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EFFECT OF DROUGHT STRESS ON GROWTH AND FLOWERING OF MARIGOLD (*TAGETES ERECTA* L.)

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Abstract

Drought is an important abiotic stress that limits the plant growth and productivity. A pot experiment was conducted by using complete randomized design (CRD) with three replications (each replication contained three plants) to evaluate morphological and physiological attributes that can be used for characterization of drought tolerance in 2 varieties of Marigold (Super Giant & Inca F1). Four drought levels at 100% (control), 80%, 70% and 60% field capacity were maintained throughout the experiment. Morphological characteristics including plant height, number of leaves/plant, leaf firing percentage, leaf area, plant quality, root length, shoot fresh and dry weight, root fresh and dry weight and root-shoot ratio for fresh and dry weights were studied. Physiological parameters studied, were net CO_2 assimilation rate (Pn), transpiration rate (E), stomatal conductance (*g*s), sub-stomatal conductance, leaf water potential, water use efficiency Pn/E and chlorophyll content. Results showed that, overall plant quality of varieties decreased with the progression of drought stress where 70% F.C can be considered appropriate for acceptable plant quality, whereas Inca F1 performed better compared to Super Giant for all attributes studied.

Introduction

Drought is an important abiotic stress that limits the plant growth and efficiency (Yuyan *et al*., 2007; Riaz *et al*., 2010, Hamayun *et al*., 2010). The severity of drought is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands and moisture storing capacity of soils (Wery *et al*., 1994).

The world's water supply is at alarming stage and going towards reduction which will become a worse in coming years due to global warming (Salinger *et al*., 2005; Cook *et al*., 2007), while future demand for rapidly increasing population pressures is likely to further aggravate the effects of drought (Somerville & Briscoe, 2001). Alike many other countries, Pakistan is also facing the severe problem of drought. Pakistan falls into arid and semi-arid regions, as about an area of 0.563 Mkm^2 out of 0.804 Mkm^2 of the total area of Pakistan is the arid land with an annual rainfall of less than 60 cm. In rain-fed areas mean annual rainfall is much below than the crop water requirements (Anon., 2003). Likewise, under irrigated conditions availability of water is not ensured for the whole year. It is estimated that $1/4^{th}$ of total cultivated land of Pakistan (4.9 million ha) is drought prone (Khan & Qayyum, 1986) and situation is getting worse. All the provinces of Pakistan particularly major parts of Sindh and Baluchistan are experiencing the water deficit conditions since last few decades (Chandio, 2012) because rainfall is erratic and river flows have dropped. The unavailability of water and low rainfall are the major factors for converting large areas into deserts (Ashraf, 2006). Water in reservoirs also reached at dead level and the reservoirs have reduced capacity due to siltation. Like many countries Pakistan is also sucking out ground water more than the required rate of replenishing 'fossil' groundwater at an alarming rate (Anon., 2012) which can turn into a disaster.

For plants, this scarcity of water is a severe environmental constraint which effects photosynthesis and growth rate ultimately limiting their productivity (Yordanov *et al*., 2003; Ramanjulu & Sudhakar, 1997; Cornic & Massacci, 1996; Mwanamwenge *et al*., 1999). Plants also exhibit many biochemical and physiological changes under water deficit conditions (Pattangual & Madore, 1999, Kidokoro *et al*., 2009). Generation of reactive oxygen species (ROS) lead to lipid per oxidation (Chen *et al*., 2000; Sreenivasulu *et al*., 1999) protein degradation (Jiang & Zhang, 2001) and nucleic acid damages (Hagar *et al*., 1996). Under current scenario there is a dire need to develop strategies especially for ornamental Horticulture. Due to shortage of water worldwide trends in gardening are changing and many water saving techniques like Xeriscaping, use of alternate water sources, use of drought tolerant plant species, water wise and desert landscaping are gaining popularity.

Among these introducing and developing drought tolerant ornamental plants species is considered the most sustainable approach to cope with drought situation. Water needs of such plant species are about 50% of the water needs of non-drought tolerant plants. Among ornamental flowering plants, Marigold (*Tagetes erecta* L.) is considered one of them that can grow well under these conditions. It belongs to the family Compositae, and a genus of 52 species of annual and perennial herbaceous plants. They are native to the area stretching from the southwestern United States to Mexico and throughout South America. It is grown as ornamental crop both for loose flowers and as a landscape plant. It is used in landscape design due to its variable height (1-3 feet) and colour shades of flowers and is planted to form a solid mass of a color in beds. They have pinnate green leaves, and white, golden, orange, yellow, to an almost red floral heads typically 0.1 to 4-6 cm diameter, generally with both ray florets and disc florets (Edward, 1999). Keeping in view the importance and beauty of marigold a study was carried out to evaluate the effect of water deficit conditions on morphological and physiological attributes of marigold. This will also give an insight to select the tolerant varieties and ultimately help in breeding of the improved varieties.

