Short Research Article

SCREENING OF SUITABLE BORO RICE (ORYZA SATIVA L.) FOR COLDTOLERANCE AT GERMINATION STAGES

Abstract

An experiment was conducted to evaluate the cold tolerance ability of tenboro ricegenotypes in the laboratory conditions for suitability of growing under low temperature stress.The experiment was laid out in CRBD (Completely Randomized Block Design) and the low temperature regimes was providedunder controlled environment (Walk-in cold chamber by Saveer Biotech Limited) for germination studies that could maintain the specific low temperature. The experiment was conducted in the Department of Crop Physiology and Agricultural Biotechnology, Assam Agricultural University, Jorhat, Assam, India. The germination tests were conducted under four different temperature regimes where seed germination under ambient (T_4=28 ± 0.2 ^oC) was considered as control. Low-temperature treatments were provided by the growth chamber at three different levels of low temperature *viz*. (T_3=10-12°C), (T_2=7-9 ^oC) and (T_1=5-6 °C).

Per cent germination, germination index, shoot length, length of longest root and seed vigour index were considered for the study.The germination percent, germination index, shoot length, length of longest root and seed vigour index was recorded highest under ambient condition (T_4) irrespective of all the genotypes. Seeds grown under T_3 showed considerable germination values only after 28 days of germination studies, whereas T_1 and T_2 showed no germination. The study revealed that amongst all the ten genotypes, Kanaklata and Sona Mahsuri revealed superior performance in germination percentage, germination index, shoot length, length of the longest root and seed vigour index.In the correlation studies,germination % was significantly correlated with germination index, shoot length, length of the longestand SVI at 5% probability level. GI was also positively and significantly correlated with SL, LLR and SVI.The present study revealed that Sona Mahsuri and Kanaklata was found to ideal genotypes for germination under low temperature (10-12 ^oC).

Keywords: Boro rice, cold tolerance, seed vigour index, germination, length of longest root, shoot length, germination index

INTRODUCTION

Rice being one of the most important cereals across the globe is consumed by more than half of the world's population. The worlds' most significant commercial species of rice is *Oryza sativa* L. (Ray *et al.* 2013).India ranks second in terms of rice production after China. More than 4000 varieties of rice are cultivated in India out of the 10,000 rice varieties grown worldwide (Motwani and Mehta 2018). In Assam rice is considered the most important staple food crop. Rice accounts for 96% of the state's entire food grain output which spans over 2.54 million hectares with a gross cultivated area of 4.16 million hectares **(**Krishi Jagran, 2021).

The term "Boro" is derived from the Sanskrit word 'borob' (Bhakta *et al*., 2021). In Assam, Boro rice is cultivated in a flood-prone area where the kharif crop is wasted due to floods. Generally, farmers grow Boro rice in the deep-water areas of Assam where productivity is very poor due to the prevalence of below normal temperature during seedling and vegetative stages that leads to improper seedling establishment. Cold tolerance at the germination and vegetative stage is an important factor since Boro rice cultivars as seedlings are raised during the colder months of November and December. Moreover, rice is very sensitive to low temperatures (Tiwari *et al*, 2009). Priyanka *et al*., (2015) have reported that boro rice being a winter cropcan escape insect infestation at seedling stage but faces low temperatures during seedling and vegetative growth. Therefore, the growth and development of the plant is adversely affected. It is challenging for the farmers to raise rice seedlings due to low night temperatures in the nursery beds. Farmers faces challenges due to low temperature. Although low temperature does not prevent germination, but it leads to delayedseedling emergence.On the other hand, Cruz *et al*. (2013) have reported that low-temperature affects rice seed germination. Performance of rice seed during germination is important for fast growth and for obtaining uniform crop stand. Therefore, the present study was performed in the laboratory to identify the cold tolerance in ten different boro rice genotypes. Therefore, cold tolerant genotypes can be utilized in future breeding programmes for the development of cold tolerant variety.

