

THERMAL HARDENING FOR SEED VIGOUR AND SALT TOLERANCE IN CABBAGE

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A healthy crop is desirable in direct seeded vegetables to ensure good quality of the produce and is affected by a number of environmental and cultural factors. A number of seed treatments had been used to enhance the performance of seeds in several crops. Alternate cycles of dry heat and chilling (thermal hardening) is one of these seed treatments, used in cereals to invigorate the seeds, but reports about such treatments in vegetable crops are scarce. Therefore, seeds of cabbage varieties Golden Acre (GA) and Green Ball (GB), varying in their germination (%) and vigour, were exposed to different temperature (-20 and 40°C) and duration (24, 36 and 48 hrs) regimes and the effect of these treatments on seed vigour was evaluated. Different thermal hardening treatments, especially H-C 24 hrs, C-H 36 hrs, C-H-C 24 hrs and C-H-C 48 hrs, increased final germination percentage (FGP), power of germination and seedling vigour. These treatments decreased the germination spread over time in cabbage variety GB. However, such response was not observed in GA that already had high seed vigour. The impact of these better performing treatments was evaluated on germination and vigour of GA seeds under saline conditions. Interestingly, mean germination time and time taken for 50% germination was less for C-H 36 hrs and C-H-C 24 hrs treated seeds at all salinity levels. These seed treatments (H-C 24 hrs, C-H 36 hrs, C-H-C 24 hrs and C-H-C 48 hrs) improved vigour index at all salinity levels except at 200 mM NaCl concentration. Overall results depicted that C-H 36 hrs and C-H-C 24 hrs were more effective than other treatments to impart salinity tolerance and can be used as a safe tool for direct seeding of cabbage crop under normal and saline conditions.

Keywords: *Brassica oleracea* var. *capitata*, dry heat, chilling, seed invigoration, salinity

INTRODUCTION

Uniform and rapid seedling emergence is essential to have a good crop stand and thus increase yield and quality (Kaur *et al.*, 2005; Unal, 2013). Uniform and rapid emergence is affected by poor seed quality as well as adverse environmental conditions; high concentration of salt in the soil is one of those threats (Sarlikioti *et al.*, 2010). Salinity in the growth medium causes significant reduction in leaf area, leaf length, and root and shoot dry weight (Ashrafuzzaman *et al.*, 2002). Cabbage (*Brassica oleracea* var. *capitata*), a biennial winter crop of the cruciferous family, is regarded as moderately sensitive to soil salinity but tolerance at germination, emergence and seedling stage is low (Maas, 1986). Increasing salinity levels negatively affect germination rate, shoot and root length, shoot and root fresh weight, number of leaves and leaf area of cabbage (Jamil *et al.*, 2005).

To reduce emergence time under normal and stress conditions as well as to eliminate diseases, seeds of some species can be safely treated with dry heat (Farooq *et al.*, 2005; Kim and Lee, 2000; Kim *et al.*, 2003; Yari *et al.*, 2012). Pre-sowing chilling treatments have also been effectively used, alone or with other invigoration techniques, to shorten the period between planting and emergence and to protect the seeds from abiotic and biotic stresses during the

critical phase of seedling establishment (Iqbal and Ashraf, 2010; Shahid *et al.*, 2013), especially in case of cabbage, lettuce, carrot and tomato, which are directly seeded in many European countries and USA (Cantliffe *et al.*, 1987).

At present, the information regarding seed invigoration techniques in cabbage and their effect on salt stress tolerance is scarce. Seed invigoration is necessary to achieve healthy uniform crop stand in direct seeded cabbage, a common practice in several European countries. Therefore, the objectives of this study were: (i) whether thermal hardening can improve germination indices and vigour of low vigour cabbage seeds and (ii) to assess the impact of seed thermal hardening technique on salt tolerance of cabbage.

MATERIALS AND METHODS

A study was conducted in the Vegetable Seed Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. Seeds of two cabbage varieties [Golden Acre (Westar Seeds International, Inc., California) and Green Ball (Hybrid, Makkah Seed Company)] were selected that differed in their germination percentage and vigour under normal growing conditions.

