

# Comparative Effect of Salinity on Antioxidant Enzymes of Two Wheat Genotypes by Foliar Application of Salicylic Acid and Potassium

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**Abstract:** *The aim of this work was to examine the ameliorative effect of foliar application of salicylic acid and potassium on antioxidant enzymes activity in wheat under saline conditions. Two wheat genotypes WL-711 (salt tolerant) and Kohistan-97 (salt sensitive) were used with salicylic acid (SA) and potassium (K<sup>+</sup>) foliar spray which were applied at both vegetative and grain filling stage under saline conditions. Various enzymatic antioxidant activities include catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and ascorbate peroxidase (APX) was assessed. Result cleared that foliar spray of salicylic acid and potassium decreased the damaged effect of salinity in both wheat genotypes especially Kohistan-97 which was more salt sensitive. Overall, foliarly applied salicylic acid and potassium reduces the adverse effects of reactive oxygen species and antioxidant enzymes on cellular metabolism that was disturbed by salt stress.*

**Keywords:** *Salinity, wheat genotypes, foliar application, antioxidant enzymes*

## 1. Introduction

Salt stress is one of the main threats for agriculture productivity worldwide. In this situation, salinity tolerance of crop plant for future food supply is required for sufficient food supply to growing population. Salinity limits the crop productivity by affecting many physiological and biochemical processes such as mineral deficiencies, ion toxicity and osmotic stress [1-2]. Various enzymatic activities and plant metabolism are affected due to such disturbances [3-4]. Oxidative stress is the amalgamation of osmotic stress, ionic stress and salt stress. Another type of stress is release of reactive oxygen species mainly produced in chloroplasts and mitochondria which includes hydrogen peroxide, superoxide anion and hydroxyl radicals [5-7]. These react with high oxygen level after capturing electrons from electron transport chain and modify the function of very sensitive organelle chloroplast where photosynthesis takes place. Reactive oxygen species (ROS) are increased which trigger toxic reactions for instance DNA mutation, lipid oxidation and protein degradation under salt stress condition [8-9]. There occur two defense systems used against the repair damage induced by oxidizing agents by plants.

Firstly the system comprises of molecules such ascorbic acid (vitamin C), asglutathione (tripeptide),  $\beta$ -carotene and alpha-tocopherol (vitamin B) are categorized as non enzymatic antioxidant system. Secondly the enzymatic antioxidant system includes catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and ascorbate peroxidase (APX) [10]. Many studies emphasize on Na<sup>+</sup> control on its transport, exclusion and accumulation of Na<sup>+</sup> within plants because it has more toxic effects rather than

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Cl<sup>-</sup> [11]. Hence, excessive anion or cation concentration in growth medium reduces crop growth and causes toxicity which varies among different species or cultivars. Saturation of vacuole Na<sup>+</sup> and Cl<sup>-</sup> accumulation in cytoplasm disturbs enzymatic activities and lead to dehydration in cell [4]. Excessive Cl<sup>-</sup> ion reduces the integrity of membranes and disturbs the photosynthetic process by inhibiting enzymatic activities [12]. Different type of antioxidants like superoxide dismutase, peroxidase and catalases are generated to alleviate these oxidative effects in plant [13]. Many other protective mechanisms include antioxidant, cations and anions homeostasis, hormonal regulation and osmoregulation presents in plants against salinity [14-16]. In the whole wheat grain, the scavenging percentage of antioxidants is affected by different environmental or growing conditions such as number of hours, growing locations and average daily solar radiation. Wheat is one of the prime crops that have the assets of anti-oxidation such as membrane protein, lipids and DNA against the oxidation of essential bio-molecules. Wheat bran extracts inhibit per oxide anion (O<sup>2-</sup>), human LDL cholesterol peroxidation, free stable radicals like DPPH (1,1-Diphenyl-2-picrylhydrazine) and ABTS<sup>+</sup> (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) [17-18]. The generation of ROS is scavenged counting antioxidant compounds by an antioxidant system and antioxidant enzymes like catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) [19]. Different wheat varieties varies in antioxidant properties [20-21] and percentage of bioactive compounds like phenolic acids [22], carotenoids [23], tocopherols [18] and anthocyanins [24]. It is eminent fact that salt stress is most important factors in decreasing wheat performance and growth. The antioxidative activity of plant extracts and foods has been widely used to judge the ability of compounds like hydrogen donors and free-radical scavengers or the DPPH radical [25]. We cannot ignore the role of wheat in the search for mechanisms of salt stress tolerance especially in a system of reactive oxygen scavenging. Salinity inhibits the production of free radicals like superoxide radical, singlet oxygen, H<sub>2</sub>O<sub>2</sub> and enhanced the antioxidant enzymes production which results to death of cells [26-27]. Salt stress suppresses the activities of antioxidant enzymes while foliar application of potassium improves the activities of antioxidant enzymes [28]. Foliarly applied potassium reduces the adverse effects of ROS (reactive oxygen species), improves metabolism and yield of plant [27]. Potassium can be vital a limiting factor and is essential for maximum yield of many crops under various ecological conditions for example salinity [29]. Salicylic acid is a plant phenolic compound and contemplated an endogenous regulator like hormones. In plants, it plays a role in defense mechanism and appears as a signal molecule on chemical messenger [30]. It is known that SA plays a regulatory role in many physiological processes such as transpiration, photosynthesis, chlorophyll synthesis, nutrient uptake and plant development [31-32].