MATERIALS AND METHODS

Ten (10)numbers of boro rice genotypeswere used for laboratory experiments *viz.* Kanaklata, Disang, Sona Mahsuri, DRR-44K, Cauvery, Lal Mahsuri, Swarnabh, Jyoti Prasad, Dinanath and No-29 (BRRI-dhan29) for studying the cold tolerance abilities of the genotypes. The seeds were soaked in the water overnight and surface sterilized with 70% ethanol for 30 seconds and 0.5 % Sodium hypochlorite for 20 min and washed with sterile distilled water six times. The seeds were then placed on filter paper containing two layers of filter paper on the Petri dishes @ 10 seeds per petri plate and 1 ml of Benomyl solution @ 2.5 ppm were added to avoid contamination. The methodology was adopted by (Priyanka *et al*., 2015). The Petri plate containing the boro rice seeds were then placed in a controlled environment (Walk-in cold chamber by Saveer Biotech Limited) for low-temperature treatment and maintained at a specific temperature. The experiment was conducted on a Factorial Completely Randomized design. The treatments consisted of four different temperature regimes as given below. T_1= 5-6 °C:, T_2= 7-9 °C:, T_3=10-12 °Cand T_4 = 28 °C(ambient condition) and was replicated thrice.

Parameters *viz.*germination percentage, germination index, Shoot length, length of longest root and seed vigour index were recorded after 7 days in T4 (ambient condition) whereas T1, T2 and T3 were kept for 28 days to record the germination and its related data.

Germination per cent was calculated by taking the average germination % of the seeds of each petri plate.Shoot length and length of the longest root were calculated by using a scale.

Per-cent reduction of germination was calculated using the following formula

$$Per-cent\ reduction = \frac{germination\ at\ control - germination\ at\ treatment}{germination\ at\ \text{control}}\ x\ 100$$

The germination Index (GI) was calculated for each genotypes. The number of seeds germinated daily were counted from the day of placing the seed in petri- plates till germination was completed. The GI was calculated as the arithmetical sum of total seeds germinating every day up to a period (Mhatre and Chaphekar, 1982).

The germination Index is computed by using the following formula

$$Germination\ index = n/d$$

Where

n= number of seedlings emerging on‘d’ day

d = days after placing on Petri plates

The seed vigour index (SVI) was calculated with the emergence of roots and shoots and the average length of seedling was taken for calculation after seven days in ambient conditions whereas it was 28 days for low-temperature treatments at T_1, T_2 and T_3. The formula to calculate the SVI is given below. (Abdul- Baki and Anderson, 1973).

$$SVI = (root\ length + shoot\ length) x\ germination\ \%$$

RESULTS AND DISCUSION

The ten rice genotypeswere germinated under cold-stress conditions in the laboratoryto evaluate their tolerant ability. The results are given below.

A significant difference in germination per-cent was noted due to low temperature treatments (Table 1). Seeds germinated under Ambient condition recorded the highest per cent germination after 7 days of placing the seeds in petriplates. The rice seed at T_3 failed to germinate at 7 DAS due to the induction of low temperature. Therefore, germination % was calculated at 28 DAS. T_3 recorded a significantly lower germination % at 28 DAS. T_1 and T_2 failed to germinate upto 28 days. Amongst the genotypes, maximum germination % was recorded in Kanaklata which was at par with Sona Mahsuri . It has been reported that rice seed stored under low temperature retains their dormancy (Roberts, 1961) .

Amongst the interaction effects, Kanaklata, Swarnabh and Sona Mahsuri recorded the highest germination at T_4, followed by Disang at T_4 Cauvery and DRR-44K which were at par.Puteh *et al.,* (2010)reported that the non-dormant rice seeds had lower base temperatures for germination. Low-temperature stress have shown to be negatively affected in rice seed germination, that might possibly be due to inactivity of enzymes related to germination (Lone *et al.,* 2018) which might be the reason for a slow growth rate during germination in boro rice below the optimum range (Yaseen *et al.,* 2020). Damarias *et al*., (2019) reported that seed germination is a tri-phasic process. The first phase being imbibition, followed by the rapid uptake of water and colloidal compounds in the cytoplasm. Within 0-24 hours, the proteins, starch, and DNA repair occurs. This is followed by activation of ATP synthesis in the glycolysis, Krebs cycle, and the respiratory chain. Within 24-48 hours after imbibition, the translation of stored mRNA occurs. The third phase is emergence of radicle that occurs after 48–72 hours.

