Seed priming improves the enzymatic and biochemical performance of rice (Oryza sativa L.) during seed germination under low and high temperatures

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Abstract

As an abiotic stress, adverse germination temperatures cause serious disruptions in physiological and biochemical processes involved in seed germination. Using a factorial experiment, we examined the effects of different seed priming treatments on enzymatic and biochemical performance of rice seeds germination under different temperatures. The results showed that the enzymatic and biochemical activities of all rice genotypes are affected by seed priming agent, especially under low germination temperature. At 15°C, seed priming with ascorbic acid was found the best agent for amylase, α-amylase, soluble sugars, catalase, peroxidase, ascorbate peroxidase, superoxide dismutase respectively with 0.095, 0.047, 29.4, 0.049, 10.9, 1.24 and 2.63 units in IRON-70 genotype and for protease and soluble proteins with 0.058 and 0.79 units in IRON-70-7053-7 genotype. Among the enzymatic activities, at low germination temperature, the superoxide dismutase and at optimum and high germination temperature, the activity of catalase, peroxidase and Protease were the most important enzymatic activity in occurrence of germination potential in terms of seedling length, vigour index, normal seedling percentage and germination rate. Under priming agents, the highest changes in normal seedling percentage were observed at low and optimum germination temperature by ascorbic acid priming in Hashemi (216.9%) and NORIN-22 (13.2%) genotypes, and at high germination temperature under KCl priming in Hashemi genotype (39.4%).

Introduction

Seed germination is a critical and irreversible process that depends on the vigour of seeds. Adverse germination temperature as abiotic stress can cause serious disruptions in physiological and biochemical processes related to seed invigoration (Pastor et al., 2013; Ellouzi et al., 2017; Hussain et al., 2019). Resistant cereals varieties have their own defense mechanisms to withstand adverse temperature germination like accumulation of compatible solutes, activation of antioxidant systems (Faroq et al., 2017; García-Caparrós et al., 2020). However, different methodologies have been employed aiming at enhancing multiple stress tolerance; some are particularly time-consuming (e.g., conventional breeding) and others are currently unacceptable in many countries around the world (e.g., plant genetic modification) (Savvides et al., 2016). As an alternative, plants can be ‘prepared’ to more successfully tolerate future biotic and abiotic stress conditions through set of certain physio-chemical, biological and integrated mechanisms by seed priming or hardening (Zheng et al., 2016; Khan et al., 2020).

Seed priming is a pre-germination treatment administered through various chemical, physical and biological agents, which induce mild stress during the early phases of germination (Strivastava et al., 2021). During priming, crops are able to generate ‘stress memory’, increase metabolic enzyme activity, develop different defense mechanisms, such as osmotic adjustments and antioxidant defenses, resulting in faster seed germination, better seedling establishment, better growth, and more crop yield, particularly in stressful environments. (Noman et al., 2018; Weeraphom and Pattanagul, 2020; Strivastava et al., 2021). Several studies successfully employed seed priming to increase the temperature tolerance range in germination stage and enhance seedling vigour, growth and yield of cereals (Kata et al., 2014; Illangakoon et al., 2016; Nawaz et al., 2016).

Priming with KCl is the most effective technique for improving growth in nursery rice seedlings, stand establishment in field and yield performance of transplanted rice (Faroq et al., 2019). The osmopriming with CaCl₂ was found in improving of germination characteristic such as root length and seedling dry weight, uniform stand establishment, growth, polyphenols, flavonoids and antioxidant activity under optimal and suboptimal conditions (Hussain et al., 2017). Reported that during priming-induced by KCl and CaCl₂ the Ca⁺ and K⁺ act as co-factor and second messenger in several signaling cascades under various stresses helps to sustain membrane integrity and regulate the production of the gibberellic acid in scutellum, the hydrolases in aleuronic layer and increase activity of α-amylase (Taiz et al., 2015) are the principal reasons to improve the rate of germination, vigourous and uniform seedling growth and improved the yield performance in direct seeded rice (Faroq et al., 2006). It has been idicated that under adverse germination temperatures, hydro-priming and Ascorbic acid priming in rice increased activity of α-amylase which in turn has resulted in better mobilization of stored carbohydrate reserves resulted in improvement of germination components such as germination percentage, speed of germination, root length, shoot length and vigour index (Kata et al., 2014). In previous studies, it has been shown that L-ascorbic acid (AsA) is the most important antioxidant. It participates in glutathione metabolism at the amino acid metabolism pathway as a key metabolite during rice seed germination, providing energy and raw materials for seed germination under low temperature (Yang et al., 2019; García-Caparrós et al., 2020). The positive effects of seed priming with Ascorbic acid are attributed to the induction of the biochemical mechanisms of redox balance and regeneration of antioxidants specifically under stress conditions (Ashraf et al., 2019; Guzmán-Ortiz et al., 2019; Seneviratne et al., 2019). Likewise studies on wheat revealed that hormonal priming with Ascorbic acid have not only improved percent germination and seedling growth, but also considerably reduced time to germination and increased germination index (GI) under saline conditions (Baig et al., 2021) and under low temperature due to late sowing date (Shah et al., 2019).

Although the role of seed priming in early plant stand and uniform seedling establishment of rice is well known under optimum or stressful conditions (Faroq et al., 2006; Ella et al., 2011; Saba Anwar et al., 2013; Illangakoon et al., 2016), the role of seed priming in improving of enzymatic and biochemical performance related to germination tolerance at lower and higher temperatures than optimum temperature(s) for germination has not been investigated. Therefore, this study investigated some physiological and biochemical parameters involved in rice germination ability under several different germination temperatures, mediated by seed priming. We aimed to address the following questions: (1) which seed priming agents are more effective at enhancing enzymatic activities and biochemical mechanisms of germination under low, high and optimum germination temperatures? (2) What enzyme is most effective at expressing rice seed germination potential under different thermal regimes?