## 2. Materials and methods

### 2.1. Plant material

In this experiment salt tolerant and sensitive wheat cultivars used to find the appropriate concentration of potassium and salicylic acid (K<sup>+</sup> and SA level is important in alleviating the toxic properties of salinity). One salinity resistant (WL-711) and one salt sensitive wheat cultivar (Kohistan-97) [33] were used for the experiments. Healthy seeds were sown in earthen pots (width 10cm × length 18 cm) having growth medium of sand. Two salinity treatments i.e., 0 and 12 dS/m after 14 sowing days were maintained throughout this experimentation. On appearance of salinity symptoms these level of SA (0, 0.01, 0.02 and 0.03%) and K<sup>+</sup> (0, 0.05, 0.1 and 0.15%) applied to thirty days old plants in two intervals to check the optimum concentration. Control plants were sprayed with distilled water. Experiment was conducted in completely randomized design (CRD) with five replicates for each treatment. Most appropriate level of SA and K<sup>+</sup> were selected on basis of their effects on relevant parameters. Three plants were removed from each replicate after ten days of application of treatment and antioxidant activity were recorded.



## 2.2. Treatment detail

Distilled water was used for control treatment while NaCl (Aurobindo Pharma Limited, Pakistan) was used to create salinity of 120 mM. K<sub>2</sub>SO<sub>4</sub> (Shiiazhoung, China) and C<sub>7</sub>H<sub>6</sub>O<sub>3</sub> (Labeyond, China) were used for foliar spray of following levels of K<sup>+</sup> (0.05, 0.1 and 0.15%) and SA (0.02, 0.03 and 0.04%) respectively.

## 2.3. Sample collection

Peroxidase, superoxide, ascorbate peroxidase and catalase were determined by spectrophotometer (Hitachi U2800, Japan). Leaves were homogenized according the method by Dixit *et al.* [34] in a medium composed of 1mM dithiothreitol (DTT) (GlycoSyn, US) and 50 mM phosphate buffer (prepared in laboratory) with 7.8 pH.

### 2.3.1. Superoxide dismutase (SOD)

Superoxide dismutase activity (Units min<sup>-1</sup> g<sup>-1</sup>FW) was estimated by Giannopolitis & Ries, [37]. SOD activity was measured by calculating the rate of inhibition of Nitroblue Tetrazolium (NBT) (Haihang, China) and reduction with xanthine oxidase as a H<sub>2</sub>O<sub>2</sub> generating agent. Spectrophotometer (Hitachi U2800, Japan) was used to read the absorbance at 560 nm. Enzyme quantity caused 50% photochemical inhibition of NBT was equal to one unit SOD activity.

### 2.3.2. Peroxidase (POD)

Peroxidase activity was measured by followed the method of Chance and Maehly, [35]. It was measured by estimating of H<sub>2</sub>O<sub>2</sub> with guaiacol as an electron donor. Reaction solution prepared for POD by mixing 50 mM phosphate buffer with pH 5, 40 mM of H<sub>2</sub>O<sub>2</sub>, 0.1 mL enzyme extract and 20 mM of guaiacol (Tianjin, China). Absorbance was increase due to the formation of tetraguaiacol and assayed at 470 nm after every 20 s. The amount of POD enzyme which caused the increase in absorbance by 0.01 in one minute was taken as one unit of enzyme. The activity of enzyme was calculated as unit min<sup>-1</sup> g<sup>-1</sup> FW.

