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Exogenous Hydrogen Peroxide (H_2O_2) Eustress to Wheat Genotypes Attenuates their Salinity Distress

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ABSTRACT: The impact of salt stress (NaCl) and ameliorative contribution of hydrogen peroxide (H_2O_2) seed pretreatment at 0, 20, 60, 100 µM concentrations in ten genotypes of wheat (*Triticum aestivum* L.) with various phenotypic characteristics was assessed in this study. Eustress wheat seeds with H_2O_2 significantly improved the germination and plant growth exhibited under normal water irrigation, such as shoot and root length, their fresh weight and water content. Seeds eustressed with H_2O_2 exhibited reduction in the damage to plant growth and physiological characteristics against the un-primed seeds (control) in all the wheat genotypes under salt stress conditions. Further, seed eustress with H_2O_2 rendered an improvement in the root/shoot ratio. However, the antioxidant activity remained unchanged in normal water irrigation treatment, but it reduced under saline condition. Among the wheat genotypes, the highest salt tolerance was exhibited by Marvi-2K, Sarsabaz and Khirman, while the most sensitive genotypes were Inqalab and Imdad. The NaCl treatment affected all the wheat seedlings parameters negatively; while antioxidative effect of H_2O_2 caused significant improvement under salinity stress conditions. The study concludes that in wheat seedlings, seed soaking with H_2O_2 at 60 µM concentration may be efficient in minimizing the harmful effect of salinity.

Keywords: *Triticum aestivum* L.; Salt tolerance; Hydrogen peroxide; Antioxidant; Reactive oxygen species

I. INTRODUCTION

Worldwide, wheat is grown over 238 million hectares, with 865 million tones production [1], and livelihood to 80 million farmers [2]. However, the world's average wheat yield is quite low being 3.20 t ha^{-1}; with the lowest range of 0.50-2.50 t ha^{-1} in the developing Asian and African countries, against the highest range of 5.00-9.00 tha^{-1} in the developed and European countries [1, 3]. This large gap in the potential grain yield of wheat, mainly in the arid and semi-arid mega-environments of these developing countries, is due to many factors, including drought, heat and soil salinity [4-6]. Due to global climate change, salinity becomes a major threat to agriculture through elevated temperatures and erratic precipitation etc. Estimates indicate that due to varying degrees of salinity, 830 million hectares of world land are affected [7], and about 50% of land will be salinized by 2050 [8]. Environmental stresses affect various developmental stages of the crop, which include growth and its related traits in wheat with 88% reduction in grain yield due to salinity [9, 10] showed that salinity not only retards and modifies the growth of wheat, but also affects the metabolism of plants. An ameliorative effect on seed germination, plant growth and physiological parameters of wheat under salinity stress of various antioxidant applications, such as beta carotene, uric acid and glutathione (individually and in combination) [11]. Plants produce reactive oxygen species (ROS) in response to stress [12-14] that could harm them. Under higher salt concentrations huge amount of (ROS) are produced in plants under higher salt concentration such as hydrogen peroxide (H_2O_2), superoxide anion (O^{2-}), hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$), but they overcome their harmful effects by increasing antioxidant enzyme activity [15, 16]. In order to exacerbate the harmful impact of these ROS, the plant has produced a powerful antioxidant production mechanism which plays a key role in stress tolerance [17-19]. In plants, two mechanisms work to detoxify the effects of reactive oxygen species, viz., enzymatic and non-enzymatic antioxidant activation [20]. To classify, choose salt tolerant plants, we can use antioxidant enzyme levels as a marker parameter and this has also shown a strong positive connection between the antioxidant system and the tolerance of plant salt [21].

Numerous studies report that the impact of various environmental stresses can be minimized by increasing antioxidant enzymes and metabolites [22, 23]. Hydrogen peroxide (H_2O_2) plays a key role in reducing the harmful

aspects of ROS on plants grown under salt- affected conditions [10]. H_2O_2 eustress has been shown to increase seed tolerance and promote the growth of wheat cultivars under seawater salinity stress [24]. H_2O_2 influences respiration, stomatal conductance, assimilation, photosynthesis, plant growth and development processes and their reaction to biotic and abiotic stresses when present in lower concentrations [25]. Whereas, its accumulation above the threshold level could cause oxidative damage, eventually leading to cell death [26]. The research work regarding various antioxidants, their role in plant systems, and accumulation of osmolytes, ions, toxins and outflow in wheat crop is limited. Therefore, this study examined the impact of salinity stress on different genotypes of wheat, and H_2O_2 was used to overcome salinity stress in plant seedlings as an antioxidant agent for seed priming.

II. MATERIALS AND METHODS

A. Plant Material, Growth Conditions and Experimental Treatments

Selected seeds of ten wheat genotypes, viz., Anmol, Bhittai, Imdad, Inqalab, Khirman, Kiran-95, Marvi-2K, Moomal, Sarsabaz, and TJ-83 were obtained from the Nuclear Institute of Agriculture, Tandojam, Pakistan and surface sterilized with sodium hypochlorite (NaOCl, Merck, Germany) to save seeds from contamination during the growing period. This seedling growth experiment was conducted in the growth chamber at Environmental Centre, University of Lancaster, UK. Seed of all the ten genotypes after sterilization were treated / primed with the solution of H_2O_2 using 0, 20, 60, 100 µM concentration for a period of 8 hours. Twenty (20) seeds were selected from each variety and put on ashless Whatman No.540 filter papers kept in Petri dishes for growth, then moistened with either 10 ml of distilled water (0 Control) or 100 mM of NaCl for salinity treatment. The experiment was performed according to completely randomized design under two factor factorial arrangement and treatments were replicated thrice. Petri dishes were held at a temperature of 25 ± 0.5 / 22 ± 0.5 ℃ in the growth chambers, relative humidity 45 ± 2 / 52 ± 2 percent with a day/night photoperiod of 16 / 8 hours respectively during the months of September to October. Seeds were allowed to germinate at 25 ℃±1 ℃ in the incubator. Data on seed germination, length, fresh weight, and moisture content of seedlings and roots were recorded after eight days and their antioxidant activity was calculated.