Material And Methods

Four rice genotypes selected from earlier screenings (Monajjem et al., 2015). Two of the genotypes by low seed vigour (Hashemi and Sadry-domsfied) and two rice genotypes by high seed vigour (IRON-70-7053-7 and NORIN-22) procured from Rice Research Institute of Iran (RRII). The surface of the seeds was sterilized with sodium hypochlorite (5%) for one minute and then washed with distilled water three to five times (Mathur, 2003). Optimal conditions in all priming agents (Hydro-hardening, Potassium chloride (KCl) priming, Calcium chloride (CaCl₂) priming and Ascorbic acid priming) were established on 200 gr
seed of each rice genotypes. Hydro-hardening involves alternating hydration of sterilized seed in water at 25°C for 24 h and dehydration without aeration; this hydration–dehydration process is repeated twice (Farooq et al., 2019). For potassium chloride (KCl) priming (-1.5 Mpa), calcium chloride (CaCl2) priming (0.5 Mpa) and Ascorbic acid priming (0.1 mmol. L-1), 200 gr of seeds per genotype were placed in solution. The ratio of seed weight to solution volume (w/v) was 1:5. After 48 h soaking in the solution, the seeds were taken out and dehydrated under fan at room temperature until the seed moisture content reduced to <12%. Non-prime seeds set as control.

1. Standard germination test

After seed priming, 25 seeds of each seed lot were put on Whatman filter paper in each Petri dish (9 cm in diameter) and 5 ml of distilled water was added. Petri dishes were covered with lids to retain moisture and transferred to growth chambers at low temperature (15°C), optimum temperature (25°C) and high temperature (35°C) with optimum lighting and a relative humidity of 70±5% (Munkvold, 2009). During the 14-day experiment, germinated seeds were counted every 8 hours when the length of the radicle was at least 2 mm (Soltani et al., 2006). The software Germin which is based on the linear interpolation of cumulative germination over time (Soltani and Maddah, 2010) was used to calculate the germination rate. Using this program, seed germination rate (R50) by calculating the time (hour) required for germination of 50% (D50) of seeds during the 14-day according to the following formulae were determined. It should be noted that the shorter the germination time, the more rate (speed) the seed germination.

\[
R_{50} = \frac{1}{D_{50}}
\]

2. Seedling growth test

In the seedling growth test, we used the between paper method to measure the percent and length of normal seedlings. Two layers of paper towels (each 30 x 45 cm) were placed under and one layer on top of the twenty-five seeds. The papers were rolled up after being wet and then placed in closed containers. The containers were transferred to a growth chamber with conditions similar to those of the germination test. The normal seedlings percentage and their length [cm] were measured after 14 days (ISTA, 2009)(ISTA, 2009 #208).

\[
NSP = \frac{\sum_{ns} N}{N} \times 100\%
\]

Where N is the total number of seeds, and ns is the number of normal seedlings at the end of the experiment.

The seed vigour index (VI) after Zhu et al. (2008) was calculated using the following equation

\[
VI= S \times \sum (Gt/Dt)
\]

Where S is seedling length [cm] fourteen days after germination, Dt is the number of days from the beginning of germination until day t, and Gt is the number of germinated seeds t days after germination.

3. Enzymatic activity assay

For enzymatic activity assay, fifty seeds from each priming treatments and non-prime in three replications applied in similar conditions of germination test. Germinated seeds (on root emergence in length of 2 mm) powdered and were used for enzymatic assay.

Based on previous method described, the activity of amylase was evaluated at wavelength of 660 nm (Braga et al., 2009) and the activity of protease (cysteine proteases) was evaluated at wavelength of 650 nm (Vidyalakshmi and Selvi, 2013). The activity of α-amylase was determined by the Blackig, Corbineau et al. 1996. while barley malt α-amylase was used as a standard curve at 620 nm (Blackig et al., 1996). Total soluble sugar was determined according to the method described by Ismail et al. (2009) (Ismail et al., 2009). The total soluble sugar content was calculated from a linear equation based on a standard curve produced from d-glucose. The absorbance of the reaction mixture was read at 620 nm. Total soluble protein content of primed rice seeds was determined according to the slightly modified method of (Bradford, 1976), while Bovine serum albumin (BSA) was used as a standard. The Proxidase and Catalase activity was evaluated according to the method of (Chance and Maehly, 1955). Superoxide dismutase activity was determined according to the previous method described (Zheng et al., 2016), so that the decrease of nitroblue tetrazolium (NBT) was evaluated by reading the absorbance change at 560 nm and the activity of SOD was expressed as U/mg−1 FW. Ascorbate peroxidase activity (APox) was evaluated according to the method described by Nakano and Asada(Nakano and Asada, 1981)by determining the absorbance decrease of oxidized ascorbate after every 15 s for 1 min at wavelength of 290 nm (ε = 2.8 mM−1 cm−1). The activity of APox was expressed in U/mg−1protein. For all enzymatic activity assays, absorbance is measured using a UV-Vis spectrophotometer (Biochrom Libra S22 UV/Vis, Cambridge, United Kingdom).

Data analysis

The data were statistically analyzed using a factorial experimental design with three factors (genotype with 4 level, seed priming treatment with 5 level and germination temperature with 3 level) laid down in a RCBD with 3 replicates. Stepwise regression was used to investigate how enzymatic and biochemical activities effective on germination characteristic including germination rate, vigour index, normal seedling percentage and seedling length at each of the
Results

In the present study, the interaction effects of genotype, seed priming and germination temperature on germination rate, seed vigour index, normal seedling percentage, seedling length, enzymatic and biochemical activities were significant (Table 1).

1. Optimum germination temperature

1.1 Germination characteristics

At optimum germination temperature the lowest germination rate (0.0104 1/h), seed vigour index (0.106) and seedling length (10.22 cm) in Sadry-domsefid genotype and the lowest normal seedling percentage (81.89 %) in Hashemi genotype was observed (Table 2). By seed priming, at optimum germination temperature, the highest germination rate (0.0333 1/h) under potassium chloride priming and the highest normal seedling percentage (98.45%), seedling length (25 cm) and vigour index (0.767) under ascorbic acid priming was observed in NORIN-22 genotype (Table 2). In this investigation the effect of seed priming on germination characteristics was notable, so that at 25°C the highest changes of germination rate (112.9%) and normal seedling percentage (13.2%) in NORIN-22 genotype were observed respectively under Kcl and ascorbic acid priming (Fig. 1).

