Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 1809-1813
Received: 17-03-2019
Accepted: 22-04-2019

M Jincy
Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

P Jeyakumar
Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

P Boominathan
Agricultural College and Research Institute, Echangkottai, Thanjavur, Tamil Nadu, India

N Manivannan
National Pulses Research Centre, Vamban, Pudukkottai, Tamil Nadu, India

S Varanavasiappan
Department of Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

V Babu Rajendra Prasad
Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
V Babu Rajendra Prasad
Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Impact of drought and high temperature stress on oxidants and antioxidants in greengram (*Vigna radiata* (L.) Wilczek)

M Jincy, P Jeyakumar, P Boominathan, N Manivannan, S Varanavasiappan and V Babu Rajendra Prasad

Abstract
Drought and high temperature often occur simultaneously due to climate change which cause devastating effects in plants due to oxidative damage. This study aimed to quantify the oxidant production and antioxidant activity during combined drought (D) and high temperature (HT) stress. A set of greengram genotypes were grown in rainout shelter by pot culture and the plants were exposed to drought and high temperature stress by sowing the seeds in such a way that the vegetative stage coincide with high temperature stress (> 36±2°C) and the control plants (< 36±2°C). Irrigation was withheld for five days by maintaining the field capacity (50%) in stressed plants and (100%) in non stressed plants. The oxidants such as hydrogen peroxide, superoxide radical and melondialdehyde content were increased under stress condition and leads to increase in membrane damage. This effect can be minimized by increase in enzymatic and non-enzymatic antioxidant. Therefore the tolerance capacity of the genotypes is based on the tolerance against the oxidative stress by antioxidant activity.

Keywords: Greengram, drought, high temperature, oxidant, antioxidant

Introduction
Greengram is one of the important pulse crop with rich source of protein, India is the largest producer. The global agricultural productivity was highly affected by various abiotic and biotic stresses. Especially the abiotic stresses such as drought and high temperature stress affected the plant growth and development (Hasanuzzaman and Fujita, 2011) [11]. Due to climate change, drought and high temperature stress occur simultaneously and this resulted in reduction of crop productivity due to oxidative damage. The oxidant production can be triggered under combined drought and high temperature stress. The oxidants such as superoxide (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2) content were enhanced under combined drought and high temperature stress this leads to peroxidation of lipids, membarane damage and even leads programmed cell death (Miller *et al*. 2008; Choudhury *et al*. 2013) [15, 8]. This oxidative damage can be regulated by the enzymatic and non-enzymatic antioxidant by quenching and neutralizing the free radicals produced under stress condition (Bohnert and Shen, 1999).
In this study the greengram genotypes were used to study the influence of combined drought and high temperature stress by quantifying the oxidants such as superoxide (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2), melondialdehyde (MDA) content and the effects of oxidants on membarane. The activity of antioxidant enzymes like catalase and non-enzymatic antioxidant like proline were quantified under drought and high temperature stress.

Materials and Methods
Plant material and Growing Condition
The greengram genotypes (Table 1) were sown in pot to study the influence of drought and high temperature stress on vegetative phase of greengram. The plants were imposed to drought (50% field capacity for 5 days) combined with high temperature stress (36 ± 2°C) during vegetative Stage (20 Days after sowing).

Table 1: Details of greengram genotypes used in this study

S. No.	Source	S. No.	Source
1.	CO 8	16.	VGG 16069
2.	COGG 1319	17.	VGG 17001
3.	COGG 1332	18.	VGG 17002
4.	COGG 1339	19.	VGG 17003
5.	LGG 607	20.	VGG 17004
6.	PUSA 9072	21.	VGG 17006
7.	TARM 1	22.	VGG 17009
8.	VBN(Gg) 2	23.	VGG 17010
9.	VBN(Gg)3	24.	VGG 17019
10.	VGG 10008	25.	VGG 17036
11.	VGG 15029	26.	VGG 17037
12.	VGG 15036	27.	VGG 17045
13.	VGG 16005	28.	VGG 17049
14.	VGG 16008	29.	VMGG 12005
15.	VGG 16027		

Quantification of oxidant content

Superoxide (O_2^-) radical content

Superoxide anion radical was quantified according to Chaitanya and Naithani, (1994) [6] by macerating the leaf samples in ice-cold sodium phosphate buffer (0.2 M, pH 7.2) containing diethyl dithiocarbamate and it was centrifuged at 3000*g* for 1 min. Absorbance was measured at 540 nm with a spectrophotometer (Eppendorf BioSpectrometer kinetic).