B. Trait Measurements

Measurement of Phenotypic Attributes. Twenty (20) seeds from each of the ten (10) wheat genotypes were soaked on filter paper in the Petri dishes for germination and kept under continuous observation. The seed was considered to be germinated when its radical (1mm) emerged from the seed and became visible. From the date of planting until the end of the experiment, the germinated seeds were counted regularly. Data on all the phenotypic characteristics were recorded after eight (08) days of sowing, and seed germination percentage was calculated as mean value by using the formula described by Al-Mudaris (1998) [17].

$$\text{Germination (\%)} = \frac{\text{Total count of germinated seeds at the end of experiment}}{\text{Total number of initial seeds grown}} \times 100 \quad \ldots(1)$$

Genotypes showing higher germination percentage were having greater seedlings population in the Petri dish (Slide. 1). Mean germination time (MGT) was determined using the formula described by Hosseini *et al.* (2002) [27], and values were expressed in days:

$$\text{Mean germination time} = \frac{\sum F.x}{\sum F} \quad \ldots(2)$$

Where, F is the no. of seeds germinated on each day. The lower the value of MGT, the faster the germination of the seed population. Phenotypic plant attributes (length, weight and moisture/water content of fresh shoot and root) of each seedling were calculated in all the three replicates under control / salinity and H_2O_2 treatments. Root and shoot water contents (%) were measured as given in the following equation [28]:

$$\text{Water content (\%)} = \frac{\text{Fresh biomass weight} - \text{Dry biomass weight}}{\text{Fresh biomass weight}} \times 100 \quad \ldots(3)$$

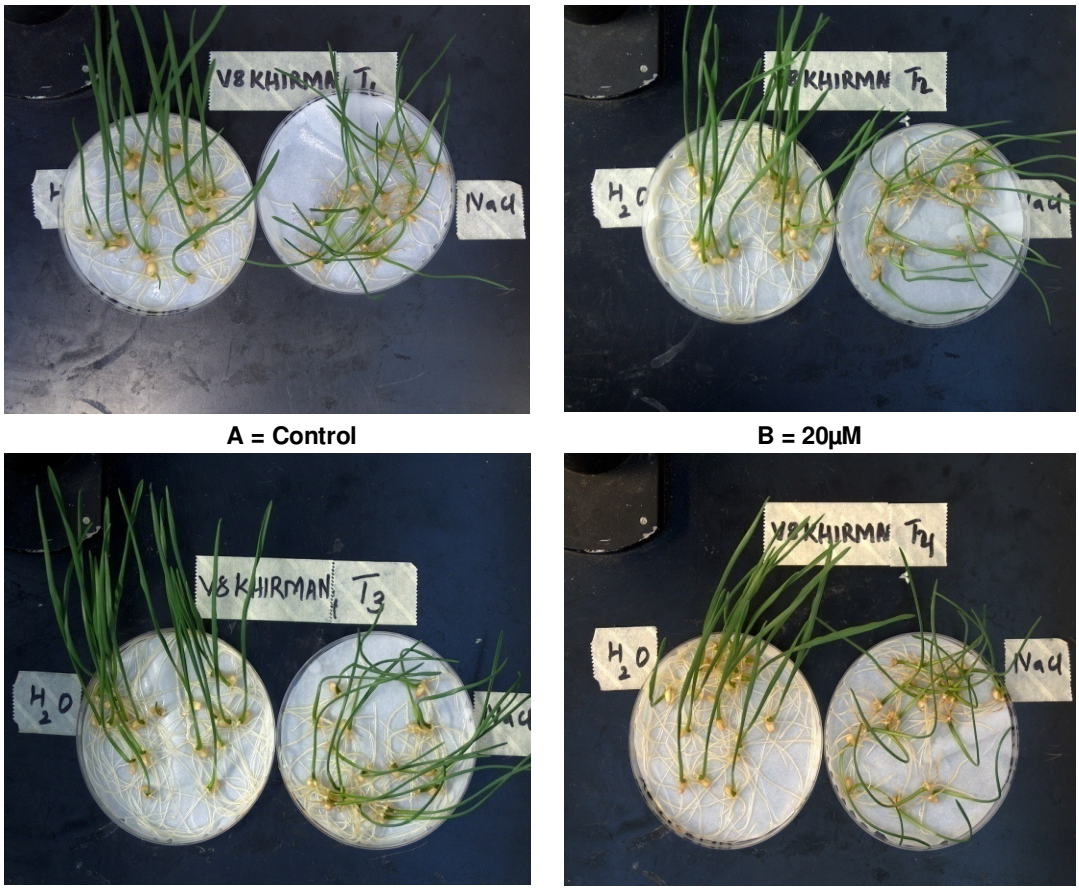
The root/shoot ratio was determined using the following formula:

$$\text{Root : shoot ratio} = \frac{\text{Dry biomass weight of root}}{\text{Dry biomass weight of shoot}} \quad \ldots(4)$$

Antioxidant assay. The content of hydrogen peroxide was determined by following the method of Velikova *et al.,* (2000) [29]. Leaf tissues have been homogenized with 0.1% (w/v) TCA on ice. Homogenate was then centrifuged for 10 minutes at 13000 × g and 0.5 ml of supernatant was applied to 0.5 ml of potassium phosphate buffer (pH 7.0) and 1 ml of 1M KI. The sample was incubated for fifteen minutes at room temperature. Absorbance of supernatant was read at 390 nm, using a spectrophotometer. From the standard curve, hydrogen peroxide content concentration was measured and the values were expressed as µmol g^{-1} of fresh weight.

C. Statistical Analysis

The data was analyzed through a two-way analysis of variance using a software STATISTIX version 8.1, The Least Significant Difference (LSD) values were used to test the differences among genotypes and the treatments at a probability of ≤ 0.05 [30]. Graphs were made using MS Excel software.



A = Control **B = 20µM**

C = 60µM **D = 100µM**

Slide 1. Germination under the influence of genotypes, salinity levels and H_2O_2 treatment on wheat genotypes.

III. RESULTS

Results on the rectal of wheat genotypes grown under salinity stress in relation to growth promoting effect of the antioxidant agent H_2O_2 have been defined in the forthcoming paragraphs.

A. Seed germination

Data on seed germination percentage and its statistical analysis indicate non-significant effect between control (water) and salinity treatments for all the ten wheat genotypes (Table 1). Khirman genotype showed the highest germination (100%) under both treatments, followed by Moomal, Bhittai and TJ-83 genotypes. Whereas, Marvi-2K exhibited 100% germination in control but very low (98.72%) with salt treatment. However, the lowest results were obtained for Inqalab in both control (97.07%) as well as under salinity treatment (98.3%). Different H_2O_2 concentrations significantly influenced the germination percentage, which improved with the increasing concentration of hydrogen peroxide both in water and NaCl treatments irrespective of cultivars (Fig. 1). Seeds primed with the solution of H_2O_2 at 60 µM concentration for 8 hours rendered the highest percentage of germination (> 99%), but at 100 µM there was no further increase, even less under salt treatment. On the overall, 60 µM H_2O_2 improved about 2% germination over control without H_2O_2 priming both with and without salt treatment.

