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Research Article

Screening of marigold (*Tagetes erecta* L.) cultivars for drought stress based on vegetative and physiological characteristics

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Abstract

Drought tolerance is an important genotypic character to be exploited for the plant cultivar selection under water deficit conditions. In the recent study, we examined the response of two marigold cultivars (Inca and Bonanza) under different regimes of drought stress. The aim was to determine the best performing cultivar under water/drought stress. Three irrigation treatments include; 4 days (T_1), 6 days (T_2) and 8 days (T_3) in comparison to control 1 day (T_0) interval were imposed. Response characters under study were morphological, physiological and anatomical. Complete Randomized Design (CRD) with four replications in two factorial arrangements was followed for experiment layout. The results revealed that increasing water stress adversely affect plant height, in both cultivars. Both cultivars showed a decreasing trend to the number of flowers under water stress. Total chlorophyll contents including a, b were also showed reduction under prolonged drought treatment in both cultivars from (2.7 mg g^{-1} FW) to (1 mg g^{-1} FW). Overall, the performance of cultivar (cv.) Inca was satisfactory under water stress regimes. These results are helpful for selecting drought tolerant marigold cultivars in water scarce areas.

1. Introduction

Drought is an abiotic stress that limits growth and development of plants by aggravating physiological disorders and reduces photosynthesis rate (Liao et al., 2012). It has most devastating effects on plant productivity and growth than any other environmental stresses (Lambers et al., 2008). Alterations in physiology, growth and development, in response to stress, change the life history of plants (Maggio et al., 2006; Rassaa et al., 2008). Drought severity is expected to be increased in the upcoming years because of changing climate scenarios (Walter et al., 2011; Handmer et al., 2012). There exists a wide opportunity for small landholders in a developing country like Pakistan to cultivate flower crops to increase profit margin (Younis et al., 2016). Because of changing climate scenario and annual low rain fall below 60cm (arid and semi-arid climate) crops should face serious drought spells during a certain period of the year (Farooqi et al., 2005). A different school of thought exists to cope with drought situation that includes; cultivars selection, efficient irrigation systems, mulches and use of media having maximum water retention (Anjum et al., 2011). Drought tolerance ability varies even amongst the member of species (Rassaa et al., 2008, Younis et al., 2017) therefore; screening of the most drought resistant plants is a realistic approach for maximum water use efficiency under changing climatic situations (Reynolds, 2006). There is also the need of time to meet the future demand of xeriscaping and water conservation.

Biological feedbacks of different plant species under water stress conditions have been studied at both organizational and molecular level (Hausman et al., 2005; Maggio et al., 2006). Marigold (*T. erecta* L.) is an important floriculture crop belonging to family Asteraceae (Kishimoto et al., 2005). It is of Mexican origin and has both ornamental and medicinal benefits

Table. 1 Treatments and their time interval.

Treatments	Time interval (Regimes)
T_0	1 day
T_1	4 days
T_2	6 days
T_3	8 days

(Cicevan et al., 2016). Divergent colours, diverse size range and long season availability provides it an opportunity for use as bedding, container and cut flower (Aguilar et al., 2009). Flowers have a potential in food processing, confectionery, poultry industry and pharmaceutical (Ram et al., 2000). It contains insecticidal, parasitic as well as nematicidal properties when intercropped in suspected crops (Wang et al., 2007). Keeping in view the importance and upcoming changing pattern of precipitation demands some strategic measures for sustainable production of this profitable crop. The main aim of the present study was to optimize the irrigation intervals according to the inherent ability of each cultivar Therefore, study was planned to assess the responses of *T. erecta* L. against different water scarcity levels.

1. Materials and methods

The present study was carried out at Floriculture Research Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad (latitude 31°300 N, longitude 73°100 E and altitude 213 m) during 2013-14. Two Marigold (*T. erecta)* cultivars; Bonanza (Pan American Seed, 1999) and Inca F1 (1982), were selected for the study. Seeds were purchased from local seed distributor and were sown in germination trays using peat moss base growing medium. After 28-days healthy marigold seedlings were transplanted in the plastic pots (24 cm x 28 cm) filled with (1:1:1:1v/v) mixture of silt, sand, leaf compost and farmyard manure as the growth medium. The moisture level was kept at pot capacity, before treatment application. Treatments were applied 10 days after transplanting and consisted of three drought levels with a control as shown in Table 1. Complete Randomized Design (CRD) having four replications in two factor-factorial arrangements was followed for trial layout.

