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# MODULATION IN ACTIVITIES OF ANTIOXIDANT ENZYMES IN SALT STRESSED AND NON-STRESSED WHEAT (*TRITICUM AESTIVUM* L.) PLANTS RAISED FROM SEED TREARTED WITH TRIACONTANOL

**SHAGUFTA PERVEEN[1], MUHAMMAD SHAHBAZ[1*], MUHAMMAD ASHRAF[1, 2]**

[1]*Department of Botany, University of Agriculture, Faisalabad, Pakistan*
[2]*Department of Botany & Microbiology, King Saud University Riyadh, Riyadh, Saudi Arabia*
**Corresponding author's email: shahbazmuaf@yahoo.com*

**Abstract**

Before sowing, the seeds of two wheat cultivars, S-24 and MH-97, were treated with three levels [(0 (water), 10 and 20 µ*M*)] of triacontanol (TRIA) for 12 h. TRIA-treated seeds were grown in full strength nutrient solution for 24 days in a greenhouse, after which time, they were supplied with two salt treatments (0 and 150 m*M* NaCl ). After 21 days of salt application, changes in the malondialdehyde (MDA), hydrogen peroxide ($H_2O_2$) and total soluble proteins contents as well as activities of some key antioxidant enzymes (CAT, POD and SOD) were measured. Salinity stress of 150 m*M* NaCl significantly decreased the activity of SOD, while increased that of CAT, and enhanced the levels of MDA and $H_2O_2$ contents in both cultivars under salt stress conditions. The outcome of salt stress was non-significant on soluble proteins and activity of POD. The effect of pre-sowing application of TRIA was non-significant on all measured attributes except that it significantly increased the activity of POD under non-saline conditions. The cultivar difference with respect to the different attributes measured in the present investigation was non-significant.

## Introduction

Triacontanol (TRIA) is an effective plant growth regulator, which has been reported to enhance growth and yield of several crop species when applied exogenously (Ries *et al*., 1977; Gatica *et al*., 2008; Naeem *et al*., 2011). Moreover, TRIA has been reported to influence enzymes which regulate metabolic (Ries & Houtz, 1983; Morre *et al*., 1991) and growth processes in plants (Ries *et al*., 1990; Chen *et al*., 2002). Several membrane bound enzyme systems have been reported to be stimulated by a direct action of TRIA (Savithiry *et al*., 1992). TRIA is also known as an antioxidizing agent because it can effectively inhibit both enzymatic and non-enzymatic peroxidative breakdown of lipids (Ramanarayan *et al*., 2000). Some reports also show that it can stimulate enzyme activities, and improve photosynthesis, membrane stability and productivity of many crop species (Naeem *et al*., 2009). It is also known that TRIA application is effective in enhancing growth and yield of various crops like rice, wheat, maize, tomato, green gram, cotton and hyacinth bean (Mamat *et al*., 1983; Ries, 1991; Shripathi & Swamy, 1994; Khan *et al*., 2006; Naeem *et al*., 2009), while a synergistic stimulation in the growth of wheat, barley and rye was reported by Welebir (1982). It is also reported that TRIA after initial application, elicits a metabolite or a secondary messenger, which rapidly induces a variety of physiological responses, and influences the activities of enzymes involved in carbohydrate metabolism (dry weight increase) (Ries & Wert, 1988; Ries, 1991; Khan *et al*., 2007; Naeem *et al*., 2009).

The salt-induced disturbance in ionic homeostasis causes a cascade of secondary effects such as oxidative stress due to reactive oxygen species (ROS) production (Ashraf, 2009; Joseph & Jini, 2011). Plants in order to escape from the damaging effects of ROS have developed an antioxidative defense system entailing some key enzymes like peroxidase, catalase and superoxide dismutase (antioxidant enzymes) (Ashraf, 2009; Azooz *et al*., 2009). However, an active antioxidative defense system comprising enzymatic and non-enzymatic antioxidants reduces the level of oxidative stress in plant cells by scavenging free radicals (Azooz *et al*., 2009; Abogadallah *et al*., 2010). At the cellular level, salt-induced oxidative damage can be determined by malondialdehyde, a lipid peroxidation product, which is accumulated in plants under saline stress (Hernández & Almansa, 2002; Aghaleh *et al*., 2009).