Materials and Methods

A pot experiment was conducted at Rose Project, Institute of Horticultural Sciences, University of Agriculture Faisalabad, in which effect of drought was observed on two varieties of Marigold (*Tagetes erecta* L.) i.e., Marigold Super giant (Hamzaden, The Netherlands) and Inca F1 (Goldsmith, USA).

The seeds were sown in seed germination trays using peat moss as growth media. Seeds were irrigated daily with tap water till germination was completed. After 24 days of sowing, seedlings were transplanted into plastic pots containing a mixture of sand, silt and leaf compost (1:1:1). Pots were arranged in complete randomized design (CRD) with three replications and each replication had three plants. Seedlings were allowed to establish for 20 days before treatment started. Three drought levels i.e., 80%, 70%, 60% along with 100% F.C (control) were maintained throughout the experiment.

Data collection was started after 18 days of drought application. Morphological characters including plant height (cm), number of leaves/plant, leaf firing percentage (Carrow & Duncan, 2003), leaf area (cm^2) (Parsons, 1982) and plant quality (Huang, 2004) were calculated every 15^{th} day, whereas net CO_2 assimilation rate (Pn), stomatal conductance (*gs*), sub-stomatal conductance (Mosaad *et al.*, 1995), transpiration rate (Subrahmanyam *et al*., 2006), water use efficiency (WUE) (Rafiq *et al*., 2005) and leaf water potential (Makela *et al*., 1998) were estimated every month using an open system LCA-4 ADC portable infrared gas analyzer (IRGA) (Analytical Development Company, Hoddesdon, England). Chlorophyll content (a, b and total) were determined using method of Aron (1995) and Taiz & Zeiger (2002) every 15^{th} day till harvesting. Plants were uprooted carefully, washed with distilled water and root length (cm) was measured. Leaves, branches and roots were separated and shoot, root fresh weight (g) and root-shoot ratio of fresh weight (Bush, 1995) was estimated. Later, plant parts were dried in an oven at 65^oC to constant weight for shoot and root dry weight (Dubey, 1997; Chaves & Oliveria, 2004). Data were analyzed statistically following analysis of variance technique (ANOVA) (Steel *et al*., 1997), using STATISTICA computer program. Means of variation were distinguished by least significance difference test at $p<0.05$.

Results and Discussion

Results showed that drought had a highly significant effect on plant height, number of leaves/plant and plant quality. Whereas it had significant effect on leaf firing percentage, leaf area and root length. Among drought stress treatments, maximum plant heights i.e., 41.9cm and 23.0cm were observed in both varieties (Super giant and Inca F1 respectively) when grown under control condition (100% F.C), while these were minimum (25.3cm, 13.5 cm) at 60% F.C (Fig. 1). Between varieties, maximum plant height (33.9cm) was recorded in Super Giant on average as it was a taller variety compared to Inca F1. In this study, better response was achieved by the Inca F1, under drought stress conditions, which showed relatively lesser declines in plant height (Table 1) with the increase in drought stress compared to Super Giant which failed to maintain the decent plant height. Significant interaction among different plant cultivars and water stress treatments has been discussed by many scientists already (Ashraf & Khan, 1993; Dhanda *et al*., 2004; Asghari *et al*., 2009).

It was also observed that drought stress decreased number of leaves in both Super Giant and Inca F1 varieties from 100% F.C (control) (18.33 and 26.0, respectively) to 60% F.C. (7.0 and 9.66, respectively) (Fig. 2). Among varieties, more average number of leaves per plant (16.5) were recorded in Inca F1 on all drought treatments compared to Super Giant (12.6) because that was short and compact variety. Although relative decrease in number of leaves was lesser in Super Giant compared to Inca F1 (Table 1). Reason for decrease in number of leaves with increase in drought might be that drought inhibits growth in association with changes in cell size and division resulting in reduced leaf production and promoting senescence and abscission (Karamanos, 1980). This reduction of leaf number under drought stress (Maqsood & Ali, 2007) could probably be one of the drought tolerance mechanisms or water conservation strategy (Jones, 1992) under the limited soil moisture available. The reduction in the number of leaves is also observed in *Conocarpus erectus* under drought stress (EI-Juhany & Aref, 2005). This inhibition of leaf formation also decreases volume and size of new leaf tissues resulting decrease in leaf area which was reduced to 30.33cm^2 and 39cm^2 at 60% F.C from 59.67cm^2 and 61.67cm^2 at 100% F.C in both Super Giant and Inca F1 varieties, respectively (Fig. 3). The increment in water stress caused the reduction in leaf area where leaf became spindle and remained in a stunted state (Warrag & Hall, 1984) to avoid the excessive transpiration with low stomatal density (Parsons, 1982) until they were re-watered. This is a special mechanism in plants to tolerate the water stress. Our result concurs with the Kozlowski (1982) whose results also show decreasing growth of forest trees due to water stress in details.

