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Induction of heat stress tolerance in barley (*Hordeum vulgare*) through thermopriming

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ABSTRACT

Present study was carried during 2021–22 and 2022–23 at ICAR-Indian Institute Seed Science, Mau, Uttar Pradesh to evaluate the effect of thermopriming on inducing heat stress tolerance in barley (*Hordeum vulgare* L.) grown under suboptimal conditions. The experimental material consisted of two location specific varieties of barley, DWRB 101 and DWRB 123, with two distinct lots, one of fresh seeds and the other of seeds aged for 1-year duration. Temperature regime between 30 to 45°C with 5°C increment and 6, 12, 24, 36 and 48 h duration, was selected as the treatment combinations along with the control (non-treated seeds). Results showed that the quantum of temperature exposure as well as the duration has significant impact on the germination and seedling growth of barley under lab conditions. The ANOVA and Dunken multiple range test enumerated that, the seeds primed at 30°C for 12 h, has positively improved the germination potential and seedling vigour index when compared to the respective lower and higher durations of exposure and revealed it as the best treatment combination. The subsequent year of evaluation of emergence potential of the treated seeds under an artificially induced high temperature stress revealed that the selected treatment was a good performer in inducing heat stress tolerance in barley, and can be chosen as solution to the high temperature stress induced through increasing atmospheric temperature at different stages of crop growth.

Keywords: Barley, Ciz priming, Emergence potential, Germination percentage, Heat stress tolerance, High temperature stress

Barley (*Hordeum vulgare* L.) is the fourth largest cereal crop globally (145.9 million tonnes), with a share of approximately 7% in the world cereal production (Pal *et al*. 2012, FAOSTAT 2022) and covering 0.45 million ha area in the subtropical conditions of India, over 13 states (Aayog NITI 2017, Banerjee *et al*. 2020, Dhanya *et al*. 2022). The IPCC indicates that the end of 21st century might witness an increase in temperature 1.5 to 2°C, over the pre-industrial levels (Djalante 2019). This can directly impact the production of barley by inducing high temperature stress (HTS) at various stages of the crop growth, adversely affecting the germinability, seedling establishment and yield (Kumar *et al*. 2014, Hatfield and Pruegar 2015, Sendhil *et al*. 2017). Studies have reported that HTS in field crops can create structural deformation of membrane proteins, disturb the protein synthesis, enhance the formation of ROS (Kumar *et al*. 2020), inhibit the photosynthetic activity (Fahad *et al*. 2016) and also diminish the activity of various metabolic enzymes (Sehgal *et al*. 2018). In barley, HTS is found to denature the D1 and D2 proteins of the Photosystem II, thereby reducing the potential for chlorophyll production (Wang *et al*. 2017). In this scenario, sustainable agriculture practices necessitate the formulation of suitable strategies that can boost immune responses in plant species towards such factors of abiotic stress. However, one of the low-cost technology to mitigate the effect of HTS in barley, is through thermopriming, which can activate the "stress memory". This activation of stress memory through thermopriming can categorically fall into the status of cis-priming since the eliciting stimulus and the response-triggering stress being the same (Hilker *et al*. 2016). Though several studies have been conducted on understanding the basic effects of cis-priming, specific studies on barley in understanding the effect of priming in inducing heat stress tolerance is lacking. Hence, a study was planned to understand the suitable thermopriming methodology that can positively impact the germinability and seedling quality in barley along with inducing heat stress tolerance under suboptimal conditions.

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MATERIALS AND METHODS

The study was carried out during 2021–22 and 2022–23 at ICAR-Indian Institute of Seed Science, Mau, Uttar Pradesh. The material selected for the experiment consisted of two location specific varieties of barley-DWRB 101 (mean maturity period of 132 days) and DWRB 123 (mean maturity period of 130 days). The seeds were separated into two specific lots with one lot being the fresh seeds while the other lot comprising of seeds aged for 1 year, viz. G_1, Fresh seed lot of DWBR 101; G_2, Aged seed lot of DWBR 101; G_3, Fresh seed lot of DWBR 123; G_4, Aged seed lot of DWBR 123 (with the hypotheses that priming may not have a significant impact on the germinability of fresh seeds). In the initial year of experiment (2021–22), the seeds were exposed to 20 different treatments of thermopriming (considering two variables of 'temperature' and the 'duration' of priming), viz. E_1, Control; E_2, 30°C-6 h; E_3, 30°C-12 h; E_4, 30°C-24 h; E_5, 30°C-36 h; E_6, 30°C-48 h; E_7, 35°C-6 h; E_8, 35°C-12 h; E_9, 35°C-24 h; E_{10}, 35°C-36 h; E_{11}, 35°C-48 h; E_{12}, 40°C-6 h; E_{13}, 40°C-12 h; E_{14}, 40°C-24 h; E_{15}, 40°C-36 h; E_{16}, 40°C-48 h; E_{17}, 45°C-6 h; E_{18}, 45°C-12 h; E_{19}, 45°C-24 h; E_{20}, 45°C-36 h; E_{21}, 45°C-48 h. Standard germination test was conducted as per ISTA (2018). Based on the ANOVA and Duncans Multiple Range Test, the best among the treatment combinations were selected for the next year, to evaluate the effect of thermopriming on the emergence potential of seeds under high temperature stress conditions.

