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Effect of foliar application of salicylic acid and thiourea on wheat grown under drought stress

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Abstract
Wheat is important staple food crop of the world, exposed to drought stress thereby reduces crop yield. Salicylic acid is an important signal molecule involved in the activation of plant defense responses against abiotic and biotic stresses and plays a crucial role for the regulation of physiological and biochemical processes. Thiourea has great implications in changing plant growth both under normal and stressful conditions. Being water-soluble and readily absorbable in living tissues, exogenous application of thiourea at appropriate concentrations is suggested to have multiple roles both under optimal and sub optimal conditions. Based on biochemical studies, foliar spray of 50 µM salicylic acid and 50 µM thiourea was found to be effective in retaining the RLWC of C-306 and NI-5439 amongst 50, 100 and 200µM concentrations of salicylic acid and thiourea. However, for wheat genotype Trimbak 200 µM salicylic acid and 100 µM thiourea was found to be effective. Foliar spray of 50 µM salicylic acid was found to be slightly effective in retaining the chlorophyll content of all the three genotypes and the effect was more prominent in Trimbak. As compared to 50,100 and 200 µM salicylic acid and thiourea concentrations foliar spray of salicylic acid and thiourea increased leaf proline content and the effect was more prominent at 50 µM salicylic acid and 200 µM thiourea. Foliar spray of salicylic acid and thiourea increased lipid peroxidation rate and the effect was more prominent at 200 µM salicylic acid and 100 µM thiourea in all the three genotypes. Antioxidative enzymes such as ascorbate peroxidase, superoxide dismutase and catalase shown significant increase under stress. The spray of 50 µM salicylic acid and thiourea increased the Antioxidative enzyme activity. The present work revealed that as compared to all the treatments to the genotypes under drought stress condition the lower concentration 50 µM salicylic acid and thiourea shown significant results.

Keywords: Wheat, drought stress, relative leaf water content, total chlorophyll, proline, antioxidative enzymes activity

Introduction
Wheat (*Triticum aestivum* L.) is the second most important cereal crop in the world after rice (Tunio *et al*., 2006) [1]. It is a staple food for more than 35% of the world population and also the first grain crops in most of the developing countries. Wheat has been reported to provide 73 % of the calories and protein requirements of the daily diet (Arif *et al*., 2010) [2]. Wheat contains more protein (8-15 %) than other cereals. The characteristic substance 'gluten', which is very essential for backers. Wheat straw is good source of feed for livestock in our country. It plays an important role in food security and poverty alleviation as a strategic crop and has an important role in economy (Khan *et al*., 2011) [3].
Wheat is grown globally in 217 Mha areas with a total production of 632 million tons. The area remained constant at about 220 Mha in the past 3 decades. India rank second in area and production in wheat crop after China. Total area under wheat crop in India was about 30.9 Mha with productivity of 2794 kg/ha. About 95% areas under *Triticum aestivum,* 4 % under *Triticum durum* and 1 % under *Triticum dicoccum.* Wheat is grown in all the states in India except Southern and North Eastern states. Uttar Pradesh, Haryana, Punjab, Rajasthan are the major wheat producing states and accounts for almost 80 % of total production in India. Only 13 % area is rain fed under wheat crop. Major rain fed wheat areas are in Madhya Pradesh, Gujarat, Maharashtra, West Bengal and Karnataka. Punjab ranks first in productivity 4283 kg/ha whereas productivity in Maharashtra is about 1461 kg/ha (Anonymous, 2015) [4].
In India, wheat sowing time varies from October to December with temperature range of 10 to 35 ˚C. Apart from genotypic variation, temperature fluctuation and moisture stress plays a crucial role in wheat growth. The stress level is mainly determined by the degree and duration of stress, developmental stages and time of stress (Gupta *et al.,* 2013) [5]. The abiotic stresses such as temperature, drought and salinity and unusual warming trends during growth stage are

