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INDUCED SEED TREATMENT WITH HYDROGEN PEROXIDE (H_2O_2) PROMOTES PHYSIOLOGICAL, BIOLOGICAL CHANGES AND SALT-TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

The present study was undertaken to study the effect of exogenously applied six hydrogen peroxide H_2O_2 concentrations (0, 20, 40, 60, 80 and 100 µM) as seed primer on two wheat varieties (Khirman and Inqalab) under salt and non-salt water levels (0 and 100 mM NaCl). The oxidizing effects were measured and estimated using the wheat index of different salt and non-salt water levels, as well as H_2O_2 soaking seed at varying fixations and concentrations. The H_2O_2 as a seed primer impacted on growth, yield and physiological and biochemical aspects such as moisture content, sodium potassium substance and sodium potassium content under H_2O_2 and NaCl levels. The results revealed that the exogenous application of hydrogen peroxide was effective in increasing tolerance of wheat under salt stress. The Khirman and Inqalab varieties could be established and cultivated under saline conditions. 60µM treatment of H_2O_2 is seen with the strongest impacts. Progress has contributed to enhance physiological and biochemical features of stress outflow, which promote growth.

Keywords: antioxidant, hydrogen peroxide (H_2O_2), reactive oxygen species (ROS), salinity, varieties

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most significant cash crop, cultivated throughout the world (Sara *et al.*, 2015). FAO's latest forecast for world cereal production in 2020 has been trimmed 2.5 million tons since the previous report in September and now stands about 2762 million tons. According to the most recent Pakistan Economic Survey (GoP., 2020), over 830,169 MT has been procured under grains procurement campaign for year 2019-2020 in order to fulfill the domestic requirement as well as for exporting. Salinity is one of the abiotic stresses affecting crop production. Over 800 million hectare of land across the world is affected by salinity (Alemán *et al*., 2009). The ion toxicity and osmotic stress due to high salinity result in generation of reactive oxygen species (ROS), cause damage to lipids, proteins and DNA (Esfandiari *et al*., 2007). Reactive Oxygen Species (ROS) such as hydroxyl radical (OH), O_2 and H_2O_2 in seed physiology are usually considered as toxic molecules (Koornneef *et al.,* 2002).

However, exogenously applied H_2O_2 ameliorates seed germination in many plants (Bailly, 2004). The H_2O_2 signals the activation of antioxidants in seed, which persists in the seedling to offset the ion-induced oxidative damage and the treatment tends to reduce plant H_2O_2 levels, which was a significant effect (Panhwar *et al.,* 2017). This has been explained by the fact that the scavenging activity for H_2O_2 is sufficiently high, resulting in the production of O_2 for mitochondrial respiration (Bailly, 2004). In contrast, H_2O_2 promotes seed germination indicating that H_2O_2 itself possibly promotes seed germination rather than O_2. In seed biology it performs important role in the growth processes, at early embryogenesis, and participate in the mechanisms underlying radical projection during seed germination and seed aging (Kibinza *et al.,* 2006). Individual effects of H_2O_2 and salt on growth and yield indicated that overall, H_2O_2 increased growth and yield, while salinity decreased it. However, the increase in yield after H_2O_2 treatment, even under well-watered conditions was very interesting (Panhwar *et al*., 2017). Antioxidant enzyme activities in various plants are correlated with

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their tolerance to salinity (Abdellaoi *et al*., 2017: Boughalleb *et al*., 2020).

Salinity influences antagonistically the metabolism in plants and causes significant changes affecting extreme loss of crop production (Hala *et al.*, 2015; Geo *et al.,* 2018). In all the development stages, variety and salt treatment impacts were watched. Salt pressure caused 33%, 51% and 82% decreases in germination life, seedling shoot dry issue and seed grain yield, separately. Saline soils when saturated with excessive dissolved salts are reduced in their metric potential therefore plant root water uptake capacity is affected (Sheldon *et al*., 2017; Tedeschi *et al*., 2017). Plants therefore face water stress in addition to ion disequalibrium when in hyper-osmolarity growth conditions it also negatively interferes with turgor pressure and cellular expansion (Alemán *et al.,* 2009).

Boughalleb *et al.* (2020) indicated that polygonum equisetiforme in response to salinity, phenolic compounds with high antioxidant activities considered a salt-tolerant species able to survive at levels up to 300 m*M* NaCl. The salinity mediated physiological drought can have its drastic impact to inhibit the stomatal conductance in addition to leaf photosynthetic activity (Tavakkoli *et al*., 2010). Plant growth in saline soils is specifically affected by ion toxicity of Na^+ or many other ions (Zhu, 2015). Soils, however, saturated with Na^+ trigger K^+ deficiency primarily due to competition through high-affinity K^+ transporters in plant root cells (Alemán *et al*., 2009). Hamna *et al*. (2019) finded the methods in which salt inhibits plant function and the correlating responses of plants to salt stress. According to their findings plants divided into two categories in regards to salt stress: glycophytes and halophytes.

Plants are devised with the pace of root and shoot water misfortune because of salt pressure displayed critical negative relationship with shoot K^+, yet not with shoot Na^+ and shoot K^+ Na^+ proportion. Plants are devised with an unpredictable cell reinforcement framework to forestall the destructive impacts of responsive and Reactive Oxygen Species (ROS) which assume a significant job in pressure resistance or in stress tolerance (Farhoudi *et al*., 2015). Mechanisms and systems of reactive oxygen species detoxification exist in all the plants and incorporate initiation of enzymatic just as non-enzymatic cell reinforcements or antioxidants (Fercha *et al*., 2014). Spike organs in treated wheat showed lower accumulations of hydrogen peroxide (H_2O_2). Compared to the flag leaf under drought stress, higher activity of antioxidant enzymes higher proline content in the spike organs were observed. The studies revealed that the improved drought stress resistance in spike organs was correlated with the elevated phenylpropanoid pathway. It could sustain a better water status for the spike and thus contribute to comparatively higher photosynthesis and lower damage to the membrane (Xiaorui *et al*., 2020)

The hydrogen peroxide pretreatment seems to assume a job in improvement of searching the produced receptive and reactive oxygen species under saline conditions (Hala *et al.*, 2015). The work with respect to the similar job of plant cancer prevention agent frameworks or antioxidants systems, osmolytes and collection of poisonous and helpful particles comparable to saltiness stress resilience in grain crops is restricted. The present study was carried out to elaborate the hydrogen peroxide (H_2O_2) induced seed treatment promotes physiological, biological changes and salt-tolerance in wheat (*Triticum aestivum* L.)

