Original Research Article

Influence of seed storage and priming on naturally aged seeds of aromatic *Joha*rice

ABSTRACT

Joha is a fine grain aromatic rice of Assam, well known in the world market. This rice is protected and tagged as a geographical indication. This study evaluated priming effects on naturally aged seeds of two varieties, *Kon Joha* and *Keteki Joha*, after 0, 3, 6 and nine months of storage under ambient conditions. Seeds were hydro-primed, osmo-primed with 5% and 10% PEG, halo primed with 1% and 2% KCl and primed with 5 ppm and 10 ppm GA_3 for 12 and 24 hours. Seed moisture percentage, germination percentage, germination index, mean germination time, seedling length and dry weight, seed vigour index, field emergence, seed reserve utilization rate, seed reserve use efficiency, seed reserve depletion percentage, and biochemical parameters like electrical conductivity, lipid peroxidation and α-amylase were observed.Seed quality was gradually deteriorating due to ageing over the storage period. The rate of deterioration was faster for seedling vigour traits than germination parameters. There was a varietal difference in the rate of deterioration; it was slower in *Kon Joha*, an indigenous variety which also showed dormancy. The priming treatments were able to ameliorate the effect of seed ageing on seed germination, seedling growth and biochemical parameters in *Kon Joha*. Treatments with 1% KCl, 10 ppm GA_3 and 5% PEG enhanced the germination parameters and vigour indicators. KCl (1%) priming was the best priming agent; 24 hours of priming for all agents was better than 12 hours.

Keywords - Aromatic rice seed, Biochemical parameters, Physiological parameters, Priming effect, Storage.

INTRODUCTION

Seed, the primary and essential input for sustainable agriculture, has the genetic information for determining the yield potential of a crop. However, being a living entity, a seed is prone to deterioration due to ageing, especially in tropical regions with high temperatures and humidity (Cortelazzo*et al*., 2005). Seed ageing, a natural change in seeds' physiological and biochemical properties, leads to vigour loss, declined and delayed germination, slower growth, stress susceptibility, and ultimately leading to seed death (Walters, 1998).It also leads to DNA damage and membrane disruption, causing lipid peroxidation and protein synthesis disruption. Lipid peroxidation-mediated membrane damage is the most significant reason for seed deterioration during seed ageing (Koostra and Harrington, 1969). The speed of the seed ageing process depends on the seed's ability to resist degradative changes and its protection mechanism (Gupta and Aneja, 2004), but with ageing, seeds become weak and unable to resist degradative changes efficiently.

The slow or poor germination problem of aged seeds can be ameliorated through many methods, and one of the best among them is seed priming. Seed priming is a pre-sowing controlled hydration treatment in which seeds are soaked in water or solution that allows them to complete the first two germination stages but does not permit radicle emergence. Priming seeds in aerated solution allows some of the metabolic processes for

germination to occur without actual germination (Bradford, 1986), thus improving the germination rate and uniformity (Basra *et al.*, 2006). Several priming treatments, such as hydropriming, halopriming, osmo-priming, hormonal priming, bio-priming, matrix priming, and thermo-priming, are used to invigorate and repair aged seeds. Priming has successfully enhanced seed performance for several crops, including cereals and vegetables (Sarika *et al.*, 2013).

Rice (*Oryza sativa* L.), of the family Poaceae, is a staple food for more than 60% of the global population. India ranks second in rice production (129.66 mt) globally (Anon, 2022-newsonair.gov.in). The world market has an excellent demand for aromatic fine-kernel rice varieties. *Keteki Joha* and *Kon Joha* are two popular aromatic varieties. *Keteki Joha*, with an average yield of 3.5-4 tons/ ha and duration of 150-160 days, is a very popular and an improved joha (scented) rice variety developed by crossing Sabitri and Badshabhog which was released by Assam Agricultural University (AAU). It has medium, slender grain, sweet aroma, good cooking quality and excellent palatability and taste. *Kon Joha* is also a popular indigenous short slender grain aromatic rice with excellent cooking qualities, 155-165 days of duration, mainly cultivated during the *Sali* season (Anon, 2019). Hence, a study was conducted to investigate the effect of seed ageing on the physiological and biochemical parameters of small-grained aromatic rice seed and assess the ameliorating effect of priming treatments on the deteriorating effect of seed ageing.

