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Research Article

# Investigating the impact of different halopriming treatments on biochemical and physiological changes in barley (*Hordeum vulgare* L.) under different salinity levels

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## Abstract

**A research experiment was carried out at, Department of Seed Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, during 2023-24 on barley to study the effect of various priming treatments on seed quality of barley under different salinity levels. The experiment was laid out in a completely randomized design with three replications having seven priming treatments *viz.,* $T_0$: Control, $T_1$: Hydropriming with water, $T_2$: Halopriming with saline water (4 dS/m), $T_3$: Halopriming with saline water (8 dS/m), $T_4$: Halopriming with saline water (12 dS/m), $T_5$: Halopriming with saline water (16 dS/m), T6: Halopriming with saline water (20 dS/m) with six salinity treatments *viz.,* $ST_0$: Control (Non-saline), $ST_1$: 4 $dSm^{-1}$, $ST_2$: 8 $dSm^{-1}$, $ST_3$: 12 $dSm^{-1}$, $ST_4$: 16 $dSm^{-1}$, $ST_5$: 20 $dSm^{-1}$. The results revealed that seed priming with saline water produce a negative effect on seed quality as it showed a reduction in all the seed quality parameters with increase in salinity of the priming solution as compared to simple water. Seed priming with water of upto 8 $dSm^{-1}$ salinity improved the seed performance as compared to control (dry seed) and from above 8 $dSm^{-1}$, the performance of seed decreased and at the level of 20 $dSm^{-1}$, it drastically reduced. Simple water priming (hydropriming) was found best with highest germination percentage, seedling length, vigour indices, emergence index and seedling establishment parameters which was found to be statistically at par with that of saline priming with a salinity level of 4 $dSm^{-1}$.**

**Keywords:** Barley, priming, salinity, germination, halopriming

## 1. Introduction

Barley (*Hordeum vulgare* L.) was domesticated from its wild progenitor *Hordeum spontaneum.* It belongs to one the earliest domesticated and most important crops of the Fertile Crescent, emerging in the Near East around 11,000 years ago (Badr *et al.,* 2000; Pourkheirandish and Komatsuda, 2007; Lister *et al.,* 2018). Over the passage of domestication, barley grain morphology changed significantly, moving from an elongated shape to a more rounded spherical one (Hughes, *et al.,* 2019). Barley varieties are classified into two-rowed and six-rowed, based on type of ear. In wild barley only the central spikelet is fertile, whereas the other two are reduced. This condition is retained in certain cultivars known as two-rowed barley. A pair of mutations (one dominant, the other recessive) results in the fertile lateral spikelets to produce six-rowed barleys (Zohary and Hopf, (2000). Two-rowed barley has a lower protein content than six-rowed barley. High-protein barley is best suited for animal feed. Lower protein barley is used for malting purpose (Adrian *et al.,* 2008).

It is the world's fourth most important cereal crop, after wheat, rice and maize with production of 145.93 million metric tons (Annonymous, 2022). It is one of the world's most important and resilient crops, able to grow in marginal environments where other crops are unable to grow, which has important implications for food security (Newton *et al.,* 2011). As it is cultivated in extremely diverse regions of the world from 330 m below sea level near the Dead Sea in the Middle East up to 4200 m on Atipano and the Andes in Bolivia. In comparision to wheat it is more tolerant, hardier and genetically equipped to adjust well under limited inputs and saline soils (Ballantyne, 1962; Sallam *et al.,* 2019).

Soil salinity has become a severe threat to ensuring food security in the dry and semi-dry regions as it negatively impacts the production of crops (El hasini *et al.,* 2019). Increasing soil salinity had significant impact on food production and more agriculture lands are expected to become salt affected due to climate change (Rengasamy, 2006). Globally, about 831 million hectares (M ha) area is affected by soil salinity and sodicity which include 397 m ha of saline and 434 m ha of sodic soils. In India, about 6.75 m ha area is salt-affected. (Mandal *et al.,* 2018). Approximately 20 percent of the total agricultural land is affected by salt, accounting for more than 7 percent of the world's total land area and most of this land is nearly unsuitable for cereal production (Parihar *et al.* 2014) and is estimated to expand by the year 2050 at a greater rate than it is presently (Central Soil Salinity Research Institute (CSSRI), 2014).

