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Analysis of antioxidant enzyme activity in various genotypes of *Helianthus annuus* L. (Sunflower) under varied irrigation regimes

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ABSTRACT

In this investigation, a pot culture experiment was conducted to estimate the effect of drought stress of *Helianthus annuus* L. (Sunflower). Sunflower is one of the most important oil crops and due to its high content of unsaturated fatty acids, a lack of cholesterol and the oil benefits from a desirable quality. 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. The present study was aimed to understand the effects of drought stress on antioxidant enzymes such as peroxidase (POX), superoxide dismutase, catalase, and ascorbate peroxidase activity on five different cultivars (SH-3322, A-110, K-618, SH-416, and S-275) of sunflower. All the sunflower cultivars showed reduced growth and altered enzymes activities under drought conditions. The antioxidant enzymes activities were increased under drought stress in all parts of the sunflower cultivars. The maximum activity was observed in K-618 and the minimum in S-275 cultivar. Among the five cultivars studied, K-618 showed better tolerance capacity in pot culture conditions under drought stress when compared to other cultivars studied.

KEY WORDS: Antioxidants enzyme, biotic stress, drought, sunflower

INTRODUCTION

The sunflower (*Helianthus annuus* L.) belonging to the family Asteraceae is the world's fourth largest oil-seed crop (Rodriguez *et al*., 2002). Sunflower is grown mainly as the oil-seed crop in most of the countries of the world. Plant growth and productivity is adversely affected by natures' wrath in the form of various abiotic and biotic stress factors. Plants are frequently exposed to many stress conditions such as low temperature, salt, drought, flooding, heat, oxidative stress, and heavy metal toxicity (Mahajan and Tuteja, 2005). Acclimation of plants to changing environmental conditions such as drought stress is essential for survival and growth. Drought stress causes the production of reactive oxygen radicals or species (ROS). The ROS are responsible for most of the oxidative damage in biological systems (Ramachandra Reddy *et al*., 2004). Mechanisms of ROS detoxification exist in all plants and can be categorized as enzymatic (superoxide dismutase [SOD], ascorbate peroxidase [APX], peroxidase [POX], glutathione reductase, etc). The level of response depends on the species, the development and the metabolic state of the plant, as well as duration and intensity of stress. Many stress situations cause an increase in the total foliar antioxidant activity (Pastori *et al*., 2000).

Water stress tolerance is seen in all plant species, but its extent varies from species to species. Improving the efficiency of water use in agriculture is associated with increasing the fraction of the available water resources that is transpired, because of the unavoidable association between yield and water use (Lawlor, 2002). For the last few decades, several scales of physiological works have been conducted under drought stress in crop plants (Shao *et al*., 2005). Drought occurs in many parts of the world every year, often with devastating effects on crop production (Ludlow and Muchow, 1990). Water deficit can be defined as the absence of adequate moisture necessary for a plant to grow normally and complete its life cycle (Zhu, 2002). The lack of adequate moisture leading to

water stress is a common occurrence in rain-fed areas, brought about by infrequent rains and poor irrigation (Wang *et al.*, 2005).

POX plays a role in decreasing H_2O_2 content accumulation, eliminating malondialdehyde resulting cell peroxidation of membrane lipids and maintaining cell membrane integrity. *Radix astragali* plants under water deficit stress showed an enhancement in POX activity irrespective of different genotypes (Tan *et al.*, 2006). An extensive study on oxidative stress has demonstrated that exposure of plants to adverse environmental conditions induces the overproduction of ROS, such as superoxide radical (O_2^-), H_2O_2, and hydroxyl radical (HO) in plant cells (Shao *et al.*, 2007). They are chloroplastic enzymes, which scavenge H_2O_2 generated primarily through SOD action (Chaitanya *et al.*, 2002). Double the amount of SOD activity was noted in water stressed citrus plants when compared to well water control plants during seedling stage (Wu *et al.*, 2006). Water stress caused an enhancement of catalase (CAT) activity in both wild and cultivated species of *R. astragali* at seedling stage (Tan *et al.*, 2006). APX is one of the most important antioxidant enzymes of plants that detoxify hydrogen peroxide using ascorbate for reduction. APX reduces H_2O_2 to water by ascorbate as the specific electron donor (Gara *et al.*, 2003). In trifoliate orange, under water stress, an increased APX activity was no significant variation in APX activity at mild water deficit in maize and wheat (Nayyar and Gupta, 2006). The objectives of the present study were to understand the effect of early season drought stress, and enzymatic antioxidant changes among drought stressed sunflower.

MATERIALS AND METHODS

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 completely randomized block design. One set of 110 pots were kept as control, and other 3 sets of 330 pots were used for 3, 4, and 5 days interval drought (DID) treatments. Seeds were sown, and the seedlings were thinned to one uniform seedling per pot on 10 days after showing (DAS). The plants were allowed to grow up to 30 DAS on alternative day irrigation. On 30th to 50th day (before flowering period), all the potted plants to be grown under the poly house. Altering the irrigation intervals as follows imposed drought stress. One set of 60 pots were irrigated at 3 days interval and another two set of pots at 4 days and 5 days interval up to 50th DAS. After the drought treatments, all the pots to be irrigated alternate day's interval.

Plants were uprooted randomly 50th, 60th, and 70th DAS, washed carefully and separated into root, stem and leaves for estimating growth, pigment, yield, biochemical, antioxidant contents, proline metabolizing enzymes, and antioxidant enzyme activities in all the 3, 4, and 5 DID, in all the treatments three replication for enzyme analysis.

