Original Article

Current Botany 2014 5: 7-13
http://scienceflora.org/journals/index.php/jp/

Drought stress-induced modification on growth and pigments composition in different genotypes of *Helianthus annuus* L.

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Received: 14.10.2014
Revised: 16.11.2015
Accepted: 21.11.2014
Published: 23.11.2014

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ABSTRACT

In this investigation, a pot culture experiment was conducted to estimate the ameliorating effect of drought stress-induced modification on various growth and pigments composition in different genotypes of *Helianthus annuus* L. (Sunflower). Economically important oil seed crop sunflower five cultivars viz., Asgrow SH 3322 (A-SH 3322), Agsun 110 (A-110), Kaveri 618 (K-618), SH 416, and Sunbred 275 (S-275) were selected for drought analysis. Plant growth and productivity is adversely affected by nature's wrath in the form of various biotic and abiotic stress factors. Water deficit is one of the major abiotic stresses, which adversely affects crop growth and yield. 3, 4, and 5 days interval drought was selected for the drought stress treatments. Drought stress causes considerable decreases in growth and pigments content of sunflower. Among the cultivar K-618 was less affected by the drought and to it had increased root length and near normal vegetative and shoot growth during stress and at stress recovery. The growth parameters and pigment total chlorophyll and carotenoid content decreased under drought stress in all the sunflower cultivars. Among the cultivars, the S-275 cultivar was severely affected when compared to other cultivars.

KEY WORDS: Growth, pigments, stress, sunflower

INTRODUCTION

Water is essential for all living organisms, and it plays very significant role in building plant metabolism. Water availability and quality can be a limiting factor in plant growth (Ceylan *et al.*, 2013). This adaptation in plant responses to water shortages can involve changes in expression of genes encoding proteins that contribute to drought adaptation. The proteins could be enzymes involved in the synthesis of hormones and changes in a plant's hormone levels, increasing inhibitors, and reducing growth promoters (Wasternack and Hause, 2013). A remarkable feature of plant adaptation to abiotic stresses is the activation of multiple responses involving complex gene interactions and crosstalk with many molecular pathways (Basu, 2012). Water deficit will be created when insufficient irrigation prevents a plant from normal growth and completion of life cycle (Zhu, 2002). In soil, insufficient moisture can be the created due to a shortage in rainfall (drought), coarse textured soils that retain little water in the root zone, or drying winds. Under water deficit conditions, plants suffer from cellular damage and this is typically accompanied by an increase in plant-body temperature. Depending on the duration and extent of drought stress, a range of plant processes occurring at molecular, biochemical, cellular, and whole-plant levels may be altered (Chaitanya *et al.*, 2003). Water stress in sunflower was shown to cause irregular seed germination, and poor and unsynchronized establishment of seedlings (Albuquerque and Carvalho, 2003). Water stress affects plant growth, productivity, caused an increase in the concentration of soluble sugar and proline content in the leaves of sunflower (Nazarli *et al.*, 2011). "Water deficit" is defined as lack of the amount of water necessary for a plant to grow normally and complete its life cycle (Manivannan *et al.*, 2008). Drought affects nearly all the plant growth processes in sunflower (Kheybari *et al.*, 2013).

The objectives of the present study were to understand the effect of early season drought stress, growth and yield, photosynthetic pigment composition, and osmaticum adjustments of sunflower genotypes.

MATERIALS AND METHODS

Seeds Collection

Economically important oil seed crop sunflower (*Helianthus annuus* L.) belonging to the family Asteraceae was selected for the present investigation. Five cultivars viz., Asgrow SH 3322 (A-SH 3322), Agsun 110 (A-110), Kaveri 618 (K-618), SH 416, and Sunbred 275 (S-275) of sunflower were obtained from Kaveri Seeds Pvt. Ltd., Andhra Pradesh, India, and used for the experiments. The experiments were conducted at the Botanical Garden and Stress Physiology Laboratory, Department of Botany, Annamalai University, Tamil Nadu, India.

The pot culture studies were conducted to measure the growth parameters, biochemical, and physiological changes and also in antioxidant potentials in the early season of sunflower cultivars. The potted plants were raised during the months of February-May, 2005-2007. The seeds were surface sterilized with 0.2% mercuric chloride solution for 5 min with frequent shaking and thoroughly washed with tap water. The experiment was laid out in a completely randomized block design (CRBD).