Hydrogen peroxide

The H_2O_2 levels was quantified by following the method of Patterson *et al.* (1984) [16]. The leaf samples were homogenized in 1 mL of cold acetone. 0.1 mL of 20% titanium reagent (20%w/v $TiCl_4$ in 12.1M HCl) and 0.2 mL of 17M ammonia solution were added in known volume of supernatant. It was centrifuged at 3000*g* for 10 min at 4ºC and discard the supernatant, 3 mL of 1M sulphuric acid was used to dissolve the pellet. Absorbance was measured at 410nm using UV-VIS spectrophotometer (Eppendorf BioSpectrometer kinetic).

Lipid peroxidation

Lipid peroxidation was estimated by malondialdehyde (MDA) content produced by thiobarbitaric acid (TBA) as described by Behera *et al.* (1999). Greengram leaf sample was homogenized in 0.1% trichloroacetic acid. The homogenate was centrifuged at 10,000*g* for 5min at 4ºC. Then it was mixed with 1.2 mL of 0.5% TBA prepared in 20% trichloroacetic acid and incubated at 95ºC for 30 min. After stopping the reaction in an ice bath for 5 min, samples were centrifuged at 10,000*g* for 10 min at 25ºC. Absorbance was measured at 532 nm with a spectrophotometer (Eppendorf BioSpectrometer kinetic).

Membrane damage

The greengram leaf sample was cut into small pieces and washed with deionized water, then incubated in 10 mL of deionized water at 25°C for 4 h in a shaker. The initial electrical conductivity (E_1) was read using a EC/TDS hydrotester. The samples were kept in water bath at 95°C for 60 min and cooled to 25°C and again the electrical conductivity (E_2) was measured. The membrane damage was estimated using the following formula: membrane injury (%) = E_1/E_2 x 100 (Chauhan and Senboku, 1996) [7].

Proline content

For assessing the proline content the greengram leaf samples were homogenized in 3% sulfosalicylic acid and centifuged at 11500 x g. The supernatant was mixed with acid ninhydrin, glacial acetic acid and phosphoric acid. Incubate the mixture at 100°C for 1 h then cool it and add toluene to separate the chromophore containing toluene and it was read spectrophotometrically (Eppendorf BioSpectrometer kinetic) at 520 nm (Bates *et al*., 1973) [4].

Catalase activity

Catalase activity was measured according to Aebi (1983) [1]. The catalytic activity of the enzyme was measured spectrophotometrically (Eppendorf BioSpectrometer kinetic) by recording the decline of absorbance at 240 nm due to decomposition of H_2O_2.

Statistical Analysis

The data was statistically analyzed using the Statistical Tool for Agricultural Research (STAR) version 2.0.1.

Results and Discussion

Effect of high temperature and drought on oxidant production, lipid peroxidation and membrane damage

Superoxide (O_2^-) radical content

The superoxide radical content was significantly (P<0.001, Fig.1.) decreased in tolerant genotypes such as VGG 16069 (0.83 change in OD min^{-1} g^{-1} FW), VGG 17003 (0.87 change in OD min^{-1} g^{-1} FW), COGG 1332 (0.87 change in OD min^{-1} g^{-1} FW) and increased in VGG 16027 (2.77 change in OD min^{-1} g^{-1} FW), VGG 17037 (2.77 change in OD min^{-1} g^{-1} FW) at 50% FC during vegetative stage (Fig. 1).