causing yield decline, especially in eastern and central India (Chatrath *et al.,* 2007) [6]. The drought stress alone commonly reduces average yield of wheat crop by more than 50 % (Bayoumi *et al.,* 2008) [7]. Plants improve the physiological and morphological adaptation to abiotic stresses (Gillham and Dodge, 1987) [8]. In this case an antioxidant defense system that includes enzymatic and non-enzymatic components is induced (Schoner and Krause, 1990) [9]. Antioxidant enzymes such as Super oxide dismutase (SOD), Peroxidase (POD) and ascorbate peroxidase (APX) play important roles in scavenging excess Reactive oxygen species (ROS), which are generated by abiotic stress (Jiang and Zhang, 2002 and Hernandez *et al.,* 2012) [10, 11]. The action of antioxidant systems under drought has been investigated by many authors in several crops, such as wheat (Sgherri *et al.,* 2000) [12]. Among the common response in plants to abiotic stresses is the production of different types of organic solutes (Serraj and Sinclair, 2002) [13] which include small molecules such as proline (Szabados and Savoure, 2010) [14]. It has been reported that plant cells achieve their osmotic adjustment by the accumulation of some kind of compatible solutes such as proline and betaine to protect membranes and proteins (Delauney and Verma, 1993) [15]. These compounds accumulate in high amounts mainly in cytoplasm of stressed cells without interfering with macromolecules and behaved as osmoprotectants (Yancey, 1994) [16].

The improvement of drought tolerance has been a principal goal of the majority of breeding programmes for a long time, as water deficit at certain stages of wheat growth is common for many wheat growing regions of the world (Farshadfar, 2012) [17]. Selection efficiency could be improved if particular physiological and morphological attributes related to yield under a stress environment could be identified and employed as selection criteria for complementing traditional plant breeding (Acevedo, 1991) [18].

Salicylic acid (SA, 2-hydroxybenzoic acid) is considered as a hormone like endogenous regulator, which influences a range of diverse processes in plants, including seed germination, ion uptake and transport, membrane permeability, and photosynthesis (Simaei *et al*., 2011) [19]. SA is an important signal molecule involved in the activation of plant defense responses against abiotic and biotic stresses and plays a crucial role for the regulation of physiological and biochemical processes (Saruhan *et al*., 2012) [20]. Exogenous SA could regulate the activities of antioxidant enzymes and increased plant tolerance to abiotic stresses (He *et al*., 2002 and Erasalan *et al*., 2007) [21, 22].

Exogenous application of various synthetic organic compounds can modify plant growth due to their biological properties (Tang *et al*., 2009) [23]. Thiourea has three functional groups; mino, amino and Thiel. By virtue of these groups, TU has great implications in changing plant growth both under normal and stressful conditions (Srivastava *et al*., 2009 and Anjum *et al*., 2011) [24, 25]. Being water-soluble and readily absorbable in living tissues, exogenous application of TU at appropriate concentrations is suggested to have multiple roles both under optimal and sub optimal conditions (Mani *et al*., 2013) [26]. The present investigation was carried out to evaluate wheat varieties for physiological and biochemical traits with the objectives to identify the effective concentration of salicylic acid and thiourea for drought stress tolerance induction in wheat and to estimate osmolytes and Antioxidative enzymes in leaves of wheat varieties with and without foliar application under drought stress.

Materials and Methods

Genotypes and treatments

The clean and sound seeds (50 g) of wheat genotypes *viz.,* NI-5439, C-306 and Trimbak were surface-sterilized with 0.1 percent (w/v) $HgCl_2$ for 2 minutes and washed 4-5 times with distilled water. The seeds were then sown in 24 pots filled with black cotton soil. These 24 pots were divided into eight treatments as T_1: Control (Irrigated up to field capacity), T_2: Drought stress (With holding irrigation), T_3: Drought stress + Foliar spray of 50 µM salicylic acid, T_4: Drought stress + Foliar spray of 100 µM salicylic acid, T_5: Drought stress + Foliar spray of 200 µM salicylic acid T_6: Drought stress + Foliar spray of 50 µM thiourea,T_7: Drought stress + Foliar spray of 100 µM thiourea and T_8: Drought stress + Foliar spray of 200 µM thiourea.

