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Evaluation of rice (*Oryza sativa* L.) genotypes for cold tolerance at seedling stage

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ABSTRACT

Cold tolerance during germination is important for ensuring fast and uniform crop establishment. Rice plants are injured at the seedling stage when they are grown in early spring in temperate or subtropical environments. The productivity of rice is particularly low during spring due to cold, which is an important abiotic constraint, where low temperature prevails below 18 ºC. The present study was taken up to evaluate cold tolerance in 86 rice genotypes based on seed and seedling parameters as per Cruz and Milach (2004). Seeds were germinated under two conditions i.e.,16^{0}C for 28 days (cold) and 28^{0}C for 7 days (control).The genotypes showed highly significant differences forparameters such as germination (%), germination index (%), percentage of seeds with coleoptile superior to 5 mm (PERCOL), percentage of reduction in coleoptile length (REDCOL) and coleoptile regrowth (COLREG, cm).Both REDCOL and COLREG seem to be the most adequate characteristics to be used to evaluate cold tolerance during the germination period in rice. Among those genotypes, AC 35548, JBT 37/164, PS 353, KMP-175 andThanu were adjudged as promising when screened for cold tolerance under laboratory conditions based on the aforementioned parameters.

Key words: *Oryza sativa L.,cold tolerance, low temperature, germination*

INTRODUCTION

Rice is a staple food for half of the human population. Unlike other cereals such as wheat and barley, rice plants are susceptible to cold stress, which often results in decreased productivity. Low temperatures can have negative impacts on rice plants during germination, vegetative growth, and reproductive stages. During the early growth stages in rice, the occurrence of low-temperature stress affects seed germination that inhibits seedling establishment and eventually leads to non-uniform crop maturation (Sharifi, 2010).

Rice plants are injured at the seedling stage when they are grown in early spring in temperate or subtropical environments. The productivity of rice is particularly low during spring due to cold, which is an important abiotic constraint, where low temperature prevails below 18 ºC. Even though temperature does not prevent rice germination, it delays beginning and, consequently, plant emergence. Optimum temperature range for rice germination lies between 20 and 35°C, and the temperature of 10°C is cited as the minimum critical value below which rice does not germinate (Yoshida, 1981).

Good performance during germination is important to guarantee fast establishment and uniform crop stand in rice. However, evaluation of this trait under field conditions is limited by environmental variation, which makes it difficult to identify genetically superior lines. Therefore, laboratory screening is more reliable as the intensity and duration of cold stress can be adjusted and it also eliminates the chances of interference of other biotic and abiotic factors (Cruz et al., 2006). In this context, the present study aimed at evaluation of rice genotypes under low temperature based on seed and seedling parameters and to identify

the genotypes showing most tolerance to low temperature.

MATERIALS AND METHODS

The material for this study consisted of 86 selected rice genotypes from NRRI, Cuttack, Orissa and popular rice varieties developed at AICRP (Rice), Zonal Agricultural Research Station, V.C. Farm, Mandya. The released varieties namely CTH-1 and CTH-3 were used as cold tolerant checks.A total of 86 genotypes were evaluated for cold tolerance based on seed and seedling parameters as per Cruz and Milach (2004). Before the beginning of the experiment, all the seeds were washed with aqueous ethanol (70%) for 30s followed by immersion in aqueous sodium hypochlorite (4%) for 20 minute to prevent contamination and then washed six times with sterile distilled water.

Two methods were used to evaluate rice genotypes for cold tolerance, described in experiments I and II.

In Experiment I, seeds of 24 rice genotypes were germinated under two conditions: 16°C for 28 days (cold) and 28°C for seven days (control). Seeds were allowed for germination following germination paper rolling method. Germination paper was made wet with distilled water containing 1 mL of Benomyl solution to avoid contamination. The experiment was conducted in a completely randomized design with three replications in the germination chamber. Each germination paper roll contained 20 seeds, and the average of these seeds was used as a replication, amounting to 60 seeds per genotype.

Seeds germinated at 16°C had their coleoptile length measured weekly for a period of 28 days and for seeds germinated at 28°C, coleoptile length was measuredseven days after the beginning of the experiment. Evaluation ofthe genotypes cold tolerance in this experiment was carried out by means of the following characteristics:

1. Germination (%)

The per cent germination was calculated by using the formula,

$$\text{Germination percent} = \frac{\text{Number of normal seedlings obtained}}{\text{Total number of seeds kept for germination}} \times 100$$

Only the seeds that had coleoptile and radicle were considered as normal for calculation.

