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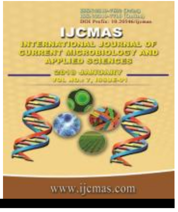

Original Research Article

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Cold Tolerance at Germination and Seedling Stages of Rice: Methods of Evaluation and Characterization of Thirty Rice Genotypes under Stress Conditions

Javeed A. Lone[1*], M. Nisar Khan[2], M. Ashraf Bhat[1], Asif B. Shikari[3], Shabir H. Wani[4], Najeeb R. Sofi[4], M. Imran Khan[5] and Rayees A. Lone[1]

[1]Division of Genetics and Plant Breeding, FoA, Sher-e- Kashmir University of Agricultural Science and Technology of Kashmir, Wadura Sopore-193201, Jammu and Kashmir, India
[2]Dryland Agriculture Research Station, Budgam, India
[3]Division of Plant Biotechnology, Sher-e- Kashmir University of Agricultural Science and Technology, Shalimar-190025, India
[4]Sher-e-Kashmir University of Agricultural Science and Technology Shalimar, Rice Research Station (MRCFC) Khudwani, India
[5]Division of Agri-Statistics, Sher-e- Kashmir University of Agricultural Science and Technology, Shalimar-190025, India

**Corresponding author*

ABSTRACT

Rice is one of the most important staple foods among the cereal crops for half of the human population. Unlike other cereals such as wheat and barley, rice plants are susceptible to cold stress, which causes decrease in productivity, especially in regions where the indica subspecies is cultivated. Low temperatures can have direct impacts on rice plants during germination, vegetative growth, and reproductive stages. This study aims to identify a suitable technique to estimate rice (*Oriza sativa* L.) cold tolerance at the germination stage and identify the variability among 30 rice genotypes. Cold tolerance was evaluated by two methods: in method I, rice seeds were kept and germinated under two conditions; 28 days in 13°C and seven days in 28°C; in experiment II, seedlings were kept at 72 hours 96 hours at 13°C and again 72 hours at 28°C. In experiment I percentages of reduction in radicle and coleoptile length and germination percent and percent decline in germination were measured. In experiment II coleoptiles growth radical regrowth after the cold period were measured. Results showed that low temperature effects in both experiments were varied among studied genotypes. Highest germination was achieved in SKAU-402 fallowed by SI-6, K-332, SI-5 and SI-3 with less percent decline in germination that is, 0.13, 0.17, 0.24 and 0.32 at 13°C. Percentage of coleoptiles growth by low temperature (13ºC) was varied from 13.8 to 86.6%. In experiment II, radicle regrowth values varied from 2.0 to 12.0 mm, and SI-6,-SI-5, Kamad, SKAU-402 and K332, were the genotypes with high radicle regrowth.

Keywords

Abiotic stress, Controlled situation, Coleoptile growth, Radical regrowth, Low temperature

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Introduction

Rice (*Oryza sativa* L.) is the most important staple food crop for more than 60 % of the global population and forms the cheapest source of food and energy. Rice occupied 39.16 million hectares with production and productivity of 85.59 million tonnes and 2185 kg ha^{-1} respectively, in India during 2012-2103. The production of rice was low during spring (48 lakh tonnes), compared to rainy season (84.1 lakh tonnes) during 2012-13 (INDIASTAT, 2013), The productivity of rice is particularly low in during spring due to cold, which is an important abiotic constraint, where low temperature prevails below 18 ºC. Datta and Datta (2006) reported that low temperature stress is one of the major abiotic factors which reduce rice yield in several countries. Losses can range from 0.5 to 2.5 ha-1 (Singh *et al.,* 2005) and grain yields can drop by up to 26% (Lee *et al.,* 2001) mostly due to low temperature during the reproductive stage, even though cold temperature can be harmful during the entire developmental stage of rice plants, from germination to grain filling (Ye *et al.,* 2009 and Cruz *et al.,* 2013). As per Cruz and Milach (2013) good cold tolerance at the seedling stage is an important character for stable rice production, especially in direct seeding fields. Cold tolerance at germination and seedling emergence and early stages is essential to obtain optimum plant population. Breeding varieties for cold tolerance could be the better solution for the problem. However selection for tolerance requires a suitable and cost effective screening technology. Breeding cold tolerant varieties in rice is essential to reduce yield loss, for which information on various traits that contribute to tolerance is necessary (Priyanka, 2015). Keeping in view of the above problem the present study is taken up to evaluate cold tolerance in rice genotypes with a suitable method to estimate cold stress in thirty rice genotypes at the germination and seedling stages under controlled and stressed conditions, as well as introduce genotypes with high tolerance to cold stress in germination stage and also choose the best characters of resistance for use in breeding programs.