Table 1. Relative reduction in morphological characters of Marigold with the progression of drought stress.

Drought treatments	Plant height (cm)		Number of leaves/plant		Leaf area (cm^2)		Plant quality	
	Super giant	Inca F1	Super giant	Inca F1	Super giant	Inca F1	Super giant	Inca F1
80% FC	4.17	1.90	4.33	8.34	7.33	6.00	0.40	0.77
70% FC	6.70	4.43	3.00	5.00	12.33	12.33	0.60	0.23
60% FC	5.70	3.20	4.33	3.00	9.67	4.33	0.67	0.43

Water is an important constituent of plant body but excess or deficit leads to death of tissues, which appear in the form of leaf firing. Leaf firing provides a good assessment of overall drought resistance (Carrow & Duncan, 2003) of ornamental plants. The comparison of means in Fig. 4 showed that the leaf firing percentage reached up to 100% in Inca F1 while it remained 71% in Super Giant at 60 % F.C. which indicates that Inca F1 possesses poor dehydration avoidance for extended periods of drought compared to Super Giant, though these varieties did not perform bad at 70% F.C. Increase in percentage of leaf firing was might be due to smaller root depth, lesser density and biomass, higher evapotranspiration rate during progressive water stress (Sifers & Beard, 2000). Leaf firing is the major characteristic considered to estimate the quality of ornamental plants along with color, height, plant health, insects/disease attack and other biotic and abiotic stress. Between the two varieties overall Inca F1 retained better quality after an evident reduction from 3.4 at 100% F.C to 2.0 at 60% F.C, (Fig. 5) while plant quality of Super Giant reduced to 1.63 at 60% F.C. Plant quality was most probably affected by the burning of tissues and these results are in the confirmation with the findings of (Jiang & Huang, 2001; Huang, 2004).

Plants take water and minerals through roots to sustain life and compete for nutrition. When plants are grown under stress i.e. salt or water stress, the competition is more pronounced (Nadeem *et al*., 2012; Riaz *et al*., 2010; Hameed *et al*., 2008) and roots define the tolerance of plants against stress under such conditions. Results in Fig. 6 show that the overall trend of root length for both the cultivars was increasing in the beginning and then decreasing as drought level increased. It was also observed that, root length between both of the varieties was highly significantly different where better response was achieved by the Inca F1, under drought stress conditions. Super Giant attained maximum root length (8.85 cm) at 100% F.C, whereas for Inca F1 it was at 80% F.C (8.40 cm). These varieties had minimum root length (6.05 cm and 7.45 cm, respectively) at 60% F.C. The reason for better performance of Inca F1 could be that its root system might have developed certain mechanism to cope with drought stress. Similar to our results, Passioura (1982) also reported that the reduction in the growth of the roots due to low water supply includes the root characteristics especially root length, root density and root thickness. Root system that enhances the ability of a plant to capture water is a fundamental adaptation mechanism to drought.

The analysis of variance revealed that overall growth of plants was effected by the drought stress, where drought had highly significant effect on shoot fresh and dry weight, root fresh and dry weight, and significant effect on the shoot-root ratio of these weights. There was again a decreasing trend of shoot fresh and dry weight as well as root fresh and dry weight with the increase in drought stress (Table 2). On average both varieties had maximum shoot fresh and dry weights at 100 % F.C. and least at 60 % F.C. Shoot fresh weight was particularly more of Super Giant, may be because it was a tall variety which produced more green biomass. However, rest of the growth parameters were more in weight in case of Inca F1, comparatively. Results in Table 2 also show that beside the less production of overall biomass, Super Giant variety remained more consistent in its growth particularly of root fresh and dry weights with the progression of drought stress, compared to Inca F1 hybrid. Production of dry mass is directly related to the amount of water transpired as there is reduction in growth of plants by alerting either the efficiency with which photosynthates aid to new growth or the rate at which they are used in maintaining the existing dry matter (Dubey, 1997). Plant growth and productivity under drought stress is strongly related to the process of dry matter partitioning and the spectral and temporal root distribution, biomass allocation to root and quantity and the length of functional roots increase under water stress (Pardo *et al*., 1998; Morgan & Candon, 2002) where higher root growth under water deficit condition can increase drought tolerance in plants (Chaves & Oliveria, 2004).