Standardization of thermopriming and induction of heat stress: Seeds in sets of 50, from each lot were placed in four replications in a thermal resistant petri dish of diameter 90 mm × 12 mm after measuring the initial moisture content of seeds, in high temperature oven dry method (ranging from 9.14%) as per ISTA 2018. Temperature regime between 30 to 45°C with 5°C increment and 6, 12, 24, 36 and 48 h duration was selected as the treatment combinations along with the control (non-treated seeds). Seeds, in two specific seed lot-one of the freshly harvested and the other, aged for 1-year duration were exposed to the combinations in a completely randomized block design (CRD). After the requisite period of priming, the germination test was conducted (ISTA 2018) and the data for percentage of germination, seedling quality parameters, and seedling vigour index were recorded. Based on the statistical evaluation of results, in the subsequent year of study, the best treatments (cardinal temperature ranges) were selected to evaluate their emergence potential in suboptimal conditions of high temperature created artificially using a plant growth chamber. Temperature stress was induced through a customized plant growth chamber set at alternating day and night temperatures with 45% RH, and 550 mmol m^2/s light intensity (Model-OLSC-116W-21, Ocean Life Science Corporation). The emergence was measured for the next 10 days and the emergence potential was estimated as the number of seeds emerged per day.

Statistical analysis: The ANOVA (Analysis of Variance) for CRD was performed using STAR (Statistical Tool for Agricultural Research) software version 2.0.1 followed by DMRT (Duncans Multiple Range Test) to identify the best treatment combination.

RESULTS AND DISCUSSION

Effect of temperature on the germination of barley seeds: Temperature (duration and quantum of exposure) is a prime factor that affects the germination process in seeds, by indirectly influencing the metabolic pathways (Guo *et al*. 2020). In our study, the variability in the duration of priming and quantum of temperature at which the seeds were exposed to, has induced a differential pattern in enhancing germinability and seedling quality across the fresh seed and the aged seed lot. Based on the ANOVA, it was found that, there was a significant difference for the thermopriming treatments (E), genotypes (G), and genotype and environments (thermopriming treatments) interaction (G × E) for all the traits under study (Table 1).

For the germination percentage in the fresh seed lot, thermopriming had no significant influence, whereas, in the case of aged seed lot it has significantly increased the percentage of germination (Supplementary Table 1 and Table 2). The thermopriming treatments, viz. 30°C for 12 h (G_1E_3-97.01, G_2E_3-94.83; G_3E_3-97.11, G_4E_3-94.88) and 35°C for 6 h (G_1E_7-89.64, G_2E_7-88.65; G_3E_7-94.81, G_4E_7-92.89) were found to be best treatments compared to all the treatments under investigation. Meanwhile, during the first count of germination test, though the fresh seed lot, was not found to have a treatment which is statistically superior with respect to control (G_1E_1-94.93%, G_3E_1-93.83%), in the aged seed lot, seeds exposed to 30°C for 12 h (G_2E_3-93.27, G_4E_3-90.84) was found to be superior with respect to all other treatments and it was on par with seeds exposed to

Table 1 Analysis of variance (ANOVA) for completely randomized design (CRD) to the traits

Source	df	GP	FC	SL	SDW	VG1	VG2	MC
E	20	5901.51**	6353.80**	420.85**	0.016**	6808456.42**	263.57**	11.90**
G	3	182.98**	225.63**	383.67**	0.049**	2005314.84**	202.38**	33.91**
E × G	60	87.55**	83.98**	2.39**	0.0006**	45434.51**	2.43**	0.229**
Error	84	3.74	7.04	1.29	0.0001	5394.92	0.33	0.027

GP, Germination percentage; FC, First seed count; SL, Seedling length; SDW, Seedling dry weight; VG1, Seedling vigour index I; VG2, Seedling vigour index II; MC, Moisture content; E, Environment; G, Genotype.