1.2 Enzymatic and biochemical activities

At optimum germination temperature (25°C), the examined genotypes mediated by seed priming differed significantly in terms of α-amylase, amylase, protease, soluble sugars, soluble proteins, catalase, peroxidase, ascorbate peroxidase, superoxide dismutase (Fig. 2). Before priming, a very high amount of α-amylase (0.027 unit), amylase (0.076 unit), protease (0.068 unit), soluble sugars (21.7 unit), soluble proteins (0.98 units), catalase (0.023 activity unit), peroxidase (4.49 activity unit), ascorbate peroxidase (0.29 activity unit) and superoxide dismutase (0.92 activity unit) was observed for NORIN-22 genotype and the lowest for Hashemi and Sadry-domsefid (Table 3, 4). After seed priming, a significant increase in this parameters was observed for genotypes. On average, the increasing of amylase, α-amylase, soluble sugars, catalase, ascorbate peroxidase and superoxide dismutase for ascorbic acid priming with 0.116, 0.065, 32.3, 0.055, 0.89 and 2.28 units respectively, the increasing of protease and soluble proteins with 0.081 and 1.79 units (mg/g seed) for potassium chloride priming and the increasing of peroxidase with 8.2 units for hydro-hardening was noted in NORIN-22 genotype (Table 3, 4). However at this temperature, the highest changes were observed under potassium chloride priming in IRON-70-7053-7 genotype for amylase (64.4%) and protease (41.3%), under Ascorbic acid priming in NORIN-22 genotype for α-amylase (139.7%), ascorbate peroxidase (200.4%) and superoxide dismutase (146.7%), under Ascorbic acid priming in Hashemi genotype for soluble sugars (62.6%) and catalase (239.8%) and under hydro-hardening in NORIN-22 genotype for peroxidase (83.1%)(Fig. 2).

2. Low germination temperature stress

2.1 Germination characteristics

At non-priming conditions, the highest germination rate, seed vigour index, normal seedling percentage and seedling length was observed in all three germination temperatures in NORIN-22 genotype (Table 2). with placing the seed at low temperature stress condition in germination temperature of 15 °C, at non-priming conditions, the lowest values of germination trait were observed in Hashemi genotype for seed vigour index (0.003), normal seedling percentage (17.4%) and seedling length (1.7 cm) and in Sadry-domsefid genotype for germination rate (0.0019 1/h) (Table 2). By seed priming, at 15°C highest germination rate (0.005 1/h) and vigour index (0.052) under ascorbic acid priming and normal seedling percentage (73.31 %) and seedling length (10.39 cm) under Kcl priming was observed in NORIN-22 genotype (Table 2). The highest changes of germination rate were observed at 15°C under Hydro-hardening in Sadry-domsefid genotype (115.2%) (Fig. 1). For normal seedling percentage the highest changes were observed at low temperature stress under ascorbic acid priming in Hashemi genotype (216.9%) (Fig. 1).

Table 1. Analysis of variance (mean squares) for seed germination characteristics of rice genotypes in priming and temperature treatments.
<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Amyl.</th>
<th>α-amyl.</th>
<th>protease</th>
<th>S. sugars</th>
<th>S. proteins</th>
<th>CAT</th>
<th>POX</th>
<th>APOX</th>
<th>SOD</th>
<th>R50</th>
<th>P.N.S</th>
</tr>
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<td>0.0001**</td>
<td>0.0005**</td>
<td>74.25**</td>
<td>0.191**</td>
<td>0.0001**</td>
<td>4.81**</td>
<td>0.03**</td>
<td>0.299**</td>
<td>0.00003**</td>
<td>803.1</td>
</tr>
<tr>
<td>Temperature</td>
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<td>0.0331**</td>
<td>0.0039**</td>
<td>0.0231**</td>
<td>2426.08**</td>
<td>17.6**</td>
<td>0.004**</td>
<td>67.6**</td>
<td>0.74**</td>
<td>1.554**</td>
<td>0.0057**</td>
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<td>0.0037**</td>
<td>0.0024**</td>
<td>1160.63**</td>
<td>2.88**</td>
<td>0.005**</td>
<td>53.7**</td>
<td>1.39**</td>
<td>5.08**</td>
<td>0.0039**</td>
<td>2241</td>
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<td>0.417**</td>
<td>0.003**</td>
<td>69.3**</td>
<td>0.22**</td>
<td>7.54**</td>
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<td>Genotype ×Temperature</td>
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<td>0.0001**</td>
<td>0.0001**</td>
<td>57.5**</td>
<td>0.224**</td>
<td>0.0001**</td>
<td>1.79**</td>
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<td>0.13**</td>
<td>0.00007**</td>
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<td>0.000001**</td>
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<td>1.81</td>
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<td>1.68</td>
<td>2.31</td>
<td>1.9</td>
<td>3.37</td>
<td>1.48</td>
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</table>

Amyl. (amylase (mg amylase per mg protein)), α-amyl. (α-amylase (mg α-amylase per mg protein)), protease (mg tyrosine per mg protein), s.sugars (soluble sugars (mg soluble sugars per gr seed)), s.proteins (soluble proteins (mg soluble protein per gr seed)), CAT (catalase (μM CAT-1 min-1 mg protein)), POX (peroxidases (μM POX-1 min-1 mg protein)), APOX (ascorbateperoxidases (μM APX-1 min-1 mg protein)), SOD (superoxide dismutase (unit mg-1 protein)), R50 (germination rate (1/h)), P.N.S. (normal seedling percentage (%)), V.I. (Vigour index) and S.L. (Seedling length (cm)).

ns: non-significant, * and **: Significant at 5% and 1% probability levels, respectively.