METERIALS AND METHODS

Pot experiment was conducted in the glass-house of Environmental Centre, University of United Kingdom. Healthy seeds of two wheat varieties (Khirman and Inqalab) were sterilized with 5% sodium hypochlorite (NaOCl) solution for three minutes and transferred to H_2O_2 solution at different concentrations (0, 20, 40, 60, 80 and 100 µM). After 8 hours of soaking, seven seeds were sown in compost filled earthen pots of 12 x10 cm size then the pots were irrigated with distilled water according to field capacity and plants were raised in pots to assess them for salt-tolerance.

After 8 days of planting (DAS) the plants were treated with 0 and 100 mM NaCl. Sodium chloride (NaCl) treatment was applied in three parts at the pace of 33.3 mM per each split to each pot at each sixth day aside from control for which refined and distilled water was applied.

The water was restored each fourth day in control conditions resembled and paralleled with treated plants. The 100 ml of water was applied by necessity of pots. The experiment was consisted of five replications and all treated and untreated pots were analyzed for H_2O_2 treatment applied against salt stress by using Complete Randomized Block Design (RBCD). The greatest day time temperature of the glasshouse was set at 22˚C and least night temperature

18˚C with light-dull pattern of 16:8 hours and light power of 600 lux.

Table 1. Physico-chemical properties of compost used

Electrical conductivity	**450 - 550µS**
Standard pH	5.3 - 5.7
Sodium (Na)	280 mg/l
Phosphoras (P)	160 mg/l
Potassium (K)	350 mg/l

Number of spikes (plant^{-1})

Number of spikes plant^{-1} of each variety were recorded after harvesting.

Spike weight plant^{-1} (g)

Spike weight plant^{-1} of each variety was recorded after harvesting.

Seed index (g)

Seed index was randomly taken from each selected sample and weighed in grain on electronic balance. It was calculated as

1000-grain wt. = 100-grains wt × 10

Plant moisture content (%)

After eight days of both treatments the shoot fresh and dry weight was calculated using electric balance before and after drying at (60^{O}C in oven for 48 days). Moisture content (%) of each plant was calculated by using the following formula (Barker *et al*., 2007)

Moisture content (%) = [(fresh mass- dry mass)/ fresh mass] × 100

Ion analysis

Leaves were isolated from shoots, which were broiler dried, powdered and prepared to get extract through dry debris strategy (Chapman and Pratt, 1961). The substance and contents were resolved in the concentrate got from leaf dry matter with fire photometry utilizing Jenway flame photometer.

Potassium content (%)

Same as above

Sodium/ Potassium (Na/K) ratio (%)

Na/K was calculated by the following given formula:

Sodium/ Potassium = NA/K × 100

Statistical analysis

The factual investigation was done through electronic programming system of 8.1 versions. The LSD value for mean examination was calculated and determined just if the overall treatment F test was huge at a likelihood of ≤ 0.05 (Gomez and Gomez, 1984).

RESULTS

Number of spikes (plant^{-1})

The response of two wheat varieties (Inqalab and Khirman) for the no spike of plant^{-1} in Figure 1 showed significant effect of salinity and non-significant effect of H_2O_2 concentrations, varieties and their interactions on of spikes plant^{-1}. It did not showed linear response to various concentration. Hence in Inqalab and Khirman the H_2O_2 at 40-60 µM will be optimum for spike plant^{-1}. The number of spikes was also significantly reduced by salinity, and as a result spike weight and grain weight were also significantly reduced.

Spike weight (plant^{-1})

The response of two wheat varieties (Inqalab and Khirman) for the spike weight plants^{-1} in Figure 2 showed significant ($P<0.05$) effect of salinity varieties and of H_2O_2 concentrations. Maximum spike weight plants^{-1} of Inqalab under H_2O and NaCl irrigation was observed when seeds were soaked with H_2O_2 at 60 µM concentration against spike weight plant^{-1} in control, while variety khirman under H_2O and NaCl irrigation resulted in maximum spike weight plant^{-1} then seed was soaked with H_2O_2 at 20 µM and 40 µM concentrations, against in control. The spike weight plant^{-1} in most cases started decreasing when H_2O_2 concentration exceeded 60 µM. However, H_2O_2 irrigation proved to be effective for higher spike weight plant^{-1} (Figure 8) as compared to NaCl irrigation; while in varieties, Inqalab showed better.

Seed index (g)

The seed index of two wheat varieties Inqalab and Khirman was examined; and showed in Figure 3. The analysis of variance illustrated significant ($P<0.05$) effect of H_2O_2 concentrations, salinity and varieties. Maximum seed index of variety Inqalab under H_2O_2 and NaCl irrigation was recorded when seed was soaked with H_2O_2 at 60 µM concentration against seed index in control, while variety Khirman under H_2O_2 and NaCl irrigation resulted in maximum seed index when seed was soaked with H_2O_2 at 60 µM and 100 µM against seed index in control, respectively. The seed index decreased when H_2O_2 concentration for seed soaking increased beyond 60 µM. However, NaCl treated irrigation resulted in higher seed index value as compared to H_2O irrigation; while in varieties, Inqalab showed higher seed index value than khirman. The results in (Figure 9) indicated a linear adverse response due to effect of hydrogen peroxide (H_2O) on the wheat varieties given normal water (H_2O_2) and positive response in saline water (NaCl) levels.