2.1 Methods

2.2 Morphological characters

Morphological characteristics under study were; plant height (cm), leaves plant^{-1}, Shoot fresh weight (g), shoot dry weight (g), root length (cm), root fresh weight (g), root-shoot ratio of fresh weight, root dry weight, root-shoot ratio of dry weight, dry weight flowers plant^{-1}, number of flowers, flower size on every 10th day calculated from the beginning to last bloom. Plant height was measured in centimetre scale. At the termination of the trial, plants were uprooted carefully and roots were washed with distilled water. Root length (cm), Root fresh weight (g), shoots fresh weight and the root-shoot ratio of fresh weight were then measured with electrical balance M.J.3000 (Japan). Shoot and root dry weight was measured by placing them in paper bags individually and then oven dried at 70^0 C to a constant weight. After that dry weight was noted by using electrical balance (M.J.3000, Japan).

2.3 Physiological characters

Chlorophyll (a, b and total) were also calculated using spectrophotometer (Davies, 1976). Fresh leaves samples taken randomly were chopped into 0.5 cm slices and then 0.5 g of chopped slices were taken and extracted in 5 in/ acetone (80%) placed overnight at 10°C. This material was centrifuged (14000-x g for 5 min) and an absorbance of supernatant was recorded at 663.645 and 453 nm on a spectrophotometer. The formula used for measuring Chlorophyll contents:

$$\text{Chl a} = [12.7(\text{OD } 663) - 2.69\ (\text{OD } 6451)\ 1 \times V/1000 \times W$$

$$\text{Chl b} = [22.9(00\ 645) - 4.68\ (\text{OD } 663)] \times V/1000 \times W$$

$$\text{Total Chl} = [\text{Chl a} + \text{Chl b}]$$

2.4 Data analysis

Data regarding morphological, physiological and anatomical attributes were gathered and analyzed using ANOVA (Steel et al., 1997). Means were compared with the least significance difference (LSD) test (Snedecor & Cochran, 1980).