Of various strategies being employed these days to mitigate the adverse effects of salinity stress, pre-sowing seed treatment with some plant growth regulators has gained much importance for being one of the most economical approaches of growing crops on salt affected soils (Iqbal *et al*., 2006; Ashraf *et al*., 2008; Kamran *et al*., 2009). For example, exogenous application of TRIA has been reported to enhance stress tolerance in common duckweed (Kilic *et al*., 2010), and *Ocimum basilicum* L. (Borowski & Blamowski, 2009), and caused significant positive changes in salt stressed *Erythrina variegata* seedlings (Muthuchelian *et al*., 1996). Similarly, along with some other growth regulators, pre-treated seed with triacontanol ameliorated the adverse effects of salt (NaCl) on barley and radish seedlings (Cavusoglu *et al*., 2008).

No information can be deciphered from the literature on the effects of triacontanol as a pre-seed treatment on wheat plants under salt stress. So the primary objective of the present study was to assess the ameliorating effects of TRIA as a seed treatment on membrane lipid peroxidation (malondialdehyde), hydrogen peroxide, total soluble proteins and activities of antioxidant enzymes of wheat plants grown under NaCl-induced saline stress.

## Materials and Methods

An experiment was conducted in the wire-house of the Botanical Garden, to examine the plausible role of TRIA applied as a pre-sowing seed treatment on wheat under salt stress during spring, 2010. The climatic conditions were: mean day and night temperature cycle of 20 °C and 6 °C, 10 and 14 h light and dark period (photoperiod with PPFD 825-1150 µmol $m^{-2}$ $s^{-1}$), and RH 54 ± 5%. Seed of two spring wheat cultivars namely S-24 and MH-97 were supplied by the Botany Department, UAF-Pakistan and AARI, Faisalabad-Pakistan, respectively. Before the start of the experiment, surface sterilization of the seed of both cultivars was done using sodium hypochlorite solution (5%) for 5 min, rinsed with

sterilized water and air-dried. Then seeds (one hundred of each cultivar) were soaked in varying concentrations of triacontanol solutions (0, 10, and 20 μ*M*) prepared in 0.1% tween-20. After 12 h of soaking, the seeds were re-dried to original weight with forced air under shade. Seeds (10 seeds per pot) were allowed to germinate in thoroughly washed river sand. After 10 days of seed germination, six plants were maintained by thinning in each pot/replicate. Twenty four day-old plants were treated with saline stress for further 21 days. There were two salt (NaCl) levels, i.e., 0 m*M* (control) and 100 m*M*. Every week Hoagland's nutrient solution (full strength) was applied @ two litters per pot. For attaining the desired level of salt, NaCl in Hoagland's nutrient solution was added in an aliquot of 50 m*M* solution to each pot every day. Salt level (150 m*M* NaCl) was applied in Hoagland's nutrient medium after every week till the end of the experiment. The sand moisture content was maintained every day by watering 200 ml of $H_2O$ to each pot. The design of the experiment was completely randomized with four replicates. From each pot, fresh leaves were collected and data for the follwoing biochemical attributes recorded.

**Malondialdehyde (MDA) estimation:** The malonyldialdehyde (MDA) contents in leaf tissues were determined by the method of Carmak & Horst (1991). Fresh leaves (0.5 g) were finely extracted in 10 ml of 0.1% (w/v) trichloroacetic acid (TCA) solution and put them for centrifugation at 12000 x *g* for duration of 10 min. To one ml of the extract added 4.5 ml of 0.5 % thiobarbituric acid. The reaction mixture was then heated in a water bath at 95°C for 30 min. Stopped the reaction by cooling the samples on ice water bath and again centrifuged. The absorbance was read at two wavelengths of 532 and 600 nm on a spectrophotometer (IRMECO U2020).

**Hydrogen peroxide:** Hydrogen peroxide in the plant samples was determined by the method of Velikova *et al*. (2000). Fresh leaf tissue (0.5 g) was finely ground with trichloroacetic acid (TCA) [5 ml of 0.1 % (w/v)], centrifuged at 12,000 x *g* for 15 min. To the supernatant (0.5 ml), added 0.5 ml phosphate buffer (pH 7.0) and potassium iodide (1 ml). Its absorbance was recorded at 390 nm after overtaxing using a UV visible spectrophotometer (IRMECO U-2020).

**Total soluble proteins:** These were estimated using the method described by Bradford (1976).

**Antioxidant enzymes activities:** Fresh leaves (0.5 g) were finely ground under chilled conditions in 10 ml of phosphate buffer (50 m*M* with pH 7.8) for the extraction of antioxidant enzymes. Centrifugation of the mixture was performed at 12000 × *g* for 20 min at 4ºC. The supernatant was recentrifuged at 15000 × *g* for 10 min and then the resultant extract stored at -20°C for determining the activity of antioxidant enzymes.