The drought stress treatment was imposed by withholding irrigation at 30 DAS and foliar spray of salicylic acid and thiourea was given after drought stress treatment in the morning at 35 DAS (i.e. at seedling stage) only one time. The uniform stress treatment was ensured with the rolling of leaf under drought stress treatment alone as compared to foliar application of salicylic acid and thiourea.

Leaf material were excised from the plants of wheat varieties five days after spraying of salicylic acid (50, 100, 200 µM) and thiourea (50, 100, 200 µM) and no spray of salicylic acid and thiourea to absolute control and drought stress. The leaf material were used for estimation of the levels of RLWC, total chlorophyll, proline, lipid peroxidation and different Antioxidative enzymes *viz*., superoxide dismutase, acerbate peroxidase and catalase activity.

Relative leaf water content

Relative leaf water content (RLWC) was estimated by the method described by Henderson and Davies-Jr. (1990) [27]. The third leaf from the top of the main stem was detached from 5 randomly selected plants and kept in sealable plastic bag in an ice box. The leaf samples were brought to a laboratory where fresh weight was recorded immediately. The leaf samples were immediately hydrated to full turgidity for 2 hours by floating on de-ionized water in a close Petri dish under room temperature. After 2 hours the samples were taken out of water and were well dried with a filter paper. They were immediately weighed to obtain fully turgid weight (TW). Samples were dried at 80 °C for 36 hours and dry weight (DW) was determined and the RLWC was calculated.

Total chlorophyll

Total leaf chlorophyll content of wheat was determined by the method described by Arnon (1949) [28]. The third leaf from the top of the tillers were cut into small pieces, known weight (0.2 g) of fresh leaf sample was macerated in mortar and pestle and extracted with 10 ml of 80 percent acetone. The contents were centrifuged at 5000 x g for 10 min and the supernatant was collected. The above steps were repeated until the residue became color less. The final volume of extract was made to 50 ml. The absorbance of chlorophyll extract was recorded at 645 and 663 nm on a Spectrophotometer and a blank was run with 80 percent acetone. The amount of total chlorophyll content was calculated by using the following formula and expressed in mg g-1 FW and results are compared on dry weight basis.

Total chlorophyll, mg g^{-1} FW = 22.2 (A_{645}) + 8.02 (A_{663}) x V/ (1000 x W).

Where, A= absorbance at specific wavelength, V= Final volume of chlorophyll extract in 80 % acetone, W= Fresh weight of tissue extracted in g.

Proline

Proline content in leaf tissues of wheat was determined using the acid ninhydrin reagent as per the method described by Bates *et al.* (1973) [29]. The proline content was expressed as μ moles per gram dry weight. Leaf samples (0.2 g) were homogenized with 2 ml of 3 per cent (w/v) aqueous solution of sulpho salicylic acid and the homogenate centrifuged at 10,000 x g for 10 min. A suitable volume (0.1 to 0.5 ml) of the filtrate was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 h at 1000 C. The reaction was terminated by placing the tubes in ice. The reaction mixture was extracted with 4 ml toluene and mixed vigorously for 15 to 20 seconds. The chromosphere containing toluene was aspirated from the aqueous phase and the absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from the standard curve and calculated on a fresh weight basis and expressed as μmoles of proline per gram fresh weight.

Lipid peroxidation rate

The level of lipid peroxidation rate was measured in terms of malondialdehyde (MDA) as thiobarbituric acid reactive substance as described by Heath and Packer (1968) [30]. Leaf sample 0.2 g was homogenized in 2 ml of 0.1 % TCA. The homogenate was centrifuged at 15000 x g for 15 min and the supernatant was used for the estimation of MDA content. The supernatant, 0.8 ml was mixed with 1.5 ml of 0.5 % TBA in 20 % TCA and was kept on boiling water bath for 30 min and then cooled in an ice bath. After cooling, the aliquot was centrifuged at 10,000xg for 10 min. The absorbance of the clear supernatant was recorded at 532 nm. Values of nonspecific absorption recorded at 600 nm were subtracted from the values recorded at 532 nm. The MDA content was calculated by using extinction coefficient (ε) of 155 $mM^{-1}cm^{-1}$.