**Table 2.** Amount of R50 (germination rate), normal seedling percentage, seedling length (cm) and Vigor index under seed priming treatments Potassium chloride (KCl), Ascorbic acid (Ascor), Calcium chloride (CaCl2), Hydro-hardening (Hydro) and non-priming (Nor), in four rice genotypes (Hashemi, Sadri-Domsef, IRON-70-7953 and NORIN-22) at germination temperature of 15°C, 25°C and 35°C.
3.2 Enzymatic and biochemical activities

3.2.1 Germination characteristics

At high germination temperature stress, the lowest values of germination characteristics were observed in Hashemi genotype for germination rate (0.013 1/h), seed vigour index (0.109) and normal seedling percentage (65.01 %) and in Sadry-domsefed genotype for seedling length (8.15 cm) (Table 2). By seed priming at 35 °C, the highest germination rate (0.0439 1/h) under ascorbic acid priming and the highest normal seedling percentage (94.45 %), seedling length (21.06 cm) and vigour index (0.802) under potassium chloride priming was observed in NORIN-22 genotype (Table 2). The highest changes of germination rate were observed at 35°C under ascorbic acid priming in NORIN-22 genotype (129.8%) (Fig. 1). For normal seedling percentage the highest changes were observed at 35°C under KCl priming in Hashemi genotype (39.4%) (Fig. 1).

3.2.2 Enzymatic and biochemical activities

The results showed that seed priming with ascorbic acid for amylase, α-amylase, soluble sugars, catalase, peroxidase, ascorbate peroxidase, superoxide dismutase respectively with 0.095, 0.047, 29.4, 0.049, 10.9, 1.24 and 2.63 units in NORIN-22 genotype and for protease and soluble proteins with 0.058 and 0.79 units in IRON-70-7053-7 genotype was found best among all the primings and genotypes (Table 3, 4). At low temperature stress condition, the effective changes by seed priming on genotypes was notable than optimum temperature (25°C). So that the highest effects was observed under ascorbic acid priming in Hashemi genotype for α-amylase (618.6%), soluble sugars (975.3%), protease (218.7%), soluble proteins (380.8), catalase (489.3%) and prooxidase (106.4%), in Sadry-domsefed genotype for ascorbate peroxidase (186.2%) and superoxide dismutase (147.9%) (Fig. 2).
the highest changes were observed under Ascorbic acid priming in Hashemi genotype for amylase (65.5%), soluble sugars (63.3%) and catalase (242.5%), in NORIN-22 genotype for α-amylase (144.4%), ascorbate peroxidase (209.7%) and superoxide dismutase (150.6%) and under hydro-hardening in IRON-70-7053-7 genotype for protease (45.2%), in Hashemi genotype for soluble proteins (134.9%) and in NORIN-22 genotype for peroxidase (85.6%) (Fig. 2).

**Table 3.** Amount of amylase (mg amylase per mg protein), α-amylase (mg α-amylase per mg protein), soluble sugars (mg soluble sugars per gr seed) and protease (mg tyrosine per mg protein) under seed priming treatments Potassium chloride (KCl), Ascorbic acid (Ascor), Calcium chloride (CaCl2), Hydro-hardening (Hydro) and non-priming (Nor), in four rice genotypes (Hashemi, Sadri-Domsed, IRON-70-7953 and NORIN-22) at germination temperature of 15°c, 25°c and 35°c.

<table>
<thead>
<tr>
<th>Treat</th>
<th>Var</th>
<th>Amylase (mg Amylase -1mg Protein)</th>
<th>α-Amylase (mg α-Amylase -1mg Protein)</th>
<th>Suger (mg Suger -1g Seed)</th>
<th>Protease (mg Tirozin Protein)</th>
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<td></td>
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<td>15 °c</td>
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<td>35 °c</td>
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<td>0.018a</td>
</tr>
<tr>
<td></td>
<td>Norin</td>
<td>0.085i</td>
<td>0.115def</td>
<td>0.118abc</td>
<td>0.039f</td>
</tr>
<tr>
<td></td>
<td>IRON</td>
<td>0.082s</td>
<td>0.109g</td>
<td>0.113i</td>
<td>0.036mn</td>
</tr>
</tbody>
</table>

*Means followed by the same letters in culms are not significantly different at 5% level of probability.

**Table 4.** Amount of soluble proteins (mg soluble protein per gr seed), catalase (μM CAT-1 min -1 mg protein), peroxidas (μM POX -1 min -1 mg protein), ascorbateperoxidases (μM APX -1 min -1 mg protein) and superoxide dismutase (unit mg-1 protein) under seed priming treatments Potassium chloride (KCl), Ascorbic acid (Ascor), Calcium chloride (CaCl2), Hydro-hardening (Hydro) and non-priming (Nor), in four rice genotypes (Hashemi, Sadri-Domsed, IRON-70-7953 and NORIN-22) at germination temperature of 15°c, 25°c and 35°c.
Table 5. Analysis of stepwise regression with consideration of germination rate, Vigor index, percent of normal seedling and Seedling length as dependent variables and enzymatic and biochemical activities as independent variables.