Salinity stress affects the various physiological and metabolic processes in plants, depending on the duration of exposure and severity, which restricts the good germination and crop production (Munns, 2005; Rozema and Flowers, 2008; Rahnama *et al.*, 2010; James *et al.*, 2011). So, seed germination, growth and development of crop plants get adversely affected by salinity stress which ultimately reduces the yield (Schleiff and Muscolo, 2011; Muscolo *et al.* 2011.

In order to revive seeds under salinity stress, a number of advanced seed priming techniques have been developed. Some of these techniques include hydro-priming, osmo-priming, halo-priming, bio-priming, hormonal-priming, chemical priming, and nutri-priming. But the extent of the effectiveness of various priming techniques varies with plant species and diverse climatic conditions (Iqbal and Ashraf, 2005; Lutts *et al.*, 2016). Hydropriming is one of the most common seed priming techniques since it is the simplest, most affordable, and most effective at improving plant stand and crop yield in both normal and stressful conditions (Kaur *et al.*, 2002; Waqas *et al.*, 2019). It pledges physiochemical processes in seeds before germination (Basra *et al.*, 2003).

## 2. Material and Methods

The study was conducted during 2023–2024 in the laboratories of the Department of Seed Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, situated in a semi-tropical region in the western zone of India. Two barley genotypes BH 393 and BH 885 were primed in water with different salininty *viz.,* $\mathbf{T_0}$**:** Control, $\mathbf{T_1}$**:** Hydropriming with water, $\mathbf{T_2}$**:** Halopriming with saline water (4 dS/m), $\mathbf{T_3}$**:** Halopriming with saline water (8 dS/m), $\mathbf{T_4}$**:** Halopriming with saline water (12 dS/m), $\mathbf{T_5}$**:** Halopriming with saline water (16 dS/m), **T6:** Halopriming with saline water (20 dS/m) with different salinity treatment *viz.,* $\mathbf{ST_0}$**:** Control (Non-saline), $\mathbf{ST_1}$**:** 4 $dSm^{-1}$, $\mathbf{ST_2}$**:** 8 $dSm^{-1}$, $\mathbf{ST_3}$**:** 12 $dSm^{-1}$, $\mathbf{ST_4}$**:** 16 $dSm^{-1}$, $\mathbf{ST_5}$**:** 20 $dSm^{-1}$.

The salinity treatments was comprises of five salinity levels dominated by $Cl^-$ salts ($Cl:SO_4^{-2}$= 7:3) and control. For creating the different salinities, different salts of $Na^+$, $Ca^{+2}$ and $Mg^{+2}$ in the ratio 4:1:3 will be dissolved.

Table 3.1: Composition of saline solutions of different salinities

| S. No. | Salts | Quantity (gram/litre) | | | | |
|---|---|---|---|---|---|---|
| | | 4 $dSm^{-1}$ | 8 $dSm^{-1}$ | 12 $dSm^{-1}$ | 16 $dSm^{-1}$ | 20 $dSm^{-1}$ |
| 1. | NaCl | 1.17 | 2.34 | 3.51 | 4.68 | 5.85 |
| 2. | $CaCl_2.7H_2O$ | 0.56 | 1.10 | 1.66 | 2.20 | 2.80 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3. | $MgCl_2$ | 0.31 | 0.61 | 0.92 | 1.22 | 1.55 |
| 4. | $MgSO_4$ | 1.48 | 2.96 | 4.44 | 5.92 | 7.40 |

These solutions were used to soak the substrata used in the germination and vigour potential test and for priming of seed.