Root-shoot ratio helps to assess the overall health of plants and is used to evaluate the stress avoidance potential of plants (Bush, 1995). Results (Fig. 7) show that overall both Super Giant Inca F1 and had maximum root-shoot ratio for fresh weight (0.60 & 0.54 respectively) at 60% F.C and least shoot-root ratio (0.25 & 0.37, respectively) at 80% F.C. Plants of many species respond to drought by increasing the proportion of assimilate diverted to growth and thus, increase the shoot-root ratio and the volume of soil water available to plant. Increase in the root-shoot ratio can due to differential sensitivities of the root and shoots to endogenous ABA, or to a greater osmotic adjustment in roots compared with shoots (Samarah *et al*., 2007) under stress conditions like drought.

Table 2. Effect of drought stress on growth of Marigold.

Drought treatments	Shoot fresh wt. (g)		Shoot dry wt. (g)		Root fresh wt. (g)		Root dry wt. (g)	
	Super giant	Inca F1	Super giant	Inca F1	Super giant	Inca F1	Super giant	Inca F1
100% FC	13.86	11.73	5.86	6.16	3.70	6.36	3.30	5.30
80% FC	10.0	9.60	5.20	6.03	2.56	5.26	2.33	3.96
70% FC	5.53	6.46	3.76	4.70	2.26	2.63	2.03	2.43
60% FC	3.76	3.90	2.60	3.40	2.26	1.46	2.00	1.33

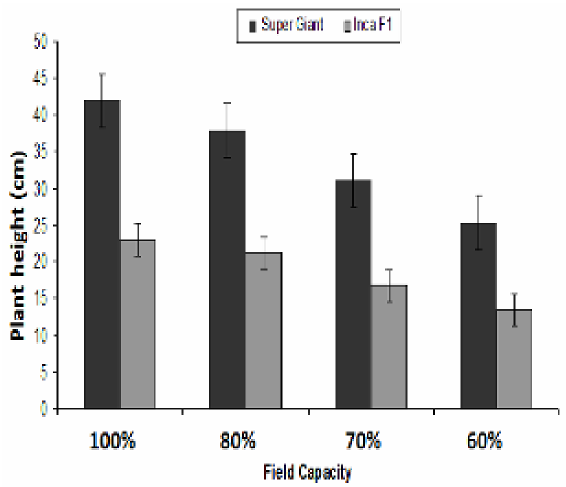

Fig. 1. Effect of drought stress on plant height (cm) of marigold varieties.

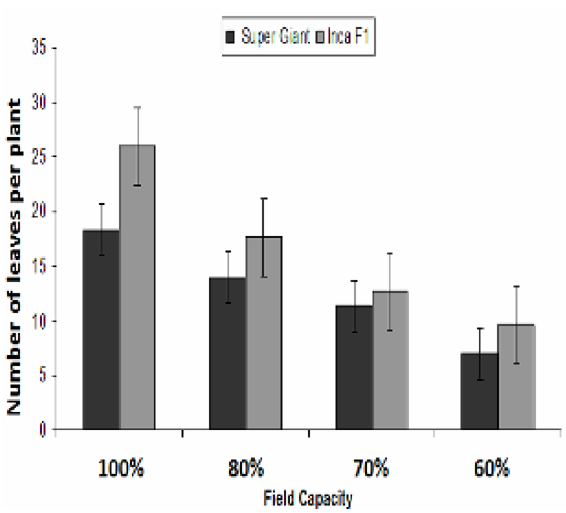

Fig. 2. Effect of drought stress on number of leaves of marigold varieties.

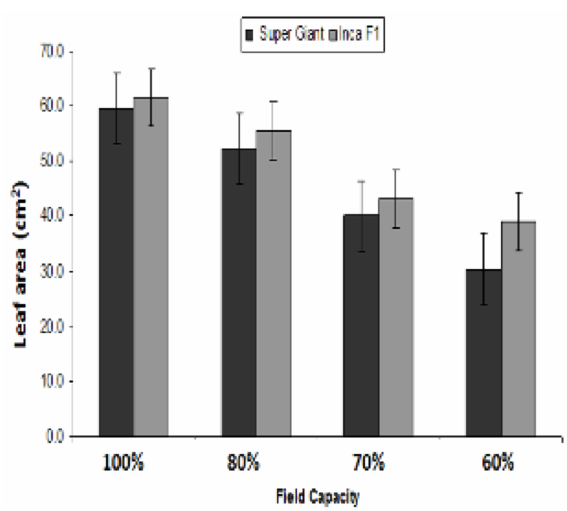

Fig. 3. Effect of drought stress on leaf area (cm2) of marigold varieties.