2. Germination index (GI)

The germination index was calculated by using the formula given by Cruz and Milach (2004)

$$GI = (N14 + N21/2) / 20 \times 100$$

where,

N14 = number of germinated seeds 14 days after the beginning of the cold treatment

N21 = number of germinated seeds 21 days after the beginning of the cold treatment

20 being the total number of seeds per genotype per replication. For the calculation of GI only the seeds bearing coleoptile and radicle were considered.

3. Percentage of seeds with coleoptile superior to 5 mm (PERCOL)

PERCOLwas obtained by considering all the germinated seeds 28 days after the beginning of the cold treatment and by counting number of seeds showing coleoptile length superior to 5 mm, according to the formula given by Cruz and Milach (2004).

PERCOL = (Number of seeds with coleoptile > 5mm) x 100/20

4. Percentage of reduction in coleoptile length (REDCOL)

This was obtained through comparison of average coleoptile length at 28 days after germination at 16°C (cold treatment) with that obtained 7 days after germination at 28°C (control) and calculating the percentage of reduction in coleoptile length by germination under cold temperature, according to the formula given by Cruz and Milach (2004).

REDCOL = [(coleoptile length under cold temperature x 100) / coleoptile length under control] - 100

where coleoptile length is the average of the 20 seeds evaluated per replication per genotype.

In Experiment II, seeds of the 86 rice genotypes were submitted to germination under the following conditions: 28°C for 72 hours, 16°C for 96 hours and again 28°C for 72 hours. This procedure was used to

simulate field conditions, where temperature variation is ordinarily observed. Sterilization of the seeds for conduction of the experiment were made according to experiment I. Coleoptile length was measured in two occasions: after the period of 96 hours at 16°C (LENGTH 1) and after the second period of 72 hours at 28°C (LENGTH 2).Cold tolerance evaluation was performed through coleoptile regrowth (COLREG, cm), which consisted in the difference between the second and the first measurements, or in how much the coleoptile grew under normal temperature after the cold treatment, according to the formulagiven by Cruz and Milach (2004),

COLREG = (LENGTH 2) - (LENGTH 1)

where coleoptile length is the average of the 10 seeds evaluated per replication.

Statistical analysis

The mean values for all the above mentioned parameters were subjected for statistical analysis and the variance for various traits was estimated following ANOVA as per Completely Randomized Design (CRD) as outlined by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Analysis of variance (Table 1) showed that there were variations among the 86 rice genotypes for traits related to germination percent, germination index (GI), percentage of seeds with coleoptile superior to 5 mm (PERCOL), percentage of reduction in coleoptile length (REDCOL) and coleoptile regrowth (COLREG). Hence, the results could be better utilized for the distinction of tolerant and sensitive genotypes of rice under low temperature. These findings were in accordance with Cruz and Milach (2004) who also reported the significant differences among rice genotypes for analysis of variance for the above parameters under cold.

Germination (%)

Germination percentage showed significant differences among the studied genotypes. The germination percentage was high in PS 391 (95.56%) followed by KMP - 175 (94.44%), JBT37/164 (93.33%), PS 229 (93.33%) which were significantly superior to the cold tolerant check varieties, CTH-1 (52.22%) and CTH-3 (73.33%).

The germination percentage under cold was reduced to an extent of more than 30 per cent in the check variety CTH-1 and other test genotypes- JBT 360, PS 399, AC 35392, AC 35006, AC 35633 and AC 35373. The reduction in germination under cold was observed because cold temperature was near the critical temperature for germination. This was supported by Yoshida (1981) who reported that critical temperature for germination of rice is 10 °C. Cruz et al. (2013) reported that low temperature stress may affect rice seed germination, avoiding development to the seedling stage. Lone et al. (2018) evaluated 30 rice genotypes 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. Germination percentage of 86 rice genotypes under normal and cold temperature is compared in Table 2.

Germination index (%)

The germination index (GI), that expresses the germination speed under cold temperature showed significant difference among the genotypes and found high in KMP - 175 (94.17%) followed by JBT 38/119 (88.33%), JBT 38/130 (86.67%) and BR - 2655 (84.17%) which were significantly superior to the cold

Table 1. Analysis of variance (ANOVA) for seed and seedling parameters of 86 rice genotypes under cold temperature.

Source of variation	Degrees of freedom	Germination (%)	Germination index (%)	PERCOL (%)	REDCOL (%)	COLREG (mm)
Genotypes	85	279.50**	1175.82**	1076.04**	1314.90**	0.220**
Error	172	29.31	15.79	32.17	8.38	0.001
CD (1%)		10.38	7.62	10.87	5.55	0.07
CV (%)		6.68	7.49	9.43	7.17	5.46

**Significance at 1% level

Table 2. Comparison of germination (%) of rice genotypes under control and cold temperature.

