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The Role of Presoaking in Hydrogen Peroxide and their Involvement in Salt Tolerance in Wheat Genotypes

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Abstract: The studies was conducted to compare two wheat genotypes (Inqalab and Khirman) for salinity tolerance under H_2O_2 seed soaking (0, 20, 40, 60, 80 and 100 µM concentrations); inducing salinity by 100 mM NaCl and the effects were compared by H_2O water treatment. The data showed H_2O_2 levels under H_2O *vs* NaCl treatment in genotype Inqalab and Khirman showed, seed emergence, plant height, leaves plant^{-1}, tillers plant$^{-1,}$ grains plant^{-1}, grains weight plant^{-1} and shoot biomass plant^{-1} improves germination, and the germination increases with increasing H_2O_2 concentration. The H_2O_2 treatment tends to cause plants to be slightly larger; they were slightly taller and had more leaves. These differences were statistically significant, but were quite small. Similarly, H_2O_2 treated plants produced the same number of tillers as controls. However, the data suggested that more of the tillers in treated plants go on to produce spikes, because spike number was significantly greater in treated plants. As a result, seed weight was also significantly increased by H_2O_2 treatment. Regardless of salinity stress, H_2O_2 seed treatment improved plant yield. Other details for grain weight, Inqalab had higher yield than Khirman and H_2O_2 treatment increased yield for both varieties, but the effect was greater in Inqalab. It could be seen that for grain weight, salt reduced yield in Inqalab when there were no H_2O_2 treatment, but treatment tends to minimize the effect of salt. That did not happen in Khirman. H_2O_2 seed treatment seemed to protect the salt-sensitive genotype, Inqalab, against yield reductions caused by salinity, but had limited effect on Khirman. 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. The most beneficial effects were seen around 60 µM H_2O_2, with yield reducing again in the plants with the highest levels of H_2O_2 treatment and results concluded that H_2O_2 treatment improved germination, and increased with increasing H_2O_2 concentration.

Keywords: Wheat, stress, genotypes, salinity, antioxidant, hydrogen peroxide (H_2O_2).

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is widely grown throughout the world (Faostat. 2014; Sara *et al.* 2015). World wheat production 2015/2016 shows 733.14 million metric tons, around 0.83 million tons more than the previous month's projection. In Pakistan it shows 25,100,000 metric tons (WASDE, 2016). The anticipated decline from 2013/14 would be mainly on account of a small reduction in plantings, outweighing expectations of above-average yields. Salinity is one of the foremost abiotic stresses negatively influence the development and plant growth as salinization of cultivated land is increasing globally It limiting crop productivity which diminished emergence and seedling growth attributes in all wheat cultivars (Khaliq *et al.* 2015). The past researches show positive impact of seed soaking on the germination and yield attributes of wheat. Jie *et al*., (2002) reported that seed soaking resulted in accelerated germination in wheat.

The imposition of biotic and abiotic stress conditions such as drought and salinity are known to raise concentrations of reactive oxygen species (ROS) such as hydrogen peroxide, super oxide and hydroxyl ions, resulting in oxidative damage at the cellular level (Zhang *et al.*, 2001) production while seed pretreatment. Hydrogen peroxide has beneficial effects on the plants. Its effect on plants can be extremely beneficial if used in the right concentrations and conditions (Williams, 2003). It produced predominantly in plant cells during photosynthesis and photorespiration and to a lesser extent in respiration processes. It plays a crucial role as a signaling molecule in various physiological processes. The potential role of H_2O_2 in the photosynthetic mode of carbon assimilation, such as C4 metabolism and CAM (Crassulacean acid metabolism) is discussed and speculate that early in the evolution of oxygenic photosynthesis on Earth, H_2O_2 could have been involved in the evolution of modern photo system II.(Slesak *et al*., 2007).

Exogenously applied H_2O_2 ameliorates seed germination in many plants and 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 as perform respiratory inhibitors, indicating that H_2O_2 itself possibly promotes seed germination rather than O_2. Hala, *et al*, (2015) showed that the primarily prominent hydrogen peroxide pretreatment appears to play a role in enhancement of scavenging the generated reactive oxygen species under saline conditions.