3. Results and discussion

Data analysis regarding vegetative characters shows significant variations in treatments and cultivars. Results regarding plant height depicted significant variations, not only among treatments but between cultivars as well. Increasing irrigation intervals during the trial, plants height decreased dramatically. Minimum mean value (23.38 cm) for plant height was recorded in T_3 (irrigation after 8 days). Inca cv. indicated superiority on Bonanza by yielding maximum plant height (44.3 cm) followed by (36.00 cm) at 4-days interval in T_2. However, for all treatment imposed, Inca cv performance regarding plant height was better as compared to cv. Bonanza. Possible cause for reduction in plant height of *Abelmoschus esculentus* under water stress conditions might be linked with the reduction in cell expansion as well as leaves senescence (Manivannan et al., 2007). Likewise, water stress effect decreased stem size in *Albizzia* seedlings (Nautiyal et al., 2002; Sundaravalli et al., 2005). It was also observed that in response to different water stress treatments number of leaves per plant varies between cultivars. The comparison regarding the number of leaves per plant shows that cv. Inca produced the maximum leaves (21.45) on average in response T_0 (control) followed by the cv Bonanza that produced (20.00) leaves per plant. Sudden decrease with respect to the number of leaves was noticed as the watering interval in treatments increased. In $T_{3,}$ both cvs. depicted few numbers of leaves (9.2) which represents the sensitivity of both cultivars to drought condition. Water stress lessened the plant growth by declining total leaf area and by encouraging leaf senescence (Kafi & Damghani, 2001). Similarly, water stress decreased the photosynthetic rate rapidly as compared to respiration rate in higher plants, as the early effect of water reduction in leaves lead to stomatal closure (Sundaravalli et al., 2005). Data presented in figures show that there are differential effects on the number of flowers in response to different treatments. Cultivars comparisons showed that the maximum number of flowers/plant (15) produce by cv. Bonanza although the cv. Inca produces (8.00) number of flowers for the treatment T_0 (control). Results also revealed that increasing irrigation time interval decrease the number of flowers per plant. Reduction in the flower number was recorded in cv. Inca under T_3 where irrigation interval was eight days, suggesting the negative impact of drought on Marigold cv. Drought condition checked plant growth by stimulating senescence and declining leaf area (Kafi & Damghani, 2001). Similarly, there is evidence that drought stress decreased much photosynthetic rate as compared to respiration rate in higher plants (Sundaravalli et al., 2005). The comparison of means clearly illustrates the significant difference of flower size among all treatments and between cultivars (Fig 1a). Maximum flower size 7.8 cm was perceived in cv Inca while cv. Bonanza remained dominant by producing 6.2cm in T_0. The similar response was observed regarding flower size in $T_{2.}$ cv Inca scoring 7.0 cm while the cv Bonanza (5.6 cm). Under T_3 regime both the cvs. displayed unsatisfactory performance regarding flower size. In T_0 both cultivars executed the best by producing the maximum size of flower diameter (6.82 cm). T_3 largely reduced flower size in both cvs. (Ashraf & O'Leary, 1996) reported that several cultivars of sunflower also demonstrate short flower diameter in water deficient conditions and the possible reason was discussed by Chaves et al. (2003) who argued about water stress at the flowering stage that it negatively affect the flower diameter and quality. Morphological variations because of water stress were also reported by Lawlor & Cornic, 2002. Regarding root length, data expose a negative variation in response to all treatments. Likewise, between the cultivars effect on root length variation was also significant where maximum root length (6.5 cm) was attained by cv. Inca in T_0 followed by cv. Bonanza that produces (6.1 cm) root length. T_2 and T_3 yield 5.6cm and 5.2cm for Inca and 5.2cm and 4.8cm for Bonanza respectively (Fig 1b). While in T_3, minimum root length was observed. Singh et al. (1973) reported that in *Avocado* varieties, water stress for a long time is responsible for decreasing the biomass as well as growth of the fibrous roots. Present study results are closed to the findings of Yin et al. (2005) in which they described reduced root growth in soil with water deficit conditions. Analysis of variance reveals shoot fresh weight significant variations among the treatments. Both the cultivars of *T. erecta* also depicted significant variations regarding shoot fresh weight against all treatments. Though, the interaction relationship between the treatments and cultivars was not significant. The shoot fresh weight of both cultivars was attained maximum in T_0 while it decreases gradually as the irrigation interval increased. Also, the shoot fresh weight decreased gradually with the increase irrigation interval. The maximum shoot fresh weight (15.5g) was produced in cv. Bonanza in T_0 while the same cv. in 4-days interval of irrigation (T_2) followed the maximum fresh weight (Fig 1c). Under reduced irrigation regime

(T_3) shoot fresh weight was decrease dramatically. Tahir & Mehdi (2001) reported