### 2.3.3. Catalase (CAT)

Catalase activity (Units min<sup>-1</sup> g<sup>-1</sup> FW) was estimated the method by Chance and Maehly, [35] and calculated by conversion rate of oxygen molecules and hydrogen peroxide (Shandong, China) to water. 3 mL reaction solution was prepared in phosphate buffer (50 mM, 7.8 pH) containing 0.1 mL sample extract and 5.9 mM of hydrogen peroxide. Activity of catalase was calculated by decline in absorbance after every 20 s at 240 nm due to consumption of H<sub>2</sub>O<sub>2</sub>. One unit catalase activity was equal to absorbance change of 0.01 unit / min.

### 2.3.4. Ascorbate peroxidase (APX)

Activity of ascorbate peroxidase was calculated by the method as described by Cakmak, [36]. It is measured by decline in absorbance of ascorbic acid in a 1 mL reaction mixture at 290 nm containing 0.1 mM Na-EDTA(Tianjin, China), phosphate buffer (50 mM, pH 7.8), 0.25 mM ascorbic acid (Fooding, China), 12 mM H<sub>2</sub>O<sub>2</sub> and the sample extract. The activity of enzyme was calculated as ABA digested g<sup>-1</sup> FW h<sup>-1</sup>.

### 2.3.5. STATISTICAL ANALYSIS

Results obtained were evaluated by using the software program Statistix 8.1. Assessment of mean values and standard errors were calculated on Microsoft Excel-2007 Version. Least significant difference (LSD) test was performed for comparison of the significant mean values at 5 percent probability level [38].

### 3. Results and discussions

#### 3.1. Superoxide dismutase activity (SOD)

Salt stress significantly increased the superoxide dismutase activity (SOD) ( $P \leq 0.001$ ) (Table 1). Plants grown under salt stressed showed highest value for SOD (200.68 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) than those under non saline conditions (160.85 Units  $\text{min}^{-1} \text{g}^{-1}$  FW). Maximum SOD was recorded at grain filling (206.73 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) in comparison to vegetative stage of plants (194.63 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) (Figure 1). Highly significant variations were exhibited among foliar application of different  $\text{K}^+$  and SA levels for SOD ( $P \leq 0.001$ ) (Table 1). Foliar spray of  $\text{K}^+$  (0.1%) lowers SOD (131.43 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) in plants as followed by SA (0.02%) foliar spray (137.53 Units  $\text{min}^{-1} \text{g}^{-1}$  FW). Kohistan-97 maintained higher SOD (228.53 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) as compared to WL-711 (206.73 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) plants (Figure 1). Overall results showed that foliar spray of  $\text{K}^+$  and SA at grain filling stage was fruitful in SOD activity in plants under saline condition. A markable increase in peroxidase activity minimizing was noted in plants expose to salt stress (Table 1). Tasgin et al. [39] also reported the ameliorative effects of salicylic acid on enzyme activity in wheat plant leaves. Salicylic acid has been assumed as imperative part in various biological phenomenons in plants under stress conditions [40]. In chloroplast, zeaxanthin, vitamin E, glutathione, vitamin C and beta carotene act as antioxidative defense mechanism against toxic oxygen derivatives.