POX (POX, EC: 1.11.1.7)

POX was assayed by the method of Kumar and Khan (1982). Assay mixture of POX contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2, and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25°C after which the reaction was terminated by adding 1 ml of 2.5 N H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 N H_2SO_4 at zero time. The activity was expressed in unit/mg protein. One unit is defined as the change in the absorbance by 0.1/min/mg/protein.

SOD (SOD, EC: 1.15.1.1)

Crude enzyme extract was prepared, for the assay of SOD by the method of Hwang *et al.* (1999). 1 g of fresh tissue was homogenized with 10 ml of ice-cold 50 mM sodium phosphate buffer containing 1 mM phenylmethanesulfonyl fluoride (PMSF). The extract was filtered through double-layered cheesecloth. The extract was centrifuged at 12,500 rpm for 20 min at 4°C. The supernatant was saved and made up to 10 ml with extraction buffer and used for estimation of the SOD enzyme activity.

SOD activity was assayed as described by Beauchamp and Fridovich (1971). The reaction medium was prepared and to 3 ml reaction medium, 1 ml of enzyme extract was added. The reaction mixture contained 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} potassium cyanide, and 5.6×10^{-5} M nitroblue tetrasodium salt (NBT), dissolved in 0.05 M sodium phosphate buffer (pH 7.8). The mixture was illuminated in glass test tubes by two sets of Philips 40W fluorescent tubes. Illumination started to initiate the reaction at 30°C for 1 h. Those without illumination saved as blank and kept in dark. The absorbance was read at 560 nm in the spectrophotometer against blank. SOD activity was expressed in units. One unit is defined as the amount of change in the absorbance by 0.1/h/mg protein under the assay condition (Cherry, 1963).

CAT (CAT, EC: 1.11.1.6)

CAT activity was assayed as described by Chandlee and Scandalios (1984). 500 mg of frozen material was

homogenized in 5 ml of ice-cold 50 mM sodium phosphate buffer (pH 7.5) containing in 1mM PMSF. The extract was centrifuged at 4°C for 20 min at 12,500 rpm. The supernatant was used for enzyme assay. The activity of enzyme CAT was measured using the method of Chandlee and Scandalios (1984) with modification. The assay mixture contained 2.6 ml of 50 ml of 50 mM potassium phosphate buffer (pH 7.0) 0.4 ml, 15 mM H_2O_2, and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in units 1 mM of H_2O_2 reduction per minute per mg protein.

APX (APX, EC: 1.11.1.11)

APX was extracted and estimated by the method of Asada and Takahashi (1987). 500 mg of fresh plant tissue was ground in a pestle and mortar under liquid nitrogen and 10 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mm EDTA, 1% PVP, and 1 mM ascorbic acid. The homogenate was filtered through a double-layered cheesecloth and centrifuged at 15,000 rpm for 20 min at 4°C. The supernatant was used as a source of enzymes. One ml of reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2, and 200 µl of enzyme extract. The absorbance was read as a decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2 (extinction coefficient 2.9/mM/cm). The enzyme activity was expressed in µg per gram dry weight.

Statistical Analysis

Each treatment and control was analyzed with at least seven replicates and a two-factor ANOVA for two factors is used the experiment and computer package Agres 2003 is used.

RESULTS

POX Activity

Root

The activity of POX increased in the root under drought stress. Among these cultivars, K-618 showed the highest POX activity, and it was 166.18%, 172.18%, and 181.18% over control, and it was lowest in S-275 cultivar, and it was 154.77%, 159.15%, and 167.13% over control, respectively, in 3, 4, and 5 DID on 50 DAS. During the recovery period, the lowest POX activity was recorded in the cultivars K-618, and it was 124.64, 127.24, and 131.14 the highest was S-275, and it was 136.32, 138.11, and 140.32 on 70DAS. This shows that the cultivar K-618 has the highest recovery potential from drought when compared to other cultivars tested (Table 1).

Stem

In the stem, POX activity increased by the drought stress in all the cultivars of sunflower. Among these cultivars, K-618 showed highest POX activity, and it was 181.21%, 188.96%, and 206.01% over control and the lowest in S-275 cultivar, and it was 169.31%, 175.36%, and 187.11% over control, respectively, in 3, 4, and 5 DID on 50 DAS. During the recovery period, the lowest POX activity was recorded in the cultivars K-618 and the highest was S-275 on 70 DAS. This shows that the cultivar K-618 has the highest recovery potential from drought when compared to other cultivars tested (Table 2).

Leaf

POX activity was increased under drought conditions in leaves in all the cultivars of sunflower. Among these cultivars, K-618 showed the highest POX activity, and it was 140.15%, 157.21%, and 166.33% over control and the lowest in S-275 cultivar, and it was 139.36%, 144.30%, and 152.60% over control, respectively, in 3, 4, and 5 DID on 50 DAS. During the recovery period, the lowest POX activity was recorded in the cultivars K-618 and the highest was S-275 on 70 DAS. This shows that the cultivar K-618 has the highest recovery potential from drought when compared to other cultivar tested. Among the organ, stem showed a higher POX activity followed by root and leaves in all treatments (Table 3).

SOD Activity

Root

Drought stress increased the activity of SOD in the root when compared with control in all the cultivars of sunflower. Among the five cultivars, K-618 showed the highest activity, and it was 155.32%, 161.14%, and 170.84% over control and the lowest was recorded S-275 cultivar, and it was 143.14%, 148.10%, and 156.59% over control, respectively, in 3, 4, and 5 DID on 50 DAS. The tendency to for recovering to normal was very high in the K-618 cultivar when compared to other cultivars (Table 4).