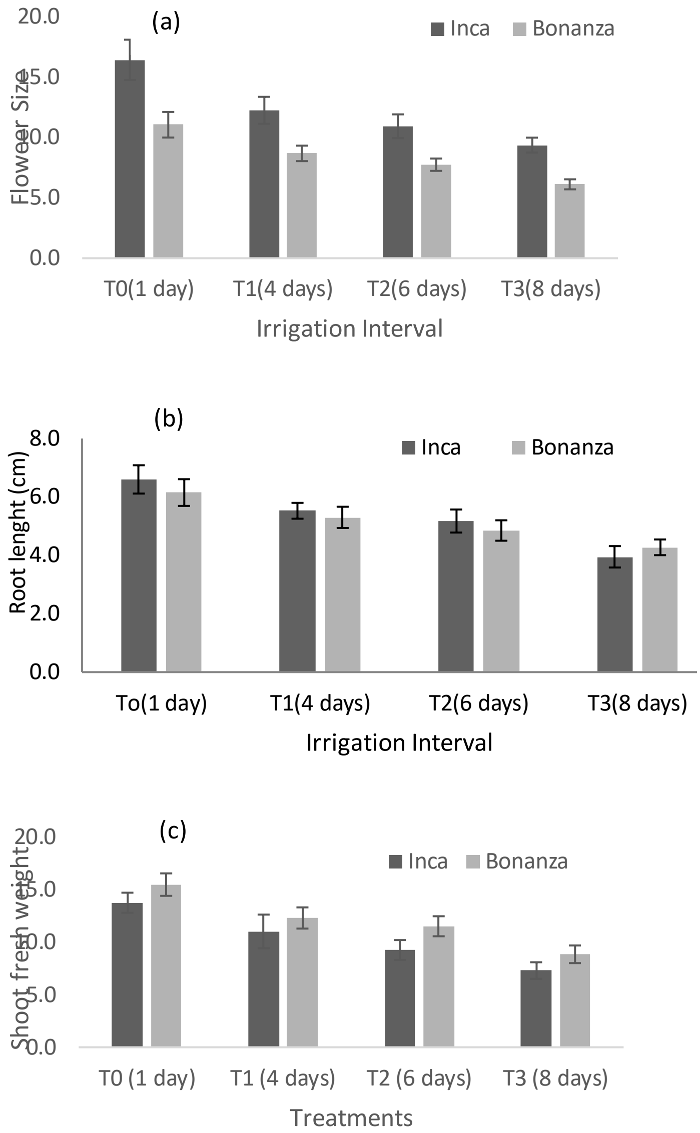

Figure 1. (a) Mean size of flowers in response to different irrigation intervals (Treatments) (b) Root length (cm) in response to different irrigation intervals (Treatments) (c) Shoot fresh weight in response to different irrigation intervals (Treatments)

that decrease in plant height and biomass linked with water shortage and prolonged drought negatively affects plant health. Results regarding root fresh weight show significance in response to all treatments. Similarly, cultivars also indicated significant results regarding root length. However, interaction among treatments and cultivars was non-significant. Statistically, it was shown that cv. Inca produced maximum root fresh weight 6.9 g while cv. Bonanza yield 5g of root fresh weight in response to T_0. Similarly, T_2 and T_3 yield 5.8 g and 4.3g for cv. Inca while 4.2g and 3.0 g for the cv. Bonanza respectively. Under moderate stress treatment (T_1) the maximum root fresh weight (5.953g) was produced. Similarly, the T_2 also exhibited better results by yielding 5.097 g root fresh weight as illustrated in Fig 2a. While T_3 produces minimum root fresh weight (2.607 g) due to prolong drought stress. Singh et al. (1973) claimed that prolonged water deficit was responsible for decreasing biomass of the fibrous roots of Avocado varieties. Similar results reported by (Riaz et al., 2013), who reported negative affect of drought on root fresh weight. Analysis of variance regarding shoot dry weight response to drought depicts the significant difference between cultivars and among treatments applied. In T_0 maximum dry weight of shoot (5.98 g) was attained by cv Inca followed by the cv Bonanza (5.3 g). Present results depicted gradual reduction in dry weight of shoot with prolonged irrigation intervals (Fig 2b). In response to T_3, cv Bonanza produces minimum (2.3 g) dry weight of shoot. Hence, overall performance in response to T_3 regarding dry shoot weight by both cv. was not satisfactory. Cultivars cumulative mean values 9.2 depict the sensitivity against water deficit. Similarly, in alfalfa crop, it was observed that lack of soil moisture negatively affects shoot dry matter weight as well as leaf area (Grewal & Williams, 2000, Mansoor et al., 2015). Root dry weight in both cvs. reveal significant variation. While non-significant interaction among treatments and the cultivars was observed. In T_0 the dry weight production was supreme in both cvs. at the termination of the experiment. It was also observed that maximum root dry weight (5.88g) was found in cultivar Inca while the same cv. in T_2 as illustrated in Fig 2c. Cv. Inca overall performance was satisfactory in all treatments in comparison with cv. Bonanza in this experiment. T_0 produce maximum cumulative root dry weight for both cvs. Under drought stress plant growth, development and productivity depend on the process of dry matter partitioning. The spectral