In the preliminary experiments of 3, 4, 5, 6, 7, and 8 days interval drought (DID) stress was used for experiments. Among these treatments, which was reduced the dry weight significant to 60%. Hence, 3, 4, and 5 DID were selected and used to all the experiments.

Plastic pots of 40 cm diameter and 45 cm height size were used for the study. The pots were filled with 10 kg of soil mixture containing red soil, sand, and farm yard manure at 1:1:1 ratio and 440 pots were arranged in CRBD. One set of 110 pots were kept as control, and other 3 sets of 330 pots were used for drought stress treatments. The sunflower seeds were sown, and the seedlings were thinned to 1 per pot on 10 days after sowing (DAS). The plants were allowed to grow up to 30 DAS. On 30th to 50th day (Before flowering period), all the potted plants were grown under the poly house. The control plants were irrigated alternative days. Mild stress (irrigation once in 3 days), moderate stress (irrigation once in 4 days), and severe stress (irrigation once in 5 days) from 30th to 50th days. After the drought period, all the pots to be irrigated alternate days up to harvest. Plants were uprooted randomly 50th, 60th, and 70th DAS, washed carefully and separated into root, stem, and leaves for estimating growth parameters and pigments.

Root and Stem Length

Root and stem length were recorded on 50, 60, and 70 DAS. Below the point of root-stem transition to the tap root and the length of lateral roots were taken as total root length. The length between stem tip and point of root stem transition region was taken as stem length. The root length and the stem length were expressed in centimeters per plant.

Fresh Weight and Dry Weights

After washing the plants in the tap water, fresh weight was determined by an electronic balance (Model–XK3190-A7M) and the values were expressed in grams. After taking fresh weight, the plants were dried at 60°C in hot air oven for 24 h. After drying, the weight was measured and the values were expressed in grams.

Chlorophyll and Carotenoid

Chlorophyll and carotenoid were extracted from the leaves and estimated by the method of Arnon (1949). Five hundred milligrams of fresh leaf material was ground with 10 ml of 80% acetone at 4°C and centrifuged at 2500 rpm for 10 min at 4°C. This procedure was repeated until the residue became colorless. The extract was transferred to a graduated tube and made up to 10 ml with 80% acetone and assayed immediately.

Chlorophyll content was estimated using three milliliters aliquots of the extract were transferred to a curette and the absorbance was read at 645, 663, and 480 nm with a spectrophotometer (U-2001-Hitachi) against 80% acetone as blank. Chlorophyll content was calculated using the formula of Arnon.

$$\text{Total chlorophyll (mg/ml)} = (0.0202) \times (A.645) + (0.00802) \times (A.663)$$

$$\text{Chlorophyll "a" (mg/ml)} = (0.0127) \times (A.663) - (0.00269) \times (A.645)$$

$$\text{Chlorophyll "b" (mg/ml)} = (0.0229) \times (A.645) - (0.00468) \times (A.663)$$

and expressed in milligram per gram fresh weight.

Carotenoid content was estimated using the formula of Kirk and Allen (1965) and expressed in milligrams per gram fresh weight.

$$\text{Carotenoid} = A.480 + (0.114 \times A.663 - 0.638 \times A.645)$$

RESULTS

Economically important oil seed crop sunflower (*H.annuus* L.) belonging to the family Asteraceae was selected for the present investigation with five cultivars viz., Asgrow SH 3322 (A-SH 3322), Agsun 110 (A-110), Kaveri 618 (K-618), SH 416, and Sunbred 275 (S-275). Pot culture experiments were conducted to identify the varietal variation in five sunflower cultivars under early season drought stress. In this experiment variation in growth, yield, photosynthetic pigments, biochemical constituents, proline metabolizing enzymes non-enzymatic antioxidant, and antioxidant enzymes under drought stress condition were studied.

Root Length

The root length increased to a larger extent in all drought treatment. However, the 5 DID increased the root length to a higher level in all cultivars than the 3 and 4 DID treatments. Among the cultivars, the root length was very high in K-618 cultivars followed by A-110, SH 416, SH 3322, and S-275 cultivars and it was 147.33%, 143.72%, 140.32%, 138.21%, and 133.60% over control on 50 DAS. The K-618 cultivars showed a tolerance to drought when compared to other four cultivars tested with regard to root growth (Figure 1).