## 3. Results and Discussion

### *3.1 Effect on physiological parameters*

The results revealed that the genotypes, salinity levels and different seed priming treatments significantly influenced the physiological parameters such as seed germination, speed of germination, seedling length, vigour indices, emergence index and seedling establishment (Table 1). Among the genotypes, BH 885 (81.03%) had the highest standard germination followed by BH 393 (79.22%). The standard germination was found to reduce continuously and significantly with the increase in salinity. Maximum germination percentage (92.17%) was recorded under non-saline conditions whereas, lowest value (63.82%) was recorded at 20 $dSm^{-1}$. Among the priming treatments (Fig. 1), highest standard germination was recorded in simple water (hydroprimed) primed seeds (87.53%) at par with 4 $dSm^{-1}$ solution (86.42%) and 8 $dSm^{-1}$ solution priming (85.36%) as compared to control (82.61%). Priming of seeds with upto 8 $dSm^{-1}$ saline solution has proved beneficial as compared to control (unprimed seeds) but the seed priming of 12 $dSm^{-1}$, 16 $dSm^{-1}$ and 20 $dSm^{-1}$ solutions has declined the seed quality. The result are in accordance with Askari *et al.* (2016) in barley, Naseri *et al.* (2012) in barley, Sayar *et al.,* 2010 in wheat, Sabagh *et al.,* 2019 in barley.

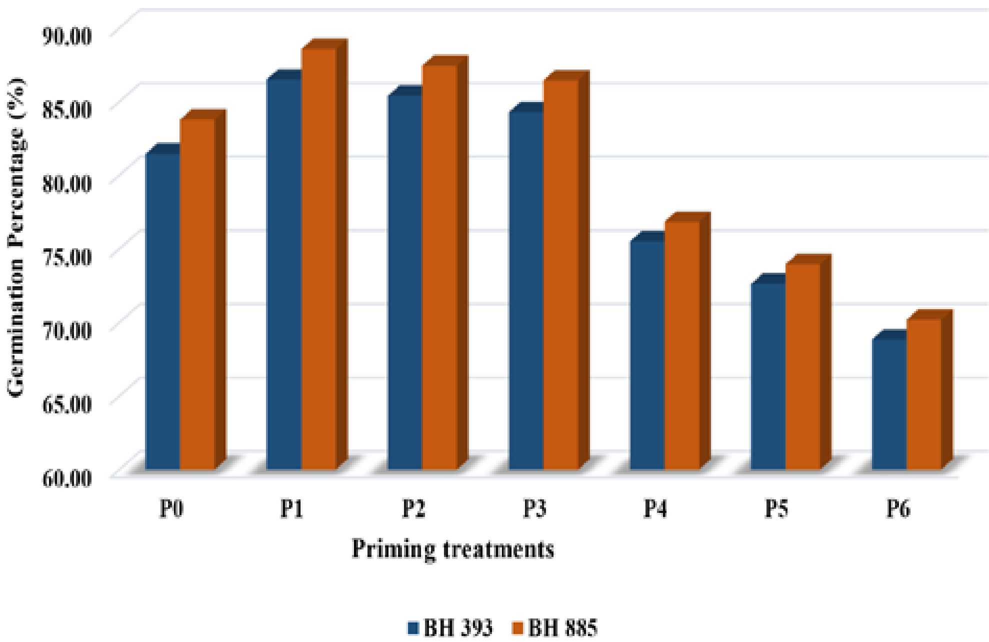


Fig. 1. Standard germination percentage of different halopriming treatments of different salinity levels.

Speed of germination was found to reduce continuously and significantly with the increase in salinity levels. Maximum speed of germination (54.59) was recorded under non-saline conditions whereas, lowest value (21.68) was recorded at 20 $dSm^{-1}$. Highest speed of germination were recorded in simple water primed seeds (61.78) followed by 4 $dSm^{-1}$ solution (55.08) and 8 $dSm^{-1}$ solution priming (46.68) ) but the seed priming of 12 $dSm^{-1}$, 16 $dSm^{-1}$ and 20 $dSm^{-1}$ solutions has declined the seed quality and reduced the speed of germination to 33.57, 28.80 and 22.52, respectively. Similar results were found in Luan *et al.,* 2014 in sunflower.