Stem

Drought stress increased SOD activity in the stem when compared with control in all the cultivars of sunflower. K-618 showed the highest activity, and it was 169.74%, 175.36%, and 184.74% over control and the lowest was recorded in S-275 cultivar when compared to other cultivars, and it was 157.14%, 162.74%, and 170.58% over control, respectively, in 3, 4, and 5 DID on 50 DAS. The tendency to for recovering for normal is very high in the K-618 and low in S-275 cultivar (Table 5).

Table 1: Drought stress induced changes in root peroxidase activity (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	Control	1.281±0.043[a]	1.363±0.047[a]	1.638±0.056[a]	1.298±0.044[a]	1.308±0.045[a]
	3 DID	2.033±0.070[b]	2.230±0.076[b]	2.722±0.093[b]	2.093±0.072[b]	2.024±0.069[b]
	4 DID	2.104±0.076[c]	2.312±0.082[c]	2.820±0.099[c]	2.171±0.080[c]	2.081±0.075[c]
	5 DID	2.211±0.085[d]	2.414±0.088[d]	2.963±0.108[d]	1.260±0.086[d]	2.186±0.083[d]
70	Control	1.666±0.057[a]	1.953±0.067[a]	2.182±0.075[a]	1.779±0.061[a]	1.671±0.057[a]
	3 DID	2.479±0.085[b]	2.964±0.102[b]	3.341±0.115[b]	2.671±0.092[b]	2.467±0.086[b]
	4 DID	2.513±0.092[c]	2.998±0.108[c]	3.394±0.123[c]	2.714±0.098[c]	2.499±0.092[c]
	5 DID	2.551±0.098[d]	3.049±0.114[d]	3.488±0.127[d]	2.745±0.104[d]	2.536±0.098[d]
90	Control	2.381±0.082[a]	2.129±0.73[a]	2.050±0.070[a]	2.133±0.073[a]	2.531±0.097[a]
	3 DID	3.153±0.098[b]	2.704±0.94[b]	2.555±0.088[b]	2.788±0.096[b]	3.450±0.118[b]
	4 DID	3.200±0.112[c]	2.769±0.110[c]	2.608±0.099[c]	2.819±0.102[c]	3.495±0.124[c]
	5 DID	3.265±0.118[d]	2.844±0.116[d]	2.688±0.107[d]	2.892±0.108[d]	3.551±0.128[d]

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). DID: Days interval drought, DMRT: Duncan's multiple range test, SD: Standard deviation

Table 2: Drought stress induced changes in stem peroxidase activity content (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	Control	1.505±0.051[a]	1.663±0.053[a]	1.808±0.064[a]	1.597±0.057[a]	1.192±0.042[a]
	3 DID	2.608±0.089[b]	2.963±0.102[b]	3.276±0.112[b]	2.816±0.096[b]	2.018±0.069[b]
	4 DID	2.772±0.091[c]	3.077±0.108[c]	3.416±0.118[c]	2.926±0.102[c]	2.090±0.076[c]
	5 DID	2.894±0.095[d]	3.303±0.114[d]	3.724±0.125[d]	3.112±0.108[d]	2.230±0.082[d]
70	Control	2.063±0.071[a]	2.363±0.82[a]	2.584±0.089[a]	2.176±0.072[a]	2.009±0.069[a]
	3 DID	3.323±0.114[b]	3.893±0.120[b]	4.335±0.146[b]	3.554±0.120[b]	3.178±0.107[b]
	4 DID	3.376±0.119[b]	3.930±0.126[b]	4.357±0.154[b]	3.601±0.129[b]	3.231±0.113[b]
	5 DID	3.470±0.123[c]	4.048±0.135[c]	4.445±0.161[c]	3.685±0.134[c]	3.303±0.119[c]
90	Control	2.665±0.91[a]	2.486±0.085[a]	2.392±0.082[a]	2.598±0.089[a]	2.908±0.100[a]
	3 DID	3.813±0.131[b]	3.431±0.118[b]	3.232±0.114[b]	3.665±0.126[b]	4.346±0.149[b]
	4 DID	3.901±0.137[b]	3.512±0.124[b]	3.279±0.120[b]	3.779±0.133[b]	4.474±0.156[b]
	5 DID	4.001±0.142[c]	3.608±0.130[c]	3.381±0.126[c]	3.852±0.140[c]	4.539±0.164[c]

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). DID: Days interval drought, DMRT: Duncan's multiple range test, SD: Standard deviation

Table 3: Drought stress induced changes in leaf peroxidase activity content (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	Control	1.074±0.037[a]	1.251±0.043[a]	1.492±0.057[a]	1.106±0.037[a]	1.009±0.036[a]
	3 DID	1.516±0.054[b]	1.854±0.063[b]	2.091±0.072[b]	1.672±0.056[b]	1.406±0.048[b]
	4 DID	1.602±0.060[c]	1.926±0.069[c]	2.345±0.079[c]	1.684±0.062[c]	1.455±0.053[c]
	5 DID	1.688±0.066[d]	2.035±0.075[d]	2.481±0.085[d]	1.762±0.069[d]	1.539±0.058[d]
70	Control	1.363±0.047[a]	1.544±0.053[a]	1.882±0.064[a]	1.476±0.050[a]	1.168±0.040[a]
	3 DID	1.787±0.061[b]	2.070±0.071[b]	2.565±0.088[b]	1.975±0.079[b]	1.518±0.052[b]
	4 DID	1.844±0.067[c]	2.138±0.077[c]	2.639±0.094[c]	2.022±0.088[c]	1.556±0.058[c]
	5 DID	1.888±0.072[d]	2.197±0.082[d]	2.714±0.099[d]	2.082±0.093[d]	1.592±0.064[d]
90	Control	2.908±0.100[a]	2.431±0.083[a]	1.538±0.053[a]	2.844±0.098[a]	2.152±0.074[a]
	3 DID	3.471±0.119[b]	2.771±0.095[b]	1.719±0.059[b]	3.345±0.115[b]	2.631±0.090[b]
	4 DID	3.527±0.126[c]	2.860±0.102[c]	1.753±0.065[c]	3.409±0.121[c]	2.696±0.099[c]
	5 DID	3.653±0.132[d]	2.941±0.108[d]	1.842±0.077[d]	3.535±0.127[d]	2.799±0.103[d]