Experiment #1: Seeds of both cabbage varieties were exposed to different high and low temperature regimes (-20 and 40°C) for 24, 36 and 48 hrs (Table 1). After respective

invigoration treatments, one hundred seeds per treatment were cultured in Petri dishes on double sheet of Whatman No. 1 filter paper, moistened with 4 ml distilled water and placed in an incubator (dark condition) at $25 \pm 2^\circ\text{C}$. Germination was recorded on daily basis, considering the seeds with 2 mm radicle protrusion as germinated. Data for final germination (%), radicle and plumule lengths, fresh and dry weights of ten seedlings per replication were recorded after seven days. The time taken to 50 percent germination [T_{50}] was calculated according Farooq *et al.* (2005). Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981). Vigour index (VI) was calculated as: $VI = \text{Final germination (\%)} \times \text{Total seedling length (cm)}$.

Experiment #2: Better treatments were selected on the basis of seed vigour and assessed for their role in alleviation of salt stress. Seeds treated with cycles of heat-cold for 24 hrs (H-C 24 hrs), cold-heat-cold for 24 hrs (C-H-C 24 hrs), cold-heat for 36 hrs (C-H 36 hrs), cold-heat-cold for 48 hrs (C-H-C 48 hrs) and untreated seeds (control) were exposed to several salt (NaCl) concentrations (0, 50, 100, 150 and 200 mM) by moistening the filter paper with 4 ml of the respective salt concentration. Data was recorded for various parameters as mentioned in experiment #1.

Statistical Analysis: Experiments were conducted in a completely randomized design under factorial arrangements and replicated four times. The recorded data was analyzed statistically using general linear model of STATISTICA (version = 5.5) and treatments means were separated using DMR test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

EXPERIMENT # 1

Germination Indices: Seeds of both cabbage varieties showed significant differences in their germination behavior. Final germination percentage (FGP), germination energy (GE) and germination index (GI) were higher in cabbage variety Golden Acre (GA) than Green Ball (GB) (Table 1). Seeds of variety GA took less time to reach 50% germination as compared to GB. Mean germination time (MGT) was halved in GA in comparison with GB.

Thermal hardening treatments improved FGP, GE and GI values; maximum germination (%) was exhibited by seeds subjected to C-H-C 24 hrs treatment. While, minimum value was recorded for H-C-H 48 hrs at par with the control (Table 1). Cabbage seeds germinated very quickly and therefore T_{50} values for most of the treatments, although statistically different, were very close ranging from 1.47 to 1.57 days. Lowest T_{50} value was recorded for untreated seeds while maximum for seeds exposed to H-C cycle for 36 and 48 hrs. MGT was minimum for H-C-H 24 hrs (1.57 days), statistically similar to H-C-H 36 hrs (1.67 days), and maximum in C-H-C 48 hrs (Table 1).

The combined effect of different thermal hardening treatments on both cabbage varieties showed significant differences for all parameters (Table 1). Thermal hardening treatments increased final germination percentage, GE and GI in cabbage variety GB but not in GA because untreated seeds of variety GA already showed 100 % germination and higher GE and GI values (Fig. 1). Thermal cycle of C-H-C

Table 1. Effect of thermal hardening treatments on germination traits of two cabbage varieties