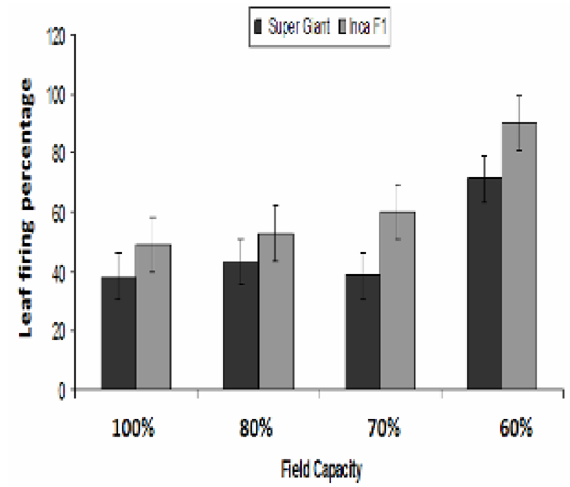

Fig. 4. Effect of drought stress on leaf firing percentage of marigold varieties.

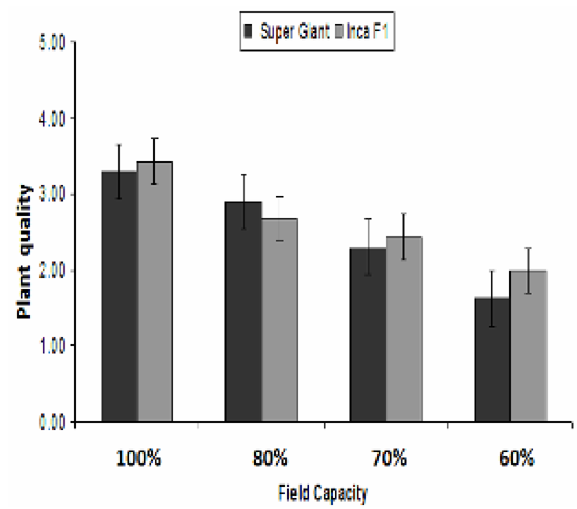

Fig. 5. Effect of drought stress on plant quality of marigold varieties.

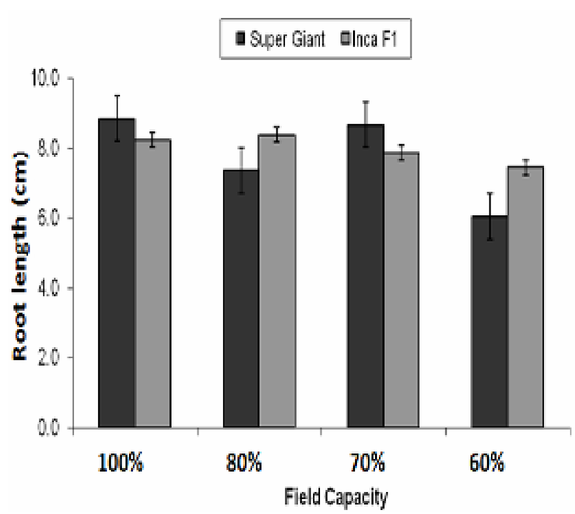

Fig. 6. Effect of drought stress on root length of marigold varieties.

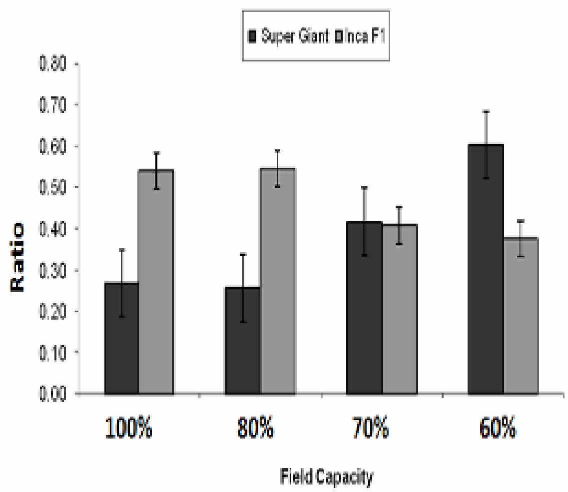

Fig. 7. Effect of drought stress on root-shoot ratio fresh weight of marigold varieties

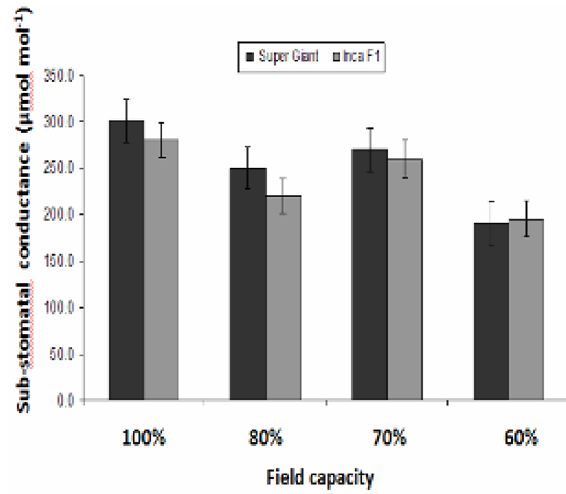

Fig. 10. Effect of drought stress on sub-stomatal conductance of marigold.