Antioxidant enzyme activity

Enzymes extraction

Antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) were extracted from leaf tissue by using the method of Costa *et al.* (2002) [31]. Leaf samples (0.2g) of control, drought, drought plus spray of salicylic acid and thiourea weighed and were homogenized with a pestle in a chilled mortar with 2 ml of an ice-cold 0.1 mM potassium phosphate buffer (pH 7.5) containing 1mM EDTA, 1mM PMSF and 5 % (w/v) PVPP. The homogenates were filtered through four layers of cheesecloth and then centrifuged at 4° C for 20 min at 15,000 xg. The supernatant was used as crude extract for enzyme activity assays.

Superoxide dismutase

Superoxide dismutase activity was determined by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium using the method described by Dhindsa *et al.* (1981) [32]. Three ml enzyme reaction mixture contained: 50 mM phosphate buffer, pH 7.8 (1.5 ml of 100 mM), 13.33 mM methionine (0.2 ml of 200 mM), 75 μM NBT (0.1 ml of 2.25 mM), 0.1mM EDTA (0.1 ml of 3 mM), 50 mM sodium carbonate (0.1 ml of 1.5 M), 100 μl enzyme extract, 0.8 ml of distilled water and 2 μM riboflavin (0.1 ml of 60 μM). The reaction was started by adding 2 μM riboflavin and placing the tubes under two 15 W fluorescent bulbs for 15 min. A complete reaction mixture without enzyme extract, which gave the maximal colour, served as irradiated control. After 15 min, the reaction was terminated by switching off light and covering the tubes with black cloth. A complete reaction mixture without enzyme extract kept in dark served as non-irradiated blank. The absorbance of the reaction mixture was red at 560 nm. One unit of SOD was defined as the amount of enzyme required to cause 50 per cent inhibition of NBT reduction per min at 560 nm. The enzyme activity was expressed in unit per mg protein.

Ascorbate peroxidase

Ascorbate peroxidase activity was measured immediately in fresh extract which was assayed as per the method described by Nakano and Asada (1981) [33]. Three milliliter of enzyme reaction mixture contained: 50 mM potassium phosphate buffer (pH 7.0) (1.5 ml of 100 mM), 0.5 mM ascorbic acid (0.5 ml of 3 mM), 0.1 mM EDTA (0.1 ml of 3 mM), 100 μl enzyme extract, 0.7 ml of distilled water and 0.1 mM hydrogen peroxide (0.1 ml of 3 mM). The reaction was initiated by the addition of 0.1 ml of 3 mM H_2O_2. The hydrogen peroxide dependent oxidation of ascorbic acid was followed by a decrease in the absorbance measured at 290 nm for three min at the interval of 30 sec. The amount of ascorbate oxidized was determined from molar extinction coefficient (ε 2.8 mM^{-1} cm^{-1}). The enzyme activity was expressed as nmoles of ascorbate oxidized mg^{-1}protein min^{-1}.

Catalase

Catalase activity was assayed as described by Aebi (1984) [34]. Three ml enzyme reaction mixture contained: 50 mM potassium phosphate buffer (pH 7.0) (1.5 ml of 100 mM), 100 μl enzyme extract, 0.9 ml of distilled water and 12.5 mM hydrogen peroxide (0.5 ml of 75 mM). The reaction was initiated with addition of 0.5 ml of 75 mM H_2O_2. For measurement of catalase enzyme activity, the decline in absorbance was recorded at 240 nm for three min at an interval of 30 sec. The amount of hydrogen peroxide decomposed was determined from molar extinction coefficient (ε 36 M-1 cm-1). The enzyme activity was expressed as μmoles of H_2O_2 decomposed mg^{-1} protein min^{-1}.

Statistical analysis

Factorial completely randomized design (FCRD) was used to analyses the data statistically.

Results and Discussion

Relative leaf water content

Foliar application of salicylic acid (SA) and thiourea (TU) and drought stress had a significant impact on flag leaf relative content (Table 1). Wheat genotype C-306 recorded minimum reduction in relative leaf water content (9.00 %) under stress followed by NI-5439 (15.73 %) and the maximum reduction in RWLC (21.24 %) was recorded in Trimbak. Foliar spray of 50 μM salicylic acid 50 μM thiourea were found to be effective in retaining the RLWC of C-306 and NI-5439. However for Trimbak 200 μM salicylic acid and

100 µM thiourea was effective concentration.