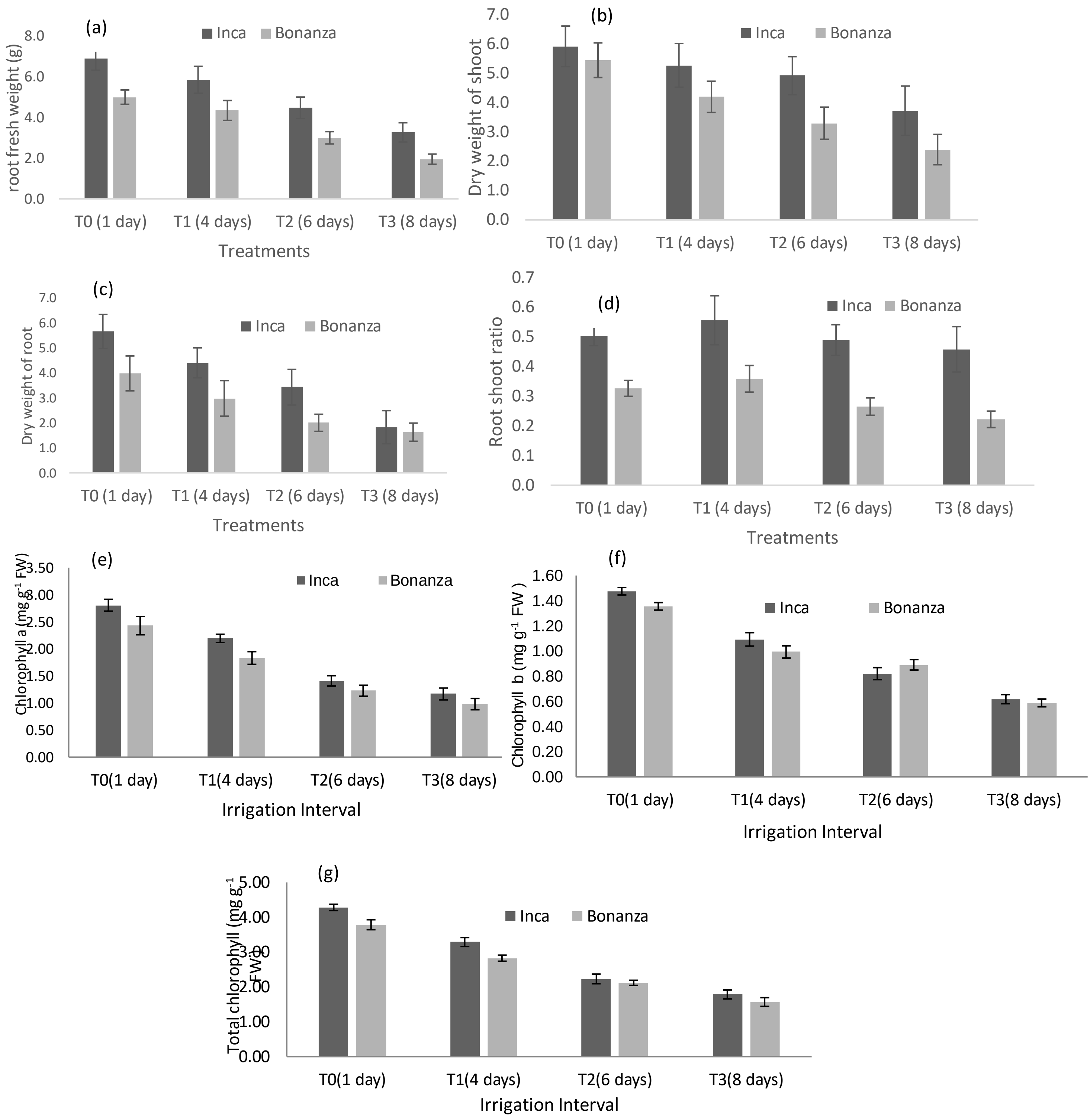

Figure 2. a) Root fresh weight in response to different irrigation intervals (Treatments); **b)** Dry weight of shoot in response to different irrigation intervals (Treatments); **c)** Dry weight of root in response to different irrigation intervals (Treatments); **d)** Root shoot ratio in response to different irrigation intervals (Treatments); **e)** Chlorophyll 'a' contents in response to different irrigation intervals (Treatments); **f)** Chlorophyll 'b' contents in response to different irrigation intervals (Treatments); **g)** Total Chlorophyll contents in response to different irrigation intervals (Treatments)

