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Biochemical Studies on Evaluation of Sunflower (*Helianthus annuus* L.) Genotypes for Heat Stress

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Crops are facing heat stress because of rapid climate change caused by global warming. We examined these issues in sunflower by exposing the plants growing with mean maximum temperature of 32.2°C (sowing to flowering) and 36.1°C (flowering to harvest) at normal temperature (S1) and 35.3°C (sowing to flowering) and 38.3 °C (flowering to harvest) at high temperatures (S2) by staggered sowings. Antioxidative responses of sunflower were also explored by studying the Superoxide dismutase, Catalase, Peroxidises and Ascorbate peroxidise activities. A significant increase was observed in antioxidant enzyme activities under high temperature stress. The final oil composition proved to be sensitive to the timing of heat stress and reduced (13%) by high temperatures. Some innovative steps should be taken on an emergency basis to prepare plants for such stressful conditions.

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1. INTRODUCTION

Rising global earth surface temperature is one of the most enthralling factor emerging from the changing climate and is the major environmental parameter which affects plant growth, development, and yield [1] Globally, annual temperature is expected to rise by 1.8–4.0°C at the end of the 21st century [2]. The heat stress is many fold high in arid to tropical zones of the globe affecting vital physiological processes of crops resulting in the reduction of food quantity and quality [3]. The high temperature is different for crops like for corn 29^{o}C, soybean is 30^{o}C, cotton 32^{o}C, sunflower 33^{o}C, and maize 36^{o}C [4].

Sunflower (*Helianthus annuus* L.) is an important lipid and protein-rich oilseed crop and the fourth largest oilseed crop in the world cultivated in more than 70 countries [5]. In *H. annuus*, previous research has demonstrated that brief period of heat stress during grain filling negatively impact on oil yield and fatty acid composition [6].

High temperatures (HT) damage the activity of membrane proteins and lipids, thus affecting the activity of chloroplast- and mitochondria based enzymes and membrane integrity [7]. Stress conditions can disturb the dynamic equilibrium of reactive oxygen species (ROS) production and elimination under normal growth in plants [1] which promotes ROS accumulation, membrane lipid peroxidation, and disrupt the structure and function of cell membrane system [1].

Different stress condition causes variations in plant metabolism and results in formation of reactive oxygen species (ROS) which is removed by antioxidant enzymes like ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) [8]. In a cell SOD enzyme is the first line of defence against reactive oxygen species which detoxifies superoxide anion to hydrogen peroxide and molecular oxygen [9]. The main objective of this study was to investigate the direct effects of constant increase of high temperature on biochemical traits in sunflower.

2. MATERIALS AND METHODS

This experiment was conducted in field at Narkhoda farm, ICAR- Indian Institute of Oilseeds Research, Hyderabad (17_1501600 N, 78_1803000 E; 542 m above sea level) during *late rabi* (February- June) 2020 in split-plot design with two temperature treatments using staggered sowings (S1- Timely sowing and S2- Delayed sowing). Fourteen cultivars of *H. annuus* named as AKSF 6-3B, CMS 17B, CMS 42B, CMS 70B, CMS 107B, CMS 125B, CMS 127B, CMS 135B, CMS 144B, ARM 243B along with four checks CO 2, CSFH 12205, DRSH 1, KBSH 44 were selected from the TIR study [10]. Each genotype was sown in 6 x 3.6 m plots with a spacing of 60 cm (between rows) x 15 cm (between plants); there were three replicates for each treatment. Sowing was done by dibbling, and the recommended fertilizer dose (60 Kg N, 90 Kg P_2O_5, and 30 kg K_2O /ha) [11] was applied; other standard practices and need-based plant protection measures were followed to ensure a healthy crop. The soil structure is sandy loam with low organic carbon (0.4%) and nitrogen (235 kg/ ha) content, and relatively high phosphorus (22 kg/ha) and potassium (405 kg/ha) content. For enzyme activity Enzyme extracts were prepared by freezing a weighed amount of leaf samples (0.1g) in liquid nitrogen to prevent proteolytic activity, followed by grinding in a 0.1 M phosphate buffer at pH 7.5 containing 0.5 mM EDTA and 1 mM ascorbic acid at a 1:10 (w/v) ratio [12]. The homogenate separated then passed through four layers of gauze, and the filtrate was centrifuged at 15,000 rpm for 20 min. The resulting supernatant was used as an enzyme source. Biochemical traits like CAT [13], SOD [14], POX [15], APX [16] and OC were studied. Cleaned seed sample of 15 gram each of fourteen lines were oven dried at 70^{o}C for 3 hours to determine the oil content using NMR (Nuclear Magnetic Resonance) spectrometer (MARS, UAS, Raichur).

2.1 Statistical Analysis

Results were analyzed by analysis of variance and LSD values were calculated for cultivars, treatments and their interactions.