<table>
<thead>
<tr>
<th>Treat</th>
<th>Var</th>
<th>Protein (mg Protein⁻¹g Seed)</th>
<th>Catalase (µM CAT⁻¹ min⁻¹ mg)</th>
<th>POX (µM POX⁻¹ min⁻¹ mg)</th>
<th>APOX (µM APX⁻¹ min⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>15 °C  25 °C  35 °C</td>
<td>15 °C  25 °C  35 °C</td>
<td>15 °C  25 °C  35 °C</td>
</tr>
<tr>
<td>KCl</td>
<td>Sad</td>
<td>0.51w  1.52m  1.63hi</td>
<td>0.013abc  0.031qr  0.035lm</td>
<td>5.58op  4.05cd  4.69wx</td>
<td>0.48im  0.29jz  0.</td>
</tr>
<tr>
<td></td>
<td>Hash</td>
<td>0.47xy  1.51m  1.60hi</td>
<td>0.010zef  0.028st  0.032sp</td>
<td>6.10mn  3.48df  4.92stu</td>
<td>0.46mno 0.23zc  0.</td>
</tr>
<tr>
<td></td>
<td>Norin</td>
<td>0.56v  1.75c  1.75bc</td>
<td>0.034mn  0.052c  0.056b</td>
<td>8.64b  6.52k  7.36h</td>
<td>0.59h  0.40gr  0.</td>
</tr>
<tr>
<td>IRON</td>
<td></td>
<td>0.64z  1.64defh  1.65efg</td>
<td>0.034mn  0.052c  0.056b</td>
<td>8.64j  4.17bc  5.02st</td>
<td>0.54i  0.35u  0.</td>
</tr>
<tr>
<td>Ascor</td>
<td>Sad</td>
<td>0.67tu  1.54m  1.57kl</td>
<td>0.032dp  0.038k  0.043i</td>
<td>8.11e  5.04ns  5.26i</td>
<td>0.96b  0.60h  0.</td>
</tr>
<tr>
<td></td>
<td>Hash</td>
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<td>0.032dp  0.038k  0.043i</td>
<td>6.85j  3.78ze  5.60dp</td>
<td>0.87d  0.52k  0.</td>
</tr>
<tr>
<td></td>
<td>Norin</td>
<td>0.76s  1.80ab  1.84a</td>
<td>0.049f  0.055b  0.060a</td>
<td>10.99a 7.93f  8.54d</td>
<td>1.25a  0.89c  0.</td>
</tr>
<tr>
<td>IRON</td>
<td></td>
<td>0.79s  1.67df  1.72cd</td>
<td>0.044hi  0.051de  0.056b</td>
<td>10.11c 7.04i  7.66g</td>
<td>0.95b  0.60h  0.</td>
</tr>
<tr>
<td>CaCl</td>
<td>Sad</td>
<td>0.41rz  1.05p  1.60i</td>
<td>0.009df  0.022e  0.025xy</td>
<td>6.18m  4.20bc  4.65wx</td>
<td>0.47imm 0.28jza 0.</td>
</tr>
<tr>
<td></td>
<td>Hash</td>
<td>0.37zab  1.16p  1.56kmm</td>
<td>0.004sf  0.017zj  0.020r</td>
<td>5.20qr  3.22zh  4.86uv</td>
<td>0.45no  0.26zab 0.</td>
</tr>
<tr>
<td></td>
<td>Norin</td>
<td>0.45sx  1.57kl  1.64sh</td>
<td>0.018zz  0.031pqr  0.034mn</td>
<td>6.77i  4.79xw  5.24q</td>
<td>0.56j  0.37h  0.</td>
</tr>
<tr>
<td>IRON</td>
<td></td>
<td>0.41rz  1.51m  1.60jk</td>
<td>0.014za  0.027ju  0.030t</td>
<td>6.25m  4.27zab  4.72xw</td>
<td>0.56i  0.37l  0.</td>
</tr>
<tr>
<td>Nor</td>
<td>Sad</td>
<td>0.22zc  0.78s  0.79s</td>
<td>0.009df  0.018zj  0.020r</td>
<td>4.38zza 3.34zgh  3.36zgh</td>
<td>0.37st  0.23zc 0.</td>
</tr>
<tr>
<td></td>
<td>Hash</td>
<td>0.13zd  0.70f  0.70i</td>
<td>0.001mn  0.011zcd  0.012zbc</td>
<td>3.32zgh 2.27zl  3.39zfg</td>
<td>0.33uv  0.19zd 0.</td>
</tr>
<tr>
<td></td>
<td>Norin</td>
<td>0.41zza  0.98l  0.93l</td>
<td>0.014za  0.024uy  0.025uy</td>
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</tr>
<tr>
<td>IRON</td>
<td></td>
<td>0.33zb  0.88f  0.90l</td>
<td>0.011zcd 0.020y  0.022x</td>
<td>5.33q  4.28zab  4.41zaz</td>
<td>0.41q  0.27zza 0.</td>
</tr>
<tr>
<td>Hydro</td>
<td>Sad</td>
<td>0.48xy  1.53mr  1.68sd</td>
<td>0.021y  0.033ro  0.036i</td>
<td>6.10mn  3.93zde  4.26zab</td>
<td>0.47imm 0.29jz 0.</td>
</tr>
<tr>
<td></td>
<td>Hash</td>
<td>0.51w  1.51m  1.65edg</td>
<td>0.014za  0.026iv  0.029as</td>
<td>5.72o  3.84zke  4.87yu</td>
<td>0.46mno 0.27zra 0.</td>
</tr>
<tr>
<td></td>
<td>Norin</td>
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<td>0.035lm  0.047y  0.050de</td>
<td>10.40b 8.23e  8.57d</td>
<td>0.64g  0.45pp 0.</td>
</tr>
<tr>
<td>IRON</td>
<td></td>
<td>0.63z  1.63ghi  1.67edf</td>
<td>0.030fr  0.043j  0.046sp</td>
<td>8.18a  6.02m  6.35j</td>
<td>0.63d  0.40gr 0.</td>
</tr>
</tbody>
</table>

*Means followed by the same letters in culms are not significantly different at 5% level of probability.

4. A stepwise regression analysis

The results of stepwise regression analysis showed that in different germination temperature conditions, the enzymatic and biochemical activities that were effective in improving germination characteristics, were different (Table 5). For vigour index and germination rate at 15°C the superoxide dismutase enzyme (Fig. 3), at 25°C the catalase enzyme (Fig. 4) and at 35°C the peroxidase enzyme (Fig. 5) was found the most important enzymes that were included in the regression model and accounted for at least 62% of the changes in seed vigour index and germination rate (Table 5). The most important enzymatic and biochemical activities affecting on the normal seedling percentage at germination temperature at 15, 25 and 35 °C were superoxide dismutase, catalase and amylase activity respectively. These factors identified as justifying for at least 64% of the variance in normal seedlings percentage (Table 5). However, for seedling length at low germination temperature stress the superoxide dismutase, at optimum germination temperature the catalase and the protease and at high germination temperature stress the catalase activity are the most important enzymes that determine seedling length (Table 5).
dependent variable  | Temperature of germination | Independent variable | Ms  | a±Es             | b±Es           | R²   | Pr-F  
--- | --- | --- | --- | --- | --- | --- | --- 
Germination rate (R50)  | 15  | SOD  | 0.00001  | 0.001+0.0003 | 0.001+0.0002 | 0.67 | 0.0001  
 | 25  | CAT  | 0.0005  | 0.0027+0.002 | 0.24+0.98 | 0.69 | 0.0001  
 | POX | 0.0002  | 0.0027+0.002 | 0.001+0.0008 | 0.75 | 0.05 |  
 | 35  | POX  | 0.001  | -0.002+0.002 | 0.004+0.0005 | 0.78 | 0.0001  
VigourIndex (V.I.) | 15  | SOD  | 3228.6  | 10.05+6.96 | 23.35+4.13 | 0.64 | 0.0001  
 | 25  | CAT  | 314.34  | 80.1+1.46 | 301.63+40.7 | 0.73 | 0.0001  
 | 35  | POX  | 1193.97  | 40.45+4.41 | 423.33+44.06 | 0.80 | 0.0001  
Normal seedling percentage (P.N.S.) | 15  | SOD  | 81.93  | 0.53+0.93 | 3.72+0.55 | 0.71 | 0.0001  
 | 25  | CAT  | 158.9  | 14.7+4.25 | 414.3+68.1 | 0.63 | 0.0001  
 | Protease  | 158.9  | 14.7+4.25 | -201.9+85.87 | 0.75 | 0.03 |  
 | 35  | CAT  | 108.04  | 7.01+1.14 | 144.1+28.9 | 0.61 | 0.0001  