Among the priming treatments, highest seedling length and seedling dry weight was recorded in simple water primed seeds (22.45cm), (12.83mg) followed by 4 $dSm^{-1}$ solution (21.39 cm), (12.30mg), respectively. Both seedling length and dry weight was found to reduce continuously and significantly with the increase in salinity. Maximum length and dry weight (29.53cm), (14.84mg) was recorded under non-saline conditions whereas, lowest value (7.74cm), (7.47mg) was recorded at 20 dSm$^{-1}$, respectively. Similar results are found by Li J *et al.,* (2011) in maize, Datta *et al.* (2009) in wheat, Hussain *et al.* (2013) in wheat.

Different seed priming treatments and salinity levels significantly influenced the vigour index I & II. The interaction of salinity with priming indicated that the vigour index I & II was found to reduce continuously and significantly with the increase in salinity. Maximum vigour index I (2739) and vigour index II (1374) was recorded under non-saline conditions, whereas lowest value (509), (484) was recorded on 20 dSm$^{-1}$ salinity level in vigour index I & II respectively. Priming of seeds with upto 8 dSm$^{-1}$ saline solution has proved beneficial as all the treatments showed higher vigour index I & II than control (unprimed seeds) but the seed priming of 12 dSm$^{-1}$, 16 dSm$^{-1}$ and 20 dSm$^{-1}$ solutions has declined the seed quality and reduced the vigour index I to 1404, 1177 and 976, and vigour index II to 843, 747 and 655 respectively. Among the priming treatments, highest vigour index I & II was recorded in simple water primed seeds (2039), (1146) followed by 4 dSm$^{-1}$ solution (1924), (1088) and 8 dSm$^{-1}$ solution priming (1737), (1016) respectively. Results are supported by Naseri *et al.* (2012) in barley.

Maximum emergence index (7.15) was recorded under non-saline conditions, whereas lowest value (0.36) was recorded on 20 dSm$^{-1}$ salinity level. Priming of seeds with upto 8 dSm$^{-1}$ saline solution has proved beneficial as all the treatments showed higher emergence index than control (unprimed seeds) but the seed priming of 12 dSm$^{-1}$, 16 dSm$^{-1}$ and 20 dSm$^{-1}$ solutions has declined the seed quality and reduced the emergence index to 2.98, 2.44 and 2.06, respectively. Among the priming treatments, highest emergence index was recorded in simple water primed seeds (5.42) followed by 4 dSm$^{-1}$ solution (5.15) and 8 dSm$^{-1}$ solution priming (4.14). Similar results are also reported by Dantas *et al.*, 2007 in beans, Othman *et al.*, 2007 in barley.

Different seed priming treatments and salinity levels significantly influenced the seedling establishment in both the genotypes. Between the genotypes, BH 885 (39.83) had the highest value of seedling establishment followed by BH 393 (37.89). The seedling establishment was found to reduce continuously and significantly with the increase in salinity (Fig. 2). Maximum seedling establishment (54.12) was recorded under non-saline conditions, whereas lowest value (20.57) was recorded on 20 dSm$^{-1}$salinity. Priming of seeds with upto 8 dSm$^{-1}$ saline solution showed higher seedling establishment than control (unprimed seeds) but the seed priming of 12 dSm$^{-1}$, 16 dSm$^{-1}$ and 20 dSm$^{-1}$ solutions has declined the seed quality and reduced the seedling establishment to 37.03, 31.56 and 26.11, respectively. Highest seedling establishment was recorded in simple water primed seeds (48.31) followed by 4 dSm$^{-1}$ solution (45.64) and 8 dSm$^{-1}$ solution priming (43.61) and minimum seedling establishment was recorded in seed priming of 20 dSm$^{-1}$ (26.11) as compared to control (dry seed) (39.75). Similar results are found in Munns *et al.,* 2012 in wheat, Debez *et al.,* 2019 in barley.