3. RESULTS AND DISCUSSION

Two sowings were taken up with timely (S1) and delayed (S2) sowing. The maximum (Tmax) and minimum temperature (Tmin) from sowing to flowering was 32.2^{o}C, 16.4^{o}C and 35.3^{o}C, 20.2^{o}C and from flowering to harvest was 36.1^{o}C, 20.8^{o}C and 38.3^{o}C, 23.8^{o}C for S1 and S2 respectively. The difference in Tmax recorded in the two

sowings was 3.1°C at sowing to flowering and 2.2°C at the flowering to harvest. The results of the biochemical study are presented below (Table 1).

Biochemical parameters: Significant variation among the genotypes along with TxG interaction to HT was observed for CAT activity at the vegetative stage, and with temperature treatments for CAT activity at the flowering stage, SOD activity at the vegetative stage, POX activity at the vegetative and flowering stages and APX activity at the vegetative and flowering stages (Table 1).

Catalase (CAT) (EC 1.11.1.6) (µmol min^{-1} g^{-1}): During the vegetative stage CAT activity varied from 0.75 to 1.27 (1.09) and from 0.76 to 1.84 (1.31) in S1 and S2. Checks DRSH 1 (1.27) being at par with checks CSFH 12205 (1.26), CO 2 (1.24), KBSH 44 (1.16), inbred CMS lines -70B (1.14), -107B (1.21), AKSF 6-3B (1.11) in S1 while inbred CMS 70B (1.84) being at par with CMS 144B (1.73) in S2 has higher CAT activity. Inbred CMS lines -70B (62%), -144B (49%), -17B (30%), -127B (30%), and AKSF 6-3B (26%) have recorded more increase in CAT activity compared to checks at the vegetative stage. At the flowering stage, CAT activity varied from 0.77 to 1.45 (1.19) and from 1.06 to 1.99 (1.58) in S1 and S2. Inbred AKSF 6-3B (1.45) being at par with checks DRSH 1 (1.45), CO 2 (1.3), CSFH 12205 (1.27), KBSH 44 (1.24) and inbred CMS 107B (1.35) in S1 while, in inbred CMS lines -70B (1.99), -107B (1.81), -144B (1.80), -17B (1.75) and checks CO 2 (1.74), DRSH 1 (1.72) in S2 has higher CAT activity. Inbred CMS lines -70B (69%), -125B (67%), -17B (51%), -144B (45%), and -42B (41%) has recorded more increase in CAT activity compared to checks at the flowering stage.

The results were in accordance with canola (Zhang *et al.,* 2015), Brassica sps (Soengas *et al.,* 2018).

The activities of CAT, POX, SOD, and APX were increased with exposure to heat stress. Production of ROS often induces the production of abscisic acid and regulates the gene expressions that control the production of enzymatic antioxidants such as SOD and CAT [17]. CAT enzyme plays a key role in maintaining H_2O_2 concentration at normal level, inhibiting the chain reaction of ROS.

Superoxide dismutase (SOD) (EC 1.15.1.1)(nmol g^{-1}): During the vegetative stage, SOD activity varied from 13.89 to 26.81 (19.3) in S1 and from 17.93 to 31.85 (23.98) in S2. Inbred CMS 70B (26.81) being at par with inbred CMS lines -42B (25.77), -144B (25.21) in S1 and CMS 42B (29.75) in S2 has higher SOD activity. At flowering SOD activity varied from 20.06 to 33.69 (25.21) and from 24.7 to 37.73 (29.67) under S1 and S2. Higher SOD activity was recorded in inbred CMS 70B (33.69 in S1 and 37.73 in S2) being at par with CMS 42B (33.33 in S1 and 36.03 in S2). Inbred AKSF 6-3B at vegetative stage (33%) and flowering (42%) recorded more percent increase in SOD activity compared to checks. The present results were also in accordance with Brassica sps (Soengas *et al.,* 2018).

Aerobic metabolism in plants releases ROS as by-products; however, environmental stresses prompt ROS production. Superoxide dismutase (SOD) is considered to be the first line of defence to safeguard plants against environmental fluctuations [18] by converting superoxide anion ($O_2^{\cdot-}$) into O_2 and H_2O_2. H_2O_2 still being toxic is converted into H_2O and oxygen by CAT [19].

Peroxidase (POX) (EC 1.11.1.7)(nmol min^{-1}g^{-1}): During the vegetative stage, POX activity varied from 14.04 to 17.91 (15.49) and from 16.08 to 27.99 (20.69) under S1 and S2. POX activity is higher in check CO 2 (17.91) being at par with inbreds AKSF 6-3B (16.91), CMS 107B (16.37), ARM 243B (16.15) in S1, and in inbred AKSF 6-3B (27.99) at par with check CO 2 (26.99) in S2. At the flowering stage, POX activity varied from 14.64 to 20.38 (17.47) under S1 and from 16.27 to 29.75 (23.19) under S2. Higher POX activity was recorded in check CO 2 (20.38) being at par with inbreds AKSF 6-3B (19.6), CMS 107B (18.44), ARM 243B (18.55), and checks DRSH 1 (19.08), CSFH 12205 (18.16) under S1 and in check CO 2 (29.75) and inbred AKSF 6-3B (29.75) under S2 respectively. Inbred AKSF 6-3B at vegetative stage (66%) and flowering (52%) recorded a more percent increase for the trait POX activity compared to checks.

