Research Article

Influence of hydro- and halo-priming on germination and seedling growth of cabbage under saline conditions

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Abstract
A study was conducted at vegetable seed Laboratory Institute of Horticultural Sciences, University of Agriculture Faisalabad to investigate the effect of halo and hydro-priming on seed germination and seedling growth of cabbage under saline conditions. Cabbage seeds were primed with five different salts i.e. KNO₃, KH₂PO₄, KCl, NaCl, Ca(NO₃)₂, and water (Hydro-priming) for 2 hours in darkness at 24±1°C temperature. Both primed and unprimed seeds were irrigated with five different saline solutions [0 mM, 50 mM, 100 mM, 150 mM and 200 mM NaCl]. Results showed that priming treatments with KCl, KH₂PO₄ and KNO₃ at different concentrations gave best results in terms of final germination percentage (FGP), time taken to 50% germination (T₅₀), germination index (GI), mean germination time (MGT) and seedling vigour index (SVI) as compared to unprimed seeds. However, enhanced salinity levels had harmful influence on almost all parameters under study. The present study exposed that under normal and saline conditions all priming treatments mainly KNO₃ can be used to increase seed enactment in cabbage. However, further studies are needed to investigate the effects of halo priming on growth and yield of cabbage.

Keywords: Cabbage; Germination energy; Halo priming; Salinity

Introduction
Drought and salinity threatens crop productivity all over the world. High salt concentrations in the soils all over the world causes great fall in crop production [1]. Salt affected cultivated land was approximately 5% [2] and now has increased up to 20% of agricultural land [3]. Almost 6.3 million hectare land that is about 14% of irrigated land is salt affected in Pakistan causing up to 64% production loss [4]. Rapid and uniform seedling emergence is necessary to increase yield and quality of vegetable crops [5]. Seedling emergence is influenced by seed quality, edaphic and environmental conditions e.g. temperature, soil moisture.
and salt concentration in the soil [6]. Salt stress hinders seed emergence and seedling establishment [7]. Excessive salinity levels may cause higher ion uptake [8], which induce oxidative stress and thus may inhibit germination [9]. Furthermore, under saline conditions carbohydrate synthesis is deteriorated due to the reduced activity of ribulose 1,5-bisphosphate carboxylase [10]. Seed priming can effectively be used to improve seed germination and emergence rate of many crops [11]. Under adverse environmental conditions seed priming encourages rapid and uniform seed germination. Seed priming can be done in a variety of ways by using drinking water, hormonal and salt solutions, sand and solid matrices. Halo-priming is a pre-sowing treatment in salt solutions that permits seeds to absorb water but limits the radicle emergence. Primed grass seeds show good results for all germination parameters [12].

Cabbage is an imperative leafy vegetable used as fresh or in cooked form. It comprises good amount of iodine, potassium, calcium, iron, phosphorous, sulfur, potassium, vitamins (A, B1, B2, B6, K, C, E), riboflavin and folic acid [13]. In the world the annual production of cabbage and other brassica during 2013-14 was 71.8 million tonnes [14]. In Pakistan, the annual production of cabbage and brassica was 77.2 thousand tonnes during 2013-14 [15]. Usually, cabbage is cultivated by raising nursery, which is transplanted in field after about one month. But, in developed countries and even in Pakistan, some growers sow it directly in the field. The crop is regarded as salt sensitive, particularly during germination and early seedling growth. But, research work on induction of salinity tolerance in cabbage is scarce. Thermal hardening of seed has been used for induction of salinity tolerance in cabbage but, seed priming reports on cabbage are scarce.

Keeping in view the scarcity of research on use of seed priming technique in cabbage, this study was planned to induce salinity tolerance in cabbage during germination and early seedling stage. Cabbage seeds were primed by using salt solutions of calcium, potassium and sodium (KNO₃, KH₂PO₄, KCl, NaCl, and CaNO₃) or water to find the best seed priming treatment(s) that can induce salt tolerance in cabbage during germination and early seedling growth.