Moisture content (%)

The moisture content of Inqalab and Khirman wheat varieties as influenced by H_2O_2 seed soaking at different concentrations under salt and non salt conditions were measured in Figure 4 that moisture content was influenced by H_2O_2 seed soaking concentrations ($P<0.05$) and varieties ($P<0.05$), while non-significance influenced by salinity ($P>0.05$). The moisture content of Inqalab variety under H_2O and NaCl treated water system as recorded was relatively high when seeds were splashed with H_2O_2 at 20 µM fixation against moisture content in charge, while variety Khirman under H_2O and NaCl water system brought about most extreme moisture content when seed was drenched and soaked with H_2O_2 at 20 µM against moisture content in charge, separately. The moisture content diminished when H_2O_2 focus for seed soaking expanded past 20 µM. Regardless of NaCl treated water system brought about higher moisture content when contrasted with H_2O irrigation; while varieties, Inqalab indicated higher moisture content than Khirman. H_2O_2 at 20 µM focuses would be sufficient so far, the moisture content is concerned. The outcomes in (Figure 10) indicated a linear adverse response because of impact of hydrogen peroxide (H_2O_2) on the wheat varieties given water (H_2O) and saline water (NaCl) levels.

Na^+ content (%)

The Na content of wheat varieties as affected by H_2O_2 seed soaking at various concentrations and salinity (H_2O and NaCl) was determined; and the data were shown in Figure 5. Statistically Na content was significantly affected by H_2O_2 seed soaking treatments and salinity ($P<0.05$), while non-significant effect for varieties ($P>0.05$). Maximum Na content of Inqalab variety under H_2O and NaCl treated irrigation was recorded when seed was soaked with H_2O_2 at 80 and 100 µM concentrations H_2O and NaCl treated irrigation in control, while Khirman variety under H_2O and NaCl irrigation resulted in maximum Na content when seed was soaked with H_2O_2 at 60 and 100 µM against control, respectively. However, NaCl treated irrigation resulted in higher Na content as compared to H_2O irrigation; while in varieties, Inqalab showed relatively higher Na content than Khirman. The results in (Figure 11) indicated a linear positive response due to effect of hydrogen peroxide (H_2O_2) on the wheat varieties given normal water (H_2O) and saline water (NaCl) levels in relation to Na content of wheat.

K content (%)

Plant's K content of as influenced by H_2O_2 concentrations and salinity (H_2O and NaCl) was determined in two wheat varieties (Inqalab and Khirman); and the data are presented in Figure 6. The analysis of variance indicated that the effect of H_2O_2 seed soaking treatments, salinity, varieties as well as their interactions on plant K content was statistically non-significant. Maximum plant K content of variety Inqalab under H_2O and NaCl treated irrigation was recorded when seed was soaked with H_2O_2 at 60 and 80 µM concentrations against equally plant K content in control; while variety Khirman under H_2O and NaCl irrigation resulted in maximum pant K content when seed was soaked with H_2O_2 at 60 and 100 µM against control, respectively. Although, the plant K content increased with application and increasing the H_2O_2 concentrations, but there was no linear trend of effectiveness. However, H_2O irrigated plants had higher K content as compared to plants given NaCl treated irrigation; while in varieties, the plants of Inqalab had relatively higher K content as compared to those of Khirman. The results in (Figure 12) indicated a linear positive response due to effect of hydrogen peroxide (H_2O_2) on the wheat varieties given normal water (H_2O) and saline water (NaCl) levels in relation to K content of both wheat varieties.

Na^+ K^+ content (%)

The Na+ K^+ content of wheat varieties as affected by H_2O_2 seed soaking at various concentrations and salinity (H_2O and NaCl) was determined; and the data are shown in Figure 7 that Na^+ K^+ content was significantly affected by H_2O_2 seed soaking treatments ($P<0.05$), while non-significant effect for varieties ($P>0.05$). Maximum Na^+ K^+ content of variety Inqalab under H_2O and NaCl treated irrigation was recorded when seed was soaked with H_2O_2 at

100 µM concentrations H_2O and NaCl treated irrigation in control, while variety Khirman under H_2O and NaCl irrigation resulted in maximum Na K$^+$ content when seed was soaked with H_2O_2 at 20 and 80 µM against control, respectively. However, NaCl treated irrigation resulted in higher Na$^+$ K$^+$ content as compared to H_2O irrigation; while in varieties, Inqalab showed relatively higher Na/K content than Khirman. The results indicated a linear positive response due to effect of hydrogen peroxide (H_2O_2) on the wheat varieties given normal water (H_2O) and saline water (NaCl) levels in relation to Na$^+$ K$^+$ content of both wheat varieties.