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However, Better understanding of physiological aspects of salinity stress tolerance mechanisms will not only help in cloning of genes involved in salt stress tolerance, development of transgenic and to chalk out accurate screening techniques ultimately aiding to crop improvement in saline soils. Thus, the present study was carried out to the intention the role of presoaking in hydrogen peroxide and their involvement in salt tolerance in wheat genotypes.

2. MATERIAL AND METHODS

The pot experiment was conducted to intention the role of presoaking of wheat genotypes in hydrogen peroxide to evaluate the physiological changes and their involvement in salt tolerance in the growth house of Lancaster Environmental Centre University of Lancaster, United Kingdom. The seeds of two wheat genotypes were selected i-e Khirman and Inqalab were obtained from Nuclear Institute Of Agriculture, Tandojam, (NIA), healthy seeds were chosen and sterilized with 5% sodium hypochlorite (NaClO) solution for three minutes and were transferred in different levels 0, 20, 40, 60, 80 and 100 µM of H_2O_2 for 8 hours. According to field capacity five seeds were sown in compost filled pots (12×10 cm) and distilled water was given. Additional plants were then insipid out leaving two plants in each pot after 8 days. Both salinity treatments (distilled water and 100 mM NaCl) were applied after 8th days of sowing in four splits to two sets of pots at every 5th day. The minimum day time temperature of the glasshouse was at 22˚C, and minimum night temperature 18˚C with light intensity of 600 µmol M-^{2} S-^{1}. Further seed emergence, plant height, leaves plant^{-1}, tillers plant^{-1},grains plant^{-1}, grains weight plant^{-1} and shoot biomass plant^{-1} respectively were recorded.

Seedling emergence (%): Seedling emergence of seedling was recorded daily by the formula of (Ellis and Roberts, 1981). After completion of germination in all pots, uniform seedlings (thinned to 2 per pot were grown for one month prior to carryout determination.

Plant height (cm): Plant height was measured from soil surface to the tip of the spike at about half grain filling stage.

Number of leaves plant^{-1}: Numbers of leaves were counted per plant after applied both treatments.

Number of tillers plant^{-1}: The number of productive tillers plant^{-1} were counted and recorded at maturity stage.

Number of grains plant^{-1}: The numbers of seeds spike-1 of each replication per genotype were recorded after harvesting.

Grain weight plant^{-1}(g): After harvesting, each plant was threshed and cleaned separately. The grains were weighed by electronic balance.

Total shoots biomass plant^{-1}(g): Total shoot biomass per plant was observed at the time of harvesting.

Statistical analysis

The statistical analysis was done through computerized soft ware programme of statistic 8.1 version. The LSD value for mean comparison was calculated only if the general treatment F test was significant at a probability of ≤ 0.05 (Gomez and Gomez, 1984)

3. RESULTS

Seed emergence (%)

The effect of seed soaking with hydrogen peroxide (H_2O_2) and given normal water (H_2O) and saline water (NaCl)] on seed emergence of wheat genotypes in figure-1 demonstrated significant effect of H_2O_2 concentrations, genotypes and salinity. Seed emergence showed improvement under seed soaking with H_2O_2 at 40µM onward concentration, resulted a linear development in seed emergence. In Inqalab under H_2O treatment increased seed emergence simultaneously with increasing concentration of H_2O_2 while soaking seed before sowing reached maximum at H_2O_2 concentration of 80µM and slightly decreased at 100 µM H_2O_2.

Plant height (cm)

Plant height in figure-2 demonstrated significant effect of H_2O_2 concentrations, genotypes and salinity. Genotypic behavior of wheat in response to H_2O and NaCl under seed soaking with different H_2O_2 concentrations varied unevenly. However, plant height in Inqalab and Khirman followed an adverse direction when H_2O_2 concentration exceeds 60 µM and 80 µM, respectively. Hence, for soaking seed in Inqalab, the H_2O_2 at 60µM and for Khirman 80µM concentration would be enough to result optimum plant height.

Number of leaves plant^{-1}

The number of leaves plant^{-1} of wheat genotypes was investigated and the data was presented in figure-3 described significant effect of H_2O_2 concentrations on number of leaves plant^{-1} and non-significant due to genotypes and salinity. However, it seemed that number of leaves plant^{-1} in Inqalab and Khirman did not show promising results when H_2O_2 concentration exceeds 40µM and 60µM, respectively. Hence, for soaking seed in Inqalab, the H_2O_2 at 40-60 µM and for Khirman 60 µM concentration will be optimum for leaves plant^{-1}.