**Materials and methods**

Petri dishes, filter papers, different salts, distilled water, seed moisture meter, aeration/air pump, incubator and cabbage seeds. Seeds of cabbage variety Golden Acre were obtained from Sidique Sons (registered) seed corporation Faisalabad. First of all, seed moisture contents were determined (7%) by placing seeds in seed moisture meter (GMK-503). Seeds were primed using distilled water and various concentrations of different salts viz., KNO₃ @ 1 and 2 %, KCl @ 1, 2 and 3 %, NaCl @ 1%, KH₂PO₄ @ 3%, CaNO₃ @ 1, 2 and 3%, in darkness at 24±1°C. Treated (primed) seeds were re-dried in laboratory at room temperature. One hundred primed and unprimed seeds per treatment in four replicates were cultured in Petri dishes (25 seeds on each Petri dish) on double sheet of filter paper for germination test. Filter paper were moistened initially with 5 mL of distilled water with these concentrations i.e. 0 mM, 50 mM, 100 mM, 150 mM and 200 mM of NaCl and later on as per requirement. These plates were kept in incubator at temperature 24±1°C in darkness.

Data were collected up to seven days for germination and other parameters viz., final germination percentage, germination index, germination energy, mean germination time (days), time taken to 50 percent germination (days), seedling length (cm), root shoot ratio and seedling vigor index.
The experiment was conducted according to completely randomized design (CRD) under factorial arrangement. The recorded data were analyzed statistically using Fisher’s analysis of variance technique [16] and treatment means were separated by Duncan’s Multiple Range test.

**Results and discussion**

Germination and related parameters were altered by the salinity and various priming treatments. There was a gradual decline in germination percentage, germination index and germination energy with increase in salinity level (Table 1 and 2). Final germination percentage (FGP) and germination energy (GE) started to decline at 150 mM NaCl while reduction in GI was evident even at 50 mM NaCl concentration. Increased final germination percentage (97.75%) was recorded at 50 mM and 100 mM NaCl salinity level that was statistically similar to the final germination percentage at 0 mM salinity level (97.42%). Final germination percentage was lowest (79.67%) at highest salinity level i.e. 200 mM NaCl. Seedling length was increased slightly at 50 mM NaCl (13.44 cm) in comparison with control (12.09 cm at 0 mM NaCl concentration) but then declined with increase in salinity upto 100 mM (11.57cm) and above (150 and 200 mM). Root shoot ratio was also significantly influenced by salinity and was statistically same at all salinity levels from 50 to 200 mM NaCl concentration. Seedling vigour index was also highest at 50 mM salinity level (1312.9), statistically greater than seedling vigour index at 0 mM salinity level. Moderate salinity level (100 mM) caused reduction in seedling vigour index as compared to control but change was not statistically significant. At 150 and 200 mM there was sudden decrease in SVI (897.5 and 579.8, respectively).

When individual effect of seed treatments on seed germination and related parameters was assessed, KNO3 (1% and 2%) showed the best results for most of the parameters under observation. Final germination percentage and germination index was maximum (97.40% and 56.53%, respectively) for seeds primed in KNO3 (1%) solution. FGP values of KNO3 (1% and 2%) primed seeds were at par with those for seeds primed in distilled water and KCl (1%) but germination index values were significantly higher for seeds primed in KNO3 (1%) than all other seed treatments and control (Table 1). Germination energy was highest in seed primed with KNO3 (2%) solution that statistically at par with CaNO3 (2%), KCl (2%) and KNO3 (3%) (Figure 1). Hydro-priming improved FGP, GI and GE over untreated seeds (control) but increased time span of seed germination (Table 1 and 2) as evidenced by higher values of T50 (2.03 days) and MGT (4.75 days) as compared to control (1.84 and 4.66 days, respectively). Our results are confirmed by the statement of [17] that hydro and halopriming enhanced seedling development of sorghum genotypes. Selvarani and Umarani [18] stated that hydro-priming enhanced germination percentage while Varier *et al.* [19] obtained uniform seed emergence of hydro-primed seeds. Priming in KNO3 (1%) significantly reduced T50 (0.71 days) and MGT (4.13 days) as compared to untreated seeds. Most priming treatments significantly increased seedling length as compared to control, maximum by KNO3 (2%) but some treatments (CaNO3 3%), distilled water and NaCl (1%) decreased seedling length. Root shoot ratio was slightly increased by various priming treatments. Our results were similar to the statement of Nerson and Govers [20] who described that seeds of muskmelon primed with (2-3%) solution of KH2PO4 + KNO3 (1:1) showed imperative rise in root shoot ratio. Seeding vigor index was maximum (1217.4) in KNO3 (2%)
primed seeds. Different seed treatments modulated seed performance indices (germination percentage, seedling length and vigour) to variable extent. There was not too much difference in FGP values among various priming treatments at 0, 50, 100 and 150 mM salinity levels (Figure 2). At 200 mM NaCl concentration KNO$_3$ (1%) significantly increased FGP (97%) over untreated seeds, even higher than FGP values of untreated seeds at 200 mM. But NaCl (1%) showed drastic reduction in FGP (55%). All priming treatments decreased time taken to 50% germination ($T_{50}$) and mean germination time (MGT) from 0 to 150 mM salinity levels except hydropriming, while at 200 mM salinity level seed primed in NaCl (1%) prolonged germination time (MGT and $T_{50}$) as compared to all other seed treatments (Figure 3 and 4). Seeds primed with KNO$_3$ (1%) significantly decreased $T_{50}$ and MGT which indicates that this priming treatment increased uniformity of germination. Thakur et al. [21] noticed a rapid increase in germination when seeds were primed with KNO$_3$ in comparison with the untreated seeds while Ahmadvand et al. [22] observed a significant increase in emergence percentage of soybean and final germination percentage when seeds were primed with potassium nitrate.