Similar results were reported in Sorghum (Gosavi *et al.,* 2014) and wheat (Sarkar *et al.,* 2016) under HT stress (30°C). Increased levels of activities of peroxidase (POX) and ascorbate peroxidase (APX) were observed in lablab (*Dolichos lablab*) seedlings (D'Souza and Devaraj, 2013). Maintenance of a high antioxidant capacity to scavenge the toxic ROS has been linked to increase in tolerance of plants to these environmental stresses [20].

Table 1. Enzymes activity of sunflower genotypes during late *Rabi*, 2020

	CAT		SOD		POX		APX		
	Vegetative	**Flowering**	**Vegetative**	**Flowering**	**Vegetative**	**Flowering**	**Vegetative**	**Flowering**	**OC (%)**
Temperature	NS	0.23	1.79	4.15	0.61	2.14	0.09	1.08	1.8
CV (%)	28	17.4	8.8	16.08	3.6	11.22	1.6	17.8	5.7
Genotypes	0.2	0.28	2.3	3.01	1.88	1.88	0.5	0.8	3.7
CV (%)	14.27	17.5	9.18	9.46	8.97	7.98	7.06	10.6	9.4
T*G	0.28	NS	NS	NS	2.66	2.66	0.71	NS	NS

CAT-catalase, SOD- super oxide dismutase, POX-peroxidase, APX- ascorbate peroxidise, OC- Oil concentration

Ascorbate peroxidise (APX) (EC 1.11.1.11) (nmol min^{-1} g^{-1}): APX activity varied from 4.12 to 7.16 (5.2) in S1 and from 5.79 to 9.22 (7.11) in S2 at vegetative stage. Inbreds CMS 42B (7.16) in S1 and ARM 243B (9.22) in S2 has higher APX activity. At the flowering, APX activity varied from 4.54 to 7.26 (5.52) under S1 while, and from 6.36 to 9.42 (7.42) under S2. Higher APX activity was recorded in inbreds CMS 42B (7.26) being at par with inbred ARM 243B (6.63) in S1. Similarly, inbred ARM 243B (9.42) being at par with check CSFH 12205 (8.92) and inbred CMS 42B (8.71) in S2 has higher APX activity. Inbred AKSF 6-3B at vegetative stage (54%) and at flowering (57%) recorded a more percent increase for the trait APX activity compared to checks.

APX efficiently scavenges H_2O_2 due to its higher affinity for H_2O_2 compared to CAT [21]. When the crop is exposed to continuous stress, the equilibrium between H_2O_2 production and the antioxidant activity becomes unbalanced, leading to excessive accumulation of H_2O_2 which further increases the levels of CAT and APX as they are continually involved in rebalancing the equilibrium.

The generation of destructive reactive oxygen species, including superoxide radical (O2−), singlet oxygen (1 O2), hydroxyl radical (OH−) and hydrogen peroxide (H2O2) was probably the reason of enhanced enzyme activities [22]. An increase in CAT, SOD, POX, APX activity may be a manifestation of the adaptive response of plants to abiotic stress without which the plant growth reduction could be more severe. An increase in temperature leads to the increased expression of these antioxidative enzymes until a pre-determined temperature after which they decline. Tolerant varieties could maintain increased activities at higher temperatures than susceptible ones [23]. This suggests that there is a common regulatory (biochemical and molecular) framework of mechanism(s) underlying the induction of enzyme activity or possibly the entire suite of changes, including other antioxidative defenses in response to the different abiotic stress conditions [24]

Increased APX activity in tomato plants proposing an effectual H_2O_2 scavenging capacity under HS. However, this enzyme activity under high temperatures could be inadequate to scavenge the surplus of H_2O_2 when the activity of CAT is not initiated, triggering oxidative damage [25].

Oil Concentration (%): OC varied from 31.9 to 39.9% (35.7 %) in S1 and from 24.8 to 37.4 % (31.1 %) in S2. Under both situations, the checks CSFH 12205 and DRSH 1 recorded the highest OC whereas inbreds CMS -135B & -17B secured the lowest OC respectively. Inbred AKSF 6-3B (5%) recorded the lowest percent reduction.

Oil concentration was significantly altered by the HT. This decrease in oil concentration with an increase in temperature was in accordance with previous reports [26]. The cause of the reduction in OC could be attributed to a shortening of the grain-filling period and reduced rate of seed maturation and duration of oil deposition in the grain at the HT [27].

4. CONCLUSION

Our results demonstrate that sunflower (*Helianthus annuus* L.) primary leaf grown under high temperature increased the activity of key enzymes and the oxidation status of the plants during leaf ontogeny. The biochemical traits under S1 and S2 differed among the sunflower genotypes studied. The inbreds and hybrids with different genetic backgrounds resulted in trait variation. Variation in the S2 condition of specific traits measured among genotypes aids the selection of these traits. These trait-specific genotypes could be used in sunflower breeding programs to develop location-specific varieties.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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