**Discussion**

**1. low germination temperature**

At the low temperature stress, the amounts of seed vigour index, normal seedling percentage, seedling length and germination rate were lower in Hashemi and Sadry-domsefif than in NORIN-22 and IRON-70-7053-7 (Table 2). By seed priming, the highest germination rate (0.005 1/h) and vigour index (0.052) under ascorbic acid priming and normal seedling percentage (73.31 %) and seedling length (10.39 cm) under Kcl priming was observed in NORIN-22 genotype (Table 2).

At this temperature condition and unprimed seeds, the activity of hydrolytic enzymes amounts of soluble sugars and soluble proteins in all genotypes were at a minimum level. The NORIN-22 and IRON-70-7053-7 by amylose content of 18.1 and 17.4%, respectively (Hori et al., 2016) have more the activity of hydrolytic and antioxidant enzymes activity than Hashemi and Sadry-domsefif by amylose content of 22.1 and 23.6%, respectively (Habibi and YahyaZadeh, 2015; Alizadeh and Habibi, 2016). There are reports indicating an activity concentration of hydrolytic enzymes varies depending on the genotype, growing conditions and germination (Kalita et al., 2017; Guzmán-Ortiz et al., 2019). Kalita et al. (Kalita et al., 2017) reported the composition of rice in amylose/amylopectin ratio can influence amylase activity. So that rice with low amylose content (12.5%) exhibited more activity in amylase than rice with optimum amylose (20.2%). This may be due to faster hydrolysis of amylose than amylpectin, since amylpectin is more complex to be hydrolyzed by enzymes (Zheng et al., 2006).

This condition, although the amounts of amylase, α-amylose, soluble sugars, catalase, peroxidase, ascorbate peroxidase, superoxide dismutase were higher in NORIN-22 genotype than other genotypes (Table 2), but the highest changes was observed under ascorbic acid priming in Hashemi genotype for α-amylose (618.6%), soluble sugars (975.3%), protease (218.7%), soluble proteins (380.8), catalase (489.3%) and proxidase (106.4%) (Fig. 2). The findings of other researchers have shown that the external use of ascorbic acid as seed priming results in improved seed physiological and biochemical properties lead to increasing seed vigour and germination (Saba Anwar et al., 2013; Shah et al., 2019; Xia et al., 2020). Likewise reported above, the improvement of seed germination and increased cold tolerance in NORIN-22 genotype by ascorbic acid priming was related to the higher constitutive superoxide dismutase, ascorbate peroxidase, peroxidase and catalase. Other researchers have shown that some rice genotypes that have higher starch and sucrrose storage in the endosperm (Basu et al., 2012) and early amounts of catalase activity in the dry seeds (before imbibition) in their embryos (Lin et al., 2006), showed markedly poten in superoxide and hydroxyl scavenging activity. It has been proposed that the germination is completed only when the ROS content is within an oxidative window that allows ROS signaling (Huang et al., 2019). Above or below the ‘oxidative window for germination’, low or high amounts of ROS would not permit progress towards germination (Bailly et al., 2008). Thus, higher level of ascorbic acid observed in primed seeds can act as signal molecule and was consistent with oxidative window concept, which resulted in better germination and faster seedling growth compared with that in unprimed and other primed seeds (Akram et al., 2017). In this regard Xia et al, (2020) reported ascorbic acid by improving the ascorbate–glutathione cycle, cytochrome c-oxidase (COX), mitochondrial malate dehydrogenase (MDH) activities, and the mitochondrial ultrastructures of the embryonic root cells were markedly improved in oat seeds (Xia et al., 2020).
In other studies, revealed that chilling stress significantly reduced the rice seed germination and seedling growth, as well as the respiration rate, ATP contents (Nie et al., 2020), GA deactivation, inhibited GA signal transduction, and increased ABA synthesis (Wang et al., 2018) in rice seeds and seedlings. While seed priming was effective to significantly enhance the respiration rate and ATP levels in rice seeds and seedlings, such positive effects might be induced by the enhanced glycolysis metabolism, as well as the repair and biogenesis of mitochondria (Nie et al., 2020). Wang et al. (2018) reported a deficiency of bioactive GAs in rice seeds exposed to low temperature conditions, the soluble sugar content in endosperm was reduced along with depression of the specific activity levels of α-amylase (EC 3.2.1.1) and β-amylase (EC 3.2.1.2), but the soluble sugar content was increased in the embryo compared with the control treatment. Low temperature treatment promoted sugar transportation from endosperm to embryo and reduced the activity levels of enzymes involved in glycolysis and the tricarboxylic acid cycle, which participated in sugar consumption to boost rice seed germination under low temperature conditions. In conclusion, a deficiency of bioactive GAs in rice seeds exposed to low temperature led to a decrement in starch hydrolysis and sugar consumption, thus inhibit seed germination (Wang et al., 2018).