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT)

Leaf

SOD activity increase under drought conditions in leaves when compared with control in all the cultivars of sunflower. K-618 showed the highest activity, and it was 184.95%, 190.47%, and 199.48% over control the lowest in S-275 cultivar with compared other cultivars, and it was 172.65%, 177.62%, and 185.96% over control, respectively, in 3, 4, and 5 DID on 50 DAS. However, the lowest SOD activity was recorded in the cultivars K-618 and the highest was S-275 on recovery period. This shows that the tendency to for recovering for normal is very high in the K-618 and low in the S-275 cultivar. Among the organ, leaves showed a higher SOD activity followed by stem and roots in all treatments (Table 6).

Table 4: Drought stress induced changes in root superoxide dismutase activity (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	Control	0.689±0.023[a]	0.753±0.025[a]	0.857±0.029[a]	0.709±0.024[a]	0.605±0.020[a]
	3 DID	1.016±0.040[b]	1.152±0.044[b]	1.331±0.051[b]	1.067±0.041[b]	0.865±0.033[b]
	4 DID	1.057±0.047[b]	1.192±0.050[b]	1.380±0.058[b]	1.112±0.047[b]	0.896±0.038[b]
	5 DID	1.114±0.053[c]	1.253±0.057[c]	1.464±0.064[c]	1.160±0.053[c]	0.947±0.044[c]
70	Control	1.163±0.058[a]	1.219±0.043[a]	1.274±0.045[a]	1.169±0.041[a]	1.011±0.034[a]
	3 DID	1.571±0.075[b]	1.684±0.062[b]	1.791±0.063[b]	1.612±0.059[b]	1.364±0.052[b]
	4 DID	1.623±0.084[b]	1.740±0.069[b]	1.841±0.070[b]	1.656±0.064[b]	1.390±0.058[b]
	5 DID	1.656±0.091[c]	1.786±0.074[c]	1.887±0.078[c]	1.701±0.079[c]	1.420±0.064[c]
90	Control	2.025±0.069[a]	1.855±0.066[a]	1.755±0.065[a]	1.945±0.069[a]	2.136±0.079[a]
	3 DID	2.497±0.092[b]	2.196±0.075[b]	2.021±0.074[b]	2.361±0.087[b]	2.722±0.100[b]
	4 DID	2.540±0.099[b]	2.254±0.082[b]	2.085±0.011[b]	2.396±0.092[b]	2.765±0.108[b]
	5 DID	2.615±0.106[c]	2.329±0.088[c]	2.160±0.009[c]	2.498±0.098[c]	2.878±0.113[c]

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c) differ significantly at $P \leq 0.05$ (DMRT). DID: Days interval drought, DMRT: Duncan's multiple range test, SD: Standard deviation

Table 5: Drought stress induced changes in stem superoxide dismutase activity (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	Control	0.873±0.031[a]	1.025±0.035[a]	1.122±0.040[a]	0.978±0.033[a]	0.662±0.023[a]
	3 DID	1.406±0.054[b]	1.701±0.065[b]	1.904±0.073[b]	1.612±0.057[b]	1.040±0.040[b]
	4 DID	1.459±0.060[b]	1.763±0.071[b]	1.967±0.079[b]	1.666±0.063[b]	1.077±0.046[b]
	5 DID	1.529±0.066[c]	1.846±0.077[c]	2.072±0.083[c]	1.739±0.06[c]	1.129±0.052[c]
70	Control	1.356±0.048[a]	1.584±0.054[a]	1.641±0.060[a]	1.460±0.052[a]	0.817±0.029[a]
	3 DID	2.054±0.073[b]	2.442±0.087[b]	2.530±0.090[b]	2.244±0.077[b]	1.232±0.045[b]
	4 DID	2.077±0.079[b]	2.474±0.093[b]	2.595±0.097[b]	2.270±0.083[b]	1.242±0.052[b]
	5 DID	2.125±0.084[c]	2.526±0.099[c]	2.653±0.104[c]	2.229±0.089[c]	1.259±0.059[c]
90	Control	2.346±0.083[a]	2.138±0.079[a]	1.676±0.062[a]	2.211±0.081[a]	2.417±0.086[a]
	3 DID	3.170±0.102[b]	2.786±0.117[b]	2.133±0.079[b]	2.945±0.109[b]	3.372±0.124[b]
	4 DID	3.222±0.109[b]	2.856±0.123[b]	2.239±0.084[b]	2.991±0.114[b]	3.387±0.131[b]
	5 DID	3.291±0.115[c]	2.923±0.128[c]	2.247±0.089[c]	3.065±0.119[c]	3.450±0.138[c]

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c) differ significantly at $P \leq 0.05$ (DMRT). DID: Days interval drought, DMRT: Duncan's multiple range test, SD: Standard deviation