Relative leaf water content is useful variable to evaluate the physiological water status of plants (Kadioglu *et al.,* 2011) [35]. RLWC of wheat cultivars expressed to water stress improved when these genotypes were subjected to foliar spray of SA and TU. This enhancement implied that SA and TU might support plants to maintain their RLWC in spite of greater water loss. Such increased RLWC in response to foliar SA in drought as previously been reported on wheat genotypes (Mohammad and Ismail, 2009) [36]. The results of present investigation are in agreement with earlier researchers. In general, water stress considerably reduced RLWC. A high amount of RLWC in leaves was maintained in some of the cowpea genotypes as a result of stomata closure and a reduction in leaf area (Anyia and Herzog, 2004) [37]. RLWC tends to decline when transpiration exceeds water absorption under drought condition (Tas and Tas, 2007) [38] leading to decrease in cell turgor. Increase in RLWC of strawberry plants treated with salicylic acid was also reported for other crops grown under salt stress including barley (El-Tayeb, 2005) [39] his phenomenon may be attributed to the fact that foliar SA application can increase the leaf diffusive resistance and lower Transpiration rates.

Total chlorophyll

Total chlorophyll content of leaves of wheat genotypes is depicted in Table 1. Wheat genotype C-306 recorded minimum reduction in chlorophyll content (2.23 %) followed by NI-5439 (18.01 %) whereas wheat genotype Trimbak recorded maximum reduction (32.06 %) under drought stress. Foliar application of 50 µM salicylic acid was found to be slightly effective in retaining the chlorophyll content of the all three genotypes and the effect was more prominent in Trimbak. Thiourea was not found to be much effective.

Singh and Usha (2003) [40] reported that the foliar application of SA increased chlorophyll content and stomata conductance possibly causing higher fresh weight. Exogenous spraying of SA successfully ameliorated leaf chlorophyll and carotenoid contents of plants grown in salt and water stresses. Very severe drought conditions result in limited photosynthesis due to a decline in Rubisco activity and reduced gas exchange (Bota *et al*., 2004) [41]. Similarly, Idrees *et al*. (2010) [42] reported that SA protected photosynthesis and enhanced Rubisco activity in water stress treated wheat. Sinha *et al*. (1993) [43] reported that chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA by lead stress. The application of SA (20 mg/ml) to the foliage of the plants of *Brassica napus*, improved the chlorophyll contents (Ghai *et al*., 2002) [44].

Proline

The leaf proline content increased significantly under stress (Table 2). The increase in proline content was 51.53 % in NI-5439 and 46.62 % in C-306 however the increase in leaf proline content was only 13.71 % in Trimbak. Foliar application of salicylic acid and thiourea increased leaf proline content and the effect was more prominent at 50 µM salicylic acid and 200 µM thiourea.

Exogenous SA-induced increase in proline level is also observed in other species of plants under abiotic stress (Yusuf *et al*., 2008) [45]. Misra and Dwivedi (2004) [46] reported that foliar spray of 300 µM SA resulted in higher accumulation of proline. In both water stress conditions, proline concentration was highly correlated with leaf temperature. A direct consequence of higher osmolyte concentration is the maintenance of comparatively higher RLWC.

Lipid peroxidation rate

The lipid peroxidation rate increased significantly under drought stress (Table 3). The increase lipid peroxidation rate was 16.02 % in Trimbak followed by 12.33 % in C-306 and 10.88 % in NI-5439. Foliar application of salicylic acid and thiourea increased lipid peroxidation rate and the effect was more prominent at 200 µM salicylic acid and 100 µM thiourea.

It was reported that exogenous application of SA and TU had 22 % less lipid peroxidation in wheat leaves as compared to 5 DS/m salinity alone, which is in agreement with its role in quenching ROS and protect the cells from lipid peroxidation (Mahatma *et al*., 2009) [47]. Asthir *et al*. (2013) [48] reported that thiourea application on wheat ameliorated the heat induced damages by stimulating the total antioxidant activity through decrease in lipid peroxidation and membrane injury.