*, ** indicates significance level at 5% and 1 % respectively.

Table 2 Analysis of variance (ANOVA) for field emergence potential of the two varieties, DWRB 101 and DWRB 123

Source	df	Field emergence potential
E	7	442.31**
G	1	218.87**
E × G	7	32.35**
Error	16	2.28

E, Environment; G, Genotype.

*, ** indicates significance level at 5% and 1 % respectively.

35°C for 6 h (G_2E_7-90.84, G_4E_7-90.37) followed by 30°C for 6 h (G_2E_2-90.02, G_4E_2-90.84) (Supplementary Table 1 and Table 2). This gradation in germination pattern can be interpreted in terms of enthalpy, where the rise in quantum of temperature exposure increases the diffusion pressure within the living system, thereby increasing its metabolic and enzymatic activity (Windaeur *et al*. 2012). The results also point to the fact that, at a particular range of temperature, there is an initial increase in percentage of germination to a maximum level (G_2E_1-85.23, G_2E_3-94.83, G_2E_5-87.72; G_4E_1-93.83, G_4E_3-94.41, G_4E_5-89.74), followed by its gradual decline, giving an "inverted bell-shape" representation of values as majorly witnessed in the aged seed lot of both the varieties (Supplementary Table 1 and Table 2). This result might be chosen as a virtue for proving the impact of thermopriming in improving the germination capacity of aged seeds, while the fresh seed lot can be considered as a target material for the activation of "stress memory" that can improve the emergence potential, under high temperature stress conditions. This increase in percentage of germination under thermopriming can be correlated with the studies of Gao *et al*. (1999) who reported that seed priming tends to activate the expression of aquaporin's leading to an increase in transport of water across the membranes thereby facilitating the activation of germination-inducing enzymes.

With respect to seedling length measured at the final count of germination test, the results indicated that in the fresh seed lot, the treatments, 30°C for 12 h (G_1E_3-32.76, G_3E_3-33.87) and 35°C for 6 h (G_1E_7-32.03, G_3E_7-33.05) were on par to each other, in both the varieties (Supplementary Table 1 and Table 2). When the seedling dry weight was measured, the results enumerated that the seeds exposed to 30°C for 12 h were performing better in both the varieties of fresh seed and aged seed lot (G_1E_3-0.19, G_2E_3-0.18; G_3E_3-0.21, G_4E_3-0.18), in comparison to control (Supplementary Table 1 and Table 2). The seedling vigour index I improved in both fresh and old seeds at thermopriming treatments, viz. 30°C for 12 h (G_1E_3-3177.7, G_2E_3-2806.1; G_3E_3-3289.0, G_4E_3-2657.8) and 35°C for 6 h (G_1E_7-3045.2, G_2E_7-2672.8) for the variety DWRB 101 whereas, DWRB 123 only 30°C for 12 h (G_3E_3-3289.0, G_4E_3-2657.8) was found better for fresh seeds and aged seeds (Supplementary Table 1 and Table 2). While in the case of vigour index II, the fresh seeds exposed at a temperature range of 30°C for 6 h to 12 h were on par to each other (G_1E_3-18.78, G_1E_2 -18.15; G_3E_3-20.65, G_3E_2-20.91), while in aged seeds, only 30°C for 12 h could induce significant improvement over the control (G_2-16.76, G_4-16.97) and is attributed to the up regulation of GA producing genes and down regulation of ABA producing genes, that holistically improve the GA/ABA ratio (Gao *et al*. 1999) (Supplementary Table 1 and Table 2). From the results it can be depicted that in order to improve the germinability of aged seeds an increase in temperature by 5°C can reduce the time required for thermopriming by half. Statistical interpretation of the results, strongly justifies this statement that in both fresh seed and the aged seed lot of the varieties under study, the seeds exposed to thermopriming at 35°C for 12 h (E_4) was on par with 30°C at 24 h (E_8) in terms of its germinability (G_1E_4-G_1E_8, G_2E_4-G_2E_8, G_3E_4-G_3E_8, G_4E_4-G_4E_8) (Supplementary Table 1 and Table 2). Hence the results give an interpolation that instead of choosing an optimum level temperature of thermopriming, it would be wise to choose a cardinal range (T_{max} - T_{min}) of the temperature duration regime, which can give the requisite germinability and vigour.