Table 6: Drought stress induced changes in leaf superoxide dismutase activity (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	Control	1.057±0.037[a]	1.539±0.054[a]	1.927±0.071[a]	1.258±0.046[a]	0.873±0.030[a]
	3 DID	1.866±0.074[b]	2.814±0.108[b]	3.563±0.137[b]	2.261±0.083[b]	1.507±0.057[b]
	4 DID	1.927±0.083[c]	2.892±0.115[c]	3.670±0.146[c]	2.330±0.092[c]	1.550±0.063[c]
	5 DID	2.015±0.089[d]	3.025±0.123[d]	3.843±0.155[d]	2.420±0.098[d]	1.623±0.071[d]
70	Control	1.533±0.056[a]	2.067±0.076[a]	2.436±0.090[a]	1.713±0.063[a]	1.355±0.052[a]
	3 DID	2.523±0.093[b]	3.467±0.123[b]	4.121±0.147[b]	2.856±0.102[b]	2.208±0.076[b]
	4 DID	2.553±0.099[c]	3.505±0.129[c]	4.186±0.154[c]	2.894±0.108[c]	2.210±0.082[c]
	5 DID	2.632±0.107[d]	3.605±0.135[d]	4.276±0.162[d]	2.957±0.115[d]	2.269±0.088[d]
90	Control	2.855±0.109[a]	2.315±0.085[a]	2.123±0.078[a]	2.566±0.088[a]	3.220±0.119[a]
	3 DID	4.172±0.143[b]	3.264±0.120[b]	2.945±0.109[b]	3.708±0.127[b]	4.866±0.167[b]
	4 DID	4.275±0.149[c]	3.376±0.128[c]	2.975±0.116[c]	3.816±0.133[c]	4.986±0.174[c]
	5 DID	4.379±0.154[d]	3.420±0.135[d]	3.063±0.121[d]	3.903±0.140[d]	5.134±0.180[d]

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). DID: Days interval drought, DMRT: Duncan's multiple range test, SD: Standard deviation

CAT

Root

The enzyme CAT activity increased under drought stress in roots all sunflower cultivars with 3, 4, and 5 DID on 50, 60, and 70 DAS. Among the five cultivars, K-618 showed the highest activity, and it was 164.58%, 170.65%, and 184.66% over control with 3, 4, and 5 DID treatments on 50 DAS. The CAT activity was lowest in S-275 cultivar when compared with other cultivars, and it was 152.36%, 157.65%, and 170.74% over control, respectively, in 3, 4, and 5 DID on 50 DAS. The lowest CAT activity was recorded in the cultivar K-618, and the highest was with S-275 on 70 DAS. This shows that the recovery from drought stress is faster in K-618 cultivar when compared to other cultivars tested (Table 7).

Stem

Drought stress increased CAT activity in the stem in all the cultivars of sunflower. Among the five cultivars, K-618 showed highest CAT activity, and it was 148.24%, 154.18%, and 169.32% over control, and it was lowest in S-275 cultivar, and it was 136.47%, 141.13%, and 155.87% over control, respectively, in 3, 4, and 5 DID on 50 DAS. The lowest CAT activity was recorded in the cultivar K-618, and the highest was with S-275 on 70 DAS. This shows that the recovery from drought stress is faster in K-618 cultivar when compared to other cultivars tested and low in S-275 cultivar (Table 8).

Leaf

CAT activity increased under drought stress in leaves of all the cultivars of sunflower. Among these cultivars, K-618 showed highest CAT activity, and it was 134.47%, 140.56%, and 154.47% over control, and it was lowest in S-275 cultivar when compared to other cultivars, and it was 118.42%, 120.17%, and 131.66% over control, respectively, in 3, 4, and 5 DID on 50 DAS. The lowest CAT activity was recorded in the cultivar K-618, and the highest was with S-275 on 70 DAS. This shows that the recovery from drought stress is faster in cultivar K-618 has the highest recovery from drought when compared to other cultivars tested. All the three plant parts, roots showed a higher CAT activity followed by stem and leaves in all treatments (Table 9).

APX Activity

Root

Drought stress increased the APX activity in the root of a sunflower. Among these cultivars, K-618 showed highest activity and the lowest was recorded in S-275 cultivar. However, the lowest APX activity was recorded in the cultivar K-618, and the highest was with S-275 on 70 DAS. This shows that the recovery from drought-induced oxidative stress is faster in cultivar K-618, and it is slower in cultivar S-275 (Table 10).

Stem

Drought stress increased APX activity in the stem of a sunflower. Among the cultivars, K-618 showed the highest

Table 7: Drought stress induced changes in root catalase activity (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	Control	3.534±0.121^{a}	4.236±0.146^{a}	4.724±0.162^{a}	3.890±0.134^{a}	3.129±0.120^{a}
	3 DID	5.523±0.190^{b}	6.830±0.235^{b}	7.714±0.268^{b}	6.202±0.221^{b}	4.767±0.170^{b}
	4 DID	5.170±0.194^{c}	7.094±0.244^{c}	8.061±0.275^{c}	6.423±0.228^{c}	4.932±0.181^{c}
	5 DID	6.271±0.196^{d}	7.704±0.250^{d}	8.723±0.282^{d}	6.985±0.237^{d}	5.342±0.188^{d}
70	Control	4.045±0.139^{a}	4.744±0.163^{a}	5.236±0.180^{a}	4.381±0.148^{a}	3.636±0.125^{a}
	3 DID	5.838±0.201^{b}	7.018±0.242^{b}	7.835±0.270^{b}	6.410±0.223^{b}	5.182±0.178^{b}
	4 DID	5.920±0.207^{c}	7.094±0.247^{c}	7.913±0.279^{c}	6.509±0.230^{c}	5.223±0.184^{c}
	5 DID	6.118±0.214^{d}	7.321±0.254^{d}	8.143±0.284^{d}	6.622±0.236^{d}	5.345±0.192^{d}
90	Control	5.860±0.202^{a}	5.163±0.178^{a}	4.785±0.165^{a}	5.460±0.188^{a}	6.344±0.218^{a}
	3 DID	7.690±0.265^{b}	6.512±0.224^{b}	6.016±0.207^{b}	7.089±0.244^{b}	8.662±0.298^{b}
	4 DID	7.890±0.273^{c}	6.698±0.233^{c}	6.023±0.214^{c}	7.297±0.256^{c}	8.865±0.311^{c}
	5 DID	8.488±0.281^{d}	7.003±0.241^{d}	6.490±0.220^{d}	7.826±0.264^{d}	9.557±0.324^{d}