Superoxide dismutase

The superoxide dismutase activity in leaves of wheat genotypes is depicted in Table 3. The superoxide dismutase activity increased under stress. The increase in SOD activity was 27.87 % in Trimbak wheat genotype followed by 13.75 % in NI-5439 and 9.94% in C-306. Foliar application of 50 µM salicylic acid and thiourea increased the SOD activity and the effect was more prominent at 50 µM salicylic acid and thiourea in all three wheat genotypes.

It is observed that SOD activity was enhanced under drought stress. Although SOD functions as the first line of defense against ROS, its end product is the toxic H_2O_2. Therefore, an efficient H_2O_2 scavenging system is required to enable rapid removal of H_2O_2 in the plant cells. Salicylic acid induced SOD activity coupled with reduction in lipid peroxidation in wheat plant was observed under drought stress (Wang *et al*., 1999) [49]. Thiourea pretreatment increased the Antioxidative enzyme activities during water stress in wheat cultivars (Nathawat *et al*., 2007) [50]. In our study, the data showed that Antioxidative enzyme activity in leaves of wheat genotypes were substantially induced by foliar application of SA and TA. The exogenous salicylic acid application significantly increased the Antioxidative enzymes activities (Saruhan *et al*., 2012) [20] which in turn prevent the plants from ROS mediated membrane damage under drought stress (Shakirova *et al*., 2003) [51].

Ascorbate peroxidase

The foliar application of SA and TU under drought stress in wheat genotypes were significantly induced APX activity (Table 4). The increase in APX activity was 45.73 % in NI-5439 wheat genotype followed by 38.36 % in Trimbak and 33.32 % in C-306. Foliar application of 50 µM salicylic acid and thiourea increased the APX activity and the effect was more prominent at 50 µM salicylic acid and thiourea in all three wheat genotypes.

APX and SOD have an influential role in plant defense against ROS (Mittler, 2002) [52]. Salicylic acid is necessary for the induction of antioxidant defenses, it has been shown to be essential for the plant protection against oxidative stress (Borsani *et al*., 2001) [53]. It was shown that due to thiourea pretreatment an increase in the activities of antioxidant

enzymes was observed during water stress in wheat (Nathawat *et al*., 2007) [50]. Singh and Usha (2003) [40] reported that the promotive effect of salicylic acid on linen plant under drought stress may be related to the induction of Antioxidative responses that protect plant from oxidative damage. In our study, the foliar application of SA and TU induced the higher activity of APX as well as SOD and CAT that limits the level of lipid peroxidation.

Catalase

The catalase activity increased significantly under drought plus SA and TU (Table 4). The increase in catalase activity was 31.84 % in C-306 wheat genotype followed by 24.87 % in NI-5439 and 12.27 % in Trimbak. Foliar application of 50 µM salicylic acid and thiourea increased the catalase activity and the effect was more prominent at 50 µM salicylic acid and thiourea in all three wheat genotypes.

It appears that SA and TU induce redox signal (H_2O_2 as a secondary messenger) and leading to increase in antioxidant activity is linked to inhibition of plasma membrane linked NADPH oxidase (Horvath *et al*., 2007) [54]. Improved performance of maize (Khodary, 2004) [55] and barley (El-Tayeb, 2005) [39] has been reported treated with salicylic acid under salt stress conditions due to higher activity of CAT, SOD and APX. Enhancement of salicylic acid-induced SOD and CAT activities coupled with reduction in lipid peroxidation and APX in flag leaves of wheat plants indicate that H_2O_2 produced by SOD is scavenged by CAT (Wang *et al*., 1999) [49]. Our results are in agreement with other findings indicating a similar increasing trend in CAT activity in wheat genotypes under drought plus foliar application of SA and TU.