#### 3.2. Peroxidase (POD)

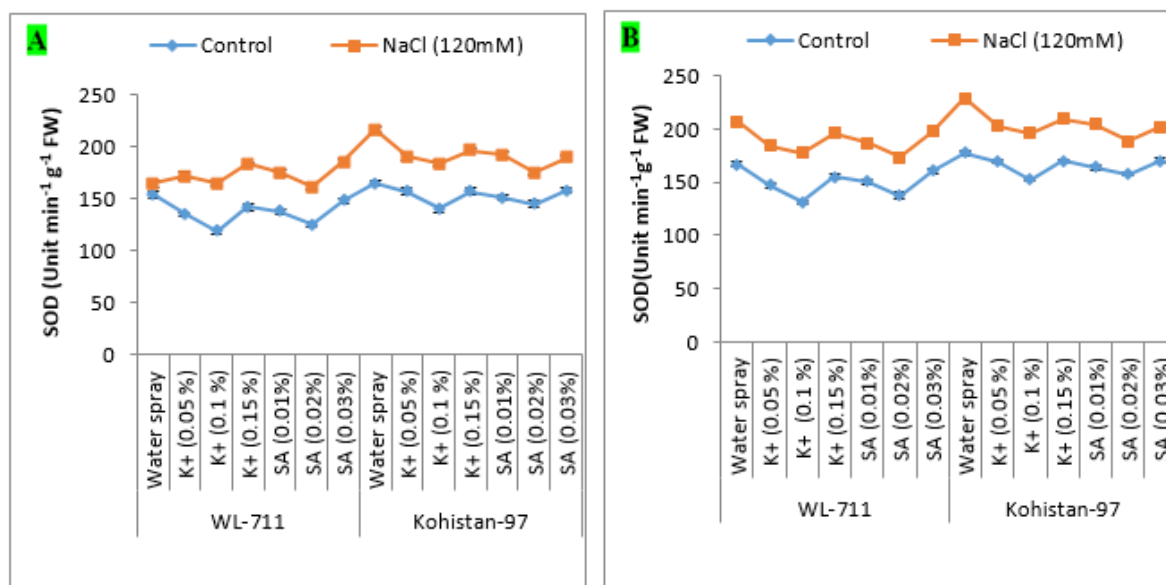
Plants showed maximum POD (256.58 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) when exposed to salt stress than those controlled plants (216.95 Units  $\text{min}^{-1} \text{g}^{-1}$  FW). Maximum value for POD was observed at vegetative (264.43 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) as compared to grain filling stage of plants (248.73 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) (Figure 2). Highly significant variations ( $P \leq 0.001$ ) were observed among foliar application of different  $\text{K}^+$  and SA levels for POD (Table 1). Foliar spray of  $\text{K}^+$  (0.15%) on plants maintained POD (264.43 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) closely followed by foliar spray of SA (0.03%) (232.2 Units  $\text{min}^{-1} \text{g}^{-1}$  FW). Wheat cultivars (WL-711 and Kohistan-97) exhibited significant difference for POD ( $P \leq 0.001$ ) (Table 1). Kohistan-97 recorded POD (253.13 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) than plants of WL-711 (275.13 Units  $\text{min}^{-1} \text{g}^{-1}$  FW) (Figure 4). Overall results showed that foliar application of  $\text{K}^+$  and SA was effective in reducing POD of plants at vegetative stage under salinity stress. Agarwal et al. [41] also reported the effect of salicylic acid on antioxidant enzyme and oxidative stress in different wheat genotypes.

**Table 1.** Mean square values for effect of potassium and salicylic acid on antioxidant enzymes at vegetative and grain filling stage in controlled and salt stressed plants of wheat cultivars