B. Mean germination time (MGT)

Mean germination time of seeds showed significant impact of salt treatment, as well as response of genotypes (Table 1). The NaCl treated seeds took more mean germination time than in control irrigated. Wheat genotypes differed significantly in mean germination time, as Marvi-2K, Moomal, Bhittai, Imdad and Sarsabaz took comparatively greater mean germination time in control treatment; while Sarsabz, Bhittai and Moomal used maximum mean germination time under NaCl treatment. The shortest mean germination time was used by Inqalab, TJ-83 and Khirman genotypes. Seed priming with hydrogen peroxide priming reduced the mean germination time in only water irrigated control up to 60 µM of H_2O_2 but increased it at 100 µM concentration. However, with saline water irrigation treatment, the MGT had increasing trend due to increased H_2O_2 concentration (Fig. 1).

Table 1: Seed germination and mean germination time of wheat genotypes eustressed with H_2O_2.

Genotypes	Seed germination (%)		Mean germination time (days)	
	Water	NaCl	Water	NaCl
Anmol	99.57 ab *	99.57 ab	1.12 cd	1.20 ab
TJ-83	99.15 abc	100.0 a	1.10 cd	1.22 ab
Moomal	100.0 a	99.57 ab	1.17 bcd	1.26 ab
Bhittai	100.0 a	99.15 abc	1.17 bcd	1.27 ab
Kiran	98.72 abc	98.75 abc	1.15 bcd	1.19 ab
Sarsabaz	99.15 abc	98.72 abc	1.14 bcd	1.36 a
Marvi-2K	100.0 a	98.72 abc	1.20 bc	1.17 bcd
Khirman	100.0 a	100.0 a	1.12 cd	1.19 ab
Imdad	99.15 abc	97.5 bc	1.14 bcd	1.22 ab
Inqalab	97.07 c	98.3 abc	1.05 d	1.15 bcd
Mean	99.28	99.03	1.13	1.22

* Values under each parameter in a column bearing different letter(s) have statistically significant difference among themselves at $P \leq 0.05$.

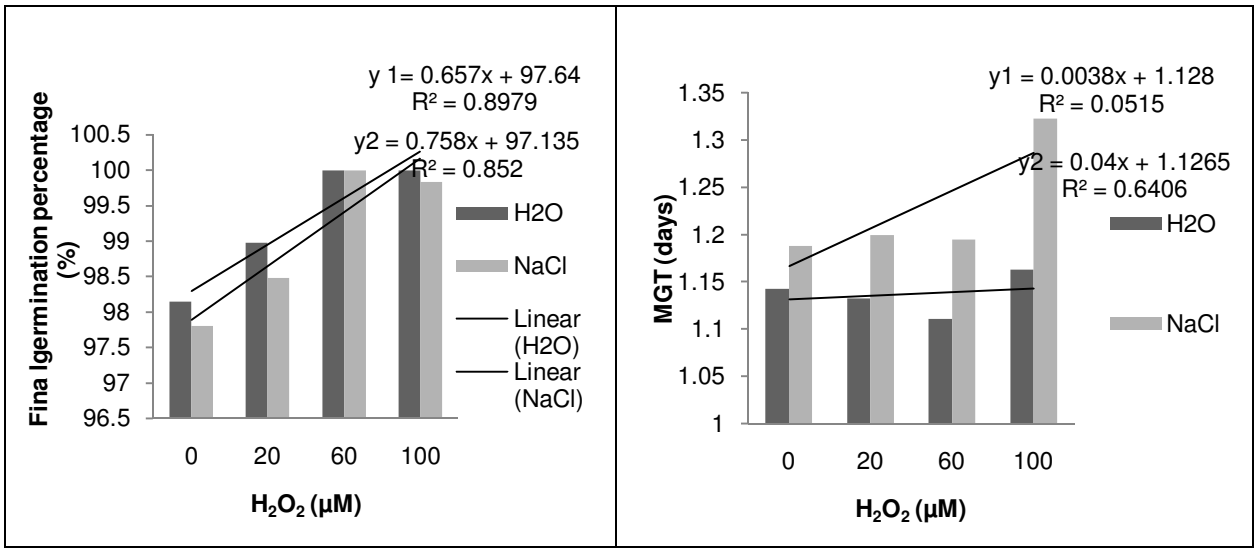


Fig. 1. Linear representation of seed germination and mean germination time (MGT) of wheat genotypes eustressed with different concentrations of H_2O_2 under normal and saline conditions.

C. Shoot and root length (mm)

Genotypic response to salt stress and treatment with hydrogen peroxide with respect to shoot length was highly significant (Table 2). With water soaking, the shoot length reached to its maximum when seeds were primed with H_2O_2 at 100 µM concentration (Fig. 2). However, the highest shoot length was noted under H_2O_2 seed soaking at 60 µM under NaCl treatment. Shoot length was significantly increased by H_2O treatment, regardless of H_2O_2 concentrations, and was severely affected by NaCl stress at all H_2O_2 concentrations. Even with NaCl treatment, increasing H_2O_2 concentrations for seed soaking resulted in a major positive impact on shoot length. Hydrogen peroxide has greatly influenced the shoot length of different genotypes. Khirman maximally tolerated salinity among the genotypes, while Imdad seemed to be the most sensitive to salinity as far as the length of the shoot was concerned.

The root length of wheat genotypes was significantly influenced by the concentration of H_2O_2 priming, salinity stress, and most of their interactions. Most of the genotypes did not differ significantly among themselves for root length except that Imdad had significantly smaller root length compared to Marvi-2K, TJ-83, Khirman and Anmol under water irrigation (Table 2). Under NaCl stress treatment, all the genotypes had statistically similar root length, with the highest value (31.2 mm) for Khirman genotype. The root length reached its limit at 60 µM H_2O_2 concentration with water irrigation treatment and fell significantly below 100 µM H_2O_2 concentration (Figure 2). However, irrespective of H_2O_2 levels, under NaCl treatment, the root length was adversely affected; while under H_2O treatment, with all H_2O_2 concentrations, the root length was substantially greater than under NaCl stress treatment. The effect of NaCl treatment on root length in all the genotypes examined was severely adverse (Table 2). Results showed that hydrogen peroxide had a linear positive response to wheat genotypes provided normal water and saline water in relation to the root length.

Table 2: Shoot and root length of wheat genotypes eustressed with H_2O_2.

Genotypes	Shoot length (mm)		Root length (mm)	
	Water	NaCl	Water	NaCl
Anmol	92.7 b	58.8 fgh	78.1 a	28.3 c
TJ-83	97.0 ab	63.8 ef	81.6 a	28.3 c
Moomal	89.0 bc	54.4 fgh	72.9 ab	25.4 c
Bhittai	105.8 a	49.8 h	74.5 ab	23.3 c
Kiran	99.3 ab	57.5 fgh	74.4 ab	25.5 c
Sarsabaz	99.0 ab	63.2 efg	69.1 ab	26.4 c
Marvi-2K	98.1 ab	53.0 fgh	81.6 a	29.6 c
Khirman	94.8 ab	79.1 cd	78.4 a	31.2 c
Imdad	75.0 de	49.2 h	62.3 b	26.7 c
Inqalab	87.7 bc	51.4 gh	70.6 ab	25.7 c
Mean	93.8	58.0	74.4	27.0

* Values under each parameter in a column bearing different letter(s) have statistically significant difference among themselves at $P \leq 0.05$.