MATERIALS AND METHODS

Materials

Breeder seeds of *Kon Joha* and *Keteki Joha* were collected from AAU, Jorhat. Seeds of both crops were divided into four parts to be stored for 0, 3, 6 and 9 months, respectively, in standard HDPE bags after treatment with malathion @ 2 g/ kg seed. After 9 months, the surface sterilized seeds of *Kon Joha* were primed with 5% and 10% PEG, 5 and 10 ppm GA_3, 1% and 2% KCl and distilled water each for 12 and 24 hours. Priming solutions were stirred at regular intervals to facilitate aeration. After the soaking, the seeds were rinsed thoroughly with distilled water and dried to their original moisture content under shade at room temperature for 4-5 days. Unprimed dry seeds were taken as control.

Observations

After each storage period and after priming, seeds were evaluated for biochemical parameters like electrical conductivity (EC), lipid peroxidation (LP) and α-amylase (AA), and physiological parameters like moisture percentage (oven dry method), germination percentage and index (GI), mean germination time (MGT), seedling length and dry weight, seed vigour index (SVI=germination percentage X average seedling length), field emergence (FE, conducted in tubs filled with field soils, FE percentage was calculated from the number of seedlings emerged at 30^{th} day). Other physiological parameters were seed reserve utilization rate (SRUR in mg/seed), seed reserve use efficiency(SRUE in mg/mg), and seed reserve depletion percentage (SRDP). SRUR is the initial seed dry weight (before germination) – seed residue dry weight (only cotyledon after germination), SRUE is the seedling dry weight without cotyledon/ SRUR, whereas SRDP is obtained by SRUR/ initial seed dry weight (Mohammadi *et al*., 2011).

Estimation of Electrical Conductivity

3 samples of 25 seeds each were weighed to 2 decimal places and then placed in beakers containing 75 ml of deionized water at room temperature for 24 hours. All beakers were covered by aluminium foil to reduce contamination. After 24 hours, the electrical conductivity of the soaking solution was determined, and the results were expressed in µS.

Estimation of Lipid Peroxidation Value (Heath and Packer,1968)

TBA-TCA reagent wasprepared by dissolving 0.5 g TBA in 100 ml of 20% TCA solution.MDA (Malondialdehyde) was extracted, first by dissolving 1g dry powdered seed sample in 7ml TBA-TCA reagent, then incubating at 95ºC for 30 minutes in capped reaction tubes; cooled in an ice bath and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected, and absorbance was read at 535 nm and 600nm against check (without test sample, keeping all other reagents intact).MDA value was calculated as:

MDA equivalent (nmol/ml) = $[(A_{535} - A_{600}) / 155000] \times 10^6$ Where,

A_{535} = 535 nm represents the maximum absorbance of the TBA-MDA complex

A_{600} = 600 nm is the correction for non-specific turbidity

155000 = molar extinction coefficient for MDA.

Estimation of α-Amylase Activity (Khan and Faust, 1967)