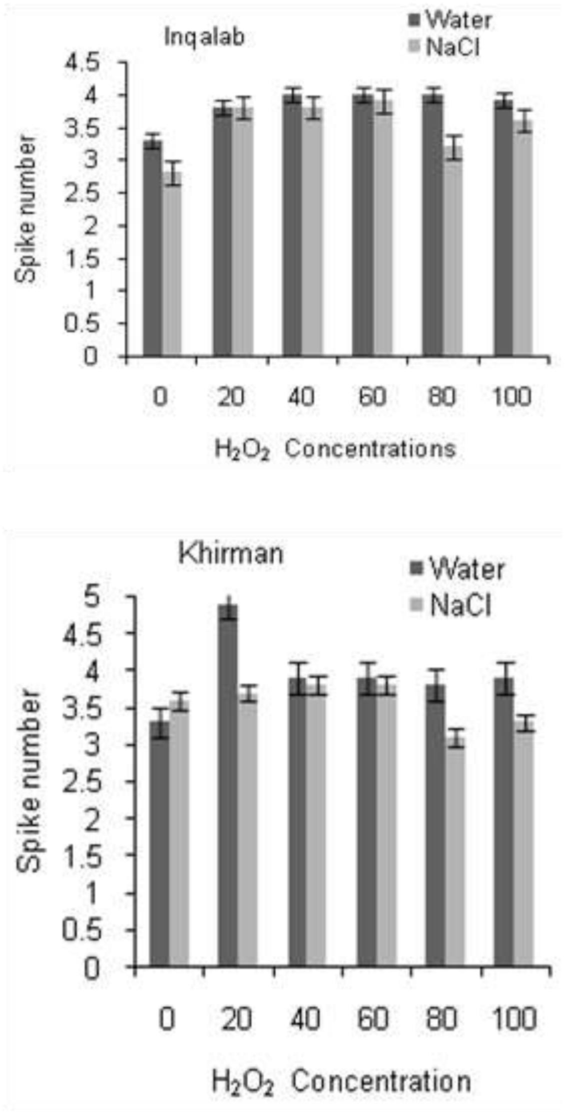

Figure 1. Effect of H_2O_2 (µM)) on spike weight (g) of two wheat varieties

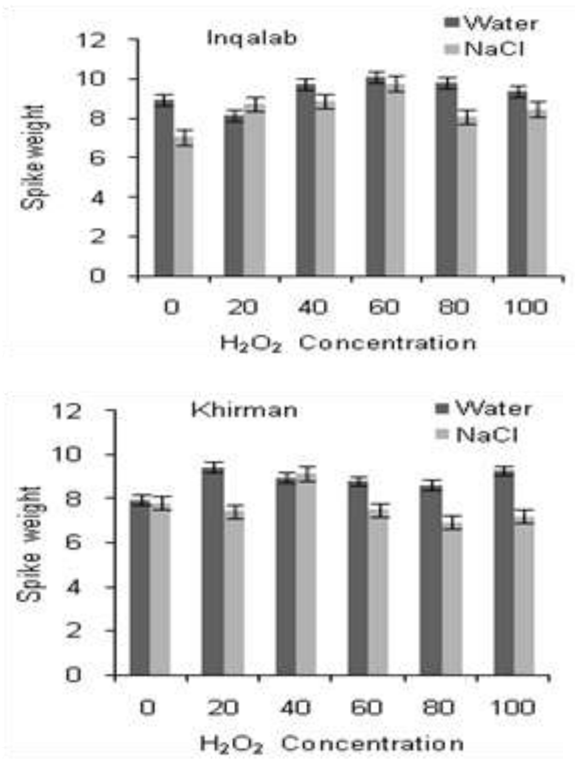

Figure 2. Effect of H_2O_2 (µM)) on spike weight (g) of two wheat varieties

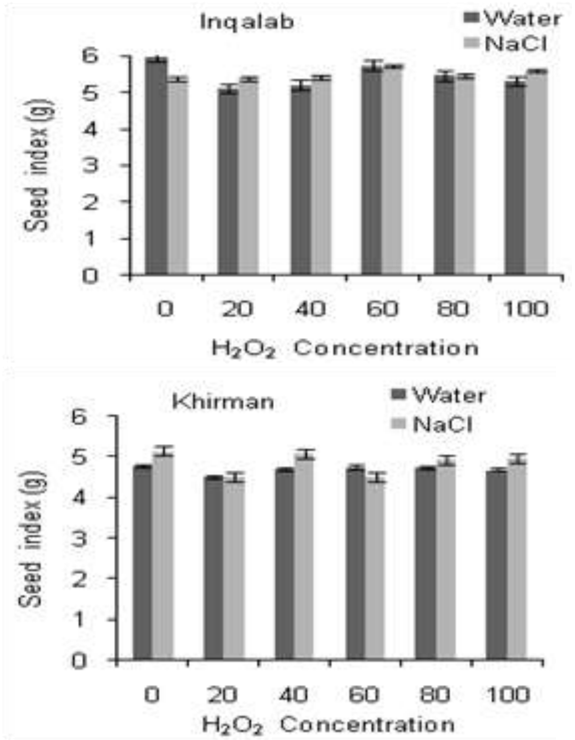

Figure 3. Effect of H_2O_2 (µM)) on seed index (g) of two wheat varieties

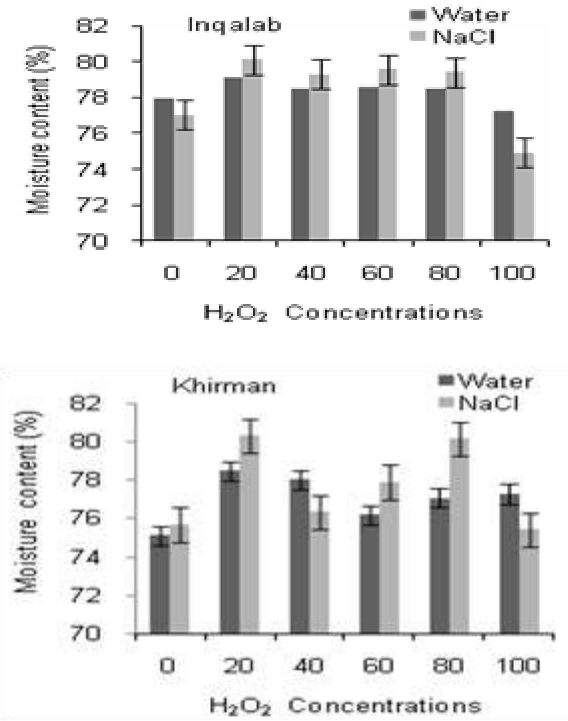

Figure 4. Effect of H_2O_2 on moisture content (%) of two wheat varieties

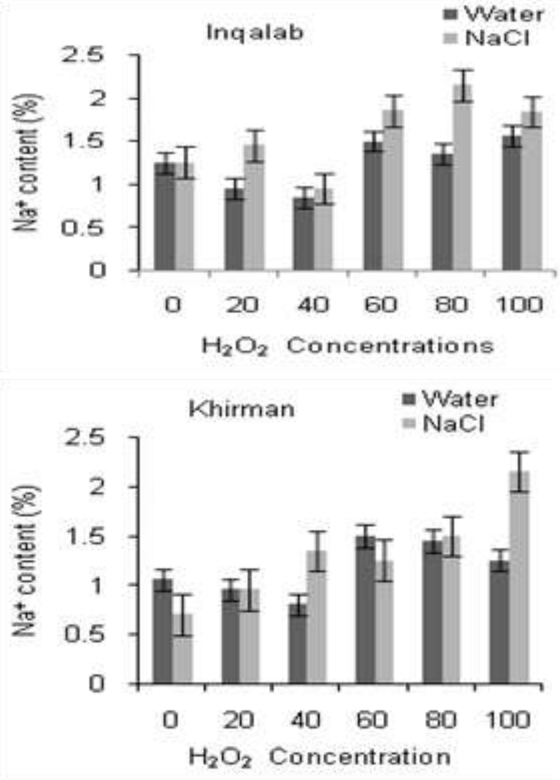