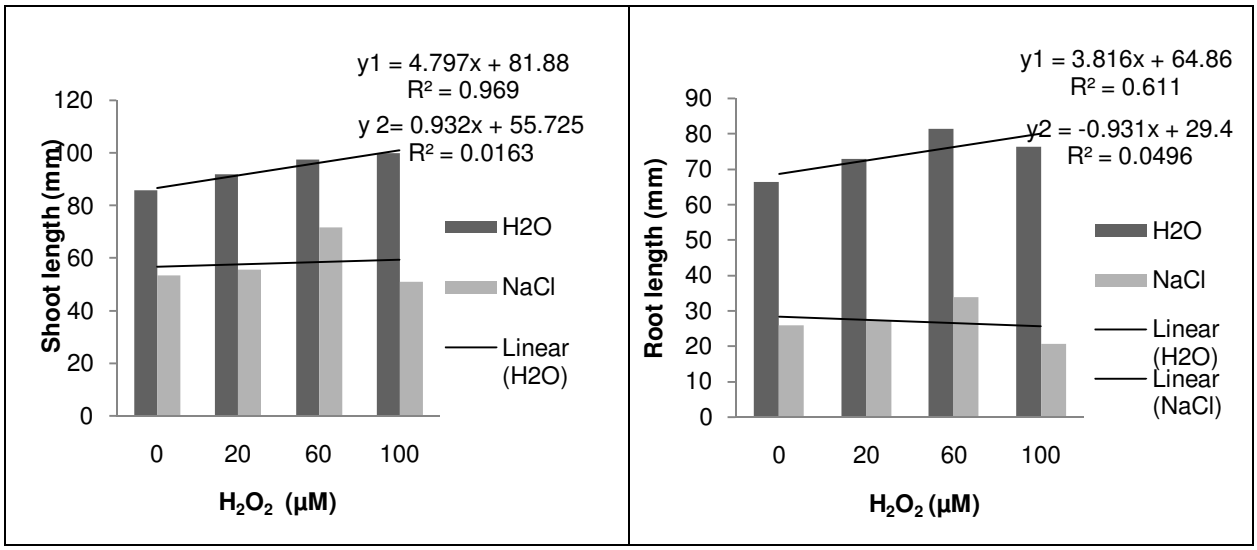


Fig. 2. Linear representation of shoot and root length of wheat genotypes eustressed with different concentrations of H_2O_2 under normal and saline conditions.

D. Weight of shoots and roots (g)

The weight of fresh shoots in wheat genotypes was significantly affected by concentrations of H_2O_2 and salinity stress. In contrast to NaCl treatment, Table 3 showed a higher shoot weight under H_2O_2 treatment. With water irrigation, the genotype Marvi-2K yielded the highest shoot weight, followed by Sarsabaz, Kiran, Bhittai and Khirman with non-significant difference, while Imdad performed the lowest. With NaCl stress, the highest shoot weight was produced by Khirman, and the lowest result was that of Bhittai genotype. The fresh shoot weight reached its limit at 100 µM H_2O_2 concentration under H_2O_2 treatment (Fig. 3). However, fresh shoot weight was markedly reduced by NaCl treatment regardless of the H_2O_2 level; whereas shoot fresh weight was markedly higher under all H_2O_2 concentrations under H_2O_2 treatment. Under H_2O_2 treatment, fresh shoot weight had a positive effect of increasing H_2O_2 concentrations up to 100 µM; while under NaCl stress, H_2O_2 concentrations up to 60 µM were found to be optimally efficient but had less impact at H_2O_2 concentrations of 100 µM. Fresh root weight of wheat genotypes was also affected by NaCl salinity stress and seed soaking with H_2O_2 at different levels as well as well as by their interactions. Higher fresh root weight was recorded for all the genotypes under H_2O_2 treatment as compared to NaCl treatment (Table 3). Marvi-2K genotype rendered the highest root weight with water but the difference was non-significant with Sarsabaz, Anmol, TJ-83, Khirman, Bhittai and Imdad; while Kiran and Inqalab produced significantly lower fresh root weight. Under salinity stress, Marvi-2K and Imdad produced the best results, while TJ-83 and Bhittai had the lowest performance. Both with H_2O and NaCl treatment, the fresh root weight reached to its maximum at 60 µM H_2O_2 concentration (Figure 3). However, regardless of the H_2O_2 levels, fresh root weight was markedly reduced by NaCl treatment. Fresh root weight was positively affected by increasing H_2O_2 concentrations up to 60 µM for soaking seed with H_2O_2 and NaCl treated water, but further increase in H_2O_2 concentrations up to 100 µM did not prove effective to improve fresh root weight of wheat genotypes. However, there was marked reduction in fresh root weight with NaCl treatment as compared to water irrigation.

Table 3: Fresh shoot and root weight of wheat genotypes eustressed with H_2O_2.

Genotypes	Fresh shoot weight (g)		Fresh root weight (g)	
	Water	NaCl	Water	NaCl
Anmol	1.17 b-e	0.82 fg	0.71 abc	0.37 ghi
TJ-83	1.18 b-e	0.79 fg	0.65 a-d	0.31 i
Moomal	1.15 cde	0.76 fg	0.62 b-e	0.35 ghi
Bhittai	1.26 abc	0.70 g	0.64 a-d	0.33 hi
Kiran	1.26 abc	0.80 fg	0.55 def	0.34 ghi
Sarsabaz	1.32 ab	0.88 f	0.73 ab	0.44 f-i
Marvi-2K	1.39 a	0.88 f	0.78 a	0.48 efg
Khirman	1.25 a-d	1.08 e	0.65 a-d	0.47 fgh
Imdad	1.08 de	0.76 fg	0.63 a-d	0.48 efg
Inqalab	1.19 b-e	0.77 fg	0.56 c-f	0.38 ghi
Mean	1.22	0.82	0.65	0.39

* Values under each parameter in a column bearing different letter(s) have statistically significant difference among themselves at $P \leq 0.05$.