Stem Length

Drought stress reduced the shoot growth significantly. Among the drought treatment, 5 DID affected the stem length to a higher level than the other two treatments. Among the cultivars, S-275 was affected by the drought to a larger extent which is followed by SH 3322, SH 416, A-110, and K-618 cultivars and it were 60.59%, 65.29%, 66.85%, 68.36%, and 71.75% over control on 50 DAS. On 70 DAS, the growth was 6.86% lesser than control while it was 14.86% on 50 DAS. The K-618 cultivars showed a fastest recovery when compared to S-275 cultivars (Figure 2).

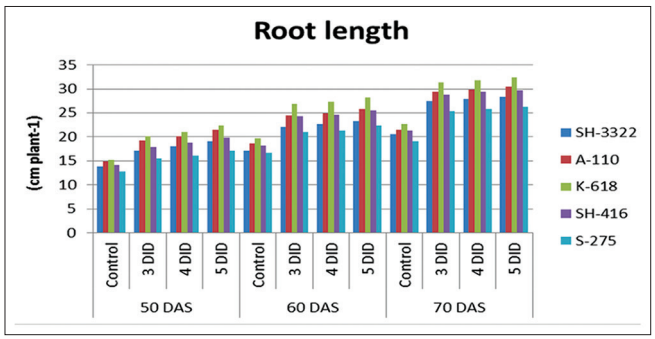

Figure 1: Drought stress-induced changes in root length (cm/plant) of five varieties of sunflower at different growth stages

Whole Plant Fresh Weight

Drought stress reduced the whole plant fresh weight significantly. The 5 DID drought treatment highly affected whole plant fresh weight than the other two treatments. Among the cultivars, the whole plant fresh weight was lowest in cultivars S-275 by the drought to a larger extent which is followed by SH 3322, SH 416, A-110 and K-618 cultivars and it was 58.04%, 63.59%, 64.28%, 66.28%, and 69.36% over control on 50 DAS. On 70 DAS, the growth was 6.83% lesser than control while it was 30.64% on 50 DAS. The K-618 cultivars showed the fastest recovery when compared to S-275 cultivars (Table 1).

Whole Plant Dry Weight

The whole plant dry weight was reduced by drought stress significantly. Among the drought treatment, 5 DID highly affected the whole plant dry weight than the other two treatments. Among the cultivars, the whole plant dry weight was highest decreased in S-275 cultivars which are followed by SH 3322, SH 416, A-110, and K-618 cultivars and it was 50.58%, 54.95%, 56.98%, 58.45%, and 61.71% over control in 50 DAS on 5 DID. On 70 DAS, the growth was 9.75% lesser than control while it was 38.29% on 50 DAS. The K-618 cultivars showed the fastest recovery when compared to S-275 cultivars (Table 2).

Effect of Water Deficit on Pigment Constituents

Chlorophyll content

Drought stress lowered the total chlorophyll content significantly. Among the drought treatments, 5 DID highest decreased total chlorophyll content as compared to other two treatments. Chlorophyll content of S-275 cultivar was severely affected by the drought and it was 67.01% over control on 5 DID in 50 DAS which was followed by SH-3322, SH-416, A-110, and K-618 cultivars and it was 72.25%, 73.21%, 75.41%, and 78.25% over control. On 70 DAS, the chlorophyll content was 7.15%

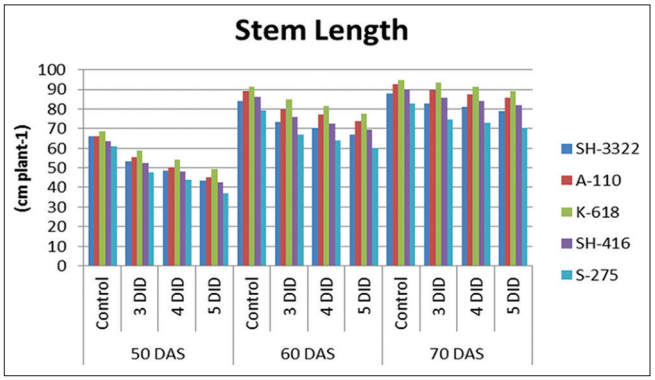

Figure 2: Drought stress-induced changes in stem length (cm/plant) of five varieties of sunflower at different growth stages

lesser than control while it was 21.75% on 50 DAS in K-618 cultivar. However, in S-275 cultivar showed the lowest recovery and it was 32.99% and 18.75% lower than control at 50 and 70 DAS, respectively. The K-618 cultivars showed the fastest recovery when compared to S- 275 cultivars (Table 3).