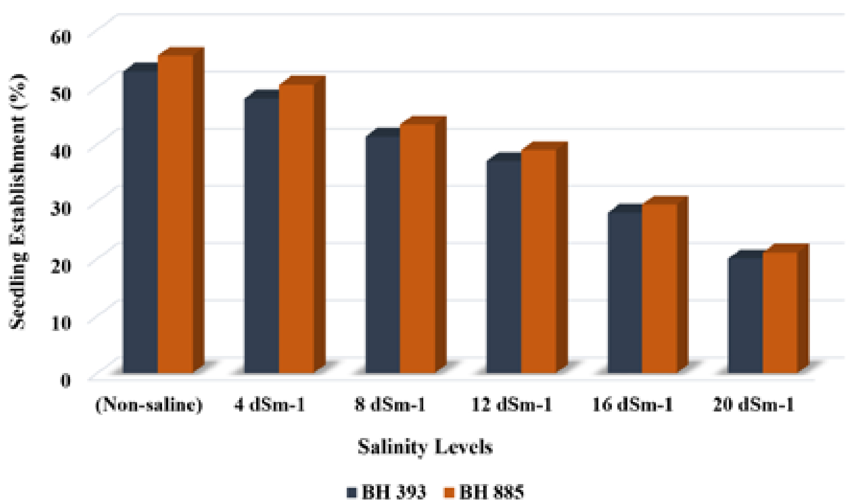


Fig. 2. Seedling establishment (%) percentage of both varieties with different halopriming treatments under different salinity levels.

Table 1. Effect of seed priming with saline water on different seed quality parameters of barley under different salinity levels

| Genotype ↓ Factors → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| BH 393 | 79.22 | 39.16 | 16.71 | 10.04 | 1419 | 829 | 3.33 | 37.89 |
| BH 885 | 81.03 | 41.66 | 19.59 | 12.22 | 1688 | 1025 | 3.99 | 39.83 |
| CD (p=0.05) | 1.628 | 0.87 | 0.31 | 0.232 | 18.56 | 19.89 | 0.118 | 1.032 |
| **Salinity ↓** | | | | **Salinity levels** | | | | |
| ST0-Control (Non saline) | 92.17 | 54.59 | 29.53 | 14.84 | 2739 | 1374 | 7.15 | 54.12 |
| ST1-4 dSm-1 | 89.12 | 52.15 | 26.67 | 13.92 | 2408 | 1251 | 6.02 | 49.21 |
| ST2-8 dSm-1 | 84.98 | 46.67 | 20.95 | 12.21 | 1807 | 1046 | 4.82 | 42.43 |
| ST3-12 dSm-1 | 79.29 | 39.36 | 13.67 | 9.61 | 1104 | 772 | 2.56 | 38.05 |
| ST4-16 dSm-1 | 71.38 | 28.01 | 10.33 | 8.74 | 754 | 635 | 1.04 | 28.76 |
| ST5-20 dSm-1 | 63.82 | 21.68 | 7.74 | 7.47 | 509 | 484 | 0.36 | 20.57 |
| CD (p=0.05) | 2.82 | 1.52 | 0.54 | 0.402 | 32.15 | 34.45 | 0.204 | 1.787 |
| **Priming ↓** | | | | **Priming Treatments** | | | | |
| P0: Control (Dry seed) | 82.61 | 34.45 | 18.48 | 11.67 | 1619 | 993 | 3.41 | 39.75 |
| P1: Simple water | 87.53 | 61.78 | 22.45 | 12.83 | 2039 | 1146 | 5.42 | 48.31 |
| P2: Saline water (4 dSm-1) | 86.42 | 55.08 | 21.39 | 12.3 | 1924 | 1088 | 5.15 | 45.64 |
| P3: Saline water (8 dSm-1) | 85.36 | 46.68 | 19.39 | 11.59 | 1737 | 1016 | 4.14 | 43.61 |
| P4: Saline water (12 dSm-1) | 76.17 | 33.57 | 17.2 | 10.67 | 1404 | 843 | 2.98 | 37.03 |
| P5: Saline water (16 dSm-1) | 73.28 | 28.8 | 15.06 | 9.8 | 1177 | 747 | 2.44 | 31.56 |
| P6: Saline water (20 dSm-1) | 69.51 | 22.52 | 13.06 | 9.06 | 976 | 655 | 2.06 | 26.11 |
| CD (p=0.05) | 3.04 | 1.64 | 0.59 | 0.434 | 34.73 | 37.21 | 0.221 | 1.93 |