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). DID: Days interval drought, DMRT: Duncan's multiple range test, SD: Standard deviation

Table 8: Drought stress induced changes in stem catalase activity (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	Control	3.067±0.105^{a}	3.545±0.122^{a}	3.990±0.137^{a}	3.371±0.116^{a}	2.619±0.093^{a}
	3 DID	4.307±0.148^{b}	5.159±0.150^{b}	5.914±0.204^{b}	4.842±0.166^{b}	3.574±0.123^{b}
	4 DID	4.487±0.154^{c}	5.372±0.158^{c}	6.151±0.213^{c}	5.041±0.172^{c}	3.690±0.130^{c}
	5 DID	4.911±0.159^{d}	5.860±0.162^{d}	6.765±0.220^{d}	5.464±0.179^{d}	4.082±0.136^{d}
70	Control	3.574±0.123^{a}	4.052±0.139^{a}	4.493±0.154^{a}	3.882±0.177^{a}	3.133±0.108^{a}
	3 DID	4.651±0.160^{b}	5.412±0.186^{b}	6.072±0.216^{b}	5.141±0.199^{b}	4.058±0.139^{b}
	4 DID	4.772±0.171^{c}	5.483±0.197^{c}	6.175±0.222^{c}	5.214±0.206^{c}	4.121±0.146^{c}
	5 DID	4.887±0.179^{d}	5.678±0.202^{d}	6.296±0.229^{d}	5.347±0.210^{d}	4.224±0.155^{d}
90	Control	5.161±0.177^{a}	5.645±0.160^{a}	4.263±0.147^{a}	4.940±0.131^{a}	5.563±0.191^{a}
	3 DID	6.214±0.214^{b}	5.435±0.187^{b}	4.886±0.168^{b}	5.939±0.157^{b}	7.014±0.242^{b}
	4 DID	6.555±0.226^{c}	5.719±0.198^{c}	5.140±0.177^{c}	6.211±0.164^{c}	7.268±0.249^{c}
	5 DID	6.732±0.235^{d}	5.876±0.207^{d}	5.469±0.189^{d}	6.338±0.169^{d}	7.534±0.255^{d}

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). DID: Days interval drought, DMRT: Duncan's multiple range test, SD: Standard deviation

Table 9: Drought stress induced changes in leaf catalase activity (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	Control	2.194±0.075[a]	2.582±0.089[a]	2.858±0.102[a]	2.395±0.082[a]	2.028±0.069[a]
	3 DID	2.778±0.095[b]	3.388±0.116[b]	3.843±0.132[b]	3.110±0.107[b]	2.491±0.085[b]
	4 DID	2.903±0.103[c]	3.551±0.124[c]	4.017±0.139[c]	3.241±0.115[c]	2.578±0.091[c]
	5 DID	3.189±0.108[d]	3.873±0.129[d]	4.414±0.145[d]	3.653±0.120[d]	2.849±0.099[d]
70	Control	2.712±0.093[a]	3.093±0.106[a]	3.361±0.115[a]	2.890±0.099[a]	2.547±0.090[a]
	3 DID	3.094±0.106[b]	3.622±0.124[b]	4.017±0.138[b]	3.370±0.116[b]	2.901±0.100[b]
	4 DID	3.209±0.112[c]	3.742±0.129[c]	4.096±0.143[c]	3.486±0.123[c]	2.963±0.107[c]
	5 DID	3.304±0.117[d]	3.869±0.134[d]	4.275±0.149[d]	3.587±0.128[d]	3.040±0.109[d]
90	Control	4.180±0.144[a]	3.823±0.131[a]	3.650±0.125[a]	3.956±0.136[a]	4.456±0.153[a]
	3 DID	4.733±0.163[b]	4.130±0.142[b]	3.849±0.132[b]	4.411±0.157[b]	5.218±0.179[b]
	4 DID	4.842±0.168[c]	4.275±0.149[c]	3.969±0.137[c]	4.475±0.162[c]	5.321±0.185[c]
	5 DID	4.995±0.174[d]	4.400±0.153[d]	4.144±0.142[d]	4.694±0.167[d]	5.531±0.192[d]

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). DID: Days interval drought, DMRT: Duncan's multiple range test, SD: Standard deviation

Table 10: Drought stress induced changes in root ascorbate peroxidase activity (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	Control	3.475±0.119[a]	3.508±0.120[a]	3.479±0.119[a]	3.666±0.126[a]	3.472±0.119[a]
	3 DID	5.895±0.203[b]	6.110±0.210[b]	6.164±0.212[b]	6.317±0.217[b]	5.735±0.197[b]
	4 DID	5.953±0.209[c]	6.337±0.216[c]	6.323±0.218[c]	6.536±0.221[c]	5.907±0.204[c]
	5 DID	6.296±0.212[d]	6.634±0.220[d]	6.788±0.222[d]	6.841±0.226[d]	6.241±0.209[d]
70	Control	4.362±0.150[a]	4.883±0.168[a]	5.059±0.174[a]	4.078±0.140[a]	3.509±0.121[a]
	3 DID	6.959±0.238[b]	7.948±0.271[b]	8.335±0.285[b]	6.572±0.225[b]	5.563±0.191[b]
	4 DID	6.930±0.244[c]	7.886±0.279[c]	8.290±0.292[c]	6.531±0.232[c]	5.530±0.199[c]
	5 DID	7.080±0.250[d]	8.064±0.284[d]	8.256±0.299[d]	6.655±0.238[d]	5.637±0.204[d]
90	Control	5.176±0.178[a]	4.978±0.171[a]	4.316±0.148[a]	5.116±0.170[a]	5.668±0.195[a]
	3 DID	6.757±0.233[b]	6.238±0.220[b]	5.283±0.186[b]	6.577±0.226[b]	7.650±0.263[b]
	4 DID	6.860±0.240[c]	6.384±0.226[c]	5.412±0.193[c]	6.905±0.233[c]	7.757±0.272[c]
	5 DID	7.060±0.244[d]	6.581±0.230[d]	5.820±0.199[d]	6.899±0.239[d]	7.831±0.279[d]