Number of tillers plant^{-1}

The response of two wheat genotypes for the number of tillers plant^{-1} in figure-4 showed significant effect of salinity and non-significant for H_2O_2 concentrations, genotypes and their interactions on number of tillers plant^{-1}. The number of tillers plant^{-1} did not show a linear response to various H_2O_2 concentrations. Hence, in Inqalab and Khirman, the H_2O_2 at 40-60µM will be optimum for tillers plant^{-1}.

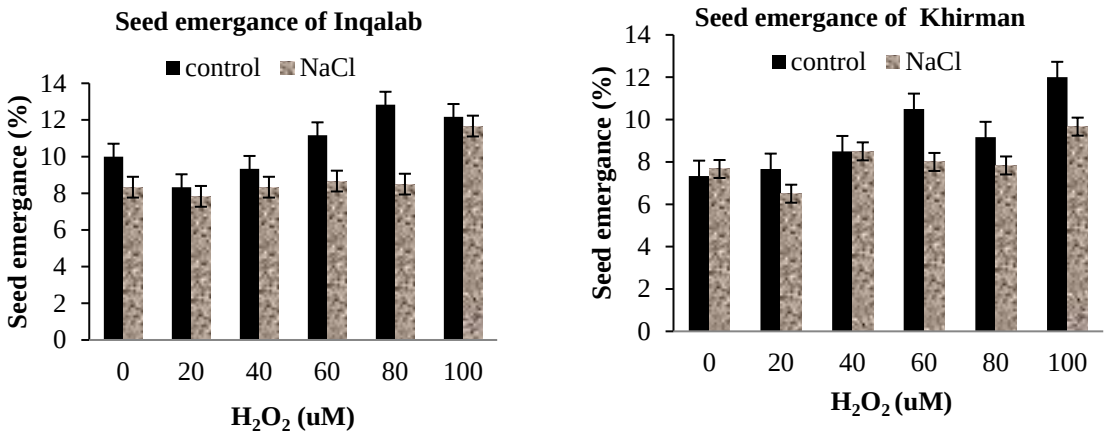

Fig. 1: Effect of H_2O_2 (µM) on seed emergence (%) on wheat genotypes.