Carotenoid

The carotenoid content decreased significantly in all the treatments. Among the drought treatments, 5 DID treated the highest reduction in carotenoid content compared to other two treatments. Among the cultivar S-275 showed the lowest carotenoid content and it was 67.30% over control in 5 DID on 50 DAS followed by SH-3322, SH-416, A-110, and K-618 cultivars and it was 69.32%, 72.20%, 75.14%, and 78.11% over control. In the cultivar K-618 on 70 DAS, the carotenoid content was only 13.76% lesser than control while it was 21.89% on 50 DAS. The K-618 cultivars showed the fastest recovery when compared to other cultivars (Table 4).

DISCUSSION

The root length increased to a larger extent with all drought treatment. 5-day interval drought (DID) increased the root length to a higher level in all cultivars than the 3 and 4 DID treatments. Among the cultivars, the root length was increased to a higher level in K-618 cultivar followed by A-110, SH 416, SH 3322, and S-275 cultivars. Drought stress increased the root length in *Eucalyptus microthea* seedlings (Li et al., 2000); *Populus* species (Yin et al., 2005) *Pearl millet* (Kusaka et al., 2005); sunflower (Manivannan et al., 2007); Olive (Bacelar et al., 2007); *Cannabis sativa* (Amaducci et al., 2008); *Oak*

Table 1: Drought stress-induced changes in fresh weight (values are the mean of seven replicates expressed in gram per plant) of five varieties of sunflower at different growth stages

Growth stages	Drought	SH-3322	A-110	K-618	SH-416	S-275
50 DAS	Control	76.58^{d}	81.60^{b}	85.31^{a}	79.56^{c}	72.36^{e}
	3 DID	61.66^{gh}	67.91^{f}	74.45^{d}	65.74^{g}	55.86^{i}
	4 DID	55.32^{i}	61.76^{gh}	66.78^{f}	58.33^{h}	50.07^{j}
	5 DID	48.69^{k}	58.08^{h}	59.17^{h}	51.14^{j}	41.99^{l}
60 DAS	Control	106.5^{d}	111.6^{b}	115.3^{a}	109.5^{c}	102.3^{e}
	3 DID	88.13^{i}	95.93^{g}	102.9^{e}	93.45^{g}	82.61^{b}
	4 DID	85.12^{j}	92.28^{gh}	99.12^{f}	88.45^{i}	78.19^{k}
	5 DID	80.31^{k}	87.60^{i}	93.94^{h}	83.61^{j}	73.00^{l}
70 DAS	Control	126.5^{d}	131.6^{b}	135.3^{a}	129.5^{c}	122.3^{e}
	3 DID	118.0^{f}	126.8^{d}	133.5^{a}	123.6^{e}	109.9^{h}
	4 DID	115.6^{g}	124.5^{e}	130.8^{b}	121.1^{f}	107.0^{hi}
	5 DID	112.2^{h}	120.1^{f}	126.0^{d}	117.3^{fg}	103.0^{j}

Group a has the best treatments and Group k has the poorest performing treatments. DID: Days interval drought, DAS: Days after sowing.
$^{b-j,l}$Values, that are not sharing a common superscript differ significantly at P £ 0.05 (DMRT)

Table 2: Drought stress-induced changes in dry weight (values are the mean of seven replicates expressed in gram per plant) of five varieties of sunflower at different growth stages

Growth stages	Drought	SH-3322	A-110	K-618	SH-416	S-275
50 DAS	Control	12.63^{c}	15.35^{b}	18.31^{a}	14.31^{b}	11.57^{c}
	3 DID	9.13^{e}	11.56^{d}	14.53^{b}	10.64^{d}	7.99^{f}
	4 DID	8.80^{e}	10.41^{de}	12.88^{d}	9.29^{e}	7.09^{f}
	5 DID	6.90^{f}	8.97^{e}	11.29^{c}	8.15^{e}	5.85^{g}
60 DAS	Control	23.63^{c}	26.35^{b}	29.31^{a}	25.31^{b}	20.57^{d}
	3 DID	18.17^{e}	20.95^{d}	24.60^{c}	19.70^{d}	15.36^{ef}
	4 DID	17.48^{e}	20.27^{d}	23.37^{c}	18.86^{de}	14.42^{f}
	5 DID	16.38^{e}	19.16^{d}	21.76^{d}	17.77^{e}	13.39^{f}
70 DAS	Control	23.63^{bc}	26.35^{b}	29.31^{a}	25.31^{b}	20.57^{d}
	3 DID	21.37^{d}	24.59^{bc}	28.05^{a}	23.38^{bc}	18.22^{e}
	4 DID	21.09^{d}	24.10^{bc}	27.46^{ab}	22.93^{c}	17.53^{e}
	5 DID	20.26^{d}	23.55^{bc}	26.45^{b}	22.02^{c}	16.69^{f}