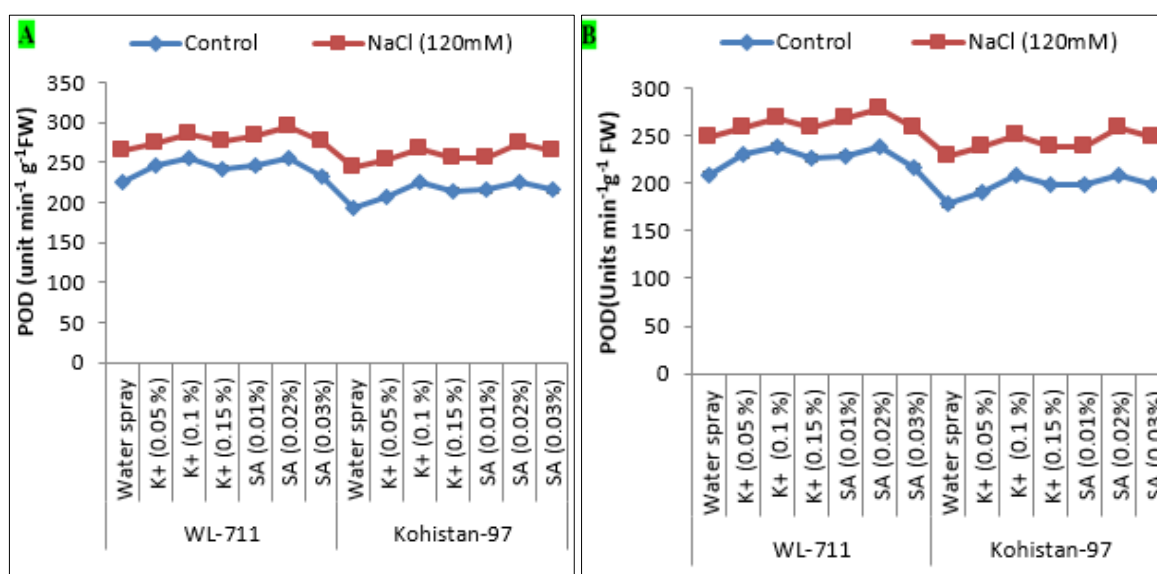
Sample	DF	Parameter			
		SOD (Units $\text{min}^{-1} \text{g}^{-1}$ FW)	POD (Units $\text{min}^{-1} \text{g}^{-1}$ FW)	CAT (Units $\text{min}^{-1} \text{g}^{-1}$ FW)	APX (ABA digested $\text{g}^{-1}$ FW $\text{h}^{-1}$ )
Variety	1	10421.8**	25806.9**	5046.86**	5.595**
Stage	1	6149.22**	10352.6**	3632.58**	2.004**
Salinity	1	63228.5**	69320.5**	67312.0**	63.110**
Treatment	6	2833.07**	2226.23**	3275.10**	1.774**
Variety*Stage	1	2.064ns	2.838ns	1.850ns	4.131ns
Variety*Salinity	1	2.675**	865.869**	44.434**	0.109**
Variety*Treatment	6	119.374**	147.539**	41.619**	0.032**
Stage*Salinity	1	9.530ns	8.679ns	4.650ns	4.392ns
Stage*Treatment	6	2.600ns	4.278ns	2.873ns	4.693ns

Salinity*Treatment	6	143.229**	107.699**	46.042**	0.060**
Variety*Stage*Salinity	1	7.547ns	1.157ns	2.359ns	3.158ns
Variety*Stage*Treatment	6	4.662ns	2.583ns	1.312ns	1.449ns
Variety*Salinity*Treatment	6	51.369**	46.981**	54.386**	0.045**
Stage*Salinity*Treatment	6	3.041ns	4.343ns	5.726ns	6.460ns
Variety*Stage*Salinity* Treatment	6	3.594ns	3.343ns	9.709ns	1.192ns
Error	112	6.113	2.936	6.391**	0.0016

\*=P<0.05; \*\*= P<0.001; ns=non-significant



**Figure 1.** Impact of potassium (K<sup>+</sup>) and salicylic acid (SA) on superoxidase dismutase (SOD) at vegetative stage (A) and at grain filling stage (B) (in controlled and salt stressed plants of wheat cultivars)



**Figure 2.** Impact of potassium (K<sup>+</sup>) and salicylic acid (SA) on peroxidase (POD) at vegetative stage (A) and at grain filling stage (B) (in controlled and salt stressed plants of wheat cultivars)