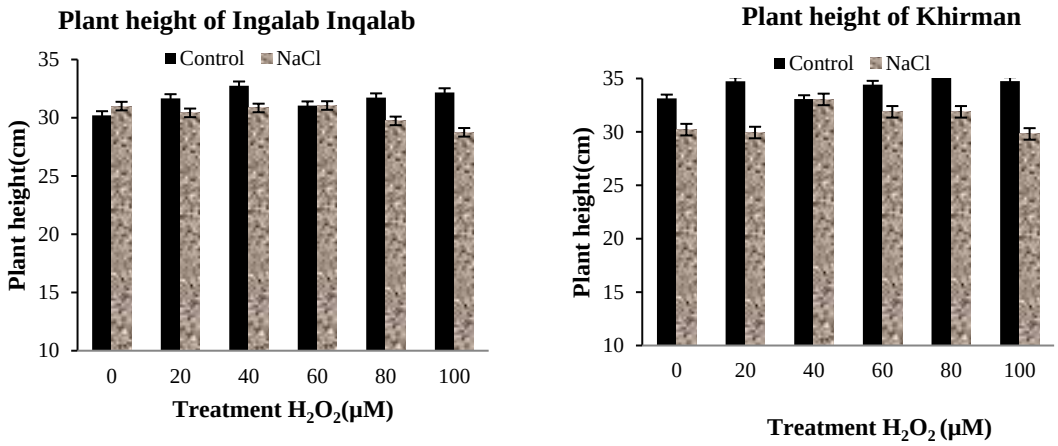

Fig. 2: Effect of H_2O_2 (µM) on plant height (cm) plant^{-1} of wheat genotypes

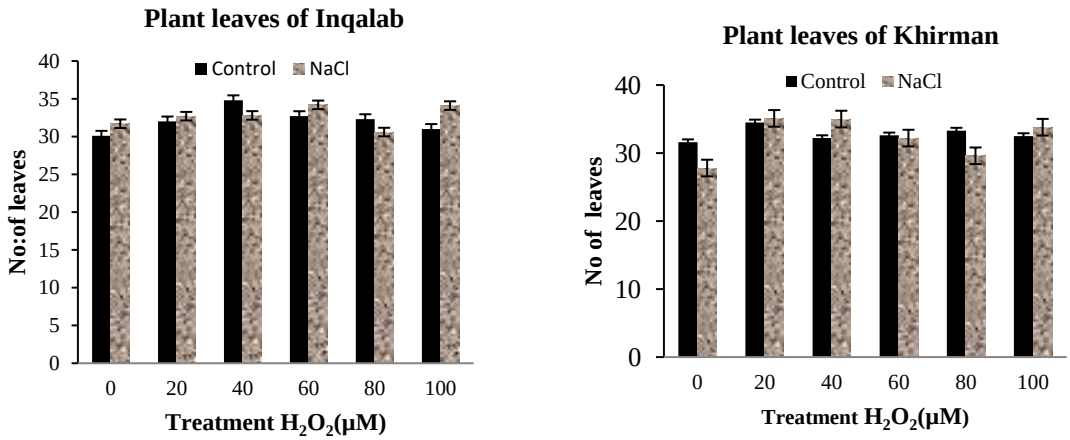

Fig. 3: Effect of H_2O_2 (µM) on No; of leaves plant^{-1} of wheat genotypes

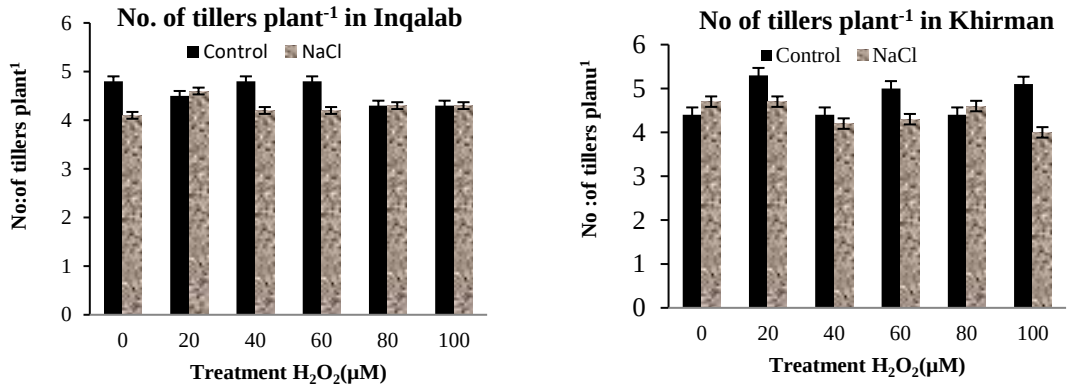

Fig. 4: Effect of H_2O_2 (µM) on No: of tiller plant^{-1} of wheat genotype

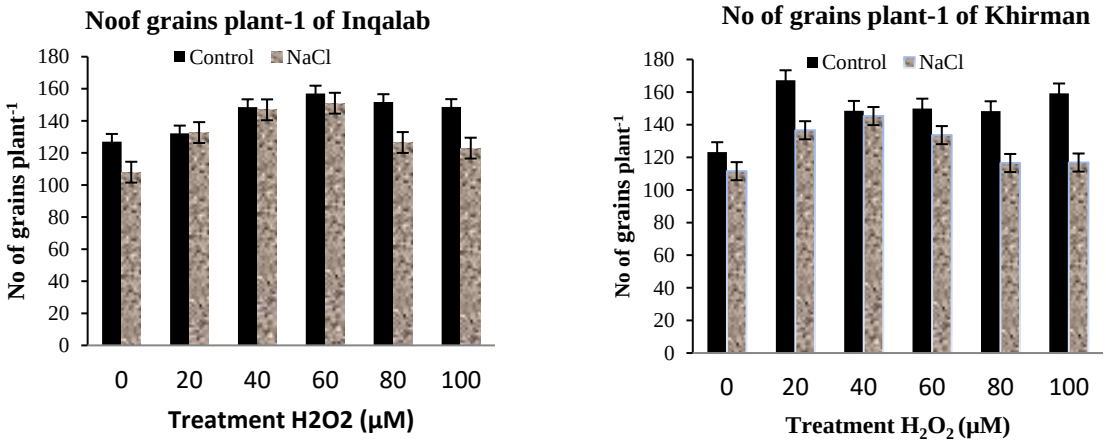

Fig. 5 : Effect of H_2O_2 (µM) on grains plant^{-1} of wheat genotypes

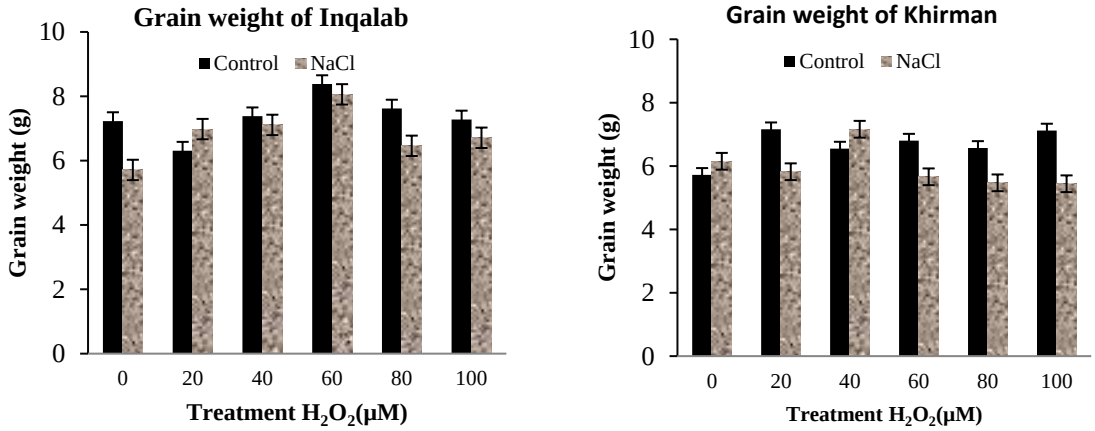

Fig. 6: Effect of H_2O_2 (µM) on grain weight plant^{-1} (g) of wheat genotypes

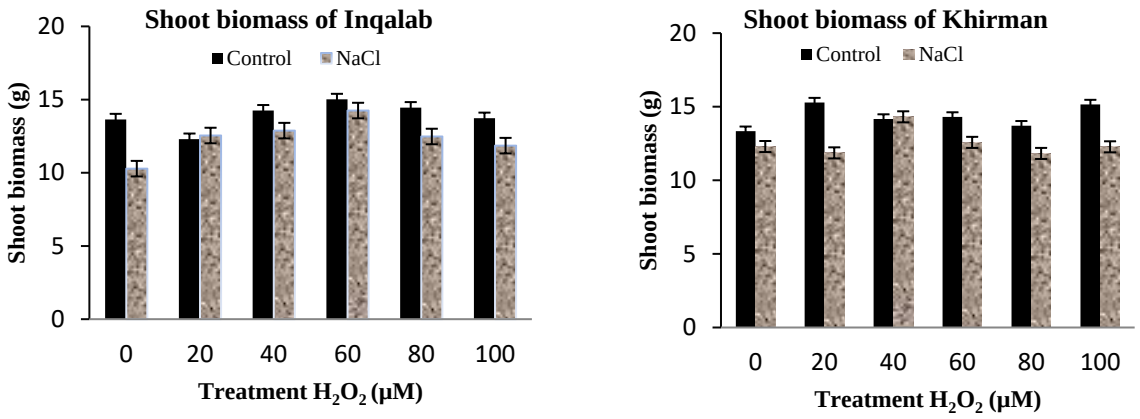

Fig. 7: Effect of H_2O_2 (µM) on shoot biomass (g) of wheat genotypes

Number of grains plant^{-1}

The number of grains plant^{-1} of wheat genotypes were examined to assess the effect of seed soaking with H_2O_2 in figure-5 and suggested significant effect of H_2O_2 concentrations and salinity on the grains plant^{-1} and non-significant due to genotypes. There was adverse response of genotypes for number of grains plant^{-1} to H_2O_2 when its concentration exceeds 40 or 60 µM. Hence, in Inqalab, the H_2O_2 at 60µM and for Khirman 40-60 µM concentration will be optimum for grains plant^{-1}.