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). DID: Days interval drought, DMRT: Duncan's multiple range test, SD: Standard deviation

activity, and it was 161.15%, 167.78%, and 176.22% over control, and it was lowest in S-275 cultivar when compared with other cultivars, and it was 149.41%, 154.14%, and 162.74% over control, respectively, in 3, 4, and 5 DID on 50 DAS. However, the lowest APX activity was recorded in the cultivar K-618, and the highest was S-275 on 70 DAS. This shows that the tendency to for retarding for normalcy is very high in the K-618 cultivar and lowest in S-275 cultivar (Table 11).

Leaf

APX activity increased under drought stress in the leaves in all the cultivars of sunflower. Among these cultivars, K-618 showed the highest activity, and it was 186.54%, 192.11%, and 201.41% over control. The APX activity was lowest in S-275 cultivar when compared with other cultivars, and it was 174.58%, 179.34%, and 187.74% over control, respectively, in 3, 4, and 5 DID on 50 DAS. However, the lowest APX activity was recorded in the cultivar K-618, and the highest was with S-275 on 70 DAS. This shows that the recovery from drought-induced oxidative stress in faster in cultivar K-618 and it is slower in cultivar S-275. Among the three plant parts leaves showed a higher APX activity followed by roots and stems in all treatments (Table 12).

DISCUSSION

Drought stress caused an increase in the POX activity in all parts of the plants to a larger extent in all the cultivars of sunflower when compared to control. Among the cultivars K-618 highest POX activity followed by A-SH-3322, A-110, SH-416, and S-275 when compared to control. Water deficit stress increased the POX activity in soybean plants (Zhang *et al.*, 2006); similar results were observed in many plants such as wheat (Lin and Wang, 2002; HongBo *et al.*, 2005); rice (Guo *et al.*, 2006); wheat (Gong *et al.*, 2005); and *Hordeum vulgare* (Acar *et al.*, 2001). Reactive oxygen scavenging is important in imparting tolerance against oxidative stress. It may be presumed that enhancement of the antioxidative system favors water stress resistance (Noctor *et al.*, 2000). POX is believed to be ubiquitous in the plant kingdom, and they are primarily associated with the enzymatic browning

Table 11: Drought stress induced changes in stem ascorbate peroxidase activity (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	Control	2.078±0.071[a]	2.107±0.072[a]	2.906±0.100[a]	2.024±0.069[a]	2.208±0.113[a]
	3 DID	3.182±0.109[b]	3.338±0.114[b]	4.683±0.161[b]	3.158±0.108[b]	3.298±0.117[b]
	4 DID	3.308±0.112[c]	3.535±0.120[c]	4.875±0.164[c]	3.285±0.112[c]	3.403±0.122[c]
	5 DID	3.475±0.117[d]	3.632±0.125[d]	5.120±0.169[d]	3.423±0.118[d]	3.593±0.128[d]
70	Control	3.157±0.108[a]	4.163±0.143[a]	4.385±0.151[a]	3.258±0.113[a]	3.007±0.103[a]
	3 DID	4.467±0.154[b]	5.999±0.208[b]	6.450±0.222[b]	4.696±0.161[b]	4.209±0.145[b]
	4 DID	4.483±0.160[c]	6.040±0.211[c]	6.455±0.229[c]	4.705±0.166[c]	4.219±0.151[c]
	5 DID	4.592±0.166[d]	5.808±0.217[d]	6.635±0.233[d]	4.826±0.172[d]	4.306±0.155[d]
90	Control	5.086±0.175[a]	4.051±0.139[a]	2.886±0.099[a]	4.103±0.141[a]	5.163±0.178[a]
	3 DID	5.934±0.207[b]	4.531±0.159	3.132±0.116[b]	4.713±0.157[b]	6.222±0.214[b]
	4 DID	6.019±0.213[c]	4.639±0.163[c]	3.372±0.124[c]	4.794±0.164[c]	5.818±0.218[c]
	5 DID	6.217±0.218[d]	4.785±0.169[d]	3.507±0.128[d]	4.986±0.168[d]	6.586±0.227[d]

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). DID: Days interval drought, DMRT: Duncan's multiple range test, SD: Standard deviation