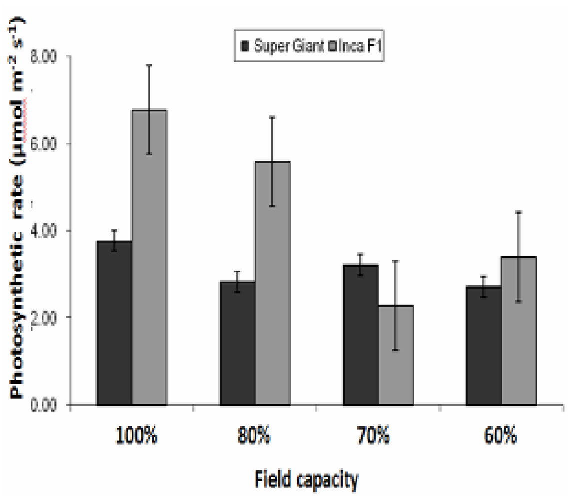

Fig. 8. Effect of drought stress on net CO2 assimilation in marigold varieties.

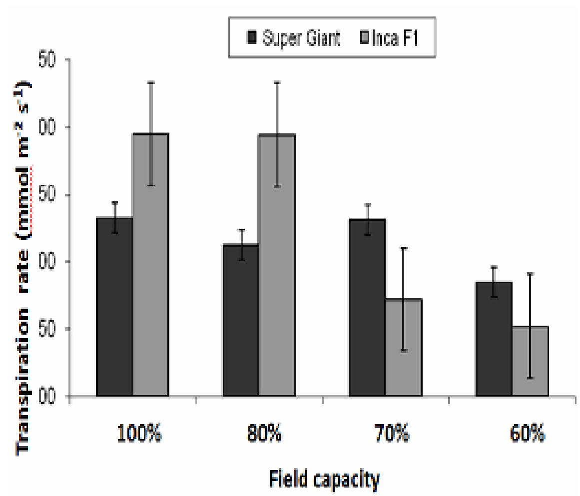

Fig. 11. Effect of drought stress on transpiration rate of marigold varieties.

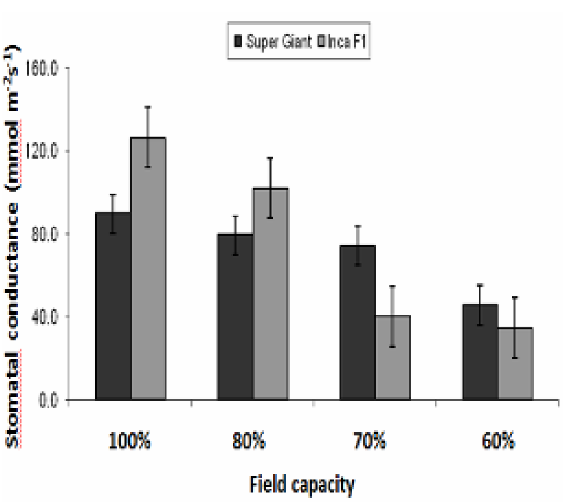

Fig. 9. Effect of drought stress on stomatal conductance of marigold varieties.

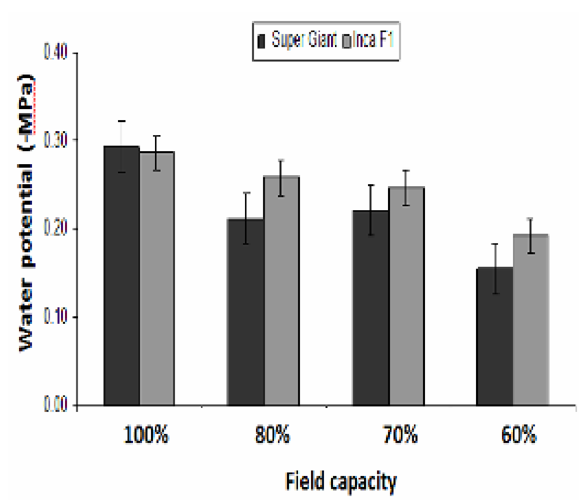

Fig. 12. Effect of drought stress on leaf water potential of marigold varieties.