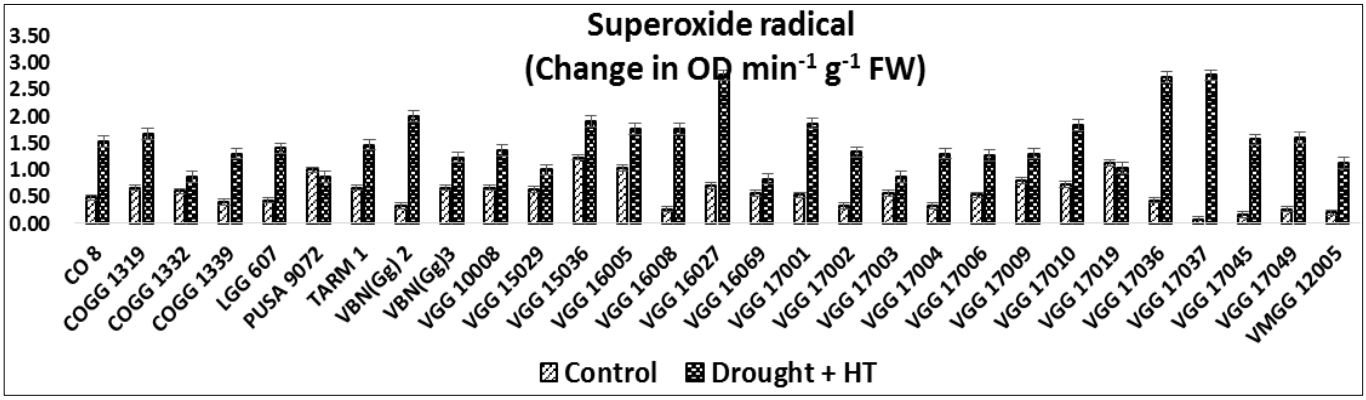

Fig 1: Superoxide radical content in greengram genotypes under drought and high temperature stress

Hydrogen peroxide

High temperature and drought stress induce the production of hydrogen peroxide quantity was significantly (P<0.001, Fig.2.) decreased in tolerant genotypes such as COGG 1332 (7.30 nM g^{-1} FW), VGG 16069 (7.77 nM g^{-1} FW) and the content was high in VGG 17037 (30.07 nM g^{-1} FW), CO 8 (23.73 nM g^{-1} FW) at 50% FC during vegetative stage (Fig. 2).

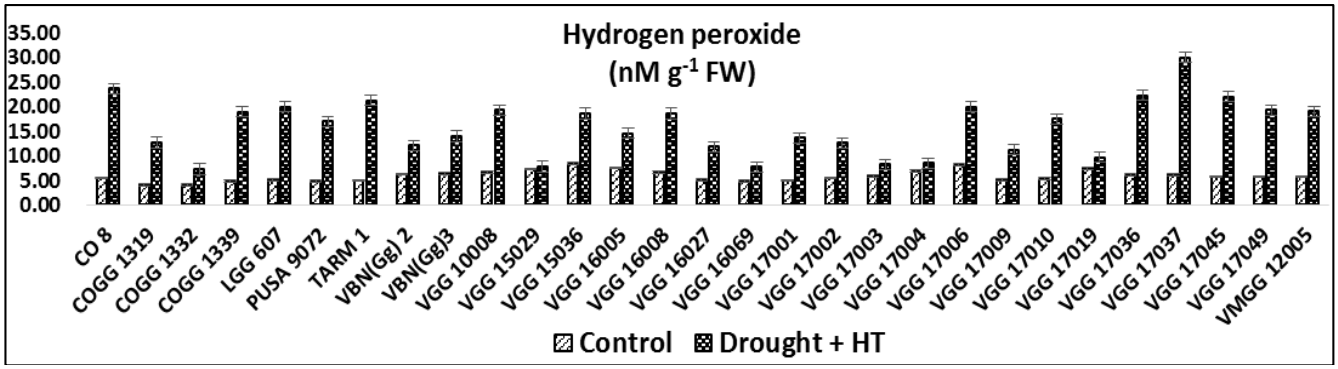

Fig 2: Hydrogen peroxide content in greengram genotypes under drought and high temperature stress

Lipid peroxidation

The malondialdehyde content was also significantly (P<0.001, Fig.3.) decreased in tolerant genotypes like VGG 17019 (13.52 nM g^{-1} FW), VGG 17003 (13.70 nM g^{-1} FW) and increased in VGG 17036 (38.28 nM g^{-1} FW), VGG 17037 (36.34 nM g^{-1} FW) at 50% FC during vegetative stage.