0.2 M Sodium Acetate buffer was prepared at pH 4.7 by adding 46 ml of Glacial acetic acid (60.05 g/mole= 1.15 ml and volume made up to 100 ml) and 54 ml of Sodium acetate (82 g/mole=1.6 g in 100 ml water). 0.1 M was prepared by adding 100 ml distilled water to 0.2 M Sodium acetate buffer solution. 1% Starch solution was prepared by dissolving 1 g starch in 100 ml of 0.1 M Sodium acetate buffer. DNS reagent was prepared by adding 1 g 3,5-Dinitrosalicylic acid, 0.2 g crystalline phenol and 0.05 g sodium sulphate in 100 ml of 1% NaOH. 0.01g Maltose in 100ml distilled water gave Maltose solution. 1 g of crushed sample (germinated seed having radicle length more than 1 mm) was used to extract alpha-amylase with 5 ml of ice-cold 10mM calcium chloride solutions (prepared by dissolving 0.15 g of calcium chloride in 1L of distilled water) overnight at 4°C or 3 hours at room temperature, and then the extract was centrifuged at 6,000 rpm at 4°C for 10minutes. The supernatant was used as an enzyme source. To prepare the enzyme assay,1ml each of starch solution and properly diluted enzyme (test sample) was pipetted out in a test tube, which was incubated at 27°C for 15 minutes. The reaction was stopped by adding 2ml DNS reagent. The solution was then heated in a hot water bath for 5 minutes. While the solutions were warm, 1 ml of 40% Potassium Sodium Tartrate (PST) was added. Then, it was cooled in running tap water. The volume was made to 10 ml by adding 5 ml water. The absorbance was read at 560nm against check (without test sample, keeping all other reagents and procedure intact). A control sample was prepared by adding all the reagents and the test sample as described before and without incubation; hence, the reaction terminated at zero time in the control tubes. This is done to reduce the error. The absorbance reading recorded in the control should be subtracted from the absorbance reading recorded in samples. For standards, a stock solution was prepared by adding 0.01g maltose in 100 ml distilled water, i.e., 100 µg/ 1 ml distilled water. A standard solution of 0, 0.1, 0.2, 0.4, 0.8 and 1ml maltose was prepared from the stock solution by adding 1, 0.9, 0.8, 0.6, 0.2- and 0-ml distilled water respectively. A standard graph with 0-100 µg maltose against absorbance reading was prepared. The unknown concentration of the test sample (after subtracting the absorbance reading of the control) was taken using the standard maltose curve in µg/ ml. The concentration was then converted into µg/ 5 ml (original extraction of 5 ml). Since 5 ml is extracted from 1 g, it can be represented as µg/ g. This value was obtained for the incubation period of 15 minutes; hence, after dividing the value by 15, the concentration will be obtained in µg/min/g.

RESULTS AND DISCUSSION

Effects of seed ageing on physiological and biochemical parameters of *Kon Joha* and *Keteki Joha* seed

Seed ageing leads to seed deterioration, which was determined by the seed's loss of viability and vigour. Significant seed quality deterioration takes place at the time of storage (Khalequzzaman et *al*., 2012. The initial seed moisture content is a crucial factor for storage.

The higher the seed moisture content, the higher the rate of deterioration. In the present study, moisture per cent and MGT for both the rice varieties (*Kon Joha and Keteki Joha*) increased with an increase in storage duration from 0 days to 9 months; this might be the reason why seeds deteriorated in the present study with the increase in storage duration (Table 1). To assess the seed quality, germination percentage, the rate, and the germination uniformity are equally important. GI shows the relationship between germination percentage and speed of germination, whereas MGT shows the rate and time spread of germination. The higher the GI and lower the MGT, the better will be the seed vigour. In this study, germination and GI decreased, but MGT increased with increased storage duration. The germination percentage in both the rice varieties was above seed certification standard, i.e., 80% till six months, but in the ninth month, it decreased drastically. This reduction may be due to the degradation of the mitochondrial membrane, leading to a reduction in the energy supply necessary for germination (Gidrol*et al*., 1998). A decrease in GI and an increase in MGT with the increase in storage period indicated a reduction in the number of seeds germinated and a slower rate of germination.

Seed vigour parameters (Table 2) like SVI, seedling length and dry weight, SRUR and SRDP decreased significantly with storage period in both *Kon Joha* and *Keteki Joha*, indicating loss of vigour of seeds with ageing, but SRUE was not affected by storage period. Though germination remained above seed certification till six months, seed vigour components decreased significantly throughout the storage period. Similar conclusions on seedling vigour as a result of ageing effects were also reported by Ellis *et al.* (1985) and Jatoi *et al.* (2001) in their study on rice and peas, respectively. SRUR, SRUE and SRDP are known as heterotrophic seedling characteristics because they are dependent on three components - initial seed weight, seedling dry weight and weight of mobilized seed reserve (Mohammadi *et al.*, 2011). Studying these traits was crucial because we can determine the fraction of mobilized seed reserve and seedling growth from these traits. SRUR represents the rate at which seed reserves are mobilized to the growing seedling tissues, whereas SRUE represents the conversion efficiency of mobilized reserves to seedling tissues. The reduction in SRUR and SRDP may be due to a lack of energy for converting mobilized seed reserve to seedling tissues because of ageing. This may indicate that SRUR and SRDP are sensitive components of seedling growth but not SRUE (Nik *et al*., 2011).