1.Standard germination (%) 2. Speed of germination 3. Seedling length (cm) 4. Seedling dry weight (mg) 5. Vigour index –I 6. Vigour index – II 7. Emergence index 8. Seedling establishment (%)

Table 2. Overall effect of seed priming with saline water on electrical conductivity (µS $cm^{-1}$ $g^{-1}$) of seed leachates, catalase activity (µmoles $mg^{-1}$ dry weight), superoxide dismutase activity (µmoles $mg^{-1}$ dry weight) and peroxidase activity (µmoles $mg^{-1}$ dry weight) in barley seed

| Genotype ↓ Factors → | electrical conductivity (µS $cm^{-1}$ $g^{-1}$) | Catalase activity (µmoles $mg^{-1}$ dry weight) | Superoxide dismutase activity (µmoles $mg^{-1}$ dry weight) | Peroxidase activity (µmoles $mg^{-1}$ dry weight) |
|---|---|---|---|---|
| BH 393 | 198.69 | 242.61 | 29.22 | 41.44 |
| BH 885 | 175.51 | 247.68 | 33.1 | 45.2 |
| CD (p=0.05) | 2.6 | 0.476 | 1.031 | 1.437 |
| **Priming ↓** | | **Priming Treatments** | | |
| P0: Control (Dry seed) | 123.87 | 242.49 | 24.36 | 42.2 |
| P1: Simple water | 107.61 | 284.77 | 45.2 | 45.06 |
| P2: Saline water (4 dSm-1) | 146.85 | 297.15 | 54.08 | 48.12 |
| P3: Saline water (8 dSm-1) | 172.11 | 265.38 | 32.49 | 44.05 |
| P4: Saline water (12 dSm-1) | 221.16 | 230.25 | 23.1 | 41.74 |
| P5: Saline water (16 dSm-1) | 253.15 | 210.35 | 20.5 | 41.22 |
| P6: Saline water (20 dSm-1) | 284.93 | 185.62 | 18.36 | 40.86 |
| CD (p=0.05) | 4.87 | 0.891 | 1.929 | 2.688 |

The Fig. 3 represents the percent reduction in standard germination and seedling establishment of both the genotypes (data pooled) under halopriming priming treatments. At salinity levels of 4, 8, 12 and 16 $dSm^{-1}$, the mean reduction is (3.31) (90.6), (7.81) (21.60), (13.98) (29.69) and (22.55) (46.85) percent in standard germination and seedling establishment as compared to non-saline conditions. Maximum reduction was observed at 20 $dSm^{-1}$ (30.77) (61.98) in standard germination and seedling establishment, respectively. The overall reduction in seedling establishment was more than that in the standard germination. The extent of reduction in the seedling establishment is higher at each salinity level than the standard germination. These results are in accordance with the findings of Okcu *et al.* (2005) in pea, Kaya *et al.* (2008) and Ozaktan *et al.* (2016) in chickpea.