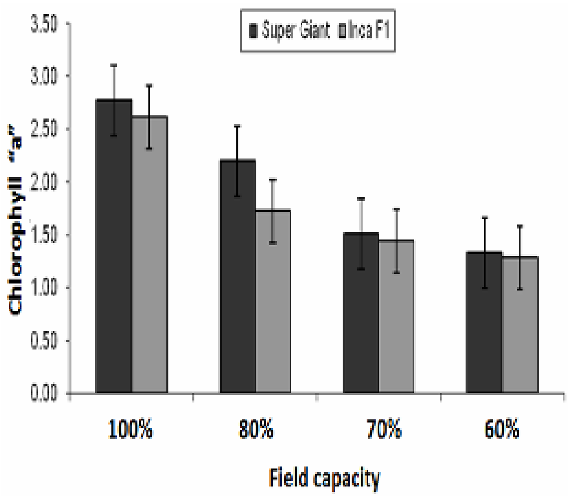

Fig. 13. Effect of drought stress on chlorophyll "a" in marigold varieties.

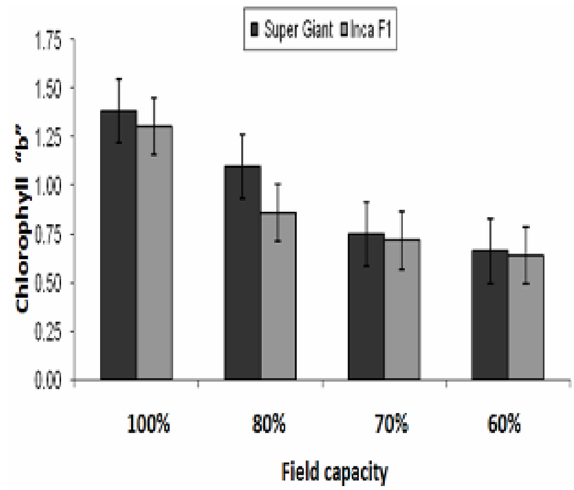

Fig. 14. Effect of drought stress on chlorophyll "b" in marigold varieties.

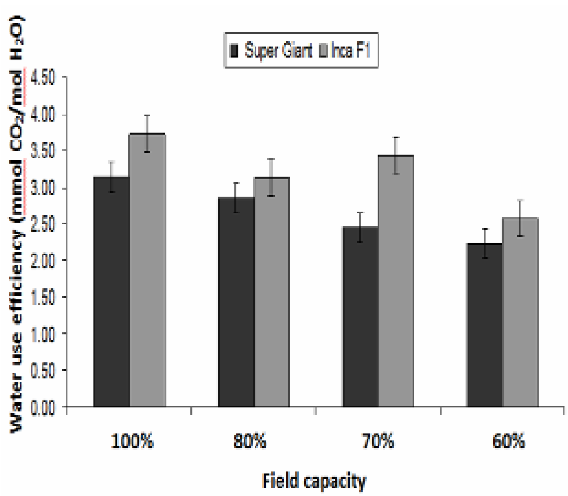

Fig. 15. Effect of drought stress on water use efficiency of marigold varieties.

Net CO_2 assimilation and stomatal conductance in marigold varieties were also significantly affected by the drought stress, whereas it had highly significant effect on transpiration rate, sub stomatal conductance and leaf water potential**,** though it remained non-significant for water use efficiency of plants. A marked reduction in net CO_2 assimilation was observed with the decrease in field capacity of growth media used in this study. Maximum plant net CO_2 was observed in plants grown under control (100% F.C) (3.77 µmol m^{-2} s^{-1} & 6.78 µmol m^{-2} s^{-1} respectively) in Super Giant and Inca F1 varieties while it was minimum (2.71 µmol m^{-2} s^{-1} & 3.40 µmol m^{-2} s^{-1} respectively) at 60% F.C (Fig. 8). Between the two varieties Inca F1 possessed better net CO_2 (3.40 µmol m^{-2} s^{-1}) under drought conditions compared to the Super Giant (2.71 µmol m^{-2} s^{-1}). The plant growth is controlled by the photosynthesis which is not only helpful in developing the structural but also non structural compounds necessary for proper plant growth. Results of present study are also in accordance with the findings in wheat crop under water stress conditions where water stress caused a significant reduction in net CO_2 (Mosaad *et al.*, 1995; Setter *et al.*, 2001; Lawlor & Cornic, 2002; Jaleel *et al.*, 2008; Farooq *et al.*, 2009) resulting in reduced the biomass of plant. Under drought stress stomatal closure (causing reduced leaf internal CO_2 concentration (*Ci*) can be considered a major reason for reduced rates of leaf photosynthetic (Chaves, 1991; Cornic, 2000; Flexas *et al*., 2004) in this study.