Sl. no.	Genotypes	Germination (%)		Difference
		Control (28^0C)	Cold (16^0C)	
1	CTH-1	91.67	52.22	39.44
2	CTH-3	81.67	73.33	8.33
3	JGL - 1798	95.00	87.78	7.22
4	IR - 30864	95.00	68.89	26.11
5	MTU - 1001	95.00	92.22	2.78
6	MTU - 1010	80.00	74.44	5.56
7	KMP - 175	98.33	94.44	3.89
8	BR - 2655	95.00	88.89	6.11
9	Raksha	91.67	83.33	8.33
10	Jaya	88.33	84.44	3.89
11	Thanu	93.33	90.00	3.33
12	KRH - 4	76.67	74.44	2.23
13	JBT 37/16	91.67	87.78	3.89
14	JBT 35/138	88.33	86.67	1.66
15	JBT 36/169	90.00	88.33	1.67
16	JBT 36/16	91.67	81.11	10.56
17	JBT 37/164	95.00	93.33	1.67
18	JBT 37/92	95.00	86.67	8.33
19	JBT 38/148	98.33	82.22	16.11
20	JBT 38/61	90.00	88.33	1.67
21	JBT 37/117	91.67	90.00	1.67
22	JBT 27/110	88.33	73.33	15.00
23	JBT 37/111	96.67	93.33	3.34
24	JBT 37/29	93.33	80.00	13.33
25	JBT 38/130	95.00	90.00	5.00
26	JBT 37/91	88.33	86.67	1.66
27	JBT 38/25	90.00	88.89	1.11
28	JBT 396	93.33	75.56	17.78
29	JBT 37/70	95.00	80.00	15.00
30	JBT 3676	85.00	82.22	2.78
31	JBT 360	95.00	67.78	27.22
32	JBT 3664	90.00	88.33	1.67
33	JBT 38/88	90.00	87.78	2.22
34	JBT 37/113	78.33	73.33	5.00
35	JBT 38/119	85.00	83.33	1.67
36	JBT 37/89	98.33	84.44	13.89
37	PS 353	91.67	90.00	1.67
38	PS 267	88.33	83.33	5.00
39	PS 225	86.67	83.33	3.34
40	PS 229	98.33	93.33	5.00
41	PS 270	93.33	78.89	14.44
42	PS 367	96.67	90.00	6.67
43	PS 329	91.67	84.44	7.22
44	PS 370	86.67	81.11	5.56
45	PS 320	83.33	81.67	1.66
46	PS 401	90.00	78.89	11.11
47	PS 399	95.00	60.00	35.00
48	PS 397	90.00	88.33	1.67
49	PS 316	93.33	86.67	6.67
50	PS 330	91.67	88.33	3.34
51	PS 253	90.00	71.11	18.89
52	PS 339	85.00	81.11	3.89
53	PS 302	98.33	87.78	10.56
54	PS 284	86.67	85.00	1.67
55	PS 384	90.00	86.67	3.33
56	PS 273	96.67	85.56	11.11
57	PS 350	95.00	85.56	9.44
58	PS 303	93.33	76.67	16.67
59	PS 332	95.00	90.00	5.00
60	PS 419	90.00	75.56	14.44
61	PS 379	95.00	93.33	1.67
62	PS 259	90.00	81.11	8.89
63	PS 276	88.33	86.67	1.66
64	PS 307	98.33	93.33	5.00
65	PS 391	96.67	95.56	1.11
66	PS 91	86.67	76.67	10.00
67	AC 35548	93.33	91.67	1.66
68	AC 35611	80.00	76.67	3.33
69	AC 39020	96.67	81.11	15.56
70	AC 39012	98.33	74.44	23.89
71	AC 35450	98.33	70.00	28.33
72	AC 35392	95.00	60.00	35.00
73	AC 36110	90.00	71.11	18.89
74	AC 39006	98.33	67.78	30.56
75	AC 35027	96.67	92.22	4.44
76	AC 35006	96.67	62.22	34.44
77	AC 35361	98.33	90.00	8.33
78	AC 39019	95.00	58.89	36.11
79	AC 35633	98.33	66.67	31.67
80	AC 35290	95.00	80.00	15.00
81	AC 35331	91.67	76.67	15.00
82	AC 35534	96.67	76.67	20.00
83	AC 35256	98.33	68.89	29.44
84	AC 35170	95.00	71.11	23.89
85	AC 35373	90.00	57.78	32.22
86	AC 35067	88.33	70.00	18.33