Treatments	FGP	T_{50} (days)	MGT (days)	GE (%)	GI
Varieties (V)					
Golden Acre (GA)	100.00 a	1.50 a	1.34 a	100.00 a	63.56 a
Green Ball (GB)	73.53 b	1.56 b	2.69 b	71.15 b	43.31 b
Thermal Hardening Treatments (T)					
Control	84.0 bcd	1.47 a	2.02 ab	83.0 cde	51.82 bcd
C-H 24 hrs	83.5 cd	1.51 abc	2.00 ab	82.0 def	51.29 cd
C-H 36 hrs	88.5 b	1.55 bc	2.21 b	87.5 abc	54.61 abc
C-H 48 hrs	88.0 bc	1.55 bc	1.95 ab	87.0 abcd	55.03 ab
C-H-C 24 hrs	93.5 a	1.51 abc	1.97 ab	92.0 a	57.40 a
C-H-C 36 hrs	87.0 bc	1.51 abc	1.97 ab	86.0 bcde	54.18 abc
C-H-C 48 hrs	88.5 b	1.55 bc	2.83 c	87.0 abcd	50.20 d
H-C 24 hrs	88.5 b	1.56 bc	1.87 ab	87.0 abcd	55.03 ab
H-C 36 hrs	88.0 bc	1.57 bc	1.97 ab	85.5 bcde	54.12 abc
H-C 48 hrs	79.5 d	1.57 c	1.85 ab	78.0 f	49.24 d
H-C-H 24 hrs	88.5 b	1.50 abc	1.57 a	88.5 ab	56.20 a
H-C-H 36 hrs	88.5 b	1.50 ab	1.67 a	88.0 abc	55.48 a
H-C-H 48 hrs	82 d	1.56 bc	2.28 b	81.0 ef	50.08 d
Interaction					
V \times T	*	*	*	*	*

The mean values carrying same letters in a column were statistically similar to each other at $P < 0.05$.