Figure 5. Effect of H_2O_2 (µM) on sodium content (%) of two wheat vrieties

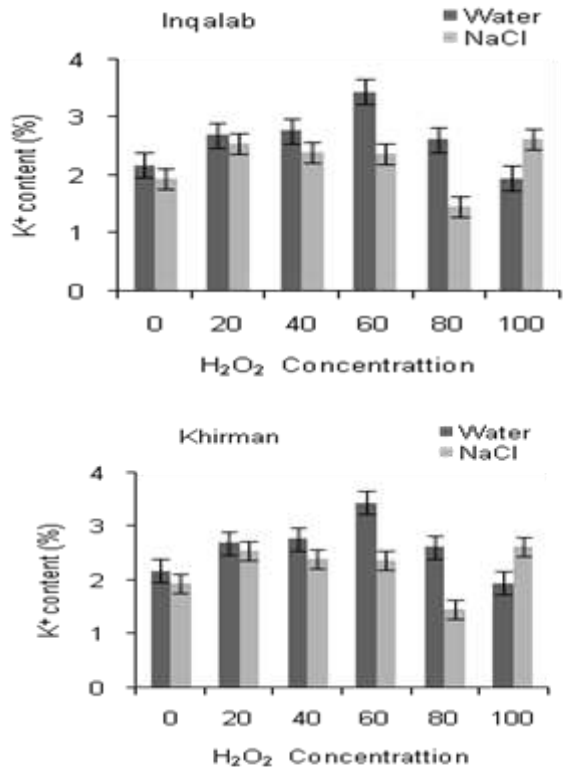

Figure 6. Effect of H_2O_2 (µM) on potassium content (%) of two wheat varieties

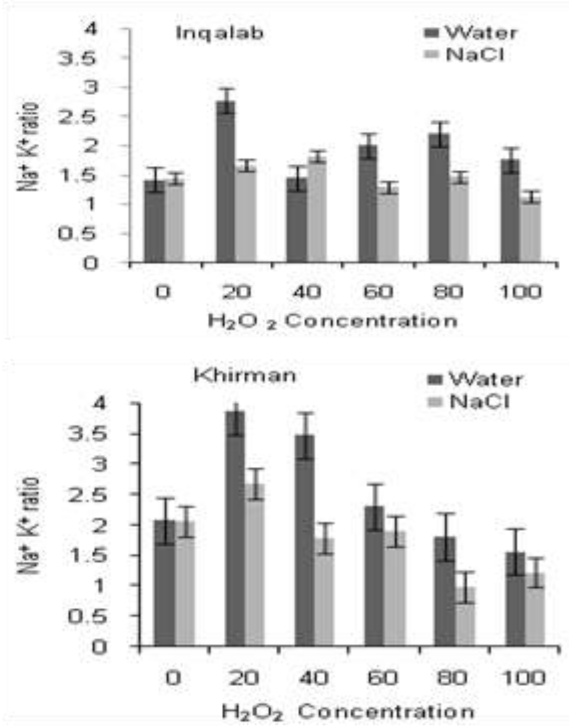

Figure 7. Effect of H_2O_2 (µM) on N/K ratio (%) of two wheat varieties

LINEAR REPRESENTATION AT DIFFERENT CONCENTRATION OF H_2O_2 OF WHEAT VARIETIES

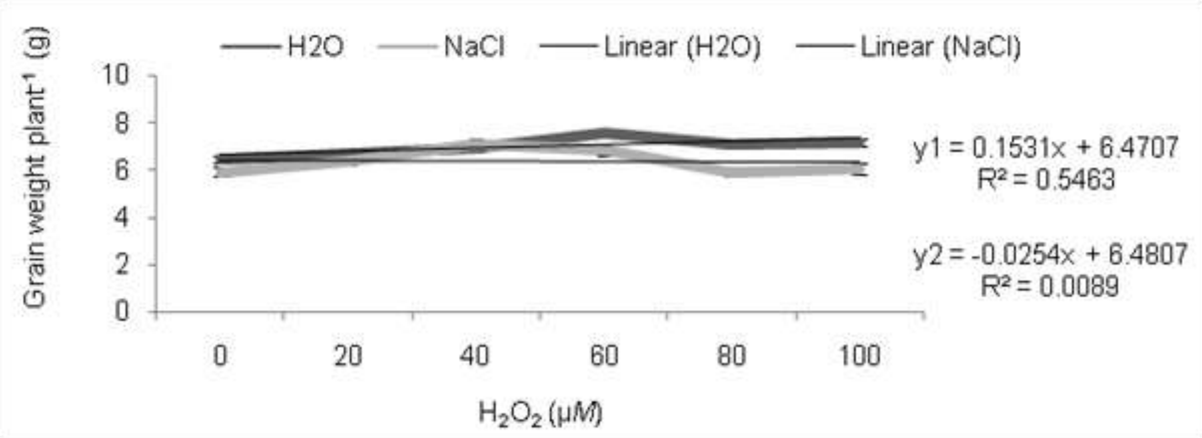

Figure 8. Linear representation of grain weight plant^{-1}(g) at different concentration of H_2O_2 (µM) of two wheat varieties