Biochemical traits are perfect indicators of seed quality. Here,EC and LP significantly increased with an increase in storage duration, whereas α-amylase decreasedfor both rice varieties (Table 3). EC measures the electrolyte leakage from the seed. The higher the seed deterioration rate, the higher will be the electrolyte leakage, hence the higher EC. The increase in EC may be due to the change in cellular membrane permeability due to ageing (Spano *et al*., 2007). LP is one of the significant causes of seed deterioration, which can be determined by estimating the seed's malondialdehyde (MDA) content (Asakaw and Matsushita, 1980). The increase in MDA content may be due to free radicle damage and the deterioration of the membrane, which is probably involved in LP and associated free radical oxidative stresses, leading to membrane leakage (Spano *et al.*, 2007). The higher MDA and total peroxide in aged seeds might also result from ageing-induced changes in peroxide-scavenging enzyme activity (Sung and Jeng, 1994). α-amylase is an enzyme found in the aleurone layer of seeds. It plays a vital role in hydrolyzing the seed reserve, which in turn provides the energy for the growth of the seedling tissues. Decreased α-amylase may be because of protein denaturation and lack of ATP due to ageing. The pooled mean over both the rice varieties revealed a significant effect of the storage period on the germination and its related parameters. However, there was also a varietal difference in the rate of deterioration, but not so conspicuous as the storage period (Table 1, 2, 3). *Kon Joha* showed better germination (72%), longer and heavier seedlings (7.91 cm and 3.12 mg), and higher SRUR

(5.96 mg) than *Keteki Joha* (66.67%, 7.25 cm and 2.78 mg, 5.45 mg respectively). EC and lipid peroxidation were less in *Kon Joha* (15.18 µS and 0.125 n moles/ ml) than *in Keteki Joha* (16.07 µS and 0.136 n moles/ ml), though α-amylase activity (8.29 µg/ min/ g) was found better than Keteki Joha (7.44 µg/ min/ g). This indicates a slower rate of deterioration in the *Kon Joha* than in the *Keteki Joha*. As indigenous aromatic rice, Kon Joha might be harder than the high-yielding fragrant*Keteki Joha*, developed by crossing Sabitri and Badshabhog (Das *et al*., 2010). As we know, indigenous varieties are harder and very well adapted to the local conditions. Hence, might have better chances of resisting the deteriorating effect of ageing. *Kon Joha* also showed dormancy, suggesting that there might be some relation between dormancy and tolerance to seed deterioration due to seed ageing. The varietal difference in rate of deterioration was also confirmed by Jatoi *et al.* (2001) in pea.

Table 1. Germination parameters of rice varieties *Kon Joha* and *Keteki Joha* as influenced by storage period

Storage periods	Moisture (%)		Mean	Germination (%)		Mean	GI		Mean	MGT		Mean	FE (%)		Mean
	Kon	Keteki		Kon	Keteki		Kon	Keteki		Kon	Keteki		Kon	Keteki	
0 days	9.65	9.59	9.62	94.67 (76.70)	94.00 (75.95)	94.33 (76.33)	14.33	15.56	14.95	3.42	3.07	3.24	92.67 (74.32)	90.67 (72.37)	91.50 (73.35)
3 months	10.80	11.58	11.19	91.33 (72.90)	89.33 (70.95)	90.33 (71.93)	12.80	14.22	13.51	3.55	3.33	3.44	88.67 (70.34)	86.00 (69.16)	87.33 (69.75)
6 months	12.53	12.65	12.59	89.33 (70.95)	83.33 (66.96)	86.33 (68.95)	11.40	9.13	10.27	4.89	5.21	5.05	79.33 (62.97)	76.00 (61.58)	77.67 (62.27)
9 months	13.95	13.99	13.97	12.67 (20.84)	0.00 (0.03)	6.33 (10.43)	1.05	0.00	0.53	6.36	0.00	3.18	0.00 (0.03)	0.00 (0.03)	0.00 (0.03)
Mean	11.73	11.95		72.00 (60.35)	66.67 (53.47)		9.90	9.73		4.55	2.90		65.17 (51.92)	63.17 (50.79)	
SE(M)	0.28	0.14	0.16	0.67	0.81	0.53	00.22	0.19	0.15	0.20	0.11	0.11	0.54	0.97	0.56
$CD_{5\%}$	0.91	0.47	0.47	2.17	2.66	1.60	0.73	0.61	0.44	0.66	0.35	0.34	1.75	3.17	1.66
$CD_{5\%(variety)}$	NS			1.13			NS			0.24			NS		