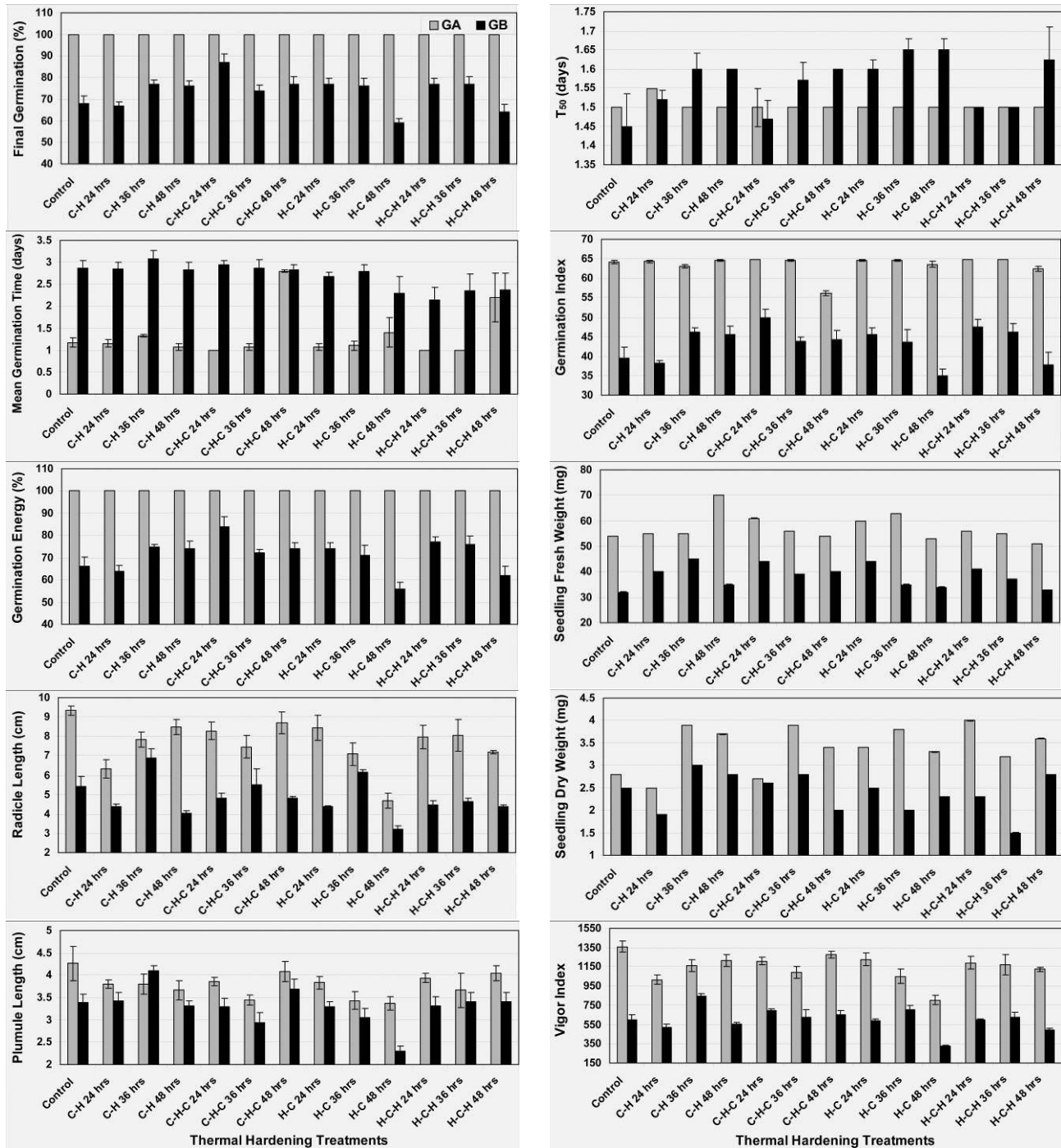


Figure 1. Seed germination and seedling vigour of two cabbage varieties in response to thermal hardening treatments

24 hrs increased FGP, GE and GI values for variety GB (Fig. 1). Some thermal hardening treatments (C-H 36 and 48 hrs, C-H-C 36 and 48 hrs, H-C 36 and 48 hrs and H-C-H 48 hrs) increased the T_{50} values of variety GB in contrast to untreated seeds while MGT values of GB decreased in

response to H-C 48 hrs, H-C-H 24 hrs, H-C-H 36 hrs and H-C-H 48 hrs. MGT and T_{50} values of variety GA remained unchanged (Fig. 1).

Seedling vigour in response to thermal hardening treatments: Radicle and plumule lengths, fresh and dry

weights and vigour index were higher for cabbage variety GA as compared to GB (Table 2). Radicle and plumule lengths of seedlings decreased in response to most of the thermal hardening treatments (Table 2). Among the thermal hardening treatments, C-H 36 hrs and H-C 36 hrs increased radicle length in GB while C-H-C 48 hrs increased plumule length (Fig. 1). Seedling fresh and dry weight was increased in cabbage variety GA and GB in response to thermal hardening treatments (Table 2); maximum increase in GA was recorded in response to C-H 48 hrs. Seedling dry weight increased in cabbage variety GA in response to all thermal hardening treatments except for C-H 24 hrs that was statistically similar to control. Dry weight values of variety GB varied greatly for different treatments; only C-H 36 and 48 hrs, C-H-C 36 hrs and H-C-H 48 hrs increased dry weight over the control (Fig. 1).

Most of the thermal hardening treatments decreased vigour index (Table 2). There was no improvement in seedling vigour of cabbage variety GA in response to thermal hardening treatments but GB responded well to thermal hardening treatments (Fig. 1). C-H 36 hrs, C-H-C 24 and 48 hrs and H-C 36 hrs significantly improved seedling vigour of cabbage variety GB as compared to vigour of untreated seeds.

EXPERIMENT # 2

Germination Indices: Seeds of cabbage variety GA were exposed to H-C 24 hrs, C-H-C 24 hrs, C-H 36 hrs and C-H-C 48 hrs to assess the effect of these treatments on alleviation of salt stress. Final germination percentage was decreased in response to thermal hardening treatments

except H-C 24 hrs (90.2%), which was at par with the control (92%; Table 3). However, time taken to 50% germination (T_{50}) and mean germination time (MGT) was decreased in response to H-C 24 hrs and C-H-C 48 hrs over the control, but was statistically similar to the control (Table 1). Final germination percentage (FGP) of seeds decreased with increasing the concentration of NaCl. Germination was maximum at 0 and 50 mM NaCl concentration i.e. 99.2% and 97.8% and then decreased gradually up to 150 mM. Germination was minimum (46.4%) at 200 mM NaCl salinity level. The same trend was recorded for T_{50} and MGT i.e., time to germination was increased with increasing the salinity level.

