthesis was seen with the increase in irrigation intervals of growth media. Maximum photosynthetic rate was observed

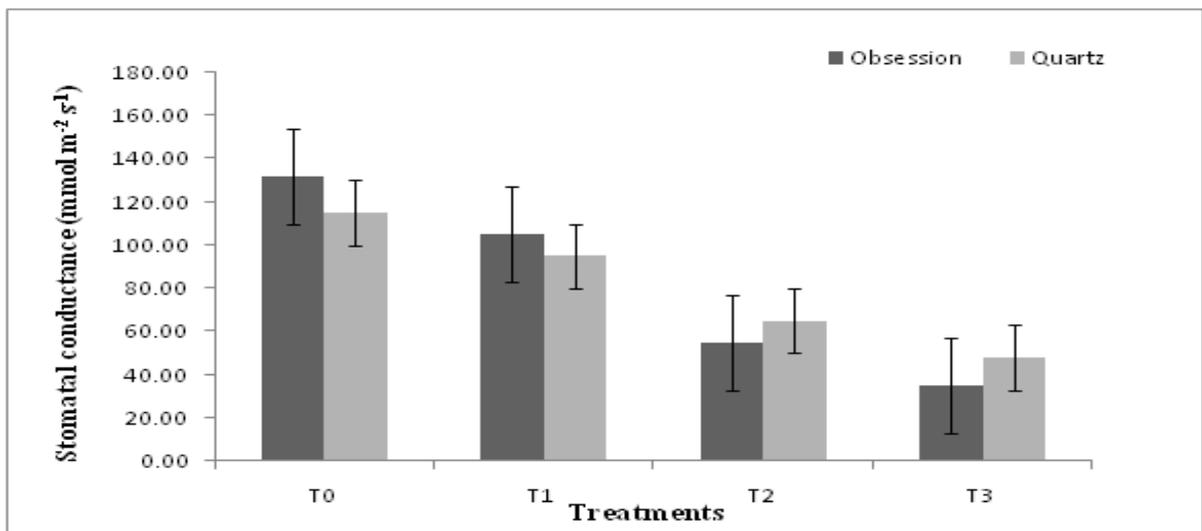
in plants experiencing every day irrigation interval (8.5, 7.0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively) in Obsession and Quartz cultivars while it was minimum (4.0, 2.8  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively) at 7 days irrigation interval (Fig. 9). Between the two cultivars Obsession possessed better photosynthetic rate (4.0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) under drought conditions contrasted with Quartz (2.8  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).



**Fig. 12.** Response of two verbena varieties regarding total chlorophyll contents to drought stress conditions.

Drought induced stomatal closure, which restricts CO<sub>2</sub> uptake by leaves, might be the major cause of reduction in leaf photosynthesis (Cornic, 2000; Flexas *et al.*, 2004).

These results are in line with the outcomes of Yokota *et al.* (2002); Jaleel *et al.* (2008); Farooq *et al.* (2009) who reported significant reduction of photosynthesis under water stress conditions.