**Superoxide dismutase (SOD):** The activity of SOD was determined following the Giannopolitis & Ries (1977) method by determining the enzyme inhibition of photochemical reduction of nitroblue tetrazolium (NBT). Then absorbance was read at 560 nm with a UV-visible spectrophotometer (Hitachi U-2100, Tokyo, Japan).

**Catalase (CAT) and peroxidase (POD):** Both CAT and POD were assayed according to the procedure described by Chance & Maehly (1955). The activity of all antioxidant enzymes was determined on protein basis.

**Statistical analysis of data:** The data for all variables were analyzed by a COSTAT computer package (Cohort Software, Berkeley, CA) and means were compared by LSD (Snedecor & Cochran, 1980).

## Results

Leaf malondialdehyde (MDA) contents significantly increased in both wheat cultivars under saline conditions (150 m*M* NaCl). The two cultivars did not differ significantly in MDA contents under saline conditions while, under non-saline conditions MDA contents were higher in S-24 than those in MH-97. The TRIA application as seed treatment did not significantly alter the MDA contents in both cultivars under non-saline or saline conditions (Table 1; Fig. 1).

Hydrogen peroxide ($H_2O_2$) significantly increased in the leaves of both wheat cultivars under saline conditions (Table 1; Fig. 1). Cultivar MH-97 was higher in $H_2O_2$ accumulation as compared to S-24. Exogenously, applied TRIA application was found to be non-effective under both saline and non-saline conditions (Table 1; Fig. 1).

Root-medium applied NaCl salinity did not influence soluble protein contents significantly, while the total free amino acids slightly increased under saline conditions in both wheat cultivars i.e. S-24 and MH-97 (Table 1; Fig. 1). The pre-sowing TRIA application did not alter the total soluble proteins or total free amino acids in both wheat cultivars under non-saline or saline conditions. The two cultivars did not differ significantly under both saline and non-saline conditions with respect to these biochemical attributes (Table 1; Fig. 1).

The activity of antioxidant enzyme, superoxide dismutase (SOD), decreased significantly in both wheat cultivars due to salinity stress (Table 1; Fig. 1). The cultivars did not differ significantly in SOD activity under saline or non-saline conditions. Exogenous TRIA application as a pre-sowing seed treatment did not alter the SOD activity significantly both under saline or non-saline conditions (Table 1; Fig 1). The activity of peroxidase (POD) remained unchanged in both wheat cultivars under saline regimes and the two cultivars showed similar response in this attribute under saline or non-saline conditions (Table 1; Fig. 1). On the other hand, the pre-sowing TRIA application increased the POD activity in both wheat cultivars under non-saline conditions. The TRIA level, 20 μ*M*, proved to be more effective in enhancing the activity of POD under non-stress conditions than the other levels tested in the present study (Table 1; Fig 1). Catalase (CAT) activity increased significantly in both wheat cultivars under saline conditions. The two cultivars did not show any difference in their response to salt stress, however, the CAT activity of S-24 was higher as compared to that of MH-97 under saline conditions (Table 1; Fig. 1).

**Table 1. Leaf malondialdihyde (MDA), hydrogen peroxide ($H_2O_2$) and total soluble proteins contents and activities of antioxidant enzymes in salt-stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol for 12 h.**

| Source of variation | df | MDA | $H_2O_2$ | Proteins | SOD |
|---|---|---|---|---|---|
| Cultivars (Cvs) | 1 | 3.456ns | 437.9* | 0.427ns | 0.010ns |
| Salinity (S) | 1 | 136.6** | 633.9** | 6.600ns | 20.90** |
| Triacontanol (TRIA) | 2 | 2.906ns | 78.41ns | 5.105ns | 4.472ns |
| Cvs x S | 1 | 39.73** | 9.514ns | 0.049ns | 0.150ns |
| Cvs x TRIA | 2 | 0.544ns | 52.408ns | 4.453ns | 1.287ns |
| S x TRIA | 2 | 3.666ns | 96.24ns | 0.117ns | 2.471ns |
| Cvs x S x TRIA | 2 | 8.104ns | 7.1429ns | 0.522ns | 0.187ns |
| Error | 24 | 4.478 | 58.60 | 1.922 | 1.600 |
| **Source of variation** | **df** | **POD** | **CAT** | | |
| Cultivars (Cvs) | 1 | 0.338ns | 6.673ns | | |
| Salinity (S) | 1 | 0.156ns | 372.5* | | |
| Triacontanol (TRIA) | 2 | 2.203* | 24.67ns | | |
| Cvs x S | 1 | 0.715ns | 80.97ns | | |
| Cvs x TRIA | 2 | 0.085ns | 32.56ns | | |
| S x TRIA | 2 | 2.507* | 41.98ns | | |
| Cvs x S x TRIA | 2 | 0.064ns | 19.64ns | | |
| Error | 24 | 0.605 | 52.72 | | |

*, **, and *** = significant at 0.05, 0.01, and 0.001levels, respectively; ns = non-significant; df = degree of freedom; MDA= malondialdehyde; $H_2O_2$ = hydrogen peroxide; SOD = superoxide dismutase; POD = peroxide dismutase, and CAT = catalase.