In the present study, a significant difference was also observed in the germination index due to different low temperature treatment(Table 1). T_4 (ambient) recorded The highest germination index was recorded in ambient condition , as compared to T_3. Out of the boro rice genotypes, Kanaklata recorded the maximum GI. Amongst the interaction effect, genotype,Kanaklata recorded the highest GI at ambient condition.Germination better index (GI) is an important parameter that expresses the speed of germination under cold temperature. GI is calculated based on the coleoptiles and radical emergence which is an important index for performance of genotypes under the cold stress (Rahul *et al.*,2017) . As compared to indica genotypes, the japonica rice recorded greater cold tolerance capability at germination stages (Cruz and Milach, 2013)

Table 1: Effect of low temperature on germination percent and germination index

	Treatment				**Treatment**		
Genotypes	**T_3**	**T_4**	**Mean**	**Genotypes**	**T_3**	**T_4**	**Mean**
Kanakalata	46.67	100.00	73.30	Kanaklata	42.63	93.66	68.14
Disang	33.30	96.60	64.90	Disang	20.59	87.56	54.07
DRR-44K	16.60	86.60	51.60	DRR-44K	15.03	81.36	48.19
Dinanath	6.60	73.30	39.95	Dinanath	2.76	74.23	38.49
Swarnabh	23.30	100.00	61.65	Swarnabh	21.63	90.43	56.03
Jyoti Prasad	10.00	80.00	45.00	Jyoti Prasad	9.097	79.53	44.31
Cauvery	20.00	93.30	56.65	Cauvery	18.89	81.36	50.12
Lal Mahsuri	3.30	43.30	23.30	Lal Mahsuri	1.66	50.50	26.08
Sona Mahsuri	43.30	100.00	71.65	Sona Mahsuri	39.89	91.23	65.56
No. 29 (BRRI-dhan29)	3.30	46.60	24.95	No. 29 (BRRI-dhan29)	2.53	54.63	28.58
Mean	20.64	81.97		Mean	17.47	78.46	
	SE.(d±)	CD (0.05)			SE.(d±)	C D (0.05)	
(T)	2.08	4.12		T	0.39	0.79	
(V)	3.27	6.52		V	0.62	1.25	
	6.50	13.04		T X V	1.25	2.50	

Statistical analysis was done by angular transformation

Due to induction of low temperature, a significant difference was noted in shoot lengthunder different low temperature treatment (Table 2).The maximum shoot length was recorded under ambient condition(T_4) when compared T_3, although T_1 and T_2 failed to show any germination.

Amongst the varieties, Kanaklata recorded the longest shoot lengthfollowed by Sona Mahsuri which were at par. In the interaction effect, Kanaklata recorded the longest shoot length at T_4, followed by Sona Mahsuri at T_4.Shoot length was found to be the best predictor of seedling vigour index(Zhang *et al.*, 2005).In the present study, low temperature significantly decreases germination ability and growth of coleoptiles and radicles in the studied genotypes. A significant difference in the length of the longest root was also noted due to different low-temperature treatments (Table 2). T_4 (ambient) recorded the maximum root length as compared to T_3 whereas T_1 and T_2 failed to germinate. Sona Mahsuri recorded the longest root length amongst the genotypes. In the interaction effect, Sona Mahsuri at T_4 recorded the longest root length which was at par with Kanaklata at T_4.