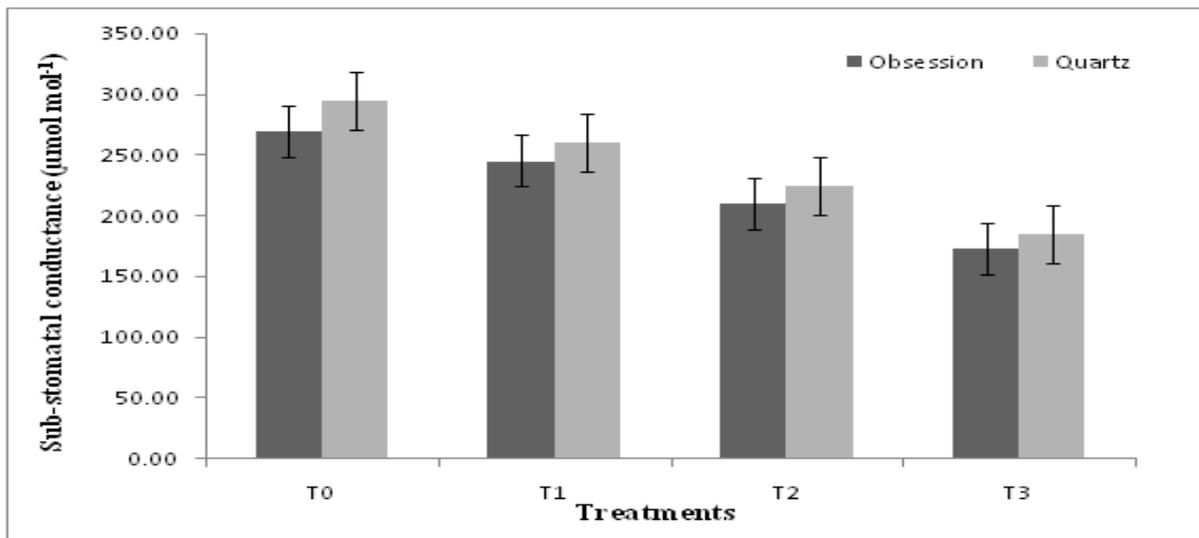


**Fig. 13.** Response of two verbena varieties regarding stomatal conductance to drought stress conditions.

*Chlorophyll contents*

Maximum amount of total chlorophyll, chlorophyll a and chlorophyll b of the cultivar Obsession (5.3 mg g<sup>-1</sup> FW, 3.1 mg g<sup>-1</sup> FW and 2.2 mg g<sup>-1</sup> FW, respectively) followed by the cultivar Quartz (4.6 mg g<sup>-1</sup> FW, 2.7 mg g<sup>-1</sup> FW and 1.9 mg g<sup>-1</sup> FW, respectively) was recorded for the treatment T<sub>0</sub> (irrigation after one day interval). As the irrigation was reduced to seven days interval (T<sub>3</sub>) the total

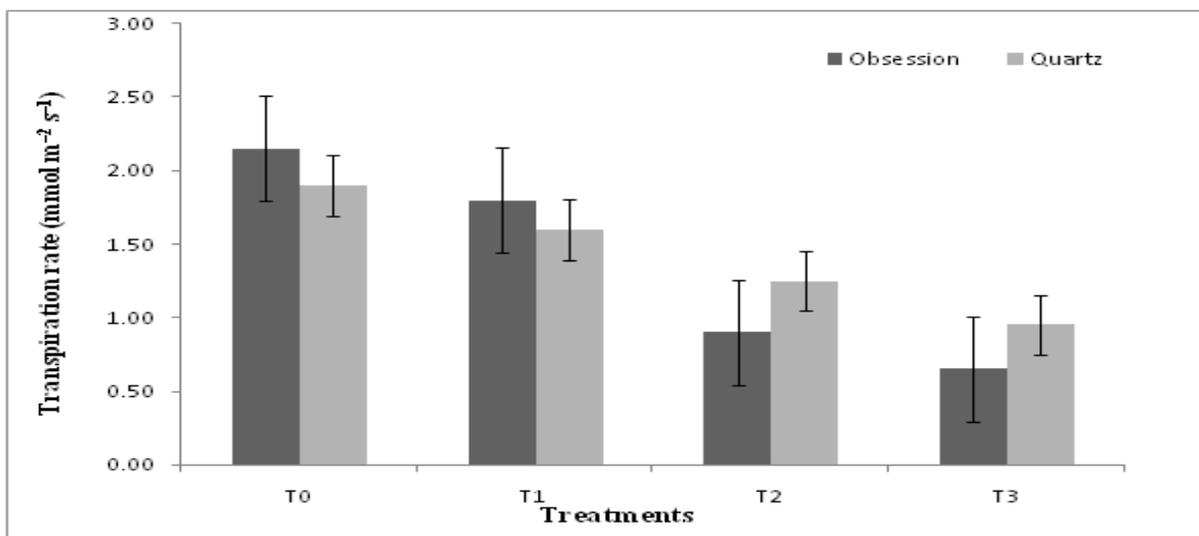
chlorophyll, chlorophyll a and chlorophyll b contents dramatically decreased leading to their least values (1.9 mg g<sup>-1</sup> FW, 1.3 mg g<sup>-1</sup> FW and 0.6 mg g<sup>-1</sup> FW, respectively) for the cultivar Obsession and (1.2 mg g<sup>-1</sup> FW, 0.8 mg g<sup>-1</sup> FW and 0.4 mg g<sup>-1</sup> FW, respectively) for the cultivar Quartz. Over all the attainment of the cultivar Obsession for these biochemical attributes was exceeding as compared to the cultivar Quartz for all treatments (Fig. 10-12).