Materials and Methods

Thirty rice genotypes including pre released, released and germplasm genotype were studied in this research (Table 1). Seeds of all the thirty rice genotypes were collected from MRCFC Kudwani, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir. The cold tolerance evaluation was carried out in, Division of Biotechnology Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar,

To evaluate genotypes for cold tolerance, two methods were used (Cruz and Milach, 2004). In method I, seeds of 30 rice genotypes were germinated in two conditions that is, 28 days in 13°C (cold stress) and seven days in 28°C (control). Seeds were sterilized and followed by washing with distilled water to protect them against any fungal attack. They were placed between the two layers of filter paper in petri plates wetted with distilled water to keep them moist. The experiment was carried out in a completely randomized design with three replications of 10 seeds. Coleoptiles growth of the germinated seeds at 13°C were measured weekly for four weeks. For 28°C one measurement was taken at the end of the experiment. To evaluate cold tolerance the following formula was used: Germination percent (GP %): = (No. of normal seedlings / total No. of seeds planted x 100). Only the seeds that had coleoptile and radicle were considered for calculation. Radicle and coleoptile length were obtained based on all the germinated seeds at the end of the experiment.

In method II, seeds of thirty rice genotypes were sown under the following conditions: 28°C for 72 hours, 13°C for 96 hours and again 72 hours at 28°C. The aim of this change in temperature was to provide field conditions because in field conditions there is a temperature variation. Length of radicle was measured two times: first, at the end of 96 hours at 13°C (LENGTH 1) and second at the end of second period of 72 hours at 28°C (LENGTH 2), according to the formula: RADREG = (LENGTH 2) – (LENGTH 1).

In this experiment, evaluation of cold tolerance was carried out through coleoptiles growth and radicle regrowth, which was shown in the distinction between the second and the first measurements.

Results and Discussion

There were variations among the rice genotypes for traits related to germination percent, reduction in radicle and coleoptiles length and regrowth. Germination percentage showed significant differences among the genotypes. The germination percentage and percent decline in germination at (13^0c) was high in SKAU-402 (83.7%) followed by K332 (80%), SI-5 (43.3%) and SI-3 (56.7%).

The germination percentage and percent decline in germination at (17^0c) was highest in K332 (86.7%) followed by SKAU-402 (88.3%), SI-6 (80.0%) and SI-3, SR-3, Jehlum with same ratio (76.7%). Yoshida (1981) reported the greatest influence of temperature on germination. Blum (1988) suggested high germination percentage is important for early season cold tolerance in rice. Temperature below 18°C causes decreases in both the speed and percentage of rice seed germination (Xu, 2008). Cruz *et al.,* (2013) reported that low temperature stress may affect rice seed germination, avoiding development to the seedling stage. In direct seedling cultivation under the low temperature, germination and early growth of seedlings take place rapidly, (Suh *et al.,* 2010). Germination percentage of rice genotypes under normal and cold temperature are compared (Table 2).

The germination percentage at (13^0c) was decreased to below 50% in the genotypes, IR-52, China-1007, SKAU-411 and IR -73 while as the lowest germination at (17^0c) was obtained again in IR-52 (33.3%) and China 1007 (36.7%) which indicated cold susceptibility at germination rate. Sharifi (2010) evaluate 68 rice germplasms for cold tolerance at germination stage and showed that germination rate, radicle and coleoptile length were reduced under cold. The low germination under cold may be due to less metabolic activity and inactive enzymes that play key role in germination. Results also showed that lowest mean reduction upon both the temperatures (13ºC and 17ºC) was observed in SKAU-402, SI-6 and K332 genotypes.

In relation to the reduction in coleoptiles, there was a significant difference at temperatures of 13ºC and 17ºC between genotypes (Table 3). Range of reduction in coleoptiles length for genotypes at 13ºC was varied between 13.8 to 86.6 percent. SKAU 402, K-332, SI-5 and SI-6 had the highest and SI-1, IR-52 and China-1007 had the lowest amount of reduction in coleoptiles length due to cold stress (13°). Similarly range in genotypes at 17^0c varies from 27.6 to 94.1 percent, SI-5 followed by SI-3, SKAU 402 and K-332. While as lowest values was obtained in SI-1, IR-52 and China 988. Among some studied characteristics in this study, reduction in coleoptiles length was one of the best characteristic for better distinction between the genotypes sensitively to cold stress (Miedema, 1982). Via comparison of coleoptile growth under normal temperature in relation to cold temperature we are able to show the genotypes response to cold stress effectively (Miedema, 1982).