When the combined effect of thermal hardening treatments and salinity was analyzed, significant differences were recorded for FGP, T_{50} and MGT (Fig. 2). FGP was improved by H-C 24 hrs treatment over the control as well as other treatments at 100 mM NaCl salinity level (Fig. 2). FGP value of control exceeded over the thermal hardening treatments at 150 and 200 mM NaCl salinity but, performance of H-C 24 hrs was better than other treatments. Thermal hardening treatments H-C 24 hrs, C-H-C 48 slightly decreased T_{50} and MGT values in comparison with control but, C-H 36 hrs and C-H-C 24 hrs increased the time significantly as compared to other treatments. Moreover, T_{50} and MGT values of H-C 24 hrs, C-H-C 48 hrs and untreated seeds remained unchanged at all salinity levels, except 200 mM NaCl salinity level in which minute increase was recorded (Fig. 2).

Table 2. Effect of thermal hardening treatments on seedling vigour of two cabbage varieties

Treatments	RL (cm)	PL (cm)	FW (mg)	DW (mg)	VI
Varieties (V)					
Golden Acre (GA)	7.68 a	3.78 a	57 a	3.4 a	1146.53 a
Green Ball (GB)	4.84 b	3.30 b	38 b	2.4 b	602.78 b
Thermal Hardening Treatments (T)					
Control	7.36 a	3.82 a	43 ab	2.7 cde	979.69 ab
C-H 24 hrs	5.35 c	3.61 ab	47 ab	2.2 e	769.33 e
C-H 36 hrs	7.35 a	3.95 a	50 ab	3.5 a	1002.90 a
C-H 48 hrs	6.26 bc	3.49 ab	53 a	3.2 abc	886.84 abcd
C-H-C 24 hrs	6.52 ab	3.57 ab	53 a	2.6 cde	954.70 abc
C-H-C 36 hrs	6.48 ab	3.19 bc	48 ab	3.3 ab	858.43 cde
C-H-C 48 hrs	6.75 ab	3.88 a	47 ab	2.7 bcde	966.40 abc
H-C 24 hrs	6.41 ab	3.56 ab	52 ab	2.9 abcd	908.83 abcd
H-C 36 hrs	6.62 ab	3.23 bc	49 ab	2.9 abcd	875.66 bcde
H-C 48 hrs	3.94 d	2.83 c	43 ab	2.8 bcde	564.32 f
H-C-H 24 hrs	6.23 bc	3.62 ab	49 ab	3.1 abc	895.15 abcd
H-C-H 36 hrs	6.34 b	3.55 ab	46 ab	2.4 de	898.45 abcd
H-C-H 48 hrs	5.78 bc	3.73 a	42 b	3.2 abc	809.87 de
Interaction					
V × T	*	*	*	*	*

RL= radicle length; PL= plumule length

The mean values carrying same letters in a column were statistically similar to each other at $P < 0.05$.

Table 3. Effect of different temperature regimes and salinity on seed invigoration in cabbage variety Golden Acre

Treatments	FGP	T ₅₀ (days)	MGT (days)	FW (mg)	DW (mg)	VI
Hardening treatments (T)						
Control	92.0 a	1.565 a	4.185 ab	35.0 c	15 b	754.14 c
H-C24 hrs	90.2 a	1.545 a	4.114 a	52.7 b	27 a	1081.29 a
C-H-C 24 hrs	79.8 b	2.125 c	4.564 c	62.5 a	28 a	971.66 ab
C-H 36 hrs	76.6 b	1.860 b	4.524 bc	60.7 a	28 a	967.54 ab
C-H-C 48 hrs	79.6 b	1.555 a	4.161 a	49.3 b	25 a	941.31 b
Salinity levels (S) (mM NaCl)						
0	99.2 a	1.630 ab	4.168 a	62.8 a	28 a	1160.32 a
50	97.8 a	1.685 ab	4.224 ab	65.0 a	29 a	1219.57 a
100	90.2 b	1.565 a	4.280 bc	56.7 b	26 a	1190.39 a
150	84.6 c	1.780 b	4.351 c	53.4 b	27 a	877.62 b
200	46.4 d	1.990 c	4.525 d	22.3 c	12 b	268.05 c
Interaction						
T × S	*	*	*	*	*	*

The mean values carrying same letters in a column were statistically similar to each other at $P < 0.05$.