(Data in parenthesis are Arc sine transformed data), GI= Germination Index, MGT=Mean germination time, FE=Field emergence

Table 2. Seedling parameters of rice varieties Kon Joha and Keteki Joha as influenced by storage period

Storage periods	SVI		Mean	Seedling length (cm)		Mean	Seedling dry weight (mg)		Mean	SRUR (mg)		Mean	SRUE (mg/mg)		Mean	SRDP		Mean
	Kon	Keteki		Kon	Keteki		Kon	Keteki		Kon	Keteki		Kon	Keteki		Kon	Keteki	
0 days	1115.3 (3.05)	1160.3 (3.06)	1137.79 (3.06)	11.69	12.05	11.87	8.66	9.67	9.17	4.17	4.28	4.23	0.480	0.490	0.485	0.65	0.75	0.70
3 months	917.3 (2.96)	840.2 (2.92)	878.75 (2.94)	10.25	9.39	9.82	7.66	6.76	7.21	4.01	3.73	3.87	0.497	0.504	0.500	0.58	0.55	0.56
6 months	762.2 (2.88)	650.4 (2.81)	706.25 (2.85)	8.53	7.56	8.05	6.24	5.37	5.81	3.54	3.10	3.32	0.513	0.520	0.517	0.47	0.43	0.45
9 months	14.9 (1.17)	0.00 (0.00)	7.43 (0.59)	1.17	0.00	0.58	1.29	0.00	0.64	0.75	0.00	0.38	0.533	0.000	0.267	0.09	0.00	0.05
Mean	702.4 (2.52)	662.7 (2.20)		7.91	7.25		5.96	5.45		3.12	2.78		0.505	0.378		0.45	0.43	
SE(M)	0.02	0.02	0.015	0.13	0.16	0.10	0.07	0.19	0.10	0.14	0.07	0.08	0.014	0.012	0.009	0.01	0.02	0.01
$CD_{5\%}$	0.06	0.08	0.04	0.41	0.52	0.30	0.22	0.62	0.30	0.47	0.24	0.24	NS	0.040	0.027	0.03	0.06	0.03
$CD_{5\%(var.)}$	0.03			0.22			0.17			0.21			0.019			NS		

SVI=Seed vigour index, SRUR= seed reserve utilization rate, SRUE= seed reserve use efficiency, SRDP= seed reserve depletion percentage

Effect of Priming treatments on aged seeds of rice variety *Kon Joha*

Seeds lose their vigour and ability to germinate during the ageing process and ultimately become less viable (Bhattacharjee *et al*., 2000). Many researchers reported improved quality of aged seeds through priming due to increased enzyme activity, such as antioxidant enzymes and amylases (Ansari *et al.*, 2012).