Group a has the best treatments and Group f has the poorest performing treatments. DID: Days interval drought, DAS: Days after sowing.
$^{b-j,l}$Values, that are not sharing a common superscript differ significantly at P £ 0.05 (DMRT)

Table 3: Drought stress-induced changes in total chlorophyll content (expressed in (mg/g fresh weight)) of five varieties of sunflower at different growth stages

Growth stages	Drought	SH-3322	A-110	K-618	SH-416	S-275
50 DAS	Control	0.341^{b}	0.374^{b}	0.419^{a}	0.362^{b}	0.317^{c}
	3 DID	0.270^{d}	0.308^{c}	0.361^{b}	0.294^{c}	0.243^{e}
	4 DID	0.260^{d}	0.296^{a}	0.344^{b}	0.278^{d}	0.233^{e}
	5 DID	0.246^{e}	0.282^{d}	0.327^{c}	0.265^{d}	0.212^{f}
60 DAS	Control	0.688^{d}	0745^{b}	0.787^{a}	0.713^{c}	0.616^{f}
	3 DID	0.575^{h}	0.636^{e}	0.709^{c}	0.601^{g}	0.500^{k}
	4 DID	0.551^{i}	0.622^{f}	0.680^{d}	0.578^{h}	0.530^{j}
	5 DID	0.524^{j}	0.590^{g}	0.646^{d}	0.549^{i}	0.507^{j}
70 DAS	Control	0.900^{d}	0.947^{b}	0.989^{a}	0.915^{c}	0.818^{gh}
	3 DID	0.830^{f}	0.901^{d}	0.959^{b}	0.862^{e}	0.723^{k}
	4 DID	0.771^{j}	0.836^{fg}	0.894^{d}	0.797^{i}	0.662^{i}
	5 DID	0.783^{j}	0.858^{e}	0.918^{c}	0.814^{hi}	0.664^{j}

Group a has the best treatments and Group l has the poorest performing treatments. DID: Days interval drought, DAS: Days after sowing.
$^{b-j,l}$Values, that are not sharing a common superscript differ significantly at P £ 0.05 (DMRT)

Table 4: Drought stress-induced changes in carotenoid content (expressed in (mg/g fresh weight)) of five varieties of sunflower at different growth stages

Growth stages	Drought	SH-3322	A-110	K-618	SH-416	S-275
50 DAS	Control	0.085^{g}	0.114^{d}	0.143^{a}	0.124^{b}	0.071^{h}
	3 DID	0.063^{i}	0.092^{f}	0.120^{c}	0.097^{e}	0.052^{j}
	4 DID	0.060^{i}	0.087^{fg}	0.114^{d}	0.090^{f}	0.049^{j}
	5 DID	0.058^{i}	0.085^{f}	0.111^{d}	0.089^{f}	0.047^{k}
60 DAS	Control	0.166^{e}	0.193^{c}	0.225^{a}	0.182^{d}	0.156^{f}
	3 DID	0.132^{i}	0.165^{e}	0.200^{b}	0.149^{g}	0.121^{j}
	4 DID	0.125^{ij}	0.157^{f}	0.190^{c}	0.142^{h}	0.114^{k}
	5 DID	0.138^{h}	0.152^{fg}	0.184^{d}	0.139^{a}	0.111^{l}
70 DAS	Control	0.206^{d}	0.135^{i}	0.267^{a}	0.222^{c}	0.193^{de}
	3 DID	0.168^{g}	0.119^{j}	0.246^{b}	0.200^{d}	0.155^{h}
	4 DID	0.163^{gh}	0.117^{j}	0.241^{b}	0.195^{de}	0.151^{h}
	5 DID	0.158^{h}	0.112^{j}	0.230^{c}	0.178^{f}	0.141^{i}

Group a has the best treatments and Group k has the poorest performing treatments. DID: Days interval drought, DAS: Days after sowing.
$^{b-j,l}$Values, that are not sharing a common superscript differ significantly at P £ 0.05 (DMRT)

species (Rodriguez-calcerrada et al., 2008), and Parsley (Petropoulos et al.,2008).The development of root system may increase the water uptake under drought stress.