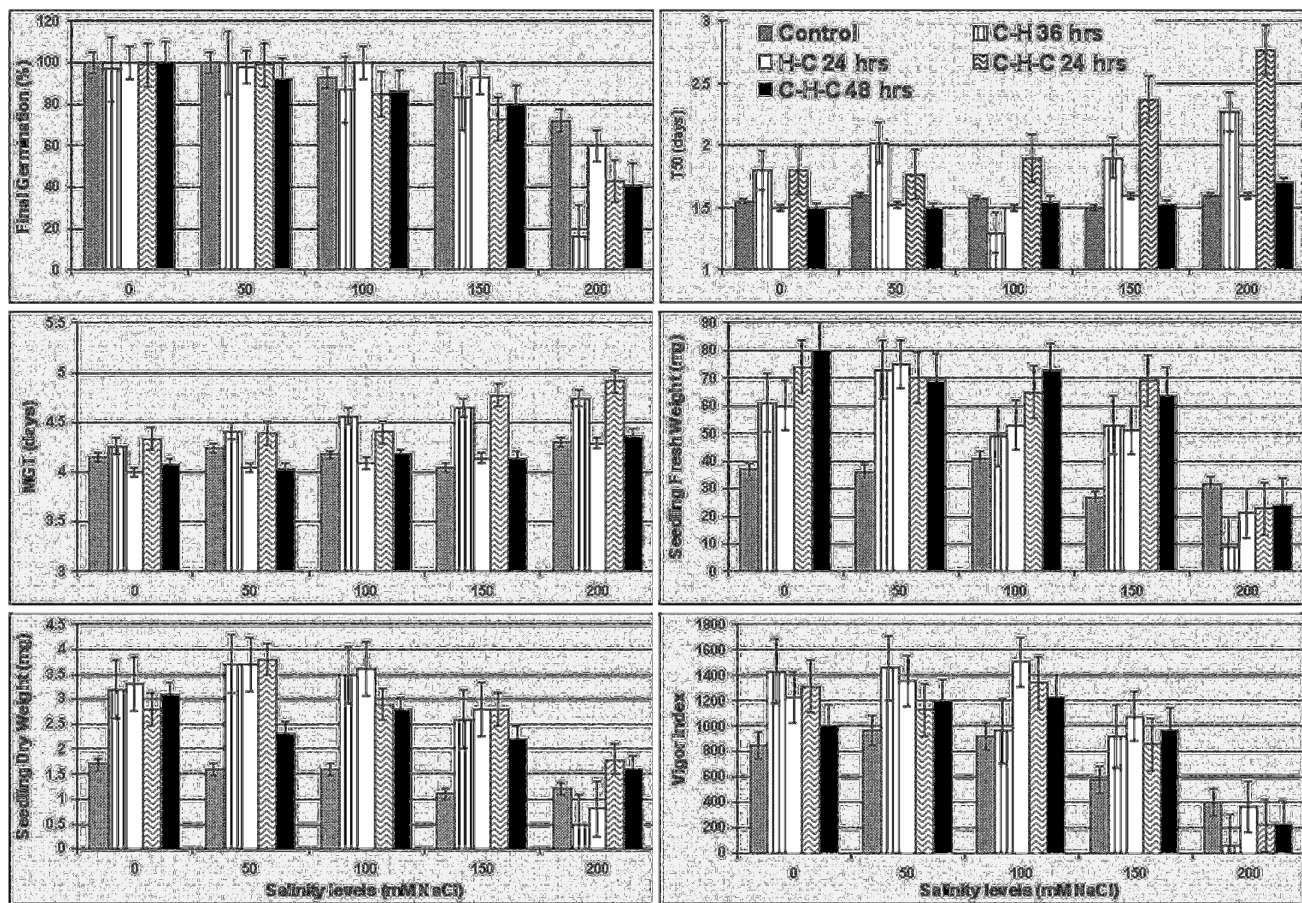


Figure 2. Effect of thermal hardening treatments on germination traits and seedling vigour indices of cabbage variety Golden Acre at various salinity levels