2. Optimum germination temperature

At optimum germination temperature condition and without priming the lowest germination characteristics and enzymatic and biochemical activities was observed in Sadyr-dosef and Hashemi genotypes, but the highest that in NORIN-22 genotype (Table 2). By seed priming, the highest germination rate (0.0333 1/h), protease (0.081 mg/g seed) and soluble proteins (1.79 mg/g seed) under potassium chloride priming was observed in NORIN-22 genotype (Table 2, 3). Reported that Potassium is not only a constituent of the plant structure but it also has a regulatory function in several biochemical processes related to protein synthesis, carbohydrate metabolism, and enzyme activation (Hasanuzzaman et al., 2018). Potassium assists in seed germination by initiating the rapid imbibition of water, and it also facilitates for improving seed germination has a promising regulatory role in increasing the germination percentage, germination rate, seedling growth and yield in plants like Oryza sativa L. (Sokht-Abandani and Ramezani, 2012), Triticum aestivum L. (Jatav et al., 2012; Hasanuzzaman et al., 2018) and Zea mays L. (Ul-Allah et al., 2020). Increase the activity of protease and soluble proteins by potassium has been stated in relation to increasing the activity of nitrate reductase in some crops as well, like Oryza sativa L. (Pandey et al., 2004), Triticum aestivum L. (Jatav et al., 2012). Because conversion of nitrate into nitrite is the first rate limiting step catalyzed by the nitrate reductase for further assimilation and the subsequent mobilization for synthesis of amino acids and proteins (Stitt et al., 2002; Ahanger et al., 2017).

In other hand reported, after priming the improved germination and normal seedling percentage are owed to starch metabolism (Hossain et al., 2015), whereas higher α-amylase and higher accumulation of soluble sugars is responsible for good stand establishment traits (Silva-Neta et al., 2015) (Silva-Neta et al., 2015 maize). Seed priming with KCl exerts moderate stress to seeds, which better equips them for future environmental conditions (Gallardo et al., 2001) (Gallardo et al., 2003 Arabidopsis). Seed priming with KCl results in the accumulation of various stress proteins, such as late embryogenesis proteins and heat shock proteins (Farooq et al., 2020) (Farooq et al., 2020 wheat), which probably increased α-amylase, dehydrogenase activities and soluble sugars to improve germination and stand establishment (Haider and Rehman, 2022).

At optimum germination temperature the seed priming with ascorbic acid priming, significantly increase the amount of amylase, α-amylase, soluble sugars, catalase, ascorbate peroxidase and superoxide dismutase so that achieved the highest normal seedling percentage (98.45%), seedling length (25 cm) and vigour index (0.767) in NORIN-22 genotype (Table 2, 3, 4). In this regard there are many reports confirming the role of ascorbic acid as a primary substrate in cyclic pathways for the detoxification and neutralization of single superoxide radicals and oxygen, in enhancing the activity of antioxidant enzymes. Results have shown that in response to environmental stresses, ascorbate peroxidase activity along with other enzymes, such as catalase, superoxide dismutase, and glutathione reductase, generally increases (Mittler, 2002; Scandalios, 2005). It has also been reported that ascorbate peroxidase isoenzymes are deactivated in the absence of ascorbic acid. Therefore, high levels of intracellular ascorbic acid are necessary to effectively protect the plant's antioxidant system from oxidative damage (Asada, 1992; Shigeoka et al., 2002).

3. high germination temperature

At high germination temperature, the normal seedling percentage and seedling length in genotypes were lower than optimum germination temperature condition (Table 2). In this regards observed during germination of rice seed, high temperature stress caused ABA accumulation, ROS (O2 and H2O2) and malondialdehyde contents increasing (Liu et al., 2019) and decreasing the abundance of proteins involved in methionine metabolism, amino acid biosynthesis, energy metabolism, reserve degradation, protein folding and stress responses (Liu et al., 2015) inhibited seed germination and seedling establishment.

By seed priming at 35 °C, the effect of KCl priming on hydrolytic enzymes; amylase and protease and germination characteristics; normal seedling percentage, seedling length and vigour index was notable. Our results are in agreement with Ella, et al. (2011), who indicated that potassium by adjusting the osmotic potential for rearrangement of membranes and repair of damaged membranes and by participating in polypeptide synthesis in ribosomes plays an important role in acclimation to high temperature stress (Ella et al., 2011). It has been shown that high protein content in the seed is needed to increase seedling vigour in cereals. However, improving the protease activity is associated with a simultaneous decrease in storage and enzymatic proteins, that under these conditions, potassium by maintaining and regulating osmotic pressure and cell elongation increases the seedling vigour of various crops (Gallardo et al., 2001). In other study reviled that KCl priming in rice seeds due to increasing the chlorophyll content led to more vigour index and seedling length (Afzal et al., 2012). Although the highest antioxidant enzymes activity such as catalase (0.06 unit), peroxidase (8.5 unit), ascorbate peroxidase (0.96 unit) and superoxide dismutase (2.35 unit) under ascorbic acid priming was observed in NORIN-22 genotype (Table 2) but hydro-hardening priming had the highest changes on protease (45.2%) in IRON-70-70S-7, soluble proteins (134.9%) in Hashemi and peroxidase (85.6%) in NORIN-22 (fig. 2). Our results confirmed the existing literature (Hussain et al., 2015; Zheng et al., 2016; Mahakham et al., 2017) which showing the application of ascorbic acid, potassium chloride and hydro-hardening priming in rice is related to the positive effect of Potassium Chloride as a catalyst for enzymatic processes (Cakmak, 2005; Siddiqui et al., 2011; Hasanuzzaman et al., 2018), ascorbic acid in direct neutralization of hydrogen peroxide and prevention of oxidation of proteins, fatty acids and other
biomolecules (Asada, 1992) and the role of hydro-hardening in the faster production of protease, α-amylase and β-amylase in the aleurone layer and increased gibberellic release from seed embryos (Gujaliah and Kumari, 2013).

Prabhu et al., (2018) reported during hydro-hardening, a number of physicochemical changes occur like the quiescent cells get hydrated and germination initiated, enhanced mitochondrial activity leading to the formation of high energy compounds, vital bio-molecules etc. and modify the protoplastic characters, increasing the embryo physiological activity and associated structures, eventually leading to higher absorption of water, increase in the elasticity of the cell and development of a stronger and efficient root system (Prabhu et al., 2018).