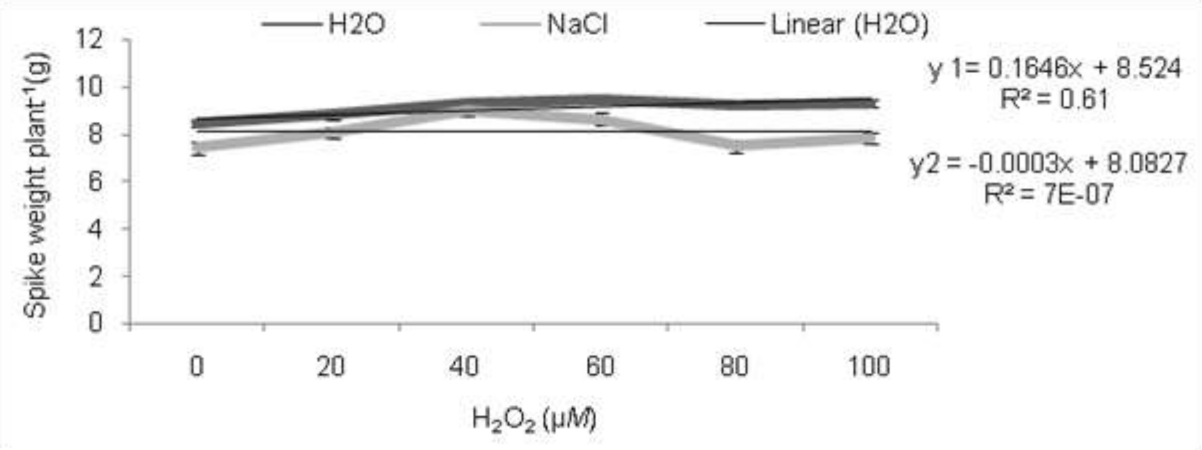

Figure 9. Linear representation of spike weight plant^{-1} (g) at different concentration of H_2O_2 of two wheat varieties

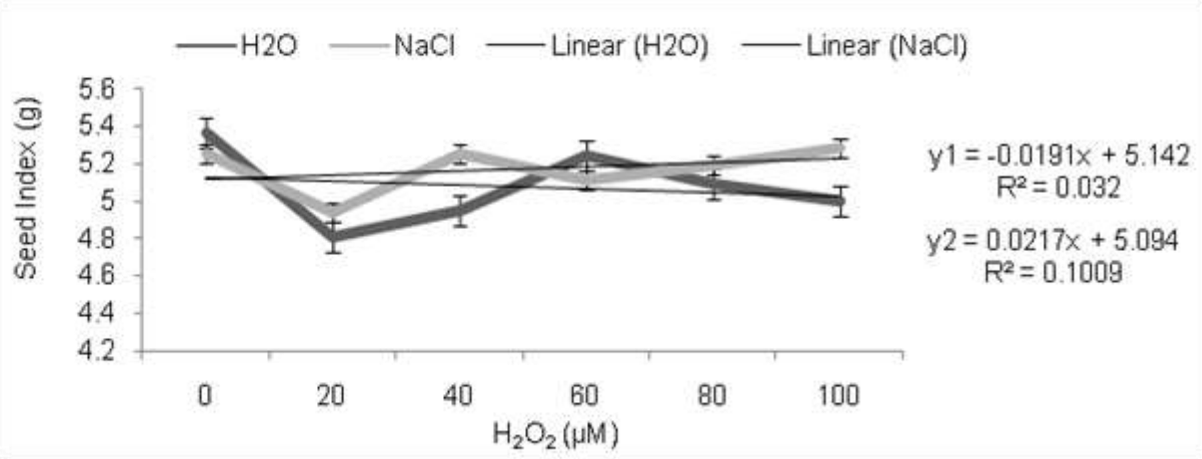

Figure10. Linear representation of seed index (g) at different concentration of H_2O_2 of two wheat varieties

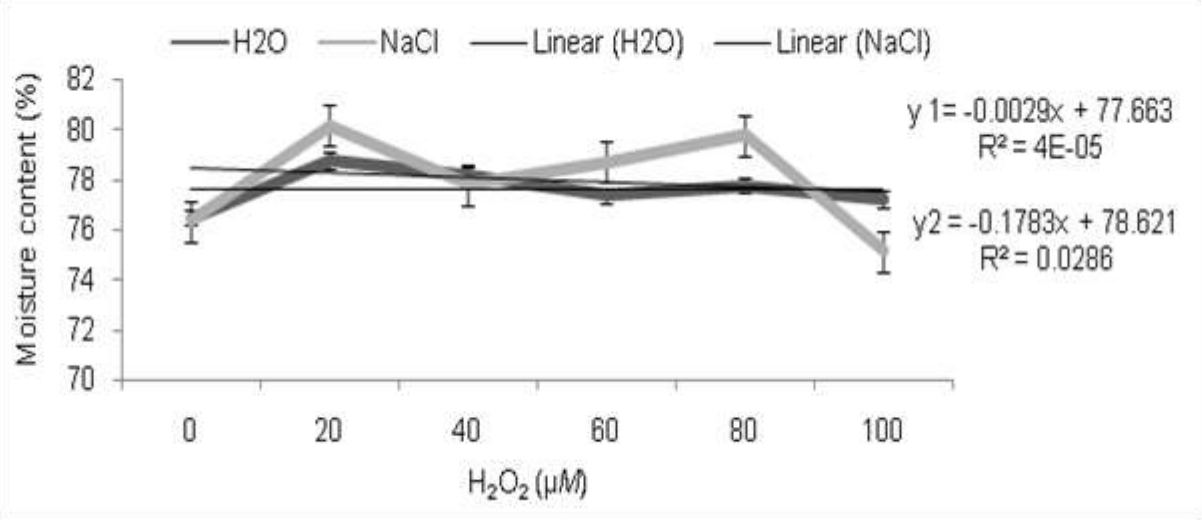

Figure 11. Linear representation of moisture content (%) at different concentration of H_2O_2 of two wheat varieties