Table 3. Biochemical parameters of *Kon Joha* and *Keteki Joha* as influenced by storage period

Storage periods	EC (µS)		Mean	AA (µg/ min/ g fw)		Mean	LP (n moles MDA/ ml)		Mean
	Kon	Keteki		Kon	Keteki		Kon	Keteki	
0 days	12.01	13.54	12.77	11.53	12.56	12.05	0.082	0.090	0.085
3 months	14.45	15.61	15.03	9.51	8.64	9.08	0.090	0.105	0.098
6 months	15.36	16.50	15.93	8.35	6.84	7.60	0.122	0.146	0.134
9 months	18.90	18.62	18.76	3.79	1.75	2.77	0.207	0.204	0.205
Mean	15.18	16.07		8.29	7.44		0.125	0.136	
SE(M)	0.15	0.13	0.10	0.098	0.15	0.09	0.006	0.006	0.005
$CD_{5\%}$	0.50	0.42	0.30	0.32	0.49	0.27	0.018	0.022	0.014
$CD_{5\%(variety)}$	0.21			0.19			0.010		

EC= electric conductivity, AA= α-amylase, LP= lipid peroxidation

Seed priming, a simple and low-cost hydration technique, is the most effective method for achieving rapid and uniform emergence and enhancing high vigour, leading to better stand establishment and yield. Four priming treatments were considered for the present study to ameliorate the effect of deterioration due to ageing. They were hydro priming (HP), halo priming (KCl), Osmo-priming (polyethylene glycol-PEG) and hormonal priming (Gibberellic acid-GA_3). Priming treatments were done after nine months of storage. *Keteki Joha* showed no germination after nine months, indicating that the seeds were dead, which was confirmed by the tetrazolium test; hence, priming treatments were ineffective in enhancing the seeds' physiological and biochemical parameters.

Priming treatments (Table 4) effectively enhanced germination, seedling vigour and biochemical parameters over control. KCl (Table 4) was most effective for successfully improving germination percentage, GI, SVI, seedling length and dry weight, SRUR, SRDP and α-amylase content. KCl priming was more effective for earlier and synchronized germination and emergence in rice (Basra *et al*., 2006; Farooq *et al*., 2006), improving seedling fresh and dry weight in rice seeds (Zheng, 2002), increasing wheat yield under greenhouse conditions (Khaing *et al*., 2020). This enhancement by KCl might be due to the enhancement of K^+ balance, thereby activating α-amylase in converting starch to reducing sugar, which in turn provides energy for the growth of seedling tissues (Farooq *et al.*, 2006). PEG effectively enhanced seedling length and dry weight, SRUR and SRDP. Osmo-priming with PEG was more effective in strengthening seedling characteristics because osmo-priming can enhance DNA replication and DNA repair and promote mobilization of reserved materials, which contribute to initiating seed germination (Aryal *et al*., 2020) and as a result of which SRUR and SRDP enhanced effectively. GA_3 enhanced GI, seedling length and α-amylase content. The results agreed with the observations of Zareh *et al.* (2006), who concluded the positive effect of GA_3 on the seedling length of wheat. The possible reason for higher seedling growth traits with GA_3 treatment may be that GA_3 is a hormone that occurs naturally in plants, affects cell growth and elongation and significantly impacts germination, vigour and nutrient uptake (Debbarma *et al*., 2018). HP was effective in enhancing SRUR, SRDP and alpha amylase content. The faster germination rate and better seedling characteristics were obtained by soaking seeds in water, probably due to more rapid water uptake and earlier initiation of the metabolism process, which determine radicle protrusion

(Ghassemi-Golezanik*et al*., 2008). However, any priming did not influence MGT, SRUE, EC, and lipid peroxidation.

Priming durations (Table 4) play an essential role in the success of priming since insufficient soaking duration will not complete the metabolic repair process (Saini *et al*., 2017), and long soaking durations may lead to radicle emergence. *Kon Joha* seeds were primed with all the priming agents for 12 hours and 24 hours. For KCl and PEG, both the duration showed similar effects except for GI in KCl and SVI in PEG, where 24 hours of priming showed better results than 12 hours. 24 hours of GA_3 priming was significantly better than 12 hours in improving all the parameters and ameliorating the ageing effect except in SRUE and lipid peroxidation. The effect of hydropriming duration was observed in MGT, seedling dry weight and SRUR, where longer time was found to be better than shorter duration, indicating 24 hours as the optimum duration for priming. Chivasa *et al*. (2000), in their study on the determination of optimum seed priming time for maize and sorghum, reported that the rate of emergence significantly increased with an increase in the priming period and 24-hour priming of maize was better in every aspect of crop emergence and early growth.