### Table 1. Effect of seed priming on final germination percent (FGP), time taken to 50 percent germination ($T_{50}$), mean germination time (MGT) and germination index (GI).

| Factor                  | FGP (%) | $T_{50}$ (days) | MGT (days) | GI (%) |
|-------------------------|---------|----------------|------------|--------|
| **Salinity (S)**        |         |                |            |        |
| 0 mM                    | 97.42a  | 0.91e          | 4.22e      | 52.66a |
| 50 mM                   | 97.75a  | 1.08d          | 4.29d      | 49.25b |
| 100 mM                  | 97.75a  | 1.36c          | 4.42c      | 43.57c |
| 150 mM                  | 93.58b  | 1.61b          | 4.56b      | 36.59d |
| 200 mM                  | 79.67c  | 2.17a          | 4.82a      | 25.73c |
| **Seed treatments (T)** |         |                |            |        |
| CaNO$_3$ 1%             | 91.40def| 1.24ef         | 4.36ef     | 44.02c |
| CaNO$_3$ 2%             | 91.80def| 1.28def        | 4.4de      | 41.76cde|
| CaNO$_3$ 3%             | 92.60cdef| 1.39cd        | 4.45cd     | 39.99e |
| Distilled water         | 96.80ab | 2.04a          | 4.75a      | 31.92g |
| KCl 1%                  | 95.80abc| 1.38cde        | 4.44cd     | 43.34c |
| KCl 2%                  | 93.60bcde| 1.48c         | 4.49c      | 40.15de|
| KH$_2$PO$_4$ 3%         | 92.00def| 1.29def        | 4.41de     | 42.49cd|
| KNO$_3$ 1%              | 97.40a  | 0.71g          | 4.13g      | 56.54a |
| KNO$_3$ 2%              | 95.00abcd| 1.21f         | 4.32f      | 47.46b |
| KNO$_3$ 3%              | 91.00ef | 1.42cd         | 4.43d      | 42.27cde|
| NaCl 1%                 | 89.20f  | 1.84b          | 4.66b      | 34.85f |
| Untreated seed (control)| 92.20cdef| 1.84b        | 4.66b      | 33.91fg|
| **S×T (Interaction)**  | *       | *              | *          | *      |
Table 2. Effect of seed priming on germination energy (GE), seedling length (SL), root shoot ratio (RSR) and seedling vigour index (SVI).