Conclusion

Based on biochemical studies as compared to 50, 100 and 200 µM Salicylic acid and thiourea concentrations. Foliar spray of 50 µM salicylic acid and thiourea was found to be effective in retaining the RLWC of C-306 and NI-5439. However for Trimbak 200µM salicylic acid and 100 µM thiourea was to be effective. Foliar spray of 50 µM salicylic acid was found to be slightly effective in retaining the chlorophyll content of the all three genotypes and the effect was more prominent in Trimbak. Antioxidative enzymes such as ascorbate peroxidase, superoxide dismutase and catalase shown significant increase under stress. The spray of 50 µM salicylic acid and thiourea increased the Antioxidative enzyme activity. The drought susceptible genotype Trimbak exhibited comparatively higher reduction in RLWC (60.59 %) and chlorophyll (1.44 mg. g^{-1} FW) under drought stress. Foliar spray with 50µM salicylic acid showed better drought recovery as evident from analysis of biochemical parameters.

Table 1: Effects of spray of salicylic acid and thiourea on relative leaf water content and total chlorophyll in leaves of wheat seedlings under drought stress

S. No.	Relative leaf water content (%)				Total chlorophyll (mg g^{-1} FW)		
	Treatments	**NI-5439**	**C-306**	**Trimbak**	**NI-5439**	**C-306**	**Trimbak**
1	T_1:Irrigated	80.98	74.03	73.19	1.61	1.34	1.84
2	T_2:Drought	68.24(15.73)	67.36(9.00)	57.64(21.24)	1.32(18.01)	1.31(2.23)	1.25(32.06)
3	T_3:50µM SA	75.23(7.10)	69.76(5.76)	60.59(17.29)	1.38(14.29)	1.33(0.75)	1.44(21.74)
4	T_4:100 µM SA	71.76(11.38)	70.65(4.56)	63.08(13.81)	1.23(23.60)	1.31(2.24)	1.23(33.15)
5	T_5:200 µM SA	60.28(25.56)	69.75(5.78)	70.26(4.00)	1.35(16.15)	1.33(0.75)	1.42(22.83)
6	T_6:50 µM TU	73.32(9.45)	69.68(5.87)	58.62(19.90)	1.33(17.39)	1.34(0.00)	1.37(25.54)
7	T_7:100 µM TU	60.70(25.04)	63.40(14.35)	64.33(12.10)	1.32(18.01)	1.30(2.99)	1.28(30.43)
8	T_8:200 µM TU	60.35(25.47)	60.63(18.10)	62.16(15.07)	1.25(22.36)	1.19(11.19)	1.30(29.35)
	Mean	68.86	67.41	64.98	1.35	1.29	1.39
	Comparison	SE ±	CD 5%		SE ±	CD 5%	
	V	0.560	1.593		0.007	0.020	
	T	0.305	0.867		0.003	0.010	
	V×T	1.585	4.506		0.020	0.056	

Figures in parenthesis are percent decrease over the irrigated and increase over drought

Table 2: Effects of spray of salicylic acid and thiourea on leaf proline accumulation in wheat seedlings under drought stress

Sr. No.	Proline (µmoles g-1 DW)			
	Treatments	**NI-5439**	**C-306**	**Trimbak**
1	T_1:Irrigated	12.44	17.49	23.65
2	T_2:Drought	25.67 (51.53)	32.77 (46.62)	27.41 (13.71)
3	T_3:50µM SA	29.56 (13.16)	37.65 (14.89)	29.47 (6.99)
4	T_4:100 µM SA	27.28 (5.90)	35.20 (7.42)	28.58 (4.09)
5	T_5:200 µM SA	26.23 (2.13)	34.28 (4.61)	28.00 (2.11)
6	T_6:50 µM TU	26.00 (1.27)	34.66 (5.77)	27.52 (0.40)
7	T_7:100 µM TU	26.22 (2.10)	33.64 (2.65)	28.40 (3.49)
8	T_8:200 µM TU	28.37 (9.52)	36.24 (10.59)	28.00 (2.11)
	Mean	22.17	25.03	22.97
	Comparison	SE ±	CD 5%	
	V	0.484	1.375	
	T	0.263	0.748	
	V×T	1.369	3.891	

Figures in parenthesis are percent decrease over the irrigated and increase over drought