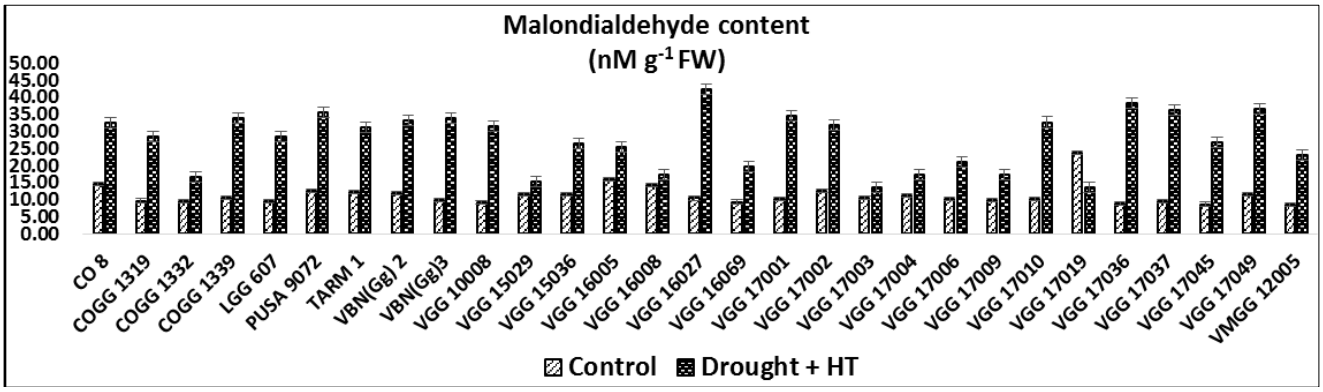

Fig 3: Malondialdehyde content in greengram genotypes under drought and high temperature stress

Membrane damage (%)

The membrane stability index was quantified to assess the influence of drought and high temperature on membrane rigidity. Membrane damage was significantly (P<0.001, Fig.4.) decreased under drought and high temperature stress in tolerant genotypes VGG 17003 (14.94%), VGG 16069 (14.99%) and increased in VGG 17036 (48.09%), VGG 16027 (45.94 %).

Under drought and high temperature stress the oxidants such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) content, melondialdehyde content and membrane damage were significantly decreased in tolerant genotypes when compared with susceptible genotypes under drought and high temperature stress. The increase in melondialdehyde content in susceptible genotypes indicates that the cell membrane integrity get severely affected (Liu and Huang 2000) [14]. The increase in oxidants under combined drought and high temperature stress leads to membarane damage by oxidising the membrane lipids and protein therefore the membrane permeability get increased (Djanaguiraman *et al*. 2009) [10].

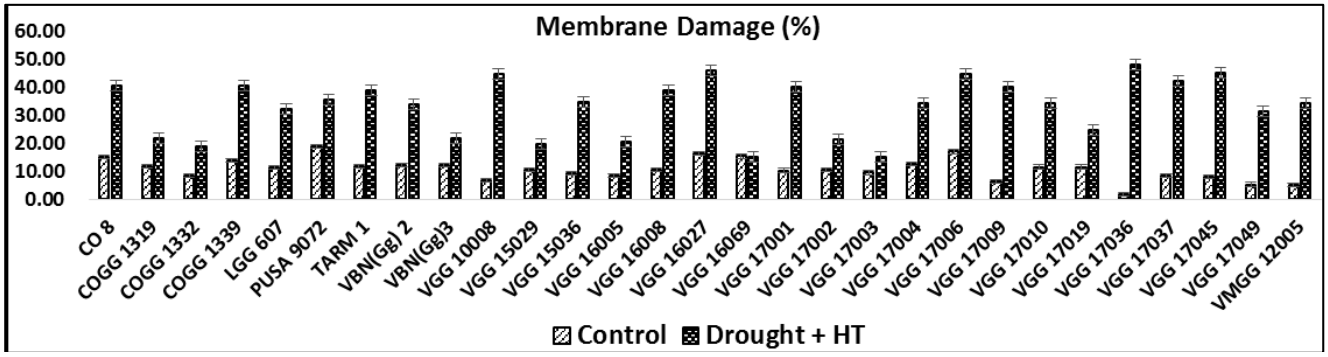

Fig 4: Membrane damage in greengram genotypes under drought and high temperature stress

Effect of high temperature and drought on proline content and catalase activity

Proline content

At 50 % field capacity the following greengram genotypes *viz*., VGG 17003 (14.34 µM g^{-1} FW), VGG 17019 (13.44 µM g^{-1} FW) has accumulated significantly (P<0.001, Fig.4.) more proline as compared to susceptible greengram genotypes VGG 16027 (1.45 µM g^{-1} FW), CO8 (2.30 µM g^{-1} FW) as shown in (Fig. 5).