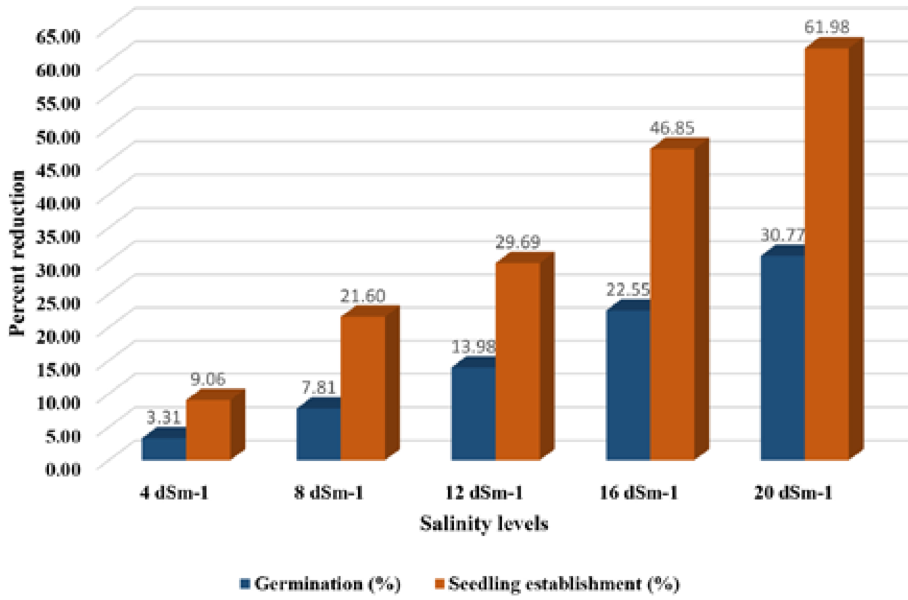


Fig. 3. Percent decrease in standard germination and seedling establishment percentage as compared to non-saline conditions in different haloprimed seeds of barley (data pooled for all the priming treatments)

The seed performance declined with the increase of salinity levels of germination media. It could be due to that it alters the imbibition of seeds as it increases the osmotic potential of germination medium, increases the toxic absorption of ions and ultimately adversely affects the germination process (Khan and Weber, 2008, Sayar *et al.,* 2010, Sabagh *et al.,* 2019, Polash *et al.,* 2019). Salinity causes toxicity for the enzymes and alters the protein synthesis of seed (Dantas *et al.*, 2007) and disrupts the hormones balance which reduces the utility of seed reserves for the developing embryo (Othman *et al.*, 2007, Bordi, 2010, Munns *et al.,* 2012, Parihar *et al.,* 2015, Debez *et al.,* 2019).

The data presented of biochemical activity in table 2 indicates that both genotypes and different priming treatments had a significant effect on enzymatic activity of barley seed. Between the genotypes BH 885 (175.51 $\mu Sm^{-1}g^{-1}$) had the minimum electrical conductivity followed by BH 393 (198.69 $\mu Sm^{-1}g^{-1}$). The electrical conductivity increased continuously and significantly with the increase in salinity of priming solutions. Minimum electrical conductivity (107.61 $\mu Sm^{-1}g^{-1}$) was recorded under simple water priming whereas, highest value (284.93 $\mu Sm^{-1}g^{-1}$) was recorded at 20 $dSm^{-1}$ priming. Priming affected the activity of this enzyme significantly in seeds and the soaking with saline water (4 $dSm^{-1}$) maximized the enzyme activity of catalase, superoxide dismutase (SOD) and peroxidise, with a value of (297.15), (54.08) (48.12) and the activity was minimum in the seeds primed with saline water of 20 $dSm^{-1}$ (185.62) (18.36), (40.86), respectively. The enzyme activity of seeds primed with 12, 16 and 20 $dSm^{-1}$ saline water was degraded and the activity was lower even than that of the unprimed seeds.

## Conclusion:

From the present study it can be concluded that simple water priming (hydropriming) gave better results in terms of germination percentage, vigour indices and other seed vigour parameters which was found to be statistically at par with that of saline priming with a salinity level of 4 $dSm^{-1}$. As the salinity of priming solution increased from above 8 $dSm^{-1}$, the performance of seed decreased and at the level of 20 $dSm^{-1}$, it drastically reduced and showed lower values of germination and seed vigour even than the unprimed (dry) seed.

## Author Contributions

All authors contributed equally for preparing the final version of the manuscript.

## Conflict of Interest

Authors declare no conflict of interest.

## Ethical Approval

The article doesn't contain any study involving ethical approval.

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