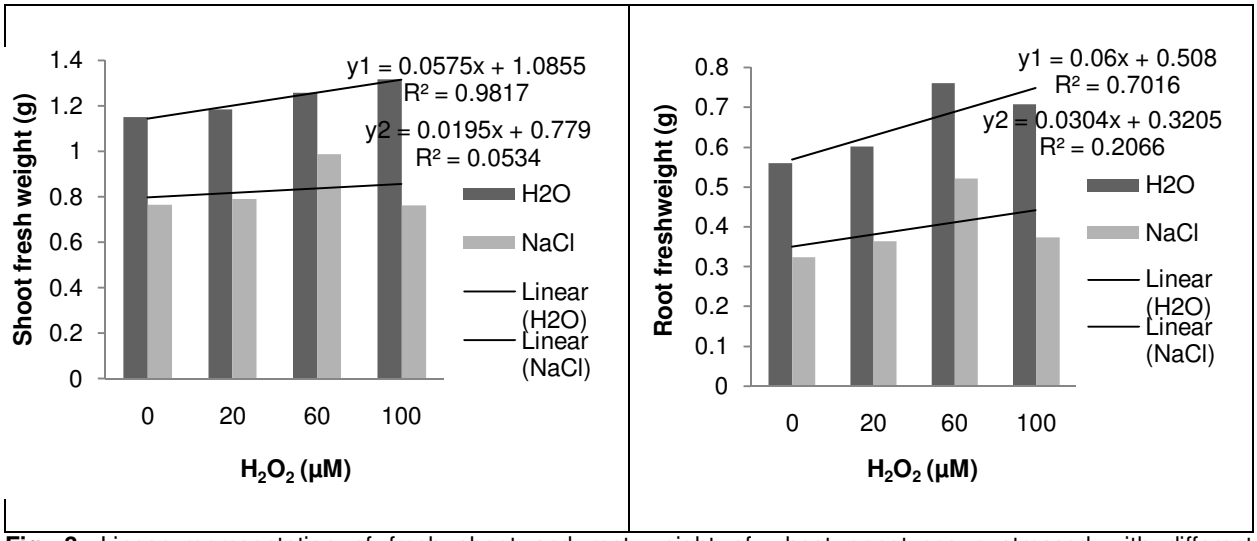


Fig. 3. Linear representation of fresh shoot and root weight of wheat genotypes eustressed with different concentrations of H_2O_2 under normal and saline conditions.

E. Moisture content in shoots and roots

Shoot moisture content had a slight significant difference ($P < 0.05$) among the wheat genotypes and H_2O_2 concentration; however, salinity rendered non-significant effect. Under H_2O_2 irrigation, the shoot water content was not substantially greater than under NaCl treated irrigation (Table 4). Genotype Anmol showed the highest shoot moisture content, which differed significantly only with Bhittai having the lowest moisture content, but both genotypes differed non-significantly with rest of the genotypes under H_2O_2 irrigation. The shoot water content of the Kiran and Khirman genotypes was comparatively higher under NaCl treated irrigation, but the difference among all the genotypes was statistically non-significant. Under NaCl treated irrigation, the shoot water content was relatively higher in genotypes Kiran and Khirman, but the difference among all the genotypes was statistically non-significant. The H_2O_2 rendered a linear increasing trend with increased concentrations except at 60 µM under H_2O_2 irrigation, but an erratic positive effect of H_2O_2 was observed under saline water conditions (Figure 4). Amazingly, at 100 µM concentration of H_2O_2 as compared to 60 µM, the shoot moisture content was higher if irrigated with normal water but it was reduced under saline conditions.

The moisture content in the roots of wheat genotypes irrigated with normal water showed non-significant difference, with Kiran having the lowest (77.5%) value (Table 4). The root moisture content was comparatively higher in the Sarsabaz, Anmol, Imdad and Khirman genotypes under H_2O irrigation, whereas the root water content was relatively higher in the Khirman, Sarsabaz and Imdad genotypes under NaCl treated irrigation. Statistical analysis found that H_2O_2 concentrations and genotypes were significantly influenced by the root moisture content ($P < 0.05$), while the salinity effects on root moisture were not important (Fig. 4). With rising H_2O_2 concentrations, a linear increase in root water content was observed. However, compared to NaCl treated irrigation, the findings were marginally better under H_2O_2 irrigation.

Table 4: Shoot and root water content of wheat genotypes eustressed with H_2O_2.

Genotypes	Shoot water content (%)		Root water content (%)	
	Water	NaCl	Water	NaCl
Anmol	84.4 a	81.6 ab	81.8 ab	77.2 b
TJ-83	84.2 ab	82.5 ab	80.4 ab	77.1 b
Moomal	83.3 ab	81.0 ab	80.4 ab	79.5 ab
Bhittai	80.1 b	80.8 ab	80.4 ab	80.3 ab
Kiran	83.4 ab	82.8 ab	77.5 b	79.6 ab
Sarsabaz	83.9 ab	82.0 ab	81.9 ab	82.9 a
Marvi-2K	84.2 ab	81.0 ab	80.6 ab	79.9 ab
Khirman	84.0 ab	82.8 ab	81.0 ab	83.3 a
Imdad	83.9 ab	80.7 ab	81.5 ab	81.9 ab
Inqalab	82.7 ab	81.1 ab	78.6 ab	79.8 ab
Mean	83.41	81.63	80.41	80.15

* Values under each parameter in a column bearing different letter(s) have statistically significant difference among themselves at $P \leq 0.05$.

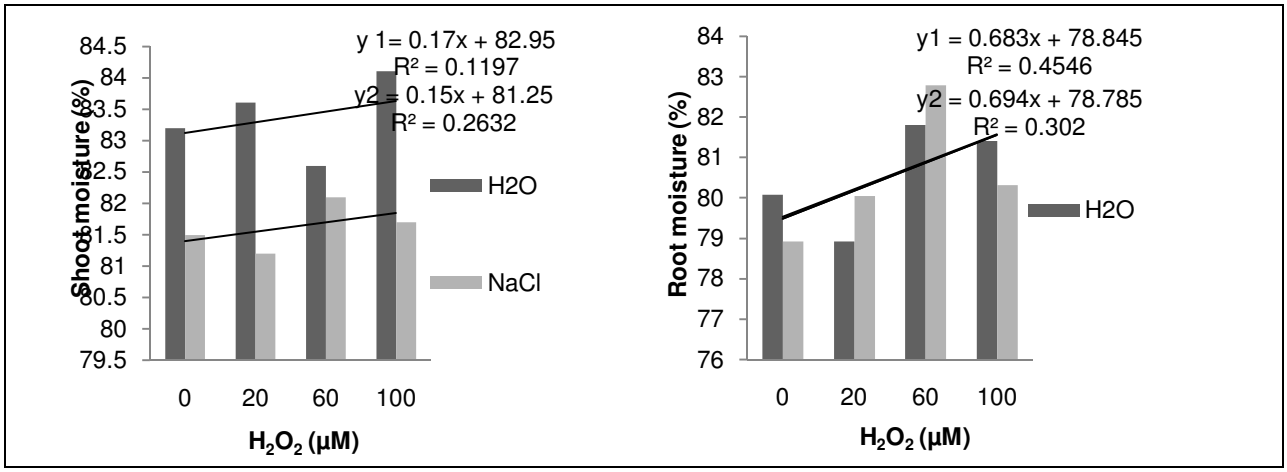


Fig. 4. Linear representation of shoot and root water content of wheat genotypes eustressed with different concentrations of H_2O_2 under normal and saline conditions.