Stomatal conductance (*gs*) and sub stomatal conductance are also important parameters to screen the different varieties for drought tolerance. Results regarding stomatal conductance shown in Fig. 9 indicate that maximum stomatal conductance was in variety Inca F1 at 100 % FC (126.29 mmol $m^{-2}s^{-1}$) which greatly reduced at 70% (40.0 mmol $m^{-2}s^{-}$) and 60% F.C (34.6 mmol $m^{-2}s^{-1}$). This was even below than Super Giant (74.20 mmol $m^{-2}s^{-}$ & 45.63 mmol $m^{-2}s^{-1}$) at 70% and 60% F.C, respectively. Sub-stomatal conductance of Super Giant remained high compared to Inca F1 except at 60% F.C (Fig. 10), which was 195 µmol mol^{-1} and 190 µmol mol^{-1} respectively for both varities. However, *gs* never fell to zero, in this study indicating that varieties maintained stomatal conductance at low water potentials. Similar response was also described by Mosaad *et al*., (1995) in wheat crop under water stress conditions which is consistent with the general behavior of closure of stomata in plants to conserve moisture inside plant body (Willmer & Fricker, 1996, Nakashima *et al*., 2000).

Transpiration rate plays a major role in estimating drought tolerance of plants. Varieties which allow less loss of water from leaves through stomata retaining more water are supposed to be more drought tolerant. Data regarding transpiration rate of two varieties influenced by water stress is shown in Fig. 11, which indicates that Super Giant had more transpiration rate (1.15 mmol m^{-2} s^{-1}) on average, compared to Inca F1 (1.28 mmol m^{-2} s^{-1}), which may be due to bigger and lose plant structure of Super Giant which exposes leaves more to the air. Though, Inca F1 transpired water on higher rate (1.95 mmol m^{-2} s^{-1}) at 100% FC when abundant of water was available to roots (de Souza *et al*., 2005) and it drastically reduced at 70% (0.72 mmol m^{-2} s^{-1})

and 60% F.C (0.52 mmol $m^{-2} s^{-1}$) respectively. Such results clearly reflect tendency of Inca F1 to conserver moisture inside the plant under excessive water stress. The similar response was also showed by wheat crop under water stress conditions (Subrahmanyam *et al*., 2006; Siddique *et al*., 1999; Jaleel *et al*., 2007). It is well documented that drought affects growth (see Introduction) and also reduces stomatal conductance (Akyeampong, 1985) which can cause reduction in transpiration under drought stress (Akyeampong, 1985; Hall & Schulze, 1980).

In case of leaf water potential decrease was more pronounced when water deficit were imposed at earlier stage in Inca F1 though it retained more leaf water potential on average compared to Super Giant (Fig. 12). The decrease in leaf water potential due to water deficit as observed in the present study has earlier been reported in sunflower by Luisa *et al*., (1995) and in wheat by Singh *et al*., (1990). Changes in leaf water potential of the varieties might be attributable to a change in osmotic pressure (Siddique *et al*., 2000). Between the varieties studied, highest water potentials were measured in Inca F1, implying a drought avoidance response (Fotelli *et al*., 2000). Pennypacker *et al*., (1990) also found a similar decrease of leaf water potential in alfalfa as result of drought stress. The simultaneous reduction in stomatal conductance with decreasing water potentials in this study reveals it as an indicator of conservative water use (Aussenac & Valette, 1982; Turner, 1986; Archer & Rambal, 1992; Castell *et al*., 1994) in marigold.

It is generally known that photosynthetic efficiency depends on photosynthetic pigments such as chlorophyll "a" and chlorophyll "b" which play an important role in photochemical reactions of photosynthesis (Taiz & Zeiger, 2002). Drought stress has capacity to inhibit the photosynthesis of plants by affecting cholorophyll components, causing changes in chlorophyll content, and damaging the photosynthetic apparatus in plants (Iturbe Ormaetxe *et al*., 1998). Effect of water stress on chlorophyll a and b was also highly significant whereas it was found significant for total chlorophyll contents in this study. When water stress was applied chlorophyll a and b were reduced to the lowest values of 1.32 mg/g and 0.66 mg/g, respectively at 60% F.C for Super Giant as well as for Inca F1 (1.28 mg/g & 0.64 mg/g, respectively) (Fig. 13 & 14). These results are in agreement with some earlier studies by Ommen *et al.*, (1999), Manivannan *et al*., (2007) and Mafakheri *et al*., 2010. Tough chlorophyll "a" was less affected than chlorophyll "b" in water deficiency. This decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff, 1995).

Water use efficiency (WUE) shows the efficient use of water by the plants in stress condition where both of these marigold varieties differed significantly with respect to water use efficiency. Between these varieties Inca F1 showed higher value (3.72 mmol CO_2/ mol H_2O) for water use efficiency on average than that of Super giant (3.13 mmol CO_2/mol H_2O) proving that it possess better drought tolerance under all drought treatments. Epron & Dreyer (1990) also concluded that species with increased water-use efficiency with increasing drought severity can be well-adapted to drought conditions. Similar results have been reported by Rafiq *et al*., (2005) and Cortazar *et al*., (1995).

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