



# Assessment of Cold Tolerance in Boro Rice (*Oryza sativa* L.) Genotypes During Germination for Enhanced Adaptability to Low Temperature Stress

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

An experiment was conducted to evaluate the cold tolerance ability of ten boro rice genotypes in the laboratory conditions for suitability of growing under low temperature stress. The experiment was laid out in Factorial CRD and the low temperature regimes was provided under controlled

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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 ( $T_4=28 \pm 0.2^\circ\text{C}$ ) was considered as control. Low-temperature treatments were provided by the growth chamber at three different levels of low temperature viz. ( $T_3=10-12^\circ\text{C}$ ), ( $T_2=7-9^\circ\text{C}$ ) and ( $T_1=5-6^\circ\text{C}$ ). 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 longest and SVI at 5% probability level. GI was also positively and significantly correlated with Shoot length (SL), Length of longest root (LLR) and Seed Vigour Index (SVI). The present study revealed that Sona Mahsuri and Kanaklata was found to be the ideal genotypes for germination under low temperature ( $10-12^\circ\text{C}$ ). Therefore, Farmers growing boro rice under low temperature regimes could take the opportunity of selecting the varieties viz. Kanaklata and Sona Mahsuri for higher yield.

**Keywords:** Cold chamber; germination; length of longest root; shoot length; germination index.

## 1. INTRODUCTION

Rice being one of the most important cereals across the globe is consumed by more than half of the world's population. The world's most significant commercial species of rice is *Oryza sativa* L. [1]. The major rice producing states in India are West Bengal, Uttar Pradesh, Odisha, Andhra Pradesh, Tamil Nadu, Chattisgarh and Bihar. About 3000 varieties are grown that takes less than 60-75 duration [2].

More than 4000 varieties of rice are cultivated in India out of the 10,000 rice varieties grown worldwide [3]. 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 [4].

The term "Boro" is derived from the Sanskrit word 'borob' [5]. 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 seedlings are raised during the colder months of November and December. Moreover, rice is very sensitive

to low temperatures [6,7] have reported that boro rice being a winter crop can 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 delayed seedling emergence [8]. On the other hand it has been reported that low temperature affects seed germination [9]. 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.

## 2. MATERIALS AND METHODS

Ten (10) numbers of boro rice genotypes were 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, with two layers per Petri dish, at a rate of 10 seeds per petri plate. Additionally, 1 ml of Benomyl solution at 2.5 ppm was added to prevent contamination". The methodology was adopted by Priyanka *et al.*, [7]. The Petri plates 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 CRD. The treatments consisted of four different temperature regimes as given below. T<sub>1</sub> = 5-6 °C.; T<sub>2</sub> = 7-9 °C.; T<sub>3</sub> = 10-12 °C and T<sub>4</sub> = 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 T<sub>4</sub> (ambient condition) whereas T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were kept for 28 days to record the germination and its related data.

Germination percent 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:

Percent to be replaced by symbol %

$$\% \text{reducton} = \frac{\text{Germnaton at control} - \text{germnaton at treatment}}{\text{Germnaton at control}} \times 100$$

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 [10].

The germination Index is computed by using the following formula

$$\text{Germination index} = n/d$$

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<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>. The formula to calculate the SVI is given below. Abdul- Baki and Anderson, [11].

$$SVI = (\text{root length} + \text{shoot length}) \times \text{germination \%}$$

## 2.1 Statistical Analysis

The design of the experiment was Factorial CRD and the statistical analysis of data was done following the method of analysis of variance (ANOVA) given by Panse and Sukhatme (1967). The critical difference (CD) values were calculated at 5 percent probability level.

## 3. RESULTS AND DISCUSION

The seeds of the ten rice genotypes were germinated under cold-stress conditions in the laboratory to assess their tolerance. The results are presented below

The ten rice genotypes were germinated under cold-stress conditions in the laboratory to evaluate their tolerant ability. The results are given below.

A significant difference in germination percent 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<sub>3</sub> failed to germinate at 7 DAS due to the induction of low temperature. Therefore, germination % was calculated at 28 DAS. T<sub>3</sub> recorded a significantly lower germination % at 28 DAS. T<sub>1</sub> and T<sub>2</sub> 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 [12].

Amongst the interaction effects, Kanaklata, Swarnabh and Sona Mahsuri recorded the highest germination at T<sub>4</sub>, followed by Disang at T<sub>4</sub> Cauvery and DRR-44K which were at par. The climate of Asian temperate regions are unstable during the growth of rice seeding therefore, it poses a challenge for rice cultivation under low temperatures conditions [13].