| Factor               | GE (%) | SL (cm) | RSR | SVI   |
|----------------------|--------|---------|-----|-------|
| Salinity (S)         |        |         |     |       |
| 0 mM                 | 97.08 a| 12.09 b | 1.66 a| 1178.5 b |
| 50 mM                | 97.50 a| 13.44 a | 1.36 bc| 1312.9 a |
| 100mM                | 97.33 a| 11.57 b | 1.36 c | 1132.2 b |
| 150mM                | 91.83 b| 9.58 c  | 1.47 b | 897.5 c  |
| 200mM                | 71.25 c| 7.14 d  | 1.38 bc| 579.8 d  |
| Seed treatments (T)  |        |         |     |       |
| CaNO₃ 1%             | 90.40 cd| 11.23 b| 1.47 abc| 1047.4 b |
| CaNO₃ 2%             | 95.40 ab| 11.02 b| 1.50 abc| 1069.1 b |
| CaNO₃ 3%             | 90.20 cd| 9.70 c | 1.51 abc| 908.7 c  |
| Distilled water      | 89.20 d| 9.53 c  | 1.43 abcd| 887.5 c  |
| KCl 1%               | 88.80 d| 11.51 b| 1.50 abc| 1069.6 b |
| KCl 2%               | 93.60 abc| 10.80 b| 1.42 bcd| 1050.0 b |
| KH₂PO₄ 3%            | 92.00 bcd| 10.85 b| 1.30 d  | 1034.8 b |
| KNO₃ 1%              | 88.40 de| 11.18 b| 1.35 cd | 1053.8 b |
| KNO₃ 2%              | 97.40 a| 12.48 a| 1.56 ab  | 1217.4 a |
| KNO₃ 3%              | 94.20 abc| 10.76 b| 1.59 a  | 1037.2 b |
| NaCl 1%              | 88.20 de| 9.12 c | 1.37 cd | 849.8 c  |
| Untreated seed (control) | 84.20 e| 10.96 b| 1.37 cd | 1016.8 b |
| S×T (Interaction)    | *      | *       | *    | *     |

Figure 1. Germination energy of cabbage cv. Golden Acre seeds in response to various priming treatments at different salinity levels.
Figure 2. Final germination percentage of cabbage cv. Golden Acre seeds in response to various priming treatments at different salinity levels.

Figure 3. Time taken to 50% (days) germination of cabbage cv. Golden Acre seeds in response to various priming treatments at different salinity levels.
Figure 4. Mean germination time of cabbage cv. Golden Acre seeds in response to various priming treatments at different salinity levels.

Seeds primed with KNO$_3$ (1%) showed maximum values for germination index (GI) at various salinity levels, even higher than germination index of untreated seeds at 0 mM salinity level (Figure 5). Seeds treated with KNO$_3$ (1%) took minimum time for germination at all salinity levels. Seeds primed with NaCl 1% and untreated (control) seeds exhibited more and almost same time for mean germination at 200 mM salinity level as compared to all other treatments i.e. 5.36 and 5.09 days, respectively (Figure 4). Ali and Kamel [23] reported that seeds treated with NaCl gave the finest germination percentage in chickpea that corroborate our results of seed priming with NaCl (1%) at 0 mM (control) salinity level. Maximum seedling length at 50 and 100 mM salinity level was recorded in seeds primed with KCl 2% (Figure 6), while maximum root shoot ratio at highest salinity level (200 mM) was recorded in KNO$_3$ (2%) primed seeds (Figure 7). Seeds primed with KCl (1% and 2%) solution showed higher seedling vigour at 50 and 100 mM salinity level, while at 200 mM, seeds primed in KNO$_3$ (1%) showed maximum seedling vigour (Figure 8).
Figure 5. Germination index of cabbage cv. Golden Acre seeds in response to various priming treatments at different salinity levels.

Figure 6. Seedling length of cabbage cv. Golden Acre seeds in response to various priming treatments at different salinity levels.
Figure 7. Root shoot ratio of cabbage cv. Golden Acre seeds in response to various priming treatments at different salinity levels.

Figure 8. Seedling vigour index of cabbage cv. Golden Acre seeds in response to various priming treatments at different salinity levels.
Conclusion
In conclusion, higher salinity levels suppressed seed germination and other parameters i.e. shoot length and root length, root shoot ratio and seedling vigor index. Almost all the treatments decreased the negative influence of salinity but to valuable extent. Highest salinity level had detrimental effect on all the parameters. Our results envisaged that all the seed priming treatments, especially KNO3 (1%), can be improve seed germination both under normal and saline conditions.

Authors’ contributions
Conceived and designed the experiments: K Ziaf & M Amjad, Performed the experiments: MM Rehman, A Batool, A Muhammad & J Latif, Analyzed the data: MM Rehman & K Ziaf, Contributed reagents/ materials/ analysis tools: M Amjad, R Ahmad & Q Zaman, Wrote the paper: MM Rehman, K Ziaf & A Batool.

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