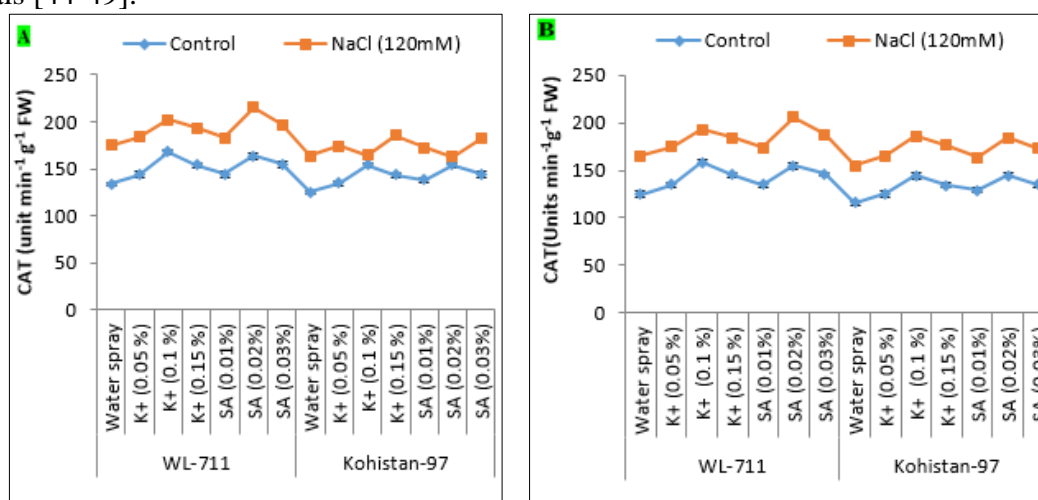


### 3.3. Catalase (CAT)

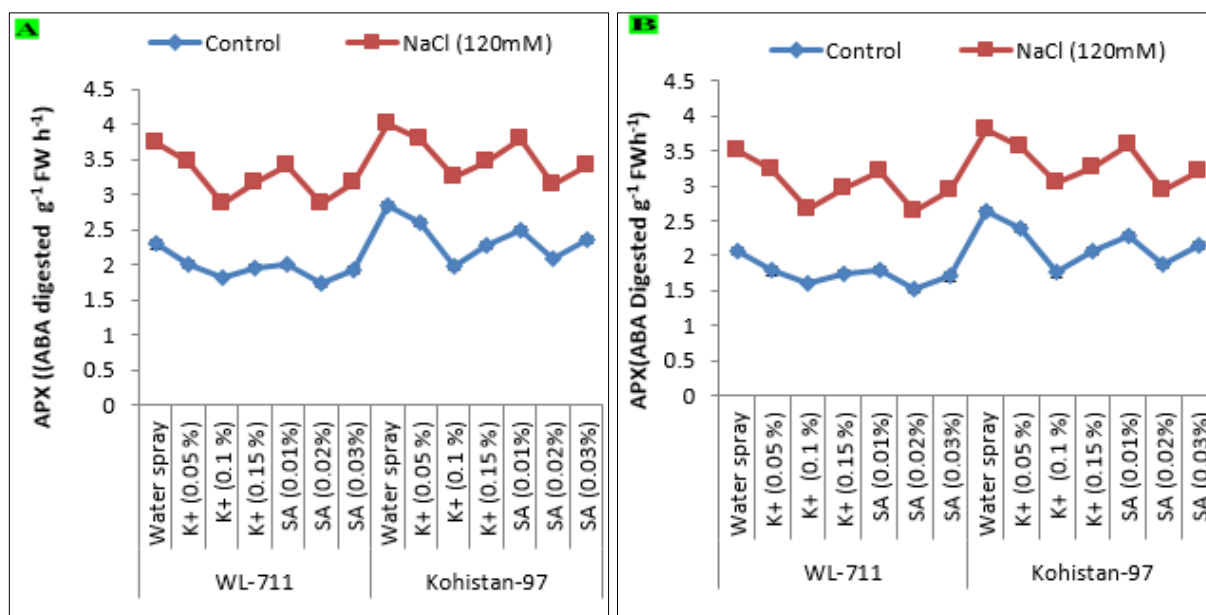
Catalase activity significantly increased in plants grown under salt stress conditions ( $P \leq 0.001$ ) (Table 1). Maximum value for catalase ( $174.9 \text{ Units min}^{-1} \text{ g}^{-1} \text{ FW}$ ) was recorded by plants exposed to salt stressed than controlled plants ( $133.83 \text{ Units min}^{-1} \text{ g}^{-1} \text{ FW}$ ). Significance difference among two growth stages revealed that plants exhibited maximum value for catalase ( $202.33 \text{ Units min}^{-1} \text{ g}^{-1} \text{ FW}$ ) at vegetative as compared to grain filling stage ( $193.03 \text{ Units min}^{-1} \text{ g}^{-1} \text{ FW}$ ). Maximum catalase value ( $167.6 \text{ Units min}^{-1} \text{ g}^{-1} \text{ FW}$ ) was observed in plants under influence of  $\text{K}^+$  foliar spray. Foliar spray of SA (0.02%) maintained catalase ( $156.36 \text{ Units min}^{-1} \text{ g}^{-1} \text{ FW}$ ) (Figure 3). Significant difference ( $P \leq 0.001$ ) was found among foliar treatment of  $\text{K}^+$  and SA levels for catalase (Table 1). Non-significant interaction, salinity  $\times$  treatment  $\times$  stages  $\times$  varieties ( $P \geq 0.05$ ) indicated that plants maintained the maximum catalase ( $202.33 \text{ Units min}^{-1} \text{ g}^{-1} \text{ FW}$ ) by foliar application of  $\text{K}^+$  (0.1%) at vegetative stage under salt stress. In wheat, the activities of CAT, SOD and POD were improved and maintained when treated with salicylic acid [42]. These results resembles to the findings of Simaei *et al.* [43] who described that salicylic acid enhanced the activity of the antioxidative system by decreasing the damaging effects.