Table 3: Effects of spray of salicylic acid and thiourea on lipid peroxidation and superoxide dismutase in leaves of wheat seedlings under drought stress

Sr. No.	Lipid peroxidation (nmol MDA g^{-1} FW)				Superoxide dismutase (Units mg^{-1} protein)		
	Treatments	**NI-5439**	**C-306**	**Trimbak**	**NI-5439**	**C-306**	**Trimbak**
1	T_1:Irrigated	212.56	203.62	227.24	6.21	6.82	6.96
2	T_2:Drought	238.53 (10.88)	232.26(12.33)	270.60(16.02)	7.20(13.75)	7.57(9.94)	9.65(27.87)
3	T_3:50µM SA	231.07(8.01)	226.30(10.02)	228.78(0.67)	13.35(46.07)	17.76(57.38)	10.52(8.27)
4	T_4:100 µM SA	232.19(8.45)	225.43(9.67)	229.00(1.01)	11.23(35.89)	10.09(24.98)	9.74(0.92)
5	T_5:200 µM SA	225.58(5.77)	227.16(10.36)	227.65(0.18)	8.60(16.28)	8.32(9.01)	9.66(0.10)
6	T_6:50 µM TU	220.38(3.55)	211.31(3.64)	215.64(0.77)	13.04(44.79)	15.23(50.30)	9.70(0.52)
7	T_7:100 µM TU	219.92(3.35)	225.68(9.77)	245.22(7.33)	10.47(31.23)	9.98(24.15)	9.72(0.72)
8	T_8:200 µM TU	219.44(3.14)	224.96(9.49)	233.84(2.82)	11.76(38.78)	9.97(24.07)	9.75(1.03)
	Mean	226.46	226.09	236.44	10.23	10.72	9.29
	comparison	SE ±	CD 5%		SE ±	CD 5%	
	V	0.665	1.892		0.012	0.036	
	T	0.362	1.029		0.007	0.020	
	V×T	1.883	5.351		0.036	0.104	

Figures in parenthesis are percent decrease over the irrigated and increase over drought

Table 4: Effects of spray of salicylic acid and thiourea on ascorbate peroxidase and catalwase in leaves of wheat seedlings under drought stress

Sr. No	Ascorbate peroxidase (nmoles min^{-1} mg^{-1} protein)				Catalase (µmoles of H_2O_2 decomposed min^{-1} mg^{-1} protein)		
	Treatments	**NI-5439**	**C-306**	**Trimbak**	**NI-5439**	**C-306**	**Trimbak**
1	T_1:Irrigated	69.65	75.34	77.91	28.42	25.36	29.31
2	T_2:Drought	128.35(45.73)	113.00(33.32)	126.41(38.36)	37.83(24.87)	37.21(31.84)	33.41(12.27)
3	T_3:50µM SA	151.00(15.00)	172.12(34.35)	132.70(4.74)	45.55(16.95)	40.42(7.94)	41.34(19.18)
4	T_4:100 µM SA	138.62(7.41)	121.11(6.70)	129.86(2.68)	42.65(11.30)	38.61(3.63)	38.39(12.97)
5	T_5:200 µM SA	131.59(2.46)	119.2(5.20)	129.26(2.20)	39.42(4.03)	37.88(1.77)	37.51(7.19)
6	T_6:50 µM TU	145.09(11.54)	141.09(19.91)	127.00(0.46)	42.76(11.45)	38.20(2.59)	36.75(9.08)
7	T_7:100 µM TU	129.57(0.89)	121.3(6.84)	127.89(1.16)	40.00(5.43)	39.57(5.96)	31.64(10.93)
8	T_8:200 µM TU	128.57(0.17)	115.47(2.14)	127.71(1.02)	39.02(3.05)	40.26(6.98)	35.40(5.62)
	Mean	120.42	111.96	106.50	32.09	25.31	30.29
	Comparison	SE ±	CD 5%		SE ±	CD 5%	
	V	2.596	7.380		0.626	1.780	
	T	1.413	4.017		0.340	0.969	
	V×T	7.345	20.874		1.771	5.035	

Figures in parenthesis are percent decrease over the irrigated and increase over drought

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