Table 12: Drought stress induced changes in leaf ascorbate peroxidase activity (mgg 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	Control	5.539±0.126[a]	3.838±0.132[a]	3.774±0.130[a]	3.924±0.135[a]	3.618±0.124[a]
	3 DID	6.306±0.217[b]	7.029±0.251[b]	7.040±0.242[b]	7.131±0.245[b]	6.316±0.217[b]
	4 DID	6.540±0.223[c]	7.223±0.258[c]	7.250±0.246[c]	7.343±0.251[c]	6.488±0.223[c]
	5 DID	6.826±0.228[d]	7.616±0.260[d]	7.601±0.255[d]	7.644±0254[d]	6.792±0.228[d]
70	Control	4.820±0.166[a]	4.951±0.170[a]	5.316±0.177[a]	4.728±0.163[a]	4.153±0.143[a]
	3 DID	8.023±0.276[b]	8.328±0.287[b]	9.112±0.314[b]	7.967±0.274[b]	6.829±0.235[b]
	4 DID	6.995±0.280[c]	7.349±0.291[c]	8.019±0.318[c]	6.965±0.278[c]	5.916±0.241[c]
	5 DID	8.229±0.287[d]	8.607±0.298[d]	9.296±0.323[d]	8.104±0.285[d]	5.895±0.246[d]
90	Control	5.748±0.198[a]	5.532±0.190[a]	5.002±0.172[a]	5.620±0.193[a]	5.950±0.205[a]
	3 DID	7.767±0.267[b]	7.215±0.248[b]	6.389±0.220[b]	7.507±0.258[b]	8.351±0.288[b]
	4 DID	7.959±0.271[c]	7.404±0.255[c]	6.468±0.226[c]	7.725±0.264[c]	8.559±0.293[c]
	5 DID	8.170±0.278[d]	7.621±0.260[d]	6.995±0.229[d]	7.950±0.267[d]	8.832±0.299[d]

Values are given as mean±SD of six experiments in each group. Values that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT). DID: Days interval drought, DMRT: Duncan's multiple range test, SD: Standard deviation

and off-flavor generation. Increasing evidence suggests that drought induces oxidative stress in various plants, in which ROS, such as superoxide radical, hydroxy radical, H_2O_2, and alkoxy radical are produced (Munne-Bosch and Penuelas, 2003). Although the general effects of drought on plants are fairly well known, the primary effects of water deficit at the biochemical and molecular levels are not well-understood (Zhu *et al.*, 2002). However, the POX activity was reduced in all cultivars during recovery period the cultivar K-618 showed a high tendency to for retarding to normalcy and it was in other cultivars.

Drought stress caused an increase in the SOD activity in all parts of the plants to a larger extent in all the cultivars of sunflower. Superoxide dismutase activity increased under drought stressed higher plants (Ramachandra Reddy *et al.*, 2004), rice (Wang *et al.*, 2005); *Phaseolus acutifolius* (Turkan *et al.*, 2005), wheat (Gong *et al.*, 2005; Shao *et al.*, 2005), and maize (Jiang and Zhang, 2002); rice (Guo *et al.*, 2006); *Pinus halepensis* (Alonso *et al.*, 2001), and *H. vulgare* (Acar *et al.*, 2001).

The SOD activity increased under drought in *Phaseolus aconitifolius* (Turkan *et al.*, 2005). An increase in SOD activity was reported in *Vigna* plants under water deficit stress and propiconazole application (Manivannan *et al.*, 2007). Water stress increased the SOD activity in *Catharanthus* plants (Jaleel *et al.*, 2007). SOD activity increased under drought stressed higher plants (Ramachandra Reddy *et al.*, 2004). A lower SOD activity was recorded in the cultivar K-618, and the highest was with S-275 on 70 DAS. This shows that the recovery from drought-induced oxidative stress in faster in cultivar K-618 and it slower in cultivar S-275.

Drought stress has increased in the CAT activity in all parts of the plants to a larger extent in all the cultivars of sunflower when compared to control. The CAT activity increased under drought in *P. halepensis* (Alonso *et al.*, 2001). Similar results were observed in higher plants (Ramachandra Reddy *et al.*, 2004); wheat (Gong *et al.*, 2005; Shao *et al.*, 2005); *P. acutifolius* (Turkan *et al.*, 2005); *Zea mays* (Jiang and Zhang, 2002); rice (Guo *et al.*, 2006).

The lowest CAT activity was recorded in the cultivar K-618, and the highest was with S-275 on 70 DAS. This shows that the recovery from drought stress is faster in K-618 cultivar when compared to other cultivar tested.

The antioxidant enzyme, APX showed an increased activity under drought stressed condition in all cultivars of sunflower. Similar results were obtained by many workers under drought stress in many higher plants (Ramachandra Reddy *et al.*, 2004); *P. halepensis* (Alonso *et al.*, 2001); maize (Jiang and Zhang, 2002); *P. acutifolius* (Turkan *et al.*, 2005), soybean (Gong *et al.*, 2005); rice (Guo *et al.*, 2006); *Catharanthus* plants (Jaleel *et al.*, 2007); and *Kentucky bluegrass* (Liu *et al.*, 2008). Drought stress induced the generation of active oxygen species is well-recognized at the cellular level and is tightly controlled at both the production and consumption levels through increased antioxidant systems (Ramachandra Reddy *et al.*, 2004). APX is a primary ROS scavenging system under stress.

CONCLUSION

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 antioxidant enzymes under drought stress condition were studied. *H. annuus* L. (Sunflower) is one of the most important oil-seed crops cultivated in many states of India. This plant has got commercial important due to its agricultural values moreover, it can be cultivated in less water available areas because it is slight drought tolerance. Due to its immense values attempt has been made to screen the drought tolerant cultivar among five cultivars of sunflower. The present study was aimed to understand the effects of drought stress on antioxidant enzymes such as POX, SOD, CAT, and APX activity on five cultivars of *H. annuus* L. Five cultivars viz., Asgrow-SH 3322 (A-SH 3322), Agsun-110 (A-110), Kaveri 618 (K-618), SH 416, and Sunbred 275 (S-275). The antioxidant enzymes such as POX, SOD, CAT, and APX activities increased under drought stress in all parts of the sunflower cultivars. The maximum activity was observed in K-618 and the minimum in S-275 cultivar.

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