**Fig. 14.** Response of two verbena varieties regarding sub-stomatal conductance to drought stress conditions.

These results are concurrence with Nyachiro *et al.* (2001), who observed reduction in chlorophyll a and b caused by water deficiency in six *Triticum aestivum* cultivars. Besides, Mafakheri *et al.* (2010) contended

that drought anxiety forced throughout vegetative development or anthesis altogether alleviated chlorophyll a, chlorophyll b and aggregate chlorophyll contents.

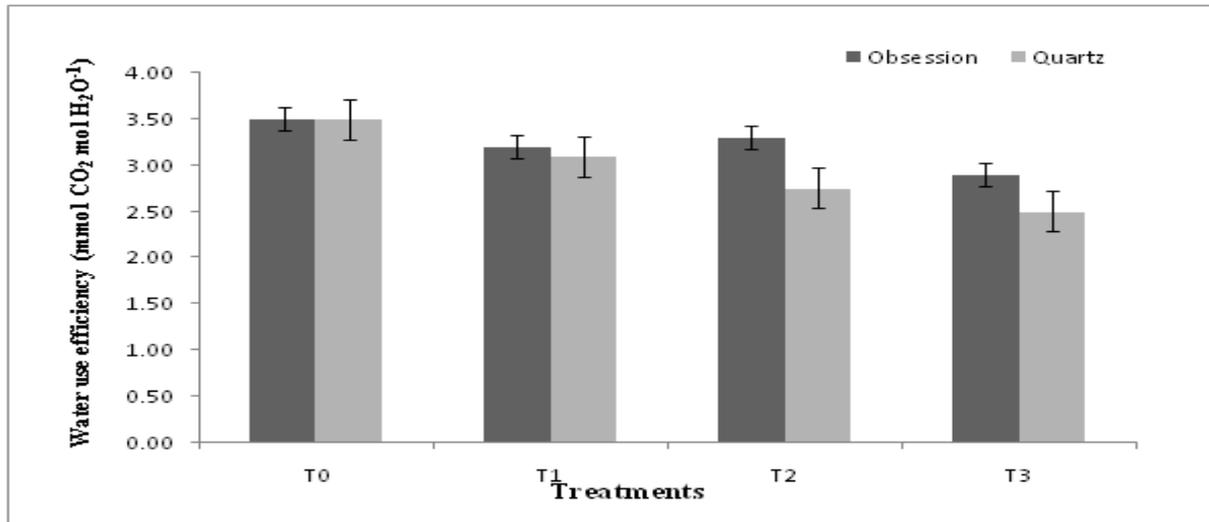


**Fig. 15.** Response of two verbena varieties regarding transpiration rate to drought stress conditions.

### Stomatal conductance ( $g_s$ ) and sub stomatal conductance ( $C_i$ )

Stomatal conductance and sub stomatal conductance characteristics are of vital importance to estimate drought tolerance of plants. Data concerning stomatal

conductance shown in Fig. 13 reflect that maximum stomatal conductance was registered in Obsession at every day irrigation interval ( $132 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) which substantially reduced at 5 and 7 days irrigation intervals ( $55$  and  $40 \text{ mmol m}^{-2} \text{ s}^{-1}$ , respectively).



**Fig. 16.** Response of two verbena varieties regarding water use efficiency to drought stress conditions.

This was even less than Quartz ( $65.20$  and  $48.63 \text{ mmol m}^{-2} \text{ s}^{-1}$ , respectively) at 5 and 7 days irrigation intervals. Sub-stomatal conductance of Quartz remained high compared to Obsession even at severe drought treatments T<sub>2</sub> (5 days irrigation interval) and T<sub>3</sub> (7 days irrigation interval) which was  $224$ ,  $185 \mu\text{mol mol}^{-1}$ , respectively for Quartz and  $210$ ,  $173 \mu\text{mol mol}^{-1}$ , respectively for Obsession (Fig. 14). It is well documented that the partial closure of stomata is the first response of plants to acute water deficit to prevent transpiration (Mansfield and Atkinson, 1990). Similar response was also depicted by many researchers like Mosaad *et al.* (1995), Willmer and Fricker (1996); Nakashima *et al.* (2000).

### Transpiration rate ( $E$ )

Water deficit in plant body impairs transpiration rate by reducing stomatal activity, which provides good assessment of drought tolerance of plants (Carrow and Duncan, 2003). Results shown in Fig. 15 clearly indicate that drought stress had significant effect on the transpiration rate of verbena cultivars.