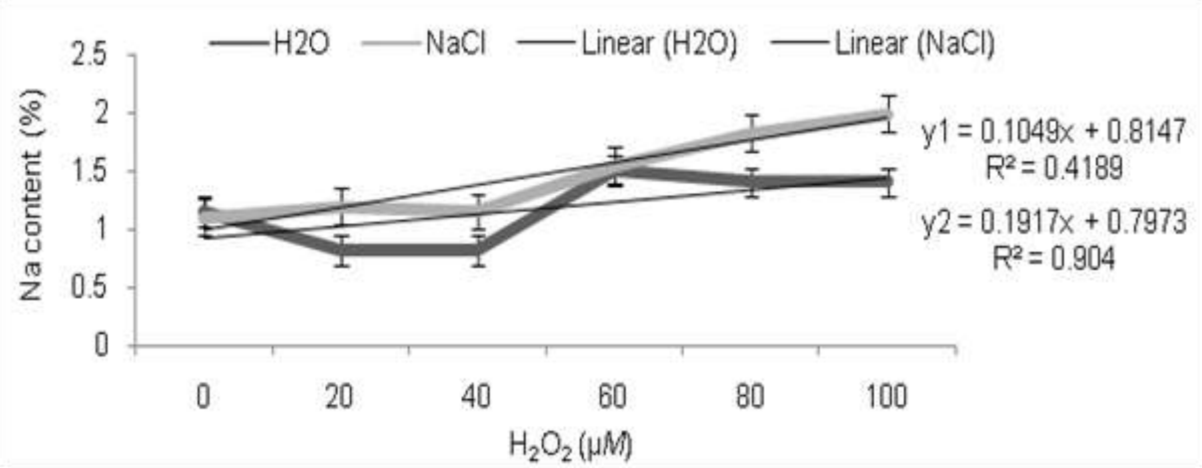

Figure 12. Linear representation of Na content (%) at different concentration of H_2O_2 of two wheat varieties

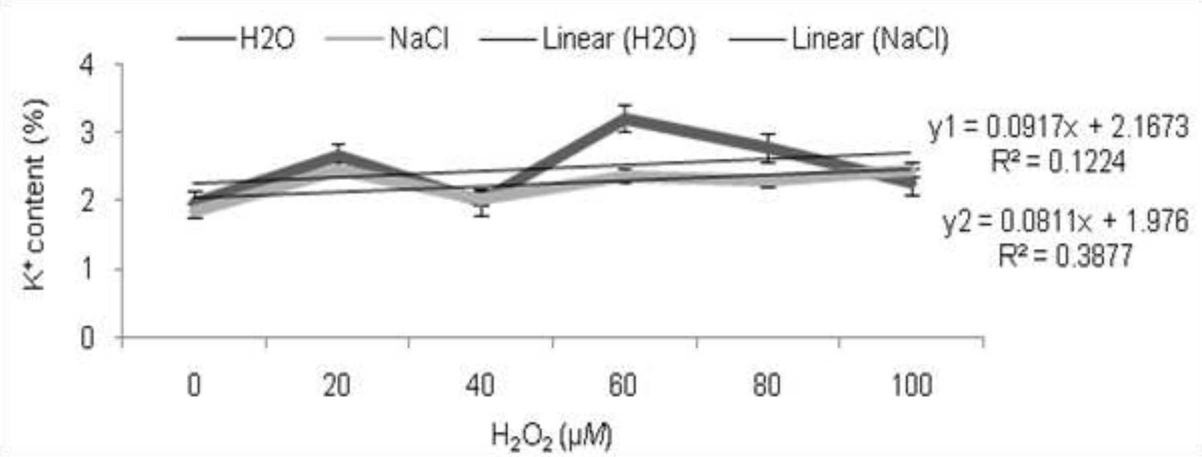

Figure 13. Linear representation of K content (%) at different concentration of H_2O_2 of two wheat varieties