Seedling vigour in response to thermal hardening treatments and salinity: Different thermal hardening treatments had significant effect on seedling fresh and dry

weights (Table 3). Maximum fresh weight (62.5 mg) was observed in seeds exposed to C-H-C 24 hrs at par with C-H 36 hrs (60.7 mg). Minimum fresh weight (35.0 mg) was

observed in untreated seeds (Table 3). Maximum dry weight was recorded in C-H-C 24 hrs (28.0 mg) treatment, at par with C-H 36 hrs (28.0 mg), H-C 24 hrs (27.0 mg) and C-H-C 48 hrs (25.0 mg). While, dry weight of seedlings raised from untreated (control) seeds was very low (15.0 mg), almost half of the thermally treated seeds. Low salinity level (50 mM NaCl), although statistically similar, but slightly increased seedling fresh weight as compared to control (Table 3). Fresh weight of seedlings started to decrease at 100 mM NaCl salinity level that was at par with 150 mM NaCl salinity level. While fresh weight was reduced significantly at 200 mM NaCl salinity; about 65% reduction was recorded as compared to the control (Table 3). In contrast to seedling fresh weight, dry weight was not much affected by salinity levels except for 200 mM NaCl in which seedling dry weight was reduced to less than half of the control (about 57% less than untreated seedlings) (Table 3). Seedling fresh weight varied significantly at various salinity levels in response to thermal hardening treatments (Table 3). All thermal hardening treatments superseded the control at various salinity levels. Maximum seedling fresh weight at 0 and 100 mM NaCl salinity levels was recorded for C-H-C 48 hrs but was at par with other treatments at 50 mM NaCl indicating that low salinity level favours fresh plant weight. Seedling fresh weight increased in response to thermal hardening treatments over the control from 0 to 150 mM NaCl salinity level but such increase was not observed at 200 mM NaCl salinity (Fig. 2).

Dry weight of seedlings at various salinity levels was significantly influenced by the thermal hardening treatments (Table 1). Seed treated with H-C 24 hrs had maximum dry weight values at 0 and 100 mM but at par with C-H-C 24 hrs at 50 and 150 mM NaCl salinity levels (Fig. 2). At 200 mM NaCl salinity levels C-H-C 24 hrs yielded more dry weight values. Moreover, seed treatment C-H 36 hrs and C-H-C 48 hrs also resulted in seedlings with more dry weight than the control but their performance was comparatively inferior to other thermal hardening treatments.

Thermal hardening treatments increased vigour of cabbage seeds as compared to untreated seeds (Table 1). Seed vigour was maximum in response to H-C 24 hrs but at par with C-H-C 24 hrs and C-H 36 hrs. Vigour decreased gradually with increase in salinity above 100 mM NaCl salinity levels. It is evident from results (Table 3) that slight increase in salinity (50 mM NaCl) enhanced the vigour. There was 76% reduction in vigour at highest (200 mM NaCl) level of salinity while only 24% at 150 mM NaCl salinity level.

Seed invigoration due to thermal hardening treatments was retained up to 150 mM NaCl salinity level and was statistically superior to control at this salinity level (Fig. 2). At 0 and 50 mM NaCl salinity level C-H 36 hrs induced more vigour than other thermal hardening treatments while at 100 and 150 mM NaCl concentrations H-C 24 hrs superseded all other thermal hardening treatments. At 200

mM NaCl salinity level, untreated seeds exhibited more vigour but were statistically similar to H-C 24 hrs.