and temporal root spread, root biomass allocation and functional root length increased under drought (Morgan and Condon, 2002). The increase in root growth can alleviate the problem of water stress (Pardo et al., 1998; Chaves & Oliveria, 2004). The root-shoot ratio for fresh weight shows significance results in response to all treatments. Likewise, the cultivar effect was also significantly in this regard. However, interaction among the treatments and cultivars was non-significant. Analysis revealed that root-shoot ratio 5.2 was depicted maximum in Inca in T_3 followed in T_0 by the same cultivar. T_2 and T_3 yield 4.9 and 4.7 in cv. Inca while 2.8 and 2.2 for the cv. Bonanza. In 1- day irrigation interval (T_0) maximum root-shoot ratio (0.457) by both cvs. was produced. Likewise, T_1 also yield better results by producing 0.415 of the root-shoot ratio. T_3 perform poorly in this regard in both cvs (Fig 2d). Plant root to shoot ratio of plants improved under water shortage because roots as compared to shoots were less sensitive to growth inhibition rate under low water availability (Wu & Cosgrove, 2000). Results revealed significant variation regarding Chlorophyll among all treatments and cultivars of Marigold. In response to T_0 maximum amount of chlorophyll observe in both the cvs. While this amount of chlorophyll affected negatively by increasing the irrigation interval. Chlorophyll a was highest in cv. Inca (2.7 mg g^{-1} FW) that was followed by cv. Bonanza in T_0 (Fig 2e). Cv. Inca performed the best regarding chlorophyll a in comparison to variety Bonanza in response to all treatments. In $T_{3,}$ the minimum value (1 mg g^{-1} FW) of chlorophyll a, recorded. T_0 produce maximum amount of chlorophyll in both cvs. Drought stress at different time intervals depicted chlorophyll contents variation that has negative effects on photosynthesis (Flexas & Medrano, 2002) by changing stomata operation (Kafi and Damghani, 2001) as well as CO_2 uptake reduction (Begg & Turner, 1976). Water stresses adversely affect chlorophyll synthesis as well as structural and functional responses in chloroplasts (Medrano et al., 2002). The chlorophyll b amount in response to all treatments depicted obvious variations. The comparison revealed that in T_0 the cv. Inca showed superiority in the amount of chlorophyll b (1.43 mg g^{-1} FW) which is followed by the cv. Bonanza (1.28 mg g^{-1} FW). Increasing time interval of irrigation decreased the amount of chlorophyll b so in T_3 which has 8- day interval of irrigation produce minimum values (0.58 mg g^{-1} FW) for the chlorophyll b in cvs. Bonanza. While T_0 produce maximum chlorophyll b (1.360 mg g^{-1} FW) in both cvs. that is illustrated in Fig 2f. In T_3 overall cumulative value of 0.593 mg g^{-1} FW was measured in both cvs. which revealed the sensitivity to water stress. Decreased chlorophyll in response to drought stress has been reported in different species (Kpyoarissis et al., 1995). Likewise, Mafakheri et al. (2010) also reported decreased chlorophyll contents in response to water stress at the vegetative and reproductive stage. Data regarding total chlorophyll contents also reveal significant variations in response to all treatments. Likewise, cultivars also show significant variation with respect to total chlorophyll contents. Treatments and cultivars interaction remained non-significant. In $T_{1,}$ total chlorophyll contents (4.32mg g^{-1} FW) was maximum yielded by cv. Inca while, in comparison cv. Bonanza produced the minimum (3.58 mg g^{-1} FW) total chlorophyll contents. Likewise, T_2 and T_3 performed significantly and yield 3.2 mg g^{-1} FW and 2.2 mg g^{-1} FW total chlorophyll contents for the cv. Inca, while the cv. Bonanza produced 2.58 mg g^{-1} FW and 2.16 mg g^{-1} FW total chlorophyll. T_1 produced the maximum (3.893 mg g^{-1} FW) total chlorophyll contents whereas. in T_2 it yielded 2.672 mg g^{-1} FW total chlorophyll contents. T_3 (eight days' irrigation interval) revealed poor performance with respect to total chlorophyll (Fig 2g). Under water stress, the lower amount of total chlorophyll suggests a dropped capacity for light harvesting thus effecting photosynthesis (Herbinger et al., 2002). In sunflower varieties, drought stress caused the reduction in the chlorophyll a, b and total chlorophyll content (Manivannan et al., 2007).

4. Conclusion

A perusal of the result shows that Inca cultivar is drought-tolerant as compared to drought-susceptible cultivar Bonanza. The parameters showed a considerable variability under drought stress conditions. This study could help to understand the genetic ability of marigold cultivars and selection of drought tolerant cultivars. Additionally, it directly relates to growers and gardeners need regarding the selection of suitable cultivar to attain the best production and aesthetic value under limited water availability conditions

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