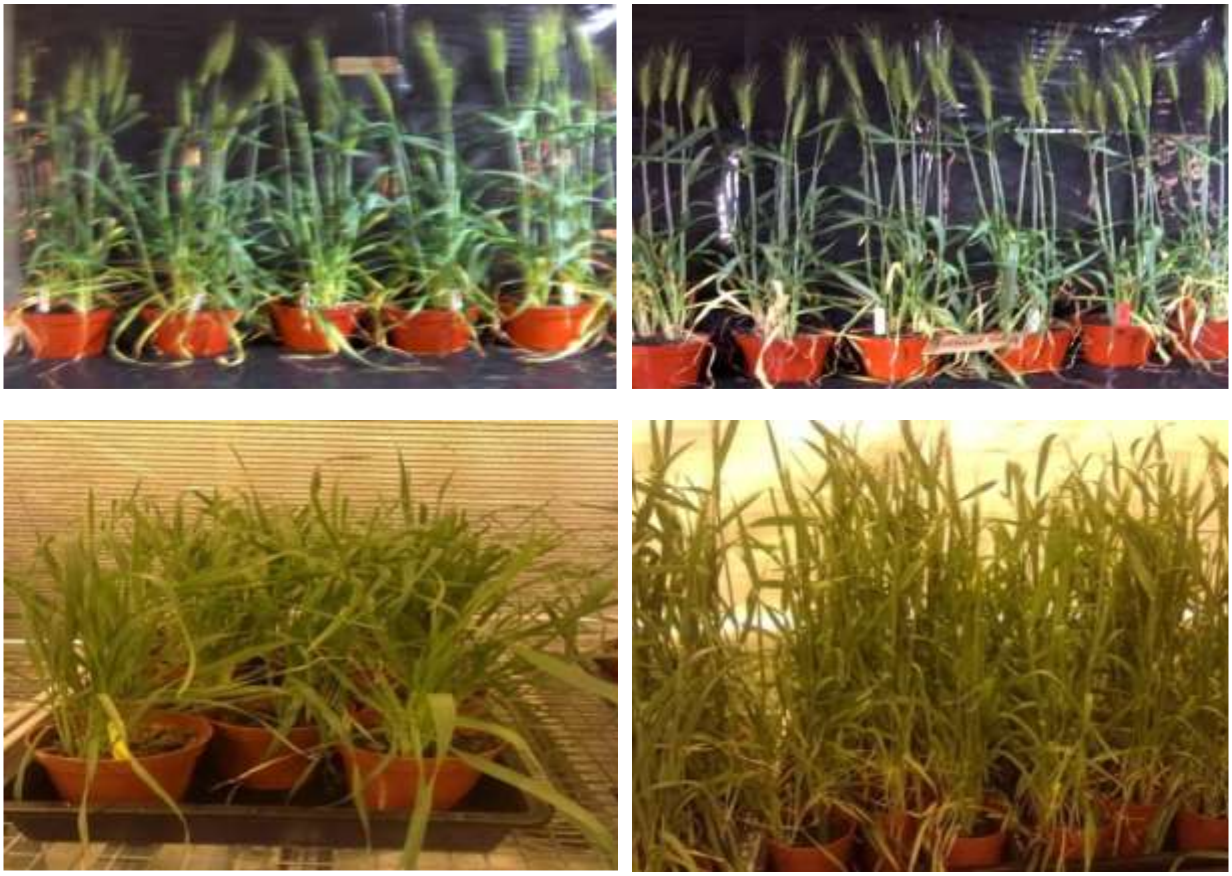

Plate 1. Effect of different contcentrations of H_2O_2 on germination of wheat genotypes Inqalab and Khirman of two wheat varieties

DISCUSSION

Studies were carried with the intention to induced seed treatment with hydrogen peroxide (H_2O_2) promotes physiological, biological changes and salt-tolerance in wheat (*Triticum aestivum* L). The data showed a clear effect of salinity stress on the growth of wheat plants. Panhwar *et al*. (2017) observed the number of spikes was also significantly reduced by salinity, and as a result spike weight and grain weight were also significantly reduced (Wael *et al.,* 2014) under heat stress Hydrogen Peroxide plays a key role in regulating the activity of antioxidant enzymes. Supporting the findings of the present research regarding varieties, (Shirazi *et al*., 2005); found enhanced growth of Bhittai and Khirman wheat varieties under saline soils resulting high K+ Na$^+$ ratio and these varieties have ability of increased K+ Na$^+$ ratio. K$^+$: Na$^+$ ratio, chlorophyll, proline, SOD, CAT and may be used as potential biochemical and physiological selection criteria for screening of salt-tolerance in wheat varieties (Honghong *et al*., 2015). Panhwar *et al*. (2017) observed the change in seed index was due to its connection with seed weight. Similarly, variation in seed weight was due to its link with spike weight. Sodium content increases in the organ but the percent of increase varies considerably among the plant organs especially at severe salinity and the opposite pattern is observed in the accumulation and distribution of ratio decreases in response to salt stress in root and shoot of wheat (Tammam *et al.,* 2008). The varieties having ability of enhanced K$^+$ Na$^+$ discrimination, might perform better under saline conditions when sufficient potassium is applied in the rooting medium (Shirazi *et al.,* 2005; Honghong *et al.,* 2015).

Data on ion content shows whether salinity resistance is identified with rejection of salt from the plant, or resilience to salt that enters the plant. The Na content expanded at the same time with expanding H_2O_2 concentrations. The plant K content expanded with application and expanding the H_2O_2 concentrations however there was no direct pattern of viability. It demonstrates that H_2O_2 increases nutrient use productivity under salt pressure condition. Panhwar *et al*. (2017) concluded that H_2O_2 treatment have beneficial effects, around 60 µM and increased with increasing H_2O_2 concentration, with yield reducing again in the plants with the highest levels of H_2O_2 treatment

Resilience to salt pressure is a complex natural marvel administered by a few physiological and hereditary variables, and it is development stage explicit (Haq *et al.,* 2010). Investigations completed under controlled conditions were not presented to those conditions that win in salt-affected soil, for example, spatial and worldly heterogeneity of soil physical and chemical properties, high diurnal temperature varieties, low mugginess and the nearness of dry spell pressure (Munns *et al.,* 2006). H_2O_2 seed treatment seemed to protect the salt sensitive varietiy; Inqalab against seed yield reductions caused by salinity, but had limited effect on Khirman (Panhwar, 2017).

A biotic stress applies huge effect on plant's development, improvement and efficiency. Profitability of harvest plants under salt pressure is lingering behind on account of our constrained information about physiological, biochemical, epigenetic and atomic instruments of salt resistance in plants. The examination meant to research physio-biochemical, atomic records and safeguard reactions of chose wheat cultivars to recognize the most differentiating salt-responsive varieties and the components related with their differential reactions. Hamna *et al*. (2019) examined the methods of salt inhabits plant function and the correlating responses of plants to salt stress.

Physio-biochemical attributes explicitly film steadiness record, cell reinforcement potential, osmo-protectants and chlorophyll substance, estimated at vegetative stage, were utilized for multivariate examination to identify the most differentiating varieties. Hereditary and epigenetic investigations showed the potential systems related with differential reaction of the wheat varieties under salt stress. Better cancer prevention agent potential, layer strength, expanded gathering of osmolytes/ phytophenolics, and higher K^+ Na^+ proportion under 200 m*M* NaCl stress recognized Kharchia-65 to be the most salt-open minded cultivar (Kumar *et al.,* 2017). A positive significant correlation between antioxidant DPPH and TPA, TFC, TPC, CAT, and APX suggests a vital protective role in controlling oxidative stress through the scavenging process.

CONCLUSION

The H_2O_2 soaking treatments increased nutrient effectiveness under salt pressure condition. Exogenous H_2O_2 application has lessened the deleterious effect of salt-stress on wheat growth characteristics. The plant Na^+ and K^+ content expanded with increasing the H_2O_2 concentrations. Those progressions prompted the declaration of stress improved physiological characteristics, which bolster development and growth under salinity. The tolerant varieties would do well to capacity to keep up stable osmotic potential, bringing about the fundamentally higher dry issue creation saw under salt pressure. The recognized salt resistant varieties could be utilized as guardians in rearing for new assortments with improved salt resistance just as in further hereditary examinations to reveal the hereditary systems overseeing salt pressure reaction in wheat varieties.

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AUTHOR’S CONTRIBUTION

M. Panhwar: Designed and conducted research

A. M. Jakhar: Conducted experiment and wrote paper

N. Soomro: Data analysis/ interpretation

S. Panhwar: Manuscript language improved

A. R. Jamali: Provided laboratory Material and seeds

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