Table.1 List of rice genotypes used for evaluation of cold tolerance

S. No	Genotypes	S. No	Genotypes	S. No	Genotypes
1	K-332*	11	Kohsaar*	21	Kamad*
2	SKAU-407**	12	Shalimar Rice-1*	22	China-1007*
3	IR-24***	13	SI-4***	23	Shalimar Rice-3*
4	IR-64***	14	IR-52***	24	SI-3***
5	IR-72***	15	SI-17***	25	HEERA*
6	SI-1***	16	China-988*	26	KOSHIHIKARI*
7	Mushkbudji ***	17	SKAU-411**	27	SI-6***
8	SKAU-5 (K-39)*	18	Jehlum*	28	SI-7***
9	Shalimar Rice-2*	19	SKAU-408**	29	IR-70***
10	SKAU-402**	20	SI-5***	30	SKAU-403***

* = Released, ** = Pre released, ***= Germplasm

Table.2 Thirty rice genotype for seed and seedling parameters and their decline in germination percent and mean reduction under normal and stress conditions

Genotypes	Germination 28deg	Germination 13deg	Germination 17deg	Per cent decline in Germination 13deg	Per cent decline in Germination 17deg	Mean reduction
K332	96.7	80.0	86.7	0.17	0.10	0.14
SKAU-407	93.3	53.3	70.0	0.43	0.24	0.33
IR-24	83.3	56.7	73.3	0.32	0.12	0.22
IR-64	66.7	36.7	56.7	0.44	0.15	0.29
IR-72	73.3	33.3	53.3	0.55	0.27	0.41
SI-1	96.7	63.3	76.7	0.34	0.20	0.27
Mushkbudji	90.0	46.7	76.7	0.48	0.15	0.31
SKAU-5 (K-39)	96.7	53.7	70.0	0.44	0.28	0.36
Shalimar Rice-2	63.3	33.3	43.3	0.47	0.32	0.39
SKAU-402	96.7	83.7	88.3	0.13	0.09	0.11
Kohsaar	93.3	60.0	76.7	0.36	0.18	0.27
Shalimar Rice-1	90.0	40.0	56.7	0.56	0.37	0.46
SI-4	93.3	40.0	40.0	0.57	0.57	0.57
IR-52	86.7	13.3	33.3	0.85	0.62	0.73
SI-17	90.0	40.0	56.7	0.56	0.37	0.46
China-988	86.7	43.3	70.0	0.50	0.19	0.34
SKAU-411	83.3	26.7	40.0	0.68	0.52	0.60
Jehlum*	96.7	60.0	76.7	0.38	0.21	0.29
SKAU-408	86.7	50.0	56.7	0.42	0.35	0.38
SI-5	56.7	43.3	53.3	0.24	0.06	0.15
Kamad	90.0	66.7	76.7	0.26	0.15	0.20
China-1007	43.0	13.3	36.7	0.69	0.15	0.42
Shalimar Rice-3	93.3	66.7	76.7	0.29	0.18	0.23
SI-3	83.3	56.7	76.7	0.32	0.08	0.20
HEERA	96.7	60.0	56.7	0.38	0.41	0.40
KOSHIHIKARI	83.3	43.3	76.7	0.48	0.08	0.28
SI-6	90.0	76.7	80.0	0.15	0.11	0.13
SI-7	80.0	50.0	76.7	0.38	0.04	0.21
IR-70	76.7	53.3	70.0	0.30	0.09	0.20
SKAU-403	83.3	56.7	50.0	0.32	0.40	0.36

Table.3 Decrease in percentage of coleoptile length of thirty rice genotypes, obtained through comparison of their germination under normal temperature with germination under stressed conditions