### 3.4. Ascorbate peroxidase (APX)

Data regarding ascorbate peroxidase activity exhibited highly significant effect of salt stress ( $P \leq 0.001$ ) (Table 1). Under salinity stress, plants exhibited maximum ascorbate peroxidase activity ( $3.62 \text{ ABA digested g}^{-1} \text{ FW h}^{-1}$ ) than normal plants ( $2.18 \text{ ABA digested g}^{-1} \text{ FW h}^{-1}$ ). At vegetative stage, maximum ascorbate peroxidase activity ( $4.00 \text{ ABA digested g}^{-1} \text{ FW h}^{-1}$ ) was recorded as compared to grain filling stage ( $3.78 \text{ ABA digested g}^{-1} \text{ FW h}^{-1}$ ) (Figure 4). Foliar spray of  $\text{K}^+$  and SA levels differed significantly ( $P \leq 0.001$ ) for ascorbate peroxidase activity (Table 1). Foliar application of  $\text{K}^+$  (0.1%) minimized ascorbate peroxidase activity ( $2.88 \text{ ABA digested g}^{-1} \text{ FW h}^{-1}$ ) in wheat plants. Ascorbate peroxidase activity ( $2.86 \text{ ABA digested g}^{-1} \text{ FW h}^{-1}$ ) was maintained by foliar application of SA (0.02%) level under salt stress conditions (Figure 4). Significant interaction was exhibited by wheat cultivars (WL-711 and Kohistan-97) for ascorbate peroxidase activity ( $P \leq 0.001$ ) (Table 1). Kohistan-97 showed maximum ascorbate peroxidase activity ( $2.84 \text{ ABA digested g}^{-1} \text{ FW h}^{-1}$ ) as compared to WL-711 ( $2.29 \text{ ABA digested g}^{-1} \text{ FW h}^{-1}$ ) plants. Salt stressed reduced the ascorbate peroxidase activity ( $2.66 \text{ ABA digested g}^{-1} \text{ FW h}^{-1}$ ) at grain filling growth stage in WL-711 with foliar spray of  $\text{K}^+$  (0.1%) (Figure 4). Overall results indicated that the foliar application of  $\text{K}^+$  and SA was effective in reducing APX in wheat plants at vegetative stage under salt stressed conditions. In above investigation about antioxidant enzymes, superoxide dismutase and peroxidase increased with salt treatments but decreased with foliar application of salicylic acid and potassium. Enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) was known to be the most efficient enzymes in the destruction of free radicals [44-49].



**Figure 3.** Impact of potassium ( $\text{K}^+$ ) and salicylic acid (SA) on catalase (CAT) at vegetative stage (A) and at grain filling stage (B) (in controlled and salt stressed plants of wheat cultivars)



**Figure 4.** Impact of potassium ( $K^+$ ) and salicylic acid (SA) on ascorbate peroxidase (APX) at vegetative stage (A) and at grain filling stage (B) (in controlled and salt stressed plants of wheat cultivars)

#### 4. Conclusions

In respect of the above results, foliar applications of  $K^+$  and SA levels had an ameliorative effect on antioxidant enzymes that ultimately enhanced the metabolic and cellular activities, disturbed by salt stress. However, foliar spray of potassium and salicylic acid levels mitigates the toxic effects of salinity at vegetative of grain filling stage in both genotypes of wheat. Overall, foliarly applied salicylic acid and potassium reduces the adverse effects of reactive oxygen species and antioxidant enzymes on cellular metabolism that were disturbed by salt stress.

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