The root/shoot ratio was significantly influenced ($P < 0.05$) by both concentrations and genotypes of H_2O_2 and salinity. Compared to NaCl treated irrigation, they had significantly higher values under H_2O_2 irrigation (Table 5). With rising H_2O_2 concentrations regardless of the water quality, the root/shoot ratio has been substantially improved on a continuous manner (Fig. 5). However, the increase in plant growth was more pronounced with simple irrigated H_2O_2 than with irrigation treated with NaCl. Salinity had a negative effect on the root/shoot ratio was clearly shown. The genotypic discrepancy suggested that the root/shoot ratio was comparatively higher with H_2O_2 irrigation in the genotypes Anmol, Marvi-2K and TJ-83; whereas the root/shoot ratio was relatively higher with NaCl handled irrigation in the genotypes Imdad, Anmol and Marvi-2K.The lowest value of root/shoot ratio was recorded for Bhittai and Kiran genotypes under normal water irrigation, and for Khirman, Kiran and Sarsabaz under NaCl irrigation stress.

F. Antioxidants activity

The antioxidants activity in wheat genotypes was studied under standard H_2O and NaCl treated irrigation, as affected by H_2O_2 seed soaking at different concentrations. Data analysis showed that the antioxidant activity differed significantly between genotypes and because of salinity (Table 5), while the effect of H_2O_2 concentrations was not significant (Table 5). Irrigation with saline water has resulted in a significant decrease in the antioxidants activity in most genotypes. However, there was, a non-linear pattern for antioxidant activity at various H_2O_2 concentrations, irrespective of water quality (Fig. 5). The genotypic response suggested that, under H_2O_2 treatment, antioxidant activity was significantly higher in the TJ-83, Inqalab, Sarsabaz and Imdad genotypes, whereas in the Kiran, Sarsabaz and Inqalab genotypes, antioxidant activity was relatively higher in the NaCl treatment. Under standard H_2O_2 and NaCl treated irrigation, the Moomal and Khirman genotypes returned the lowest antioxidant activity. In saline and non-saline conditions, there was a linear trend in antioxidant activity under saline and non-saline conditions, regardless of the genotypes as well as the H_2O_2 concentrations.

G. Correlations Analysis of phenotypic traits in the wheat

Analyses of correlation coefficient and linear regression among various phenotypic traits of wheat genotypes under the influence of salinity distress and H_2O_2 eustress have been presented in Table 6 and Fig. 6 (A-D), respectively.

Table 5: Root to shoot ratio and antioxidants activity of wheat genotypes eustressed with H_2O_2.

Genotypes	Root to shoot ratio		Antioxidants activity (µmol g^{-1})	
	Water	NaCl	Water	NaCl
Anmol	0.70 a	0.56 def	0.166 bcd	0.083 ab
TJ-83	0.67 ab	0.49 f-i	0.347 a	0.103 ab
Moomal	0.63 a-d	0.48 gj	0.088 d	0.027 b
Bhittai	0.54 e-h	0.47 g-j	0.136 cd	0.103 ab
Kiran	0.54 e-h	0.43 ij	0.227 a-d	0.186 a
Sarsabaz	0.57 c-f	0.45 ij	0.243 abc	0.148 ab
Marvi-2K	0.67 ab	0.55 efg	0.183 bcd	0.113 ab
Khirman	0.60 b-e	0.41 j	0.110 cd	0.065 ab
Imdad	0.64 abc	0.60 b-e	0.239 abc	0.093 ab
Inqalab	0.57 cde	0.47 hij	0.286 ab	0.120 ab
Mean	0.613	0.491	0.202	0.104

* Values under each parameter in a column bearing different letter(s) have statistically significant difference among themselves at $P \leq 0.05$.

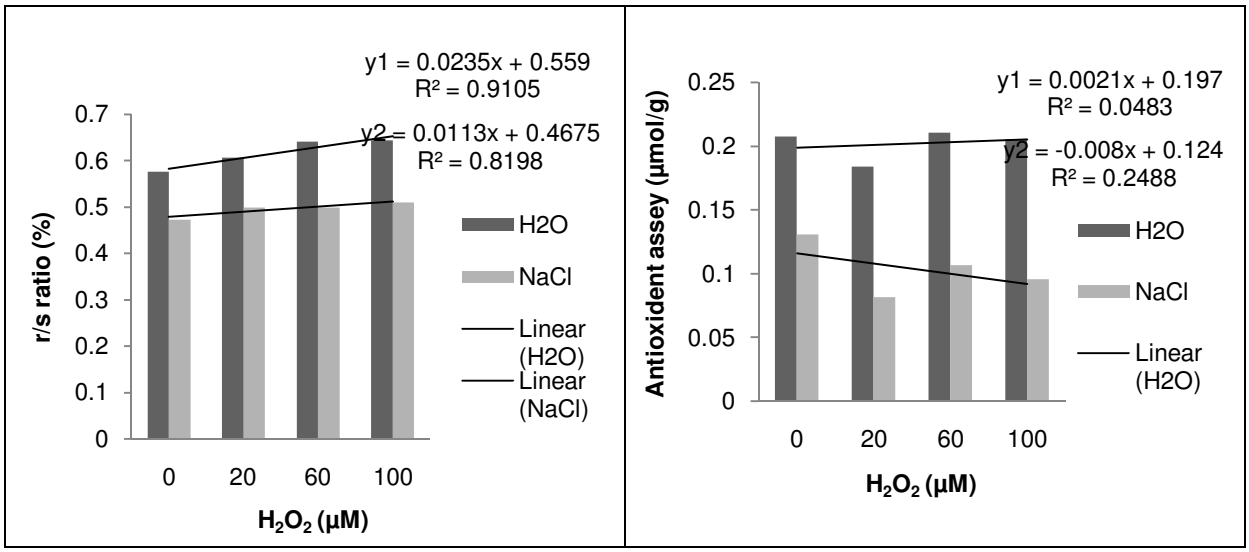


Fig. 5. Linear representation of root/shoot ratio and antioxidants activity of wheat genotypes eustressed with different concentrations of H_2O_2 under normal and saline conditions.