Moisture content impacting the germination potential of thermoprimed seeds: The experiment was conducted with the aim of analyzing, how thermopriming impacts the moisture content of seeds in lieu of the hypothesis that increased moisture content in seeds is a predictor of seed mortality (Tangey *et al*. 2019). Under ageing, the seed coat losses its ability to act as a covering agent or thermo protectant to the atmospheric moisture and it increases the percentage of absorption of moisture from the immediate surroundings. The results depicted that the non-treated seeds, were found to have higher percentage of moisture both in fresh seed lot (G_1E_1-11.39, G_3E_1-11.50) and aged seed lot (G_2E_1-14.00, G_4E_1-14.11), which can be due to the fact that the increase in immediate heat energy might drastically remove the free water molecules attached to the outer layers of the seed (Supplementary Table 1 and Table 2). When the duration of temperature exposure was increased in the fresh seed lot in both the varieties, a regular pattern of decreasing gradation in the moisture content within seeds was seen due to the fact that, the increasing hours of thermopriming, contributes to the breaking-off of hydrogen bonds within the water molecule, leading to its faster dissipation (G_1E_2-11.17, G_1E_6-10.12; G_3E_2-11.30, G_3E_6-10.18) (Supplementary Table 1 and Table 2). At the higher regime of temperature (E_{13}-E_{21}), the faster dissipation of moisture from the living cells might have resulted in the shutdown of the cell metabolism, thereby showing a rapid decline in germination to zero percentage at a temperature exposure of 45°C (G_1E_{21}-8.14, G_2E_{21}-8.97; G_3E_{21}-8.31, G_4E_{21}-8.85) (Supplementary Table 1 and Table 2).

Influence of thermopriming on emergence potential at field level: In the next year of study, the treatments that showed significantly higher germinability and seedling vigour index in comparison to control, were selected (E_1, E_2, E_3, E_4, E_5, E_7, E_8) and the fresh seed lot was exposed

(Table 2 and Supplementary 3). As per the results, the exposure of seeds to 30°C for 12 h (E_3), could significantly improve the emergence potential in the subsequent year of study (F_1E_3-82.99, F_2E_3-82.00) (F_1, Field emergence potential of DWRB 101; F_2, Field emergence potential of DWRB 123) in compassion to all other treatments and was found to be on par with control (F_1E_1-80.5, F_2E_1-79.67). As per Gurusinghe *et al*. (1999), this can be attributed to the activation of metabolic activities in the embryo (synthesis of nucleic acids), production of proteins and the synthesis of cell membranes in the seeds exposed to thermo priming. Further, the activation of α-amylase (degrading enzyme that activates the metabolic processes) and the enhanced activity of mitochondria might have attributed to this improvement in suboptimal conditions. After the requisite period of priming, the improvement in germination and seedling emergence rate, is attributed to increase in starch metabolism (Hossain *et al*. 2015) and accumulation of soluble sugars (Silva-Neta *et al*. 2015). Inducing stress tolerance through thermal-hydropriming treatment has reported in the production of heat shock proteins (Farooq *et al*. 2017) in wheat. Henceforth, the result paves light to the basic understanding of the activation of "stress memory" in plants through cis priming which is having a high correlation with priming induced thermo tolerance in barley. This might be due to the production of HSP's (Heat Shock Proteins) and molecular chaperones via priming that provides an additional insurance to the crop during situations of stress. While Kumari *et al*. (2021) has reported that under HTS, germination can be accelerated through seed priming, as it provides for trans generational memory from the parental population to the progeny and later it has been termed as meiotic stress memory (Khan *et al*. 2022).

Through this study, it could be concluded that the thermopriming can be chosen as suitable strategy in improving the germination potential of aged seeds in barley while to induce heat stress tolerance, the fresh seeds can be exposed to thermopriming. Among the variables of duration and quantum of temperature exposure, the parameter of moisture absorption shall be taken into cognizance. In consideration to that, rather than selecting a "super value" of temperature-duration, it would be wise enough to opt for a cardinal range of temperature-duration regime that can give the desired emergence potential under high temperature stress conditions. In our study, the range of temperature-duration has been identified as 30°C for 12 h which is on par with 35°C for 6 h, as a cardinal range for improving the heat stress tolerance in barley.

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