Table: 2 Effect of low temperature on shoot length and length of longest root

Shoot length				**Length of longest root**			
Genotypes	**T_3**	**T_4**	**Mean**	**Genotypes**	**T_3**	T_4	**Mean**
Kanaklata	0.86	2.56	1.71	Kanaklata	1.80	3.40	2.60
Disang	0.56	2.30	1.43	Disang	1.23	3.20	2.21
DRR-44K	0.23	1.46	0.85	DRR-44K	0.33	2.90	1.61
Dinanath	0.16	1.20	0.68	Dinanath	0.06	2.36	1.21
Swarnabh	0.63	2.03	1.33	Swarnabh	0.93	3.06	1.99
Jyoti Prasad	0.36	1.40	0.88	Jyoti Prasad	0.10	2.60	1.35
Cauvery	0.53	1.96	1.25	Cauvery	0.66	3.03	1.84
Lal Mahsuri	0.03	0.86	0.45	Lal Mahsuri	0.03	2.06	1.04
Sona Mahsuri	0.83	2.46	1.65	Sona Mahsuri	2.83	3.56	3.19
No. 29 (BRRI-dhan29)	0.16	1.16	0.66	No. 29 (BRRI dhan29)	0.04	2.30	1.17
Mean	0.44	1.74		Mean	0.82	2.85	
	SE.(d±)	CD(0.05)			SE.(d±)	CD(0.05)	
T	0.08	0.17			0.02	0.05	
V	0.13	0.27			0.03	0.08	
T X V	0.27	0.54			0.08	0.17	

The coleoptile development is severely inhibited under cold stress as compared to the control condition (Dien *et al.,* 2019).Early and fast root extension is crucial for demonstrating resilience to abiotic stresses including drought and cold (Sharifi, 2008).A reduction in temperature could cause a delay in germination thereby causing slower seedling growth..Reducing temperatures was unfavourable which caused delayed germination and slower growth of seedlings leading to partial development of radicals Asignificant difference was also observed in SVI due to low-temperature treatmentand the data is presented in (Table 3). The genotype Kanaklata recorded the highest SVI which was at par with Sona Mahsuri . In the interaction effect, Kanaklata recorded the maximum SVI at T_4 followed by Sona Mahsuri and Disang at T_4 which were at par. Lal Mahsuri recorded the lowest of all the parameters.Ranawake and Nakamura (2011) reported seedling vigour as a metric used to assess a plant's resistance to stress circumstances of any kind and is negatively impacted by temperatures below 25°C which reflected the potential of seed germination. Foolad *et al*. (2007) also reported that genotypes having good SVI can contribute to improved field emergence and crop performance .