Table 4. Germination, seedling, heterotrophic and biochemical parameters of *Kon Joha* as influenced by different priming treatments

Treatments	Germination (%)	GI	MGT (days)	SVI	Seedling length (cm)	Seedling dry weight (mg)	SRUR (mg/ seed)	SRUE (mg/ mg)	SRDP (%)	EC (µs)	α-amylase (µg/ min/ g fw)	LP (nano moles MDA/ ml)
Control	12.67	1.05	6.36	14.87	1.17	0.75	1.29	0.533	0.093	18.90	3.79	0.207
Treatment Mean	24.48	2.42	5.74	154.64	6.18	2.07	4.04	0.497	0.295	18.27	4.55	0.181
HP	22.67	2.26	5.84	131.98	5.82	1.91	4.05	0.468	0.305	18.29	4.50	0.182
KCl	27.00	2.68	5.54	181.08	6.50	2.21	4.24	0.510	0.313	18.23	4.92	0.177
PEG	23.50	2.14	5.76	147.08	6.21	2.14	4.13	0.492	0.303	18.29	4.29	0.182
GA_3	23.83	2.53	5.86	147.10	6.02	1.96	3.75	0.503	0.265	18.28	4.46	0.183
HP 12 hours	24.00	2.37	6.15	138.57	5.75	1.79	3.91	0.460	0.287	18.17	4.67	0.178
HP 24 hours	21.33	2.15	5.53	125.38	5.88	2.03	4.20	0.477	0.323	18.40	4.33	0.187
KCl 12 hours	26.67	2.50	5.64	173.88	6.35	2.19	4.21	0.508	0.310	18.25	4.89	0.178
KCl 24 hours	27.33	2.85	5.44	188.27	6.65	2.22	4.27	0.512	0.317	18.21	4.95	0.176
PEG 12 hours	22.33	2.09	5.94	134.63	6.02	2.10	4.05	0.497	0.312	18.36	4.26	0.184
PEG 24 hours	24.67	2.19	5.59	159.54	6.40	2.18	4.20	0.487	0.293	18.21	4.33	0.180
GA_3 12 hours	18.67	2.03	6.14	102.40	5.47	1.47	3.03	0.492	0.192	18.56	4.16	0.192
GA_3 24 hours	29.00	3.02	5.58	191.79	6.57	2.45	4.46	0.515	0.338	18.01	4.76	0.175
KCl 1%	33.00	3.52	5.54	243.78	7.36	2.87	5.05	0.558	0.373	18.02	5.50	0.164
KCl 2%	21.00	1.84	5.54	118.37	5.64	1.54	3.42	0.462	0.253	18.43	4.33	0.190
PEG 5%	24.67	2.31	5.86	162.60	6.51	2.44	4.29	0.523	0.335	18.08	4.33	0.179
PEG 10%	22.33	1.97	5.67	131.57	5.90	1.83	3.97	0.460	0.270	18.49	4.26	0.184
GA_3 5 ppm	21.00	2.06	6.10	121.74	5.70	1.77	3.40	0.495	0.250	18.39	4.25	0.189
GA_3 10 ppm	26.67	2.99	5.62	172.45	6.34	2.15	4.09	0.512	0.280	18.18	4.67	0.178
SE(M)	1.03	0.10	0.16	6.06	0.21	0.07	0.07	0.022	0.012	0.15	0.17	0.006
$CD_{5\%}$	2.98	0.30	0.47	17.50	0.62	0.23	0.21	0.065	0.037	0.45	0.48	0.018

GI= Germination Index, MGT=Mean germination time, SVI=Seed vigour index, SRUR= seed reserve utilization rate, SRUE= seed reserve use efficiency, SRDP= seed reserve depletion percentage, EC= electric conductivity, LP= lipid peroxidation