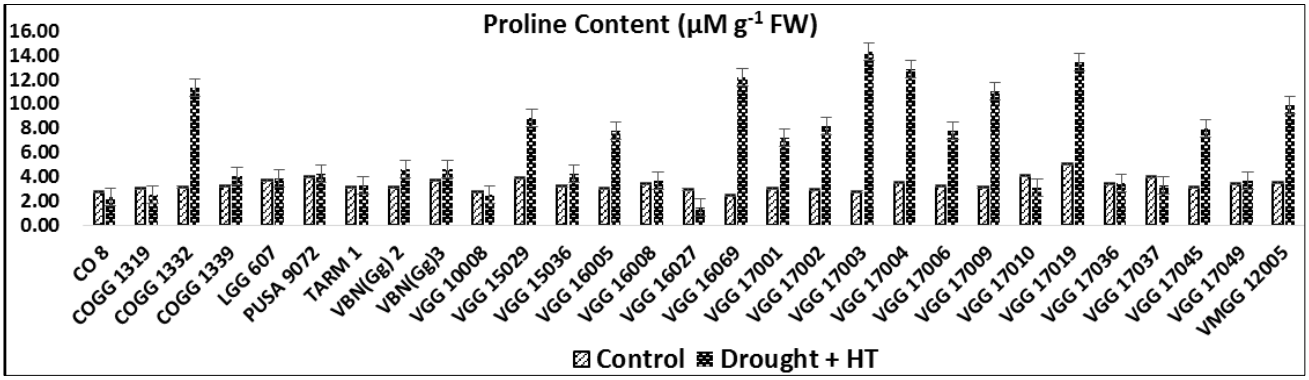

Fig 5: Proline content in greengram genotypes under drought and high temperature stress

Catalase activity

Catalase activity was significantly (P<0.001, Fig.6.) higher in VGG 15029 (30.75 µM H_2O_2 destroyed min^{-1} g^{-1} FW), VGG 17003 (28.48 µM H_2O_2 destroyed min^{-1} g^{-1} FW) and lower in VGG 17036 (6.22 µM H_2O_2 destroyed min^{-1} g^{-1} FW), VGG 17037 (6.22 µM H_2O_2 destroyed min^{-1} g^{-1} FW) at 70% FC during vegetative stage.

The plant cells have the capacity to protect itself from ROS damage by the enzymatic and non-enzymatic antioxidants such as catalase and proline content. Catalase enzyme scavenge the hydrogen peroxide and breakdown into water (Scandalios 1993) [18]. The increase in tolerant capacity of plants is associated with increase in antioxidant enzyme activity (Sairam *et al*. 2000; Snider *et al*., 2010) [17, 19]. In this study the catalase enzyme activity get increased in tolerant genotypes and decreased in susceptible genotypes under combined drought and high temperature stress, this might be due to the toxic effects of oxidants produced under drought and high temperature stress. The proline content also increased in tolerant genotypes than the susceptible genotypes under combined drought and high temperature stress. Proline is a compatible solute act as an osmoprotectant, it will reduce the stress induced cellular acidification and it mainatain the osmoregulation under stress condition (Hasegawa *et al*. 2000) [12]. Proline stabilizes the macromolecules and it also prevent the water loss and mainatain the turgidity of the plants (Ahmed *et al*. 2011; Khedr *et al*. 2003; Ashraf and Foolad 2007) [2, 13, 3].

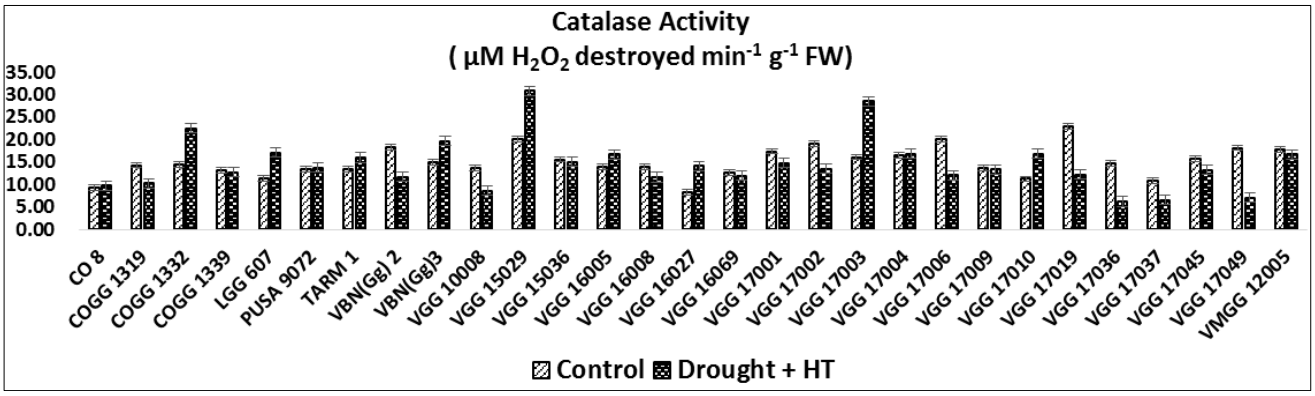

Fig 6: Catalase activity in greengram genotypes under drought and high temperature stress