**A stepwise regression analysis**

Our results depicted that seed priming was effective in increasing the thermal niche by changes the enzymatic and biochemical activities related to germination. Based on the results, it can be stated that at low germination temperature, superoxide dismutase is the most important enzyme that determines seed vigour index, germination rate, normal seedling percentage and seedling length. At this temperature, per unit increase in superoxide dismutase activity, be increased the amount of germination rate, vigour index, normal seedling percentage and seedling length with 0.001, 0.02, 23.36 and 3.72 units, respectively. Recently, reported that primed seeds exhibited more robust antioxidant system than unprimed ones during germination (Van Nghiep and Gaur, 2005; García-Caparós et al., 2020), so that the high amount of germination characteristics observed in primed seeds was related to induction by antioxidant enzymes especially SOD, which is a tightly controlled mechanism to balance ROS in oxidative window range for stimulating seed germination as discuss above (Zheng et al., 2016; Guzmán-Ortiz et al., 2019; Yang et al., 2019). However, other studies have shown that low temperature tolerance in rice genotypes depends on the activity of catalase and ascorbate peroxidase in seedling shoots (Kang and Saltveit, 2002).

In optimum and high germination temperature, the activity of catalase, peroxidase and Protease were the most important enzymatic activity in occurrence of germination potential in terms of seedling length, vigour index and germination rate. In this regard, it was found that increasing protease activity and conversion of protein to polypeptides and amino acids has a positive relationship with germination rate because of utilization of this products for a large proportion (39.1–93.9%) from seed imbibition (stage 1) to the elongation radicle (stage 3) and even the early seedling stage (Zhao et al., 2018).

Under seed priming, investigation the normal seedling percentage “as the most important assessable trait in connecting seed quality to the early seedling stage” showed that high amount of this trait at low, optimum and high germination temperature related on the activity of the SOD, Catalase and Amylase respectively. According to Grohs et al. (2016), when seeds are exposed to low temperatures, SOD is an enzyme in the first line of the plant defense, transforming the superoxide into H₂O₂ while APX and CAT detoxify H₂O₂ (Grohs et al., 2016). Moreover, the lower presence of H₂O₂ in the seedlings decreased H₂O₂ production and likely reducing the participation of CAT and APX enzymes. These changes eventually can optimize defense mechanisms during seed germination and seedling elongation through a decrease in H₂O₂ production (Grohs et al., 2016; Hsu and Hsu, 2019). Based on results, in high germination temperature, increase the normal seedling percentage of primed seeds could partly be explained as a consequence of increased activity of amylase allowing faster rate of starch hydrolysis in germinating primed seeds. This resulted in more availability of soluble sugars necessary for generating the energy required for growth and maintenance processes, thereby leading to increased speed of germination and normal seedling percentage (Zheng et al., 2016; Zhao et al., 2018).

**Conclusion**

From the present study, it can be concluded that all rice genotypes are affected by seed priming agent under all temperatures but the changes in germination characteristics and enzymatic and biochemical activities was notable at low germination temperature than other temperatures. Among the seed priming, the highest germination rate at high and low germination temperature under ascorbic acid priming and at optimum germination temperature under potassium chloride priming was observed in NORIN-22 genotype while the highest normal seedling percentage, seedling length and vigour index at optimum and low germination temperature under ascorbic acid priming and at high germination temperature under potassium chloride priming was observed in NORIN-22 genotype. The highest changes of germination rate were observed at low germination temperature under hydrohardening in Sadry-domsefid genotype (115.2%), at optimum germination temperature under KCl priming in NORIN-22 genotype (112.9%) and at high germination temperature under ascorbic acid priming in NORIN-22 genotype (129.8%). For normal seedling percentage the highest changes were observed at low germination temperature under ascorbic acid priming in Hashemi genotype (216.9%), at optimum germination temperature under ascorbic acid priming in NORIN-22 genotype (13.2%) and at high germination temperature under KCl priming in Hashemi genotype (39.4%). The most important enzymatic and biochemical activities affecting on the normal seedling percentage at germination temperature of low, optimum and high germination temperature were superoxide dismutase, catalase and amylase activity respectively. For seed vigour index and germination rate at low germination temperature the superoxide dismutase enzyme, at optimum germination temperature the catalase enzyme and at high germination temperature the peroxidase enzyme was found the most important enzyme. Based on the current results, for sowing rice at optimum germination temperature and below optimum temperature, seed priming with ascorbic acid and at germination temperature above optimum temperature, seed priming with KCl can be suggested.

**Declarations**

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datasets analysed during the current study are available from the corresponding author on reasonable request.

References


Figures
Figure 1

Percentage of changes in germination characteristics of rice genotypes (Hashemi, Sadri-Domsefid, IRON-70-7953 and NORIN-22) affected by seed priming (Potassium chloride (KCl), Ascorbic acid (Ascor), Calcium chloride (CaCl2), Hydro-hardening (Hydro)) compared to non-priming at three germination temperature of 15°C, 25°C and 35°C.

Figure 2

Percentage of changes in enzymatic and biochemical activities of rice genotypes (Hashemi, Sadri-Domsefid, IRON-70-7953 and NORIN-22) affected by seed priming (Potassium chloride (KCl), Ascorbic acid (Ascor), Calcium chloride (CaCl2), Hydro-hardening (Hydro)) compared to non-priming at three germination temperature of 15°C, 25°C and 35°C.
Figure 3
Linear relationship between SOD versus vigour index (V.I.), Germination rate (R50), Seedling length (S.L.) and normal seedling percentage (P.N.S.) at germination temperature of 15°C.

Figure 4
Linear relationship between CAT versus vigour index (V.I.), Germination rate (R50), Seedling length (S.L.) and normal seedling percentage (P.N.S.) at germination temperature of 25°C.

Figure 5
Linear relationship between POX versus vigour index (V.I.) and Germination rate (R50), between Amylase versus normal seedling percentage (P.N.S.) and between CAT versus Seedling length (S.L.) at germination temperature of 35°C.