Drought stress inhibited the shoot growth significantly in all sunflower cultivars. Among the drought treatments, 5 DID treatment highly affected the stem length than the other two treatments. Among the cultivars S-275 was most affected by the drought, which was followed by SH 3322, SH 416, A-110, and K-618 cultivars. Shoot length decreased in seedlings *Eucalyptus* seedlings under drought stress (Li et al., 2000). Similar results were observed in avocado (Chartzoulakis et al., 2002); soybean (Ohashi et al., 2002); and *Populus* species (Yin et al., 2005). *Abelmoschus esculentus* (Sankar et al., 2007), and in olive (Bacelar et al., 2007).

Drought stress decreased the whole plant fresh weight in all sunflower cultivars significantly. The 5 DID drought treatment most affected the whole plant fresh weight than the other two treatments. Among the cultivars, the whole plant fresh weight was very low in S-275 cultivars under drought.The whole plant fresh weight was reduced under drought condition in *Pearl millet* (Kusaka et al., 2005). Similar results were observed in higher plants such as *Vicia faba* (Wu and Wang, 2000); cowpea (Anyia and Herzog, 2004), and *Catharanthus roseus* (Jaleel et al., 2008). The reduction in fresh weight under drought condition might be due to suppression of cell expansion and cell growth due to the low turgor pressure, and partial root drying caused a significant reduction in shoot biomass when compared to control as observed in wheat (Shao et al., 2005).

The whole plant dry weight was reduced by drought stress in all sunflower cultivars. Among the drought treatments, 5 DID treatment reduced the whole plant dry weight than the other two treatments. Among the cultivars, the whole plant dry weight was very highly decreased in S-275 cultivar the reduction was low in the cultivar K-618 as compared to its control. Drought stress decreased the plant biomass in *Arachis hypogaea* (Nautiyal et al., 2002), *Asteriscus maritimus* (Rodriguez et al., 2005), and wheat (Pan et al., 2003; Shao et al., 2007). Decreased total dry weight may be due to the considerable decrease in plant growth, photosynthesis and canopy structure as indicated by leaf senescence during drought stress in *Abelmoschus esculentum* (Bhatt and Srinivasa Rao, 2005); *Vicia faba* (Wu and Wang, 2000); wheat (Gong et al., 2003), and cowpea (Anyia and Herzog, 2004), Severe water stress may result in arrest of photosynthesis, disturbance of metabolism, and finally drying (Liang et al., 2006).

Drought stress caused decrease chlorophyll content when compared to their control in all the cultivars of sunflower. Among these cultivars, S-275 showed more reduction in the chlorophyll with 5 DID treatment and lower reduction was observed in K-618 cultivars. A reduction in chlorophyll content was reported in drought stressed *H. annuus* (Gimenez *et al.*, 1992 and Pankovic *et al.*, 1999); rice (Widodo *et al.*, 2003); Cherry (Centritto, 2005); Wheat (Sawhney and Singh, 2002; Gong *et al.*, 2005); *Pinus halepensis* (Alonso *et al.*, 2001), and Soybean (Heerden and Kruger, 2002; De Ronde *et al.*, 2004; Zhang *et al.*, 2007).

The carotenoid content decreased in all the drought stressed sunflower cultivars when compared to their control. Reduced carotenoid content under drought was reported in Cherry (Centritto, 2005); sunflower (Gimenez *et al.*, 1992); *Nicotiana tabacum* (Delgado *et al.*, 1992); Prairie grasses (Heckathorn *et al.*, 1997); rice (Widodo *et al.*, 2003);Wheat (Sawhney and Singh, 2002; Gong *et al.*, 2005), and Soybean (De Ronde *et al.*, 2004; Zhang *et al.*, 2007). Drought stress significantly reduced total chlorophyll and carotenoid content, however, when normalcy in irrigation cycle was restored all the cultivars recovered from drought stress to a larger extent of which the cultivar K-618 recover faster and return to near normal conditions in plants had 3 DID treatment. The lowest recovery was recorded in S-275 cultivar and the differences between the control and drought stressed plants were nearly 20% when compared to control. This shows cultivar variations exist among the sunflower cultivars, and some cultivars are more adopted and tolerant to drought stress.

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