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 [13]. A reduction in  $\alpha$ -amylase activity and low soluble sugar might be the reason for a slow growth rate during germination in boro rice below the optimum range as reported by Li and Yang, [14], Damarias *et al.*, [15] 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 [16].

In the present study, a significant difference was also observed in the germination index due to different low temperature treatment (Table 1). T<sub>4</sub> (ambient) recorded the highest germination index was recorded in ambient condition, as compared to T<sub>3</sub>. 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 [17]. As compared to indica genotypes, the japonica rice recorded greater

cold tolerance capability at germination stages [9].

Due to induction of low temperature, a significant difference was noted in shoot length under different low temperature treatment (Table 2). The maximum shoot length was recorded under ambient condition (T<sub>4</sub>) when compared T<sub>3</sub>, although T<sub>1</sub> and T<sub>2</sub> failed to show any germination.

Amongst the varieties, Kanaklata recorded the longest shoot length followed by Sona Mahsuri which were at par. In the interaction effect, Kanaklata recorded the longest shoot length at T<sub>4</sub>, followed by Sona Mahsuri at T<sub>4</sub>. Shoot length was found to be the best predictor of seedling vigour index [18]. 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<sub>4</sub> (ambient) recorded the maximum root length as compared to T<sub>3</sub> whereas T<sub>1</sub> and T<sub>2</sub> failed to germinate. Sona Mahsuri recorded the longest root length amongst the genotypes. In the interaction effect, Sona Mahsuri at T<sub>4</sub> recorded the longest root length which was at par with Kanaklata at T<sub>4</sub>.

The coleoptile development is severely inhibited under cold stress as compared to the control condition [19]. Early and fast root extension is crucial for demonstrating resilience to abiotic stresses including drought and cold [20].

**Table 1. Effect of low temperature on germination percent and germination index**

Germination % Genotypes	Germination Index			Germination Index		
	T <sub>3</sub>	T <sub>4</sub>	Mean	T <sub>3</sub>	T <sub>4</sub>	Mean
Kanakalata	46.67	100.00	73.30	42.63	93.66	68.14
Disang	33.30	96.60	64.90	20.59	87.56	54.07
DRR-44K	16.60	86.60	51.60	15.03	81.36	48.19
Dinanath	6.60	73.30	39.95	2.76	74.23	38.49
Swarnabh	23.30	100.00	61.65	21.63	90.43	56.03
Jyoti Prasad	10.00	80.00	45.00	9.097	79.53	44.31
Cauvery	20.00	93.30	56.65	18.89	81.36	50.12
Lal Mahsuri	3.30	43.30	23.30	1.66	50.50	26.08
Sona Mahsuri	43.30	100.00	71.65	39.89	91.23	65.56
No. 29 (BRRI-dhan29)	3.30	46.60	24.95	2.53	54.63	28.58
Mean	20.64	81.97		17.47	78.46	
	SE.(d±)	CD (0.05)		SE.(d±)	C D (0.05)	
(T)	2.08	4.12		0.39	0.79	
(V)	3.27	6.52		0.62	1.25	
(T X V)	6.50	13.04		1.25	2.50	

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

Genotypes	Shoot length (cm)			Length of longest root (cm)		
	T <sub>3</sub>	T <sub>4</sub>	Mean	T <sub>3</sub>	T <sub>4</sub>	Mean
Kanaklata	0.86	2.56	1.71	1.80	3.40	2.60
Disang	0.56	2.30	1.43	1.23	3.20	2.21
DRR-44K	0.23	1.46	0.85	0.33	2.90	1.61
Dinanath	0.16	1.20	0.68	0.06	2.36	1.21
Swarnabh	0.63	2.03	1.33	0.93	3.06	1.99
Jyoti Prasad	0.36	1.40	0.88	0.10	2.60	1.35
Cauvery	0.53	1.96	1.25	0.66	3.03	1.84
Lal Mahsuri	0.03	0.86	0.45	0.03	2.06	1.04
Sona Mahsuri	0.83	2.46	1.65	2.83	3.56	3.19
No. 29 (BRRI-dhan29)	0.16	1.16	0.66	0.04	2.30	1.17
Mean	0.44	1.74		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	

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. A significant difference was also observed in SVI due to low-temperature treatment and 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<sub>4</sub> followed by Sona Mahsuri and Disang at T<sub>4</sub> which were at par. Lal Mahsuri recorded the lowest of all the parameters. Ranawake and Nakamura [21] 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.* [22] 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°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°C [23]. Tolerant genotypes, that have evolved under a strong selection pressure, 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.* [24] Nagamine [25] 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 transporters [26]. 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 [27].