Higher rate of transpiration was observed in Obsession cultivar in treatments T<sub>0</sub> ( $2.15 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) and T<sub>1</sub> ( $1.80 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) when water was available in abundance and it drastically reduced with the progression of water stress in T<sub>2</sub> ( $0.90 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) and T<sub>3</sub> ( $0.65 \text{ mmol m}^{-2} \text{ s}^{-1}$ ).

This ability of Obsession to retain moisture inside the plant under dry spell stress makes it more drought resistant as compared to Quartz. Alike reaction was also observed in wheat crop under dry spell (Siddique *et al.*, 1999; Subrahmanyam *et al.*, 2006).

### Water use efficiency ( $WUE$ )

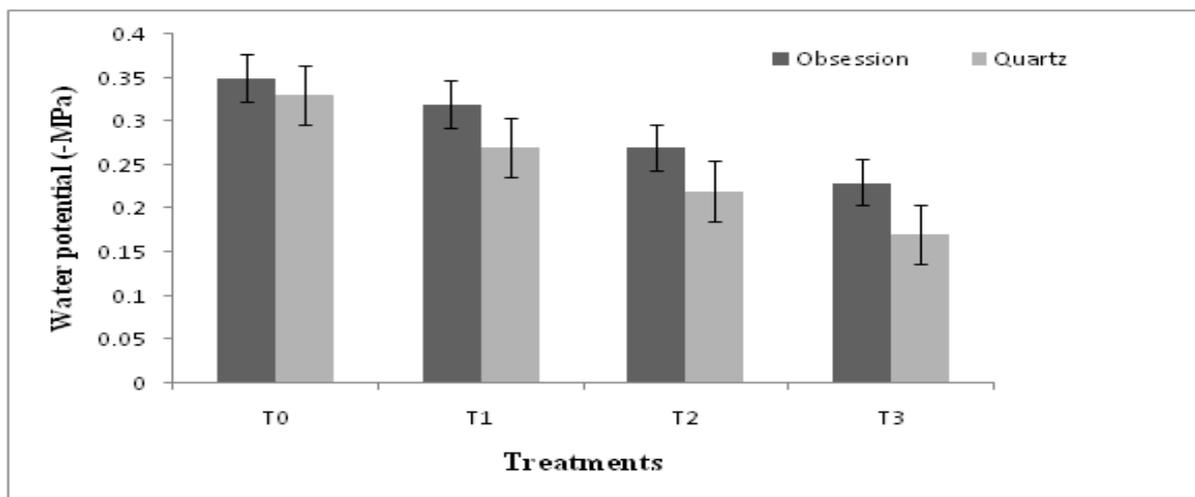
Water use efficiency is a ratio between dry matter produced and the water consumed (Monclus *et al.*, 2006). Statistically higher values of Obsession cultivar ( $3.72 \text{ mmol CO}_2 \text{ mol H}_2\text{O}^{-1}$ ) for water use efficiency as compared to Quartz cultivar ( $3.13 \text{ mmol CO}_2 \text{ mol H}_2\text{O}^{-1}$ ) further justify that it possesses better stress endurance under all treatments (Fig. 16).

High water use efficiency can be correlated to stomatal closure to reduce transpiration (Abbate *et al.*, 2004). These results are in accordance with the finding of Lazaridou *et al.* (2003); Lazaridou and Koutroubas (2004) and Rafiq *et al.* (2005).

#### Leaf water potential ( $\Psi_{wpd}$ )

In case of leaf water potential, it can be estimated from the results that more pronounced decrease

in  $\Psi_{wpd}$  is observed in Quartz cultivar as compared to Obsession cultivar with exceeding water deficit (Fig. 17). Changes in  $\Psi_{wpd}$  can be ascribed to a change in osmotic pressure (Siddique *et al.* 2000). These observations are in agreement with the findings of Archer and Rambal (1992), Castell *et al.* (1994), Luisa *et al.* (1995) and Fotelli *et al.* (2000).



**Fig. 17.** Response of two verbena varieties regarding water potential to drought stress conditions.

#### Conclusion

Our study clearly illustrates that drought stress negatively affect plant morphological and physiological attributes. Among the cultivars of *Verbena hybrida* F1 Obsession is more drought tolerant as compared to F1 Quartz.

However, a further detailed study is needed to elucidate the underlying anatomical and genetic parameters which are responsible for differential responses to drought.

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