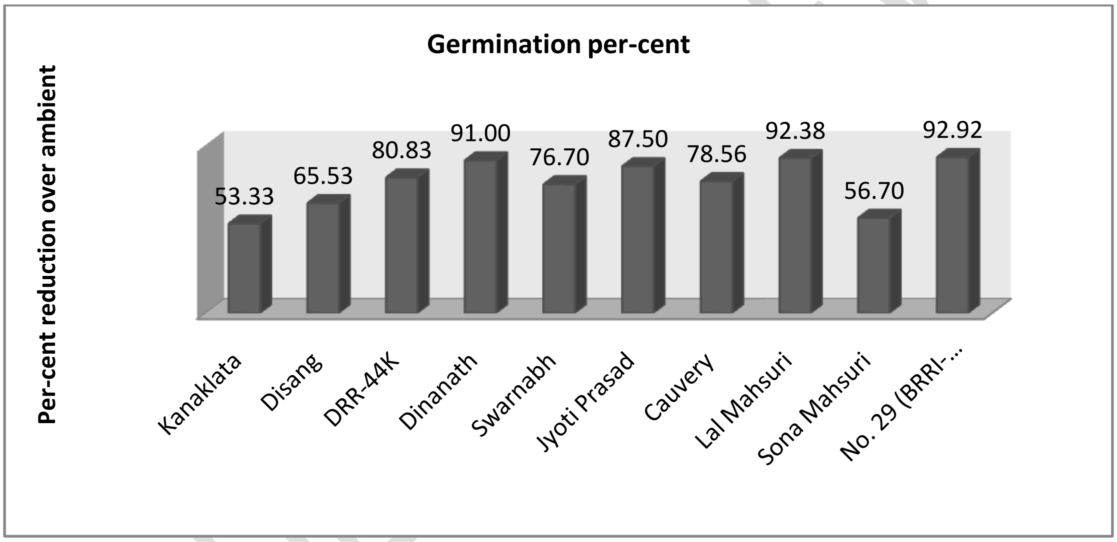
Boro rice plants encounter a lower threshold temperature of 10-13^{o}C for damages in the early stages *viz*. germination and vegetative stages. It has been reported that during reproductive stages a higher threshold temperature i.e., 18-20^{o}C (Yosida, 1981). Tolerant genotypes, that have evolved under a strong selection presure,might rely on diverse gene products at different growth stages for cold tolerance. Therefore, tolerance to environmental stresses, ensures species survivability even with one or two tolerance genes are lost(Nishiyama *et al* ., 2013). Nagamine (1991) suggested that rice tolerance at seedling stage is controlled by a single dominant gene. The mechanism of cold tolerance includes adjustment of cell metabolism due to low temperature by changes in structural, catalytic properties,, functioning of enzymes and membrane transportes(Kubien *etal*., 2003). The response to temperature stress by modifications of metabolism are mainly linked to increased tolerance mechanisms. The genotypes, Kanaklata and Sona Mahsuri showed better germination under low temperature stress, which might be due to their inherent cold acclimation characteristics probably by signaling and regulation of transcripts. The receptors of cold stress is unknown. Amongst the signalling components, calcium, ROS, protein cascades, protein phosphatases, lipid signalling cascades, ABA signalling are important factors(Yadav, 2010).

Per cent reduction in germination percentage

Fig. 2. Correlation studies amongst the various seed germination related parameters

As compared to ambient condition (T4) the maximum per cent reduction in % germination was noted in the genotype No. 29 (BRRI-dhan29) (92.92), followed by Lalmahsuri (92.38) and Dinanath (91.00) which were at par (Fig 1.). The lowest reduction in germination percentage was recorded in Kanaklata, and Sona Mahsuri which were at par.

Fig 1: Graphical representation of per cent reduction of germination percentage



Correlation studies amongst the parameters

Germination % (GP) was positively significantly correlated with germination index (GI)(0.778*), shoot length (SL) (0.959*), length of the longest(LLR) (0.906*) and SVI(0.973*) at 5% probability level. GI was also positively and significantly correlated with SL(0.861*), LLR(0.824*) and SVI(0.881*). Moreover, SL was positively and significantly correlated with LLR(0.943*) and SVI(0.970*). LLR was also positively and significantly correlated with SVI(0.925*). This showed that SVI was positively and significantly correlated with GP, GI, SL and LLR at 5% probability (Fig 2).

	GP	GI	SL	LLR	SVI
GP	1.00				
GI	0.778*	1.00			
SL	0.959*	0.861*	1.00		
RL	0.906*	0.824*	0.943*	1.00	
SVI	0.973*	0.881*	0.970*	0.925*	1.00
*** Significant at 5% probability**					

CONCLUSION

From the present experiment, it was revealed that low-temperature stress negatively impacted on boro rice germination and seedling growth. However some genotypes showed tolerance ability to low- temperatures in the laboratory condition. The genotype Kanaklata and Sona Mahsuri had the least adverse effect when grown under low-temperature stress at 10-12^{o}Cas compared to other genotypes. In the correlation studies, a significant correlation amongst the parameters were noted.This information can be valuable for selecting suitable rice genotype for cultivation in the regions where low temperature is encountered during the germination stages. Based on our study,germination under low temperature from 5-9^{o}C are not suitable for growing under low temperature stress. Our results revealed that the genotypes Kanaklata and Sona Mahsuri are suitable for growing at 10-12^{o}C.

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