## Discussion

Since plant hormones are involved in regulating various physiological and biochemical processes, the investigation of the role of new plant growth regulators in crop abiotic stress tolerance is being much focused these days (Peleg & Blumwald, 2011). Of various priming agents used for seed priming, plant growth regulators have gained much importance due to their consistent effects on germination and growth of various plant species (Iqbal & Ashraf, 2007; Pérez-García, 2009). However, various priming agents have variably under a variety of abiotic stresses and in different crop species (Iqbal & Ashraf, 2010). Triacontanol is a plant hormone that naturally occurs in plant epicuticular waxes and acts as a growth promoter (Ries, 1991; Naeem *et al*., 2011). Several membrane bound enzyme systems have been reported to be stimulated by a direct action of TRIA (Lesniak *et al*., 1989; Morre *et al*., 1991; Savithiry *et al*., 1992). Furthermore, TRIA after initial application elicits a metabolite or a secondary messenger (9-β-L(+)-adenosine), which rapidly induces a variety of physiological responses.

Triacontanol has been shown as a powerful antioxidizing agent in recent research as it produces antioxidant compounds (Grzegorezyk *et al*., 2006; Khan *et al*., 2009). It possesses anti-inflammatory properties that might be mediated through its capability to inhibit lipid peroxidation (Ramanarayan *et al*., 2000; Swamy *et al*., 2009). However, pre-sowing TRIA treatment did not significantly affect the MDA and $H_2O_2$ contents in the two wheat cultivars examined in the present study (Table 1; Fig. 1).

In our study, total protein contents remained unaffected in both wheat cultivars under saline conditions. The presowing application of TRIA did not alter the total proteins significantly in both wheat cultivars under control or saline conditions (Table 1; Fig. 1). Similarly, in another study (Perveen *et al*., 2010) TRIA has been reported ineffective to enhance growth when used as a seed priming agent on wheat plants. Contrarily, Krishnan & Kumari (2008) reported increased total protein contents in the *n*-triacontanol treated plants under saline (20 m*M* NaCl) stress. Furthermore, application of TRIA significantly enhanced the accumulation of soluble proteins in green gram under normal growth conditions (Kumaravelu *et al*., 2000).

It has been frequently reported that one of the major causes of oxidative damage to plant tissues is salt stress (Jalali-e-Emam *et al*., 2011). However, plants can escape the damaging effects of reactive oxygen species (ROS) by developing a strong defense system including antioxidant enzymes like CAT, POD and SOD (Joseph & Jini, 2011). In our work, salinity stress decreased the activity of SOD, increased that of CAT, while the POD activity remained unaffected (Table 1; Fig. 1). The activities of antioxidant enzymes vary not only under different salt levels and different plant species, but also in different genotypes of a same crop species. For example, SOD activity decreased with increase in salt level from 100 to 150 m*M* in the shoots of both salt sensitive and salt tolerant genotypes of canola (*Brassica napus* L.). However, this decrease was more in the sensitive cultivar as compared to that in the tolerant one (Jalali-e-Emam *et al*., 2011). An increase in the SOD activity has been reported in two wheat cultivars (Banysoif 1 and Sakha 68), while a decrease in the activity of the enzyme was found in another cultivar (Seds 1) of wheat. Such a differential expression of SOD was ascribed to different expression of SOD isozymes under control or saline conditions (Abdel Latef, 2010). Kant & Turan (2011) reported a decrease in CAT activity with increasing level of salt stress in bean (*Phaseolus vulgaris* L.). On the other hand, Abdel Latef (2010) reported an increase in the CAT activity in two wheat cultivars, Banysoif 1 and Sakha 68, while a decrease in the activity of the enzyme was found in cv. Seds 1 under salt stress. In our findings, exogenous TRIA application as pre-sowing seed treatment significantly increased the POD activity in both wheat cultivars, MH-97 and S-24, under control conditions. On the other hand, under saline conditions, the activity of POD decreased significantly in both cultivars with the increasing level of TRIA. Henry & Gordon (1980) also reported an increase in POD activity in pea

varieties (Little Marvel' and 'Alaska') under normal growth conditions. The same authors also reported that TRIA effect might be a cultivar-specific on growth attributes and peroxidase activity. Borowski & Blamowski (2009) reported the ameliorating effect of TRIA on reducing the chilling stress in *Ocimum basilicum* L. plants by increased activity of catalase.