Conclusion

The present study revealed that under combined drought and high temperature stress the production of oxidant level get increased and cause oxidative damage to the susceptible genotypes but in tolerant genotypes the level of oxidant get decreased due to increased in catalase enzyme activity and the proline content as compared to the susceptible genotypes. Therefore the tolerant genotypes showed tolerant traits to withstand under drought and high temperature stress and can be used for further investigation.

Acknowledgement

We thank Department of Science and Technology (DST) - Science and Engineering Research Board (SERB), New Delhi for financial support. We would like to thank National Pulses Research Centre (NPRC), Vamban, Pudukottai for providing the greengram seed material.

References

1. Aebi H. Catalase. In: Methods of enzymatic analysis, H.U. Bergmeyer (Eds.), 2nd edition, Verlag Chemie, Weinheim, 1983.
2. Ahmed CB, Magdich S, Rouina BB, Sensoy S, Boukhris M, Abdullah FB. Exogenous proline effects on water relations and ions contents in leaves and roots of young olive. Amino Acids. 2011; 40(2):565-573.
3. Ashraf MFMR, Foolad M. Roles of glycine betaine and proline in improving plant abiotic stress

resistance. Environmental and Experimental Botany. 2007; 59(2):206-216.
4. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and soil. 1973; 39(1):205-207.
5. Behera TH, Panda SK, Patra HK. Chromium ion induced lipid peroxidation in Bohnert HJ, Shen BO. Transformation and compatible solutes. Scientia Horticulturae. 1998; 78(1-4):237-260.
6. Chaitanya KSK, Naithani SC. Role of superoxide, lipid peroxidation and superoxide dismutase in membrane perturbation during loss of viability in seeds of Shorea robusta Gaertn. f. New Phytologist. 1994; 126:623-627.
7. Chauhan YS, Senboku T. Thermostabilities of cell membrane and photosynthesis in cabbage cultivars differing in heat tolerance. Journal of Plant Physiology. 1996; 149(6):729-734.
8. Choudhury S, Panda P, Sahoo L, Panda SK. Reactive oxygen species signaling in plants under abiotic stress. Plant Signaling & Behavior. 2013; 8(4):23681.
9. Developing wheat seedlings: role of growth hormones. Indian Journal of Plant Physiology. 1999; 4:236-238.
10. Djanaguiraman M, Annie Sheeba J, Durga Devi D, Bangarusamy U. Cotton leaf senescence can be delayed by nitrophenolate spray through enhanced antioxidant defence system. Journal of agronomy and crop science. 2009; 195(3):213-224.
11. Hasanuzzaman M, Fujita M. Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. Biological Trace Element Research. 2011; 143(3):1758-1776.
12. Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. Plant cellular and molecular responses to high salinity. Annual review of plant biology. 2000; 51(1):463-499.
13. Khedr AHA, Abbas MA, Wahid AAA, Quick WP, Abogadallah M. Proline induces the expression of salt stress responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt-stress. Journal of Experimental Botany. 2003; 54(392):2553-2562.
14. Liu X, Huang B. Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. Crop Science. 2000; 40(2):503-510.
15. Miller G, Shulaev V, Mittler R. Reactive oxygen signaling and abiotic stress. Physiologia Plantarum. 2008; 133(3):481-489.
16. Patterson BD, MacRae EA, Ferguson IB. Estimation of hydrogen peroxide in plant extracts using titanium (IV). Analytical Biochemistry. 1984; 139(2):487-492.
17. Sairam RK, Srivastava GC, Saxena DC. Increased antioxidant activity under elevated temperatures: a mechanism of heat stress tolerance in wheat genotypes. Biologia Plantarum. 2000; 43(2):245-251.
18. Scandalios JG. Oxygen stress and superoxide dismutases. Plant physiology. 1993; 101(1):7.
19. Snider JL, Oosterhuis DM, Kawakami EM. Genotypic differences in thermotolerance are dependent upon pre stress capacity for antioxidant protection of the photosynthetic apparatus in *Gossypium hirsutum*. Physiologia plantarum. 2010; 138(3):268-277.