Priming concentration is also an essential factor for the success of priming, which had variable effects on the traits (Table 4). In KCl priming, 1% was better than 2% for enhancing germination per cent, GI, SVI, seedling length and dry weight, SRUR, SRDP, alpha-amylase content and reduction of lipid peroxidation, whereas in GA_3 higher concentration, i.e., 10 ppm was better than 5 ppm for enhancing germination, GI, SVI, seedling length and dry weight, SRUR and reduced MGT. 1% KCl also enhanced anaerobic germination and its related traits in rice (Doley *et al.*, 2018), enhancing seed yield and quality in green gram (Devi *et al.*, 2019). Khan *et al*. (2014) in sorghum concluded that soaking seeds with low concentrations of priming agents like KCl and KNO_3 enhances germination and rapid seed emergence. In their study on rice, Ruttanaruangboworn et *al*. (2017) suggested that priming rice seeds with 1% KNO_3 is better than priming with 2% KNO_3 for maintaining plant vigour. For PEG priming, 5% was better than 10% for enhancing GI, SVI, seedling dry weight, and SRUR. Better performance in lower concentrations might be due to a faster imbibition rate than in higher concentrations; the seeds could complete the two phases of germination faster at lower concentrations (Ruttanaruangboworn*et al.*, 2017).

CORRELATION

GP was positively correlated with SVI, GI, seedling length and dry weight, SRUR, SRDP, field emergence and AA activity but negatively correlated with moisture content, EC and LP; hence, these parameters are good indicators to estimate the quality of seed and their performance in the field (Table 5). High seed moisture led to delayed and poor germination (high MGT and SRUE), and a high rate of deterioration (High LP and EC) showed a positive correlation. Hence, there was a gradual deterioration of seed quality due to ageing during storage.

CONCLUSION

It can be concluded that at the initial stage of storage, the rate of deterioration was slower but faster at a later period, especially after 6 months and was faster in seedling vigour traits than germination parameters. Reduction in heterotrophic seedling characteristics, viz., SRUR and SRDP, led to a reduction in seedling length, but seed ageing did not affect the mobilized reserve's conversion efficiency. There was varietal difference in the rate of deterioration. The rate of deterioration in *Kon Joha*, an indigenous variety, was slower than in *Keteki Joha*. The priming treatments were able to ameliorate the effect of seed ageing on seed germination, seedling growth and biochemical parameters in *Kon Joha*. Treatments with 1% KCl, 10 ppm GA_3 and 5% PEG enhanced the germination parameters and vigour indicators. KCl (1%) priming was the best priming agent; 24 hours of priming for all agents was better than 12 hours.The seed germination, seedling growth and biochemical parameters varied their performance concerning different priming agents, concentration and duration. The treatments could enhance the vigour, but once the seeds are dead, treatments cannot ameliorate the effect of deterioration.

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Table 5. Pearson's correlation coefficients among the physiological and biochemical parameters of *Kon Joha* during storage

	MP	GP	SVI	GI	MGT	SL	SW	SRUR	SRUE	SRDP	FE	EC	AA
GP	-0.81												
SVI	-0.93	0.97**											
GI	-0.89	0.99**	0.99**										
MGT	0.97**	-0.90	-0.96**	-0.94									
SL	-0.92	0.97**	1.00**	1.00**	-0.96**								
SDW	-0.87	0.99**	0.99**	1.00**	-0.94	0.99**							
SRUR	-0.93	0.97**	1.00**	0.99**	-0.97**	1.00**	0.99**						
SRUE	1.00**	-0.83	-0.94	-0.91	0.96**	-0.94	-0.89	-0.94					
SRDP	-0.93	0.97**	1.00**	0.99**	-0.97**	1.00**	0.99**	1.00**	-0.94				
FE	-0.85	1.00**	0.98**	1.00**	-0.93	0.99**	1.00**	0.98**	-0.87	0.98**			
EC	0.97**	-0.89	-0.97**	-0.95*	0.94	-0.97**	-0.93	-0.97**	0.98**	-0.97**	-0.92		
AA	-0.96**	0.94	0.99**	0.98**	-0.96**	0.99**	0.96**	0.99**	-0.97**	0.99**	0.96**	-0.99**	
LPV	0.93	-0.97**	-0.99**	-0.99**	0.98**	-1.00**	-0.99**	-1.00**	0.93	-1.00**	-0.98**	0.95*	-0.98**

**/ * = significant at 1% and 5% respectively