It is evident that the shoot length of the wheat had a very highly significant positive relationship with the root length (r = 0.88) at $P \leq 0.01$. Likewise, positive relationships between fresh shoot weight and fresh root weight (r = 0.86) were also recorded. The correlation value was non-significant for root/shoot ratio and shoot length (r = 0.38), but was positively significant for root/shoot ratio and root length (r = 0.63) at $P \leq 0.01$. Regression coefficient (b) values suggest that the shoot length was correspondingly increased by a unit increase in the root length (0.783 mm). Similarly, a unit increase in the corresponding increased shoot fresh weight of the root fresh weight (0,009 g). Whereas, the root/shoot ratio was consistently increased by 0.002 and 0.003 respectively by a unit increase in shoot and root volume. Coefficient of determination (R^2) suggested that the shoot length difference was due to its root length association (78 %) and the change in fresh shoot weight was due to its root fresh weight coalition (68 %). The difference disparity in the root/shoot ratio was due to its relation with the length of the shoot (15%) and the length of the root (40%). On these wheat growth parameters, the student's t-test was performed, which showed correlations between them. Student's *t*-test was performed these growth parameters of wheat, which showed correlation between them, the student's *t*-test was performed. The *t*-values measured were as follows: shoot length *vs.* root length (29.13), fresh shoot weight *vs.* fresh root weight (24.16). Root/shoot ratio vs. length of shoot (6.42), and ratio of root/shoot. *vs.* length of root (12.64). As measured at the 5% probability level, these t-values are higher than the book value, which means that the correlations are highly significant.

Table 6: Correlations of various seedling traits of wheat genotypes under the influence of salinity distress and H_2O_2 eustress.

Seedling traits	Correlation coefficient (r)	Regression coefficient (b)	Coefficient of determination (R^2)	Student's t-value
Shoot length and root length	0.88 **	0.783	0.78	29.13
Fresh shoot and fresh root weight	0.86 **	0.009	0.68	24.16
Root/shoot ratio and shoot length	0.38 NS	0.002	0.15	6.42
Root/shoot ratio and root length	0.63 *	0.003	0.40	12.64

** and * = Correlation is statistically significant at 1% and 5% probability level, respectively.
NS = Non-significant

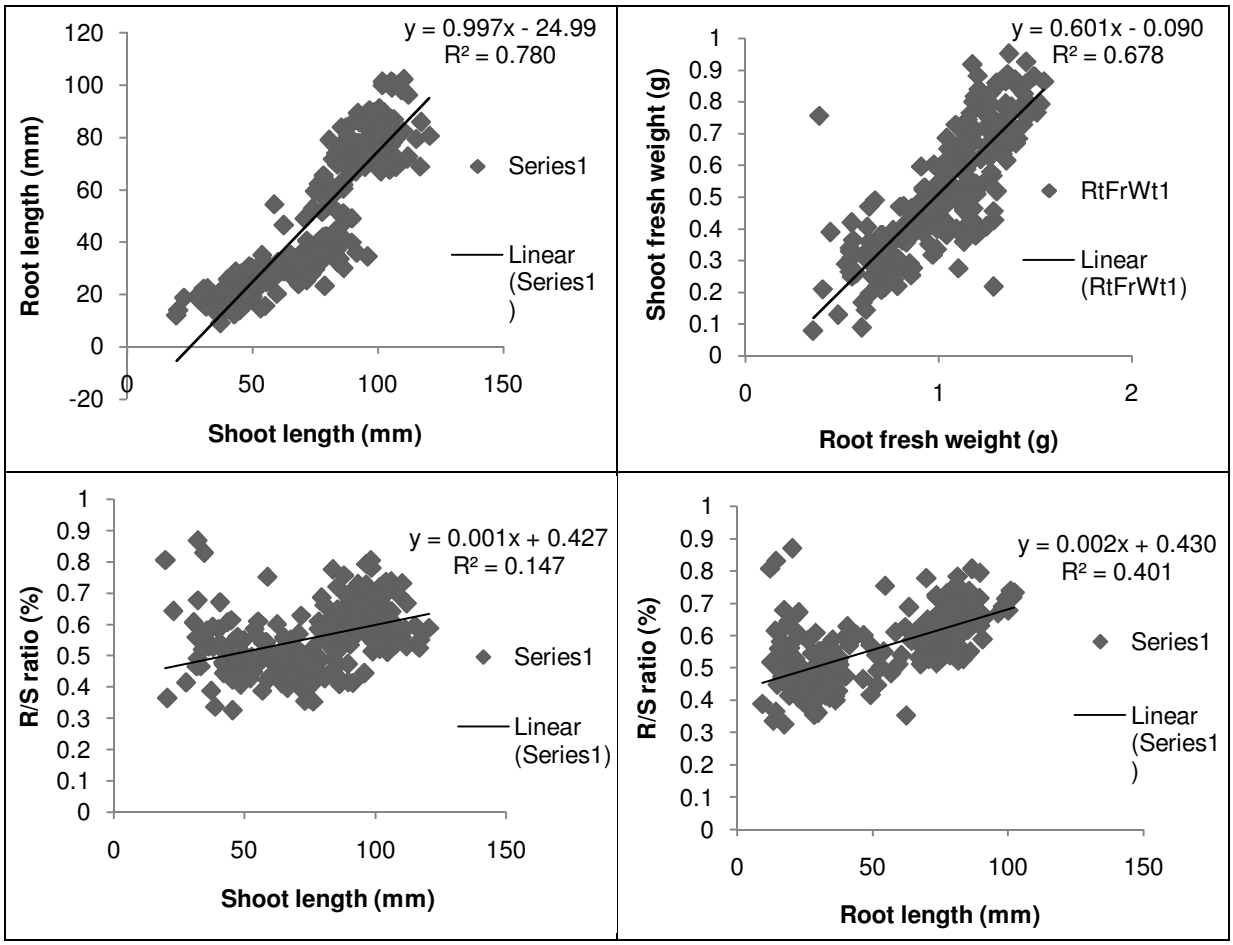


Fig. 6. Linear regression among various phenotypic traits of wheat genotypes under the influence of salinity distress and H_2O_2 eustress.

IV. DISCUSSION

Hydrogen peroxide acts as an osmotic adjustment agent, increasing the activity of starch hydrolyzing enzymes and providing better carbohydrates [14, 31]. H_2O_2 seed pretreatment alleviates germination delay and seedling growth by preventing the effects of salt by infusion into the seeds and by removing abscisic acid blockage (ABA). The deleterious effects of salinity on plant growth could therefore be reduced by H_2O_2 seed pretreatment [32]. With both saline and natural water irrigation, seed pretreatment of wheat genotypes with H_2O_2 at 60 µM concentration yielded satisfactory performance. Moomal, Bhittai, Marvi-2K and Khirman demonstrated a positive response under non-saline irrigation and had the highest germination (100 %). TJ-83 and Khirman achieved 100 % germination when under NaCl irrigation. These results indicated that H_2O_2 is an important antioxidant to increase seed germination under salinity conditions when added at a concentration of 60 µM, while lower (0 and 20 µM) or higher H_2O_2 (100

µM) concentrations resulted in lower germination of seeds compared to 60 µM.