Grain weight plant^{-1} (g)

Grain weight plant^{-1} of wheat genotypes in figure-6 illustrated significant effect of H_2O_2 concentrations, genotypes and salinity. However, H_2O treatment proved to be effective for higher grain weight plant^{-1} as compared to NaCl treated treatment. Hence, in Inqalab, the H_2O_2 at 60µM and for soaking seed of Khirman, H_2O_2 at 60µM concentration under H_2O treatment and 40µM under NaCl treated treatment may be optimum for grain weight plant^{-1}.

Shoot biomass plant^{-1}(g)

Shoot biomass plant^{-1} of wheat in figure-7 indicated significant effect of H_2O_2 concentrations and salinity, while the shoot biomass plant^{-1} remained unaffected under genotypes. The shoot biomass plant^{-1} followed decreasing trend when H_2O_2 concentration for seed soaking exceeded 60 µM. However, H_2O treatment proved to be effective as compared to NaCl treated treatment; while in Inqalab showed better shoot biomass plant^{-1} than Khirman.

4. DISSCUSION

In the present scenario of ever increasing food demands, it becomes highly important to tailor crop genotypes inheriting the tolerance ability against the salinity stresses by adopting various physiological mechanisms towards the sustainable agriculture strategies, the research was conducted to explore the genotypic response of wheat under various salinity levels by exogenous application of anti-oxidant hydrogen peroxide (H_2O_2).

The H_2O_2 treatment had tend to cause plants to be slightly larger; they were slightly taller and had more leaves. These differences were statistically significant, but were quite small and did not follow a clear dose-response. Similarly, H_2O_2 treated plants produced the same number of tillers as controls, and related to the straw weight was not significantly different between treatment and control. However, the data suggested that more of the tillers in treated plants go on to produce spikes, because spike number was significantly greater in treated plants. As a result, spike weight and seed weight were also significantly increased by H_2O_2 treatment. Regardless of salinity stress, H_2O_2 seed treatment improves plant yield. Other details for grain weight, Inqalab had higher yield than Khirman and H_2O_2 treatment increases yield for both genotypes, but the effect was greater in Inqalab. It had been reported that seed soaking improves emergence, tillering, grain and straw yields in wheat (Farooq *et al*., 2008). The effects of hydrogen peroxide on plants could be extremely beneficial if used in the right concentrations and conditions (Williams, 2003).

The salinity reduced germination, especially in Inqalab; plants grown in saline soil were significantly shorter and had significantly fewer tillers than controls. The data showed a clear effect of salinity stress on the growth of wheat plants. The shoot biomass plant^{-1} followed decreasing trend when H_2O_2 concentration for seed soaking exceeded 60 µM. However, H_2O treatment proved to be effective with higher shoot biomass plant^{-1}

as compared to NaCl treated treatment; while in genotypes, Inqalab showed better shoot biomass plant^{-1} than Khirman. Salinity stress had detrimental effects on biomass yield (Hala *et al*., 2015).

It could be seen that for grain weight, salt reduced yield in Inqalab when there were no H_2O_2 treatment, but treatment tends to minimize the effect of salt that did not happen in Khirman. H_2O_2 treatment improved grain yield in control plants, but not in salinity. H_2O_2 seed treatment seemed to protect the salt-sensitive genotype, Inqalab, against yield reductions caused by salinity, but had limited effect on Khirman. The most beneficial effects were seen around 60 µM H_2O_2, with yield reducing again in the plants with the highest levels of H_2O_2 treatment. Impact of H_2O_2 priming on physiology indicated the effects of H_2O_2 and salt on growth and yield. Priming also enhances the activities of anti-oxidative enzymes in treated seeds (Hsu *et al*., 2003).

5. CONCLUSION

The individual effects of H_2O_2 and salt on growth and yield, we could say that overall, H_2O_2 increases growth and yield, while salinity decreases it, even under well-watered conditions. H_2O_2 seed treatment seemed to protect the salt-sensitive variety; Inqalab, against yield reductions caused by salinity, but had limited effect on Khirman. The most beneficial effects were seen around 60 µM H_2O_2, with yield reducing again in the plants with the highest levels of H_2O_2 treatment.

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