DISCUSSION

Various seed invigoration techniques (humidification, priming, wet/dry hardening and thermal hardening) have been employed in several crops resulting in improved germination indices (T_{50} , MGT, germination energy and germination index) and vigour of seeds (Farooq *et al.*, 2005, 2008; Khan *et al.*, 2012; Rehman *et al.*, 2012). We analyzed the effect of thermal hardening treatments on performance of two cabbage varieties with differential seed quality. Variety Golden Acre (GA) had higher germination percentage as compared to variety Green Ball (GB) under normal germination conditions. Thermal hardening treatments improved final germination percentage, GE, GI and vigour of GB seeds and thus corroborates previous findings of Yari *et al.* (2012) and Rehman *et al.* (2014). But, significant increase in values of these parameters were not recorded for cabbage variety GA that can be attributed to genotypic difference for response to thermal hardening as reported earlier by Lee *et al.* (2002) and Farooq *et al.* (2005). Delayed germination (MGT) and speed of germination (T_{50}) observed in thermally hardened seeds has been reported previously (Farooq *et al.*, 2005). Our results were partially contrary to the findings of Farooq *et al.* (2005) who reported that thermal hardening treatments did not affect germination percentage and radicle length. This observation of Farooq *et al.* (2005) might be due to good quality of seeds of both species of rice (*indica* and *japonica*) used in that study similar to cabbage variety GA in our study. They recorded higher germination energy in treated seeds similar to the results of cabbage variety GB. This promoting effect of seed hardening treatment can be attributed to enlargement of the embryo before imbibition (Austin *et al.*, 1969) and enhancement of the germination rate (Gray and Steckle, 1977). Moreover, there was no significant difference in GE, GI and VI values of cabbage variety GB seeds exposed first to dry heat treatment compared with seeds subjected to chilling first as reported by Farooq *et al.* (2005).

Plant establishment in most crops is limited by environmental constraints, such as extremes of temperatures, drought and salinity, resulting in poor crop stands. Poor emergence rate under these conditions is the cause of uneven plant stands (Cantliffe *et al.*, 1987). Most abiotic stresses have similar physiological consequences of inducing cellular damage and induce genes of similar signalling pathways (Shinozaki and Yamaguchi-Shinozaki, 2007). Therefore, we exposed the cabbage variety GA seeds to salt stress because, it has both osmotic (drought related) and ionic (toxicity due to salts) effects. FGP decreased with increase in exposure time of thermal cycles and salt concentration in the germination medium. But, there was no drastic change in T_{50}

and MGT values in response to salinity, which indicates that these parameters behave independently of FGP. Thermal hardening treatments failed to improve FGP at higher (150 and 200 mM NaCl) salinity levels. Moreover, it is elucidated from the results that satisfactory germination is possible at 100 mM NaCl salinity level but further increase in salinity significantly decreases the germination percentage. Our results are consistent with the findings of Jamil *et al.* (2005) who reported increase in time taken to 50% germination and reduced germination percentage in cabbage (*Brassica oleracea* var. *capitata* L.) and cauliflower (*Brassica oleracea* var. *botrytis* L.) with increase in salt concentrations (0, 4.7, 9.4, 14.1 ds m⁻¹).

Reduction in fresh and dry weights of plants in response to salt stress has been reported in many crops (Azevedo-Neto *et al.*, 2004; Jamil *et al.*, 2005 and 2007). But, this reduction in fresh and dry weights was comparatively less in response to thermal hardening treatments as compared to control. Moreover, the enhanced vigour due to thermal hardening treatments at higher salinity levels (up to 150 mM NaCl) indicated that thermal seed hardening can be used as a safe tool for raising cabbage crop in saline soil with moderately high level of salts. Overall results depicted that alternate cycles of cold and heat for 24 and 36 hours (C-H 36 hrs, H-C 24 hrs and C-H-C 24 hrs) and to some extent for C-H-C 48 hrs induced more salinity tolerance than other treatments depicting short duration exposures were more beneficial than long duration exposures to high and low temperatures. Our these findings were in accordance to Farahani *et al.* (2011) who reported that increasing exposure time of thermopriming (heating) decreased germination and vigour. But, responses to exposure time vary from crop to crop as for *Trigonella*, exposure to high temperature for 20 minutes improved germination and vigour as compared to 10 minutes (Behzad *et al.*, 2011).

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