Kilic and Kahraman (2016) [33] reported that the salt-induced inhibition of seed germination and seedling development was significantly alleviated by seed pretreatment with H_2O_2. Under saline water irrigation, growth attributes also reflected remarkable positive impact of H_2O_2 at 60 µM concentration with respect to germination percentage, mean germination time, length / weight of shoots and roots, and antioxidant activity. Earlier studies have shown that seed soaking enhances enzyme antioxidant activity by increasing glyoxysome activity in seeds [33, 34]. Hence, it has been significantly effective to improve seed germination and antioxidant defense system under salinity conditions [34]. The mean germination time (MGT) for water irrigation was relatively higher for Marvi-2K, but the lowest for TJ-83 and Inqalab; the highest MGT for Sarsabaz and the lowest for Inqalab was noted for NaCl irrigation. It was reported that seed presoaking enhanced seedling establishment with ascorbic acid, salicyclic acid and H_2O_2 [17, 35, 36].

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Seedlings growth attributes of wheat genotypes differed significantly under salinity condition due to its negative influence, but their seed priming with H_2O_2 reversed the salt effect. Under H_2O_2 irrigation, shoot length was highest in Bhittai and lowest in the Imdad genotype. The shoot length under NaCl irrigation was higher in the genotype [44]. For TJ-83 and Marvi-2K, the root length in H_2O_2 irrigation was highest, but lowest in Imdad genotype; however, the maximum root length in NaCl irrigation was in Khirman and lowest with non-significant difference in Bhittai genotype. Fresh shoot and root weight differed significantly among wheat genotypes for plain H_2O_2 and NaCl treated irrigation water. Under H_2O irrigation, fresh shoot weight was highest in Marvi-2K and lowest in Imdad; while under NaCl irrigation, higher fresh shoot weight was observed in Khirman and lowest in Bhittai. In addition, for Marvi-2K, fresh root weight was noted highest in only H_2O_2, and the lowest in Kiran and Inqalab; whereas the highest fresh root weight was in Marvi-2K and lowest in TJ-83 under NaCl irrigation. Rehman & Ali [35] suggested that sodium nitro prusside (SNP) and hydrogen peroxide (H_2O_2) seed priming as a single or combination treatment minimized the harmful impact of water stress on the growth of plants and other parameters.

The shoot moisture content was lower under NaCl irrigation, where Khirman and Kiran had the highest values, while Imdad had the lowest. The shoot moisture content was highest for Anmol and lowest for Bhittai under plain H_2O_2 irrigation. Root moisture content under H_2O_2 irrigation was highest in Sarsabaz and Anmol, and the lowest value was noted in Kiran and Imdad. However, with saline water irrigation, higher root moisture content values were obtained for Khirman and Sarsabaz, and the lowest for TJ-83 and Anmol genotypes. Salt stress-induced water shortage triggers oxidative stress by enhancing the production of reactive oxygen species (ROS), resulting in cell damage by oxidizing nucleic acids, lipids and proteins [37]. Hydrogen peroxide is a non-radical and relatively stable ROS that controls low concentrations of certain metabolic processes, such as assimilation, stomatal conductance, cell growth and development and plant reaction to biotic and abiotic stresses [25]; but above the threshold level, it promotes oxidative damage resulting in cell death [26].

The root/shoot ratio with H_2O_2 irrigation was highest for Anmol and lowest in the genotypes of Bhittai and Kiran, while it was higher in Imdad and lowest in Khirman under NaCl irrigation. However, with an increased H_2O_2 concentration for seed priming regardless of the water content, the root/shoot ratio was substantially improved in a steady-state manner. Researchers found a highly positive influence on the agronomic and physiological characteristics of wheat from seed soaking [4, 39]. Hydrogen peroxide has shown to diminish the effect of salinity in wheat seedlings by increasing proline concentration. Antioxidants activity in the seedlings was greater in TJ-83 with only H_2O_2 irrigation, and in Kiran genotype under saline water irrigation. It was lowest in Khirman under plain water and in Moomal genotype with saline water treatment.

The antioxidant mechanism induced by the treatment with hydrogen peroxide resulted in higher fresh and dry biomass weight of roots and shoots inducing drought tolerance of maize plants [14, 40]. The results of seed soaking are correlated with the repair and construction of nucleic acids, increased protein synthesis and membrane repair, which enhances the function of antioxidative enzymes and increases the activity of glyoxysome enzymes [34, 41]. More recently, positive effects of hydrogen peroxide under drought stress condition have been reported, and it has been found that H_2O_2 increases the antioxidant activities thus reducing drought effect [17, 38, 42] concludes that the extent of relationship showed a positive correlation between straw weight and shoot biomass. Soaking seeds with 60 µM of H_2O_2 could mitigate the deleterious effect of salinity in wheat seedlings. Antioxidant activation of the H_2O_2 signal seed continued to compensate for ion-induced oxidative damage. As correlation genotypes of wheat represented distinct agronomic characteristics of gene effects, statistical analysis of agronomic traits in wheat could be informative [17, 43].

V. CONCLUSION

The effect of seed soaking with H_2O_2 at different concentrations (0 to 100 µM) on salinity tolerance of wheat genotypes was explored in this investigation. Best results of seed soaking in terms of germination and seedling development were obtained with H_2O_2 at 60 µM concentration under both saline and normal water irrigation. Plant growth at 100 µM did not further enhance than that with 60 µM H_2O_2 concentration. Genotypes differed significantly for their response to salinity as well as seed priming with H_2O_2. Under NaCl treatment, Khirman performed the highest and Inqalab did the lowest. Fresh shoot and root weight differed significantly with normal H_2O_2 irrigation and NaCl treatment. Under normal water irrigation, wheat seeds eustressed with H_2O_2 significantly improved the germination and plant growth attributes. Whereas in salt stress conditions, seeds eustressed with H_2O_2 reduced the damage to plant growth and physiological traits against the un-primed seeds (control) in all the wheat genotypes. Antioxidative activity remained unchanged in normal water irrigation treatment, but it reduced under saline condition. The NaCl treatment affected all the wheat seedlings parameters negatively; while antioxidative effect of H_2O_2 caused significant improvement under salinity stress conditions. It concludes that seed soaking with H_2O_2 at 60 µM concentration could be effective to minimize the damaging effect of salinity in wheat seedlings.

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