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tolerant check varieties, CTH-1 (25.83%) and CTH-3 (35%). These results indicate that more seeds of aforementioned genotypes had germinated 14 and 21 days after the beginning of the cold experiment. Germination speed is important for crop establishment, however, it does not necessarily hold any relation with the ability of a genotype to elongate coleoptile and radicle under cold temperature. As a matter of fact, for the GI calculation only seeds that possessed coleoptile and radicle were considered, independent of their length. Germination speed, and consequently GI, are related to a high seed vigour and this may be the cause of the better performance of genotypes for GI (Cruz and Milach, 2004).

Percentage of seeds with coleoptile superior to 5 mm (PERCOL)

Percentage of seeds with coleoptile length greater than 5 mm after 28 days of germination at 16°C revealed the wide variation present among the genotypes. The PERCOL was found high in KMP - 175 (90%) followed by Thanu (88.33%), JBT 37/111 (86.67%), AC 35548 (85.00%) and JBT 38/61 (83.33%) which were significantly superior to the cold tolerant checks, CTH-1 (26.67%) and CTH-3 (25.00%). The genotypes, KMP-175 and AC 35548, superior for GI, also exhibited high capacity of coleoptile growth, with values greater than 80% for PERCOL. As per Cruz and Milach (2004), this characteristic reflects the coleoptile elongation capacity under cold temperature thus, it can be used as a good criterion to distinguish cold-tolerant and cold-sensitive rice genotypes. In a study carried out to evaluate rice germplasm for cold tolerance at the germination stage, Sharifi (2010) reported that the low temperature causes delay in the growth of coleoptile and radicle compared to control, showing a strong inhibition of coleoptile and radicle lengths because of decrease in the temperature.

Percentage of reduction in coleoptile length (REDCOL)

Reduction of coleoptile length was inversely proportional to cold tolerance, i.e., higher the reduction in coleoptile length the lower was the cold tolerance (Cruz et al., 2006). There was a significant difference among the genotypes for REDCOL. The percentage of reduction in coleoptile length was significantly low in PS 353 (5.81) followed by JBT 37/164 (6.29), Thanu (6.44), BR - 2655 (6.69) over the highest performing cold tolerant varieties CTH-1 (72.47) and CTH-3 (76.64). This character is an expression of the amount of reduction in genotypes coleoptile length by germination under cold in relation to normal temperature. The results in this study are also in accordance with Farzin et al. (2013) wherein there was a reduction in the length of the coleoptile due to exposure to cold temperature of 15^0C which helped in distinguishing the tolerant genotypes and sensitive ones.

Coleoptile regrowth (COLREG)

This trait was considered for screening to know the effect of varying temperature on the genotypes. Coleoptile regrowth (COLREG) had a wide variation among studied genotypes, indicating that the germination process after the cold period differently affected the genotypes. The genotypes were first exposed to control temperature followed by cold and again to normal temperature depicting the field conditions where the temperature is not stable (Cruz and Milach, 2004). In this study, highest COLREG was recorded in genotype PS 332 (1.34cm) followed by JBT 35/138 (1.27 cm), AC 39006 (1.25 cm), JBT 37/164 (1.24 cm) and JBT 36/169 (1.22 cm) superior over the cold tolerant checks, CTH-1 and CTH-3, which recorded COLREG of 0.58 cm. The genotypes, which showed superior performance with respect to germination percentage, germination index, PERCOL and REDCOL *viz*., KMP-175, AC 35548, Thanu, PS 353 also showed moderate COLREG i.e., 0.45cm, 0.77 cm, 0.69 cm and 0.55 cm respectively. Therefore, differences in COLREG among genotypes may be an indicative of a distinct capacity of recovery of the germination process after a cold period, and of different degrees of cold tolerance.

CONCLUSION

Significant variations for cold tolerance existed among the genotypes studied for different parameterssuch as germination per cent, germination index, PERCOL, REDCOL and COLREG. Among those genotypes, JBT 37/164, PS 353, Thanu, KMP-175 and AC 35548 proved to be cold tolerant under laboratory conditions. Though tolerance at laboratory level is assured based on the above parameters, the seed and seedling cold tolerance may not correlate with field cold tolerance (Blum, 1988). Hence, these genotypes are to be further evaluated under field conditions for yield and other traits under the prevailing temperature of around 15^0C.

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