Genotypes	Coleoptile growth (%) (13 °C for 28 days)	Coleoptiles growth (%) (17 °C for 28 days)	Genotypes	Coleoptile growth (%) (13 °C for 28 days)	Coleoptiles growth (%) (17 °C for 28 days)
K332	82.8	89.7	China-988	32.0	48.0
SKAU-407	42.9	57.1	SKAU-411	76.9	80.8
IR-24	68.0	88.0	Jehlum*	62.1	79.3
IR-64	80.0	85.0	SKAU-408	57.7	65.4
IR-72	45.5	72.7	SI-5	76.5	94.1
SI-1	13.8	27.6	Kamad	74.1	85.2
Mushkbudji	51.9	85.2	China-1007	31.0	85.3
SKAU-5 (K-39)	55.5	72.4	Shalimar Rice-3	71.4	82.2
Shalimar Rice-2	52.6	68.4	SI-3	68.0	92.0
SKAU-402	86.6	91.4	HEERA	62.1	58.6
Kohsaar	64.3	82.2	KOSHIHIKARI	52.0	72.0
Shalimar Rice-1	44.4	63.0	SI-6	85.2	88.9
SI-4	42.9	42.9	SI-7	62.5	86.0
IR-52	15.4	38.5	IR-70	56.6	64.4
SI-17	44.4	63.0	SKAU-403	68.0	60.0

Table.4 Radicle regrowth of 30 rice genotypes submitted to germination for 96 hours at 13°C and 72 h at 28°C

Genotypes	96 h at 13^{0}c (Length 1)	72 h at 28^{0}c (Length 2)	Radicle Regrowth (L2-L1)	Genotypes	96 h at13^{0}c (Length 1)	72 h at 28^{0}c (Length 2)	Radicle Regrowth (L2-L1)
K332	1.0	1.9	0.9	China-988*	0.5	0.8	0.3
SKAU-407**	1.5	2.0	0.5	SKAU-411**	1.3	1.8	0.5
IR-24***	0.5	0.7	0.2	Jehlum*	1.6	1.9	0.3
IR-64***	0.7	1.2	0.5	SKAU-408**	2.1	2.9	0.8
IR-72***	1.0	1.3	0.3	SI-5***	1.1	2.2	1.1
SI-1***	1.0	1.5	0.5	Kamad*	1.5	2.6	1.1
Mushkbudji ***	1.8	2.2	0.4	China-1007*	0.4	1.0	0.6
SKAU-5 (K-39)*	2.1	2.9	0.8	Shalimar Rice-3*	0.9	1.7	0.8
Shalimar Rice-2*	1.9	2.2	0.3	SI-3***	1.7	2.6	0.9
SKAU-402**	0.6	1.6	1.0	HEERA*	1.0	1.5	0.5
Kohsaar*	1.8	2.2	0.4	KOSHIHIKARI*	1.4	2.2	0.8
Shalimar Rice-1*	1.7	2.2	0.5	SI-6***	2.3	3.5	1.2
SI-4***	1.7	2.5	0.8	SI-7***	2.0	2.4	0.4
IR-52***	0.5	0.7	0.2	IR-70***	0.5	0.8	0.3
SI-17***	1.9	2.1	0.2	SKAU-403***	1.2	1.7	0.5

There was a significant difference in the radicle regrowth rate in the rice genotypes (Table 4). Radicle regrowth for all genotypes was varied from 2.0 to 12.0 mm, and SI-6,-SI-5, Kamad, SKAU-402 and K332, were the genotypes with high radicle regrowth. In the current study, radicle regrowth ability under cold stress in some of genotypes was remarkable. In this way, SI-6,-SI-5, Kamad, SKAU-402 and K332, genotypes had the highest and IR-24 and IR-72 had the lowest level of radicle regrowth. Measurement of radicle regrowth after a period of chilling stress is an important indicator of crop resistance to chilling stress (Rab and Saltveit, 1996a; 1996b). In general, there is a cold period between periods of high temperature in field conditions that leads to cold stress damage. Under these premises, and in order to simulate field conditions, experiment II was performed. Yoshida (1981a) demonstrated that the most influence of temperature on germination stage occurs in the phases of coleoptiles and radicle activation and growth. The reduction in coleoptile growth during these phases may be attributed to the direct effect of cold temperature on cellular elongation and division, or to its indirect effect leading to a metabolic unbalance (Lyons, 1973). The reduction in the length of the coleoptile under cold temperature of 18 ^{o}C, helped in distinguishing the tolerant genotypes and sensitive ones (Farzin *et al.*, 2013). Priyanka *et al.*, (2015) evaluated cold tolerance of rice genotypes in terms of REDCOL. Cruz and Milach (2004) suggested that both REDCOL and COLREG seem to be the most adequate characteristics to be used to evaluate cold tolerance during the germination period in rice

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