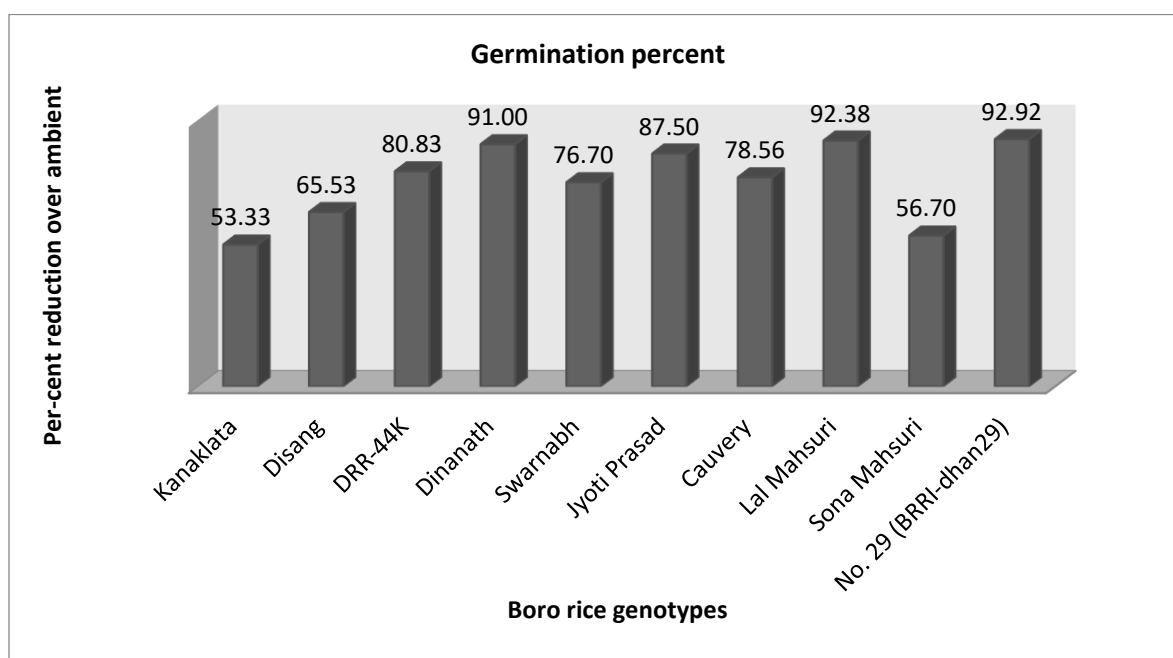
As compared to ambient condition (T<sub>4</sub>) the maximum percent reduction in % germination was noted in the genotype No. 29 (BRRI-dhan29) (92.92), followed by Lal mahsuri (92.38) and Dinanath (91.00) which were at par (Fig 1.). The lowest reduction in germination percentage was recorded in Kanaklata, and Sona Mahsuri which were at par [28].

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 correlated with GP, GI, SL and LLR at 5% significantly correlated with SVI (0.925\*). This probability (Fig. 2). showed that SVI was positively and significantly

**Table 3. Effect of low temperature on seed vigour index**

Varieties	T <sub>3</sub>	T <sub>4</sub>	Mean
Kanaklata	124.0	613.3	368.65
Disang	49.6	532.6	291.10
DRR-44K	11.6	384.3	197.95
Dinanath	3.5	306.6	155.05
Swarnabh	31.3	478.3	254.80
Jyoti Prasad	4.86	352.3	178.58
Cauvery	23.6	451.0	237.30
Lal Mahsuri	1.3	18.6	9.95
Sona Mahsuri	133.6	586.0	359.80
No. 29 (BRR1 dhan29)	2.3	213.0	107.65
Mean	38.6	403.7	
	SE.(d±)	CD (0.05)	
T	13.9	27.8	
V	22.1	44.0	
T X V	44.2	88.1	



**Fig. 1. Percent reduction in germination percentage**

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

	Germination %	Germination Index	Shoot Length	Length of Longest Root	Seed Index	Vigour
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

#### 4. 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 genotypes Kanaklata and Sona Mahsuri had the least adverse effect when grown under low-temperature stress at 10-12°C as 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 below 10°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°C.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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