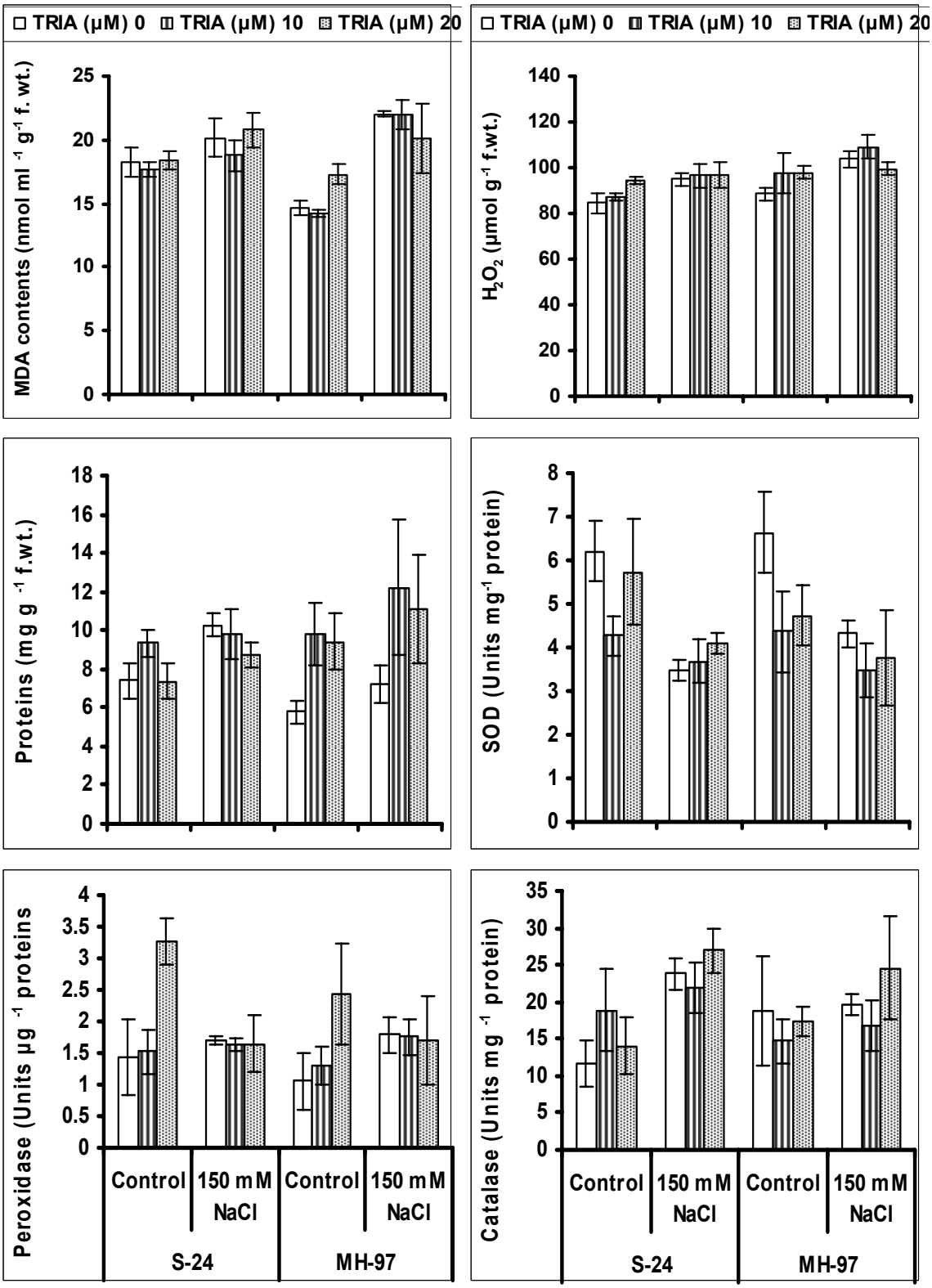


Fig. 1. Leaf malondialdehyde, hydrogen peroxide, total soluble proteins and anxidants activities in salt-stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed primed with triacontanol for 12 h.

In conclusion, salinity stress significantly increased the leaf MDA and $H_2O_2$ contents and CAT activity, while activity of SOD in both cultivars. Pre-sowing seed treatment of TRIA did not alter the studied attributes except that under non-saline conditions TRIA application increased the POD activity significantly in both cultivars.

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