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Evaluation Eighteen Rice Genotypes in Cold Tolerance at Germination Stage

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Abstract: Eighteen rice genotypes were used for cold tolerance evaluation at the germination stage in laboratory. The experiment was carried out in a factorial based on complete block design in three blocks. Seeds of rice genotypes germinated at four different temperatures containing 13, 17, 21 and 25°C. Analysis of variance revealed that low temperature had a significant effect on germination rate, coleoptile length and radicle length. Germination rate was strongly affected by the low temperature treatments. Stress of low temperature at germination stage on tested rice genotypes caused a reduction in final germination rate and lengths of coleoptile and radicle. The normal and healthy seeds began to germinate within 36 h after imbibition and germination were completed on the 7th day in the control (25°C), but delayed under other temperature treatments. The low temperature treatments delayed the growth of coleoptile and radicle compared to the control and the average lengths of coleoptile and radicule were strongly inhibited with the decreasing temperature in all of tested genotypes. Taichung and sadri showed a significantly higher germination rate, bigger coleoptile and radicle length at three low temperature treatments.

Key words: Rice · Cold tolerance · Germination rate · Coleoptile length · Radicle length

INTRODUCTION

Rice is one of the most important staple crops in the world and its consistent production is vital for food security. Most rice growing countries are faced with climate-induced stresses that significantly reduce rice productivity, such as droughts, floods, low temperatures and winds. Yield loss caused by low temperature is a worldwide problem in rice production, because it is a temperature-sensitive field crop. In the breeding programs, the establishment of new rice culture in such areas requires an extensive knowledge of varietal chilling sensitivity, which in turn can only be accurately determined by screening under controlled conditions [1].

During the early growth stages of rice, the occurrence of low temperature stress restrains seedling establishment and leads to non-uniform crop maturation. The optimum temperature for seed germination and early seedling growth is from 25°C to 35°C. The occurrence of low temperature stress below 15°C at this stage usually results in poor seedling establishment or seedling mortality [2]. Selection and development of cold-tolerant varieties is one of the most effective ways to impede the damage of low temperature [3]. Good performance during germination is important to guarantee fast seedling establishment and uniform crop stand [4].

One of the first objectives in breeding programs for stability to cold stress is use of tolerant parents from different origins. Denotation tolerant parents at germination stage can be performed under controlled temperature condition with adjusted intensity and duration of temperature. In this condition, greater precision resulting will be obtain due to absence of other abiotic and biotic factors that may interfere in field data [5]. Numbers of methods are available for evaluation rice genotypes to low temperature at germination stage. Bertin *et al.* [6] proposed put rice seeds at temperatures varying from 10 to 25°C for periods of 3 to 35 days and measure characteristics such as germination rate and speed and the lengths of coleoptile and radicle. The mode of inheritance for cold tolerant traits has been reported by Sharifi [7] that indicated the reduction in of radicle length, coleoptile length and germination rate were affected by non-additive gene action. Cruz *et al.* [8] also showed that both additive and non-additive gene action were involved in cold tolerance traits at germination stage, such as

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percentage of reduction in coleoptile length and coleoptile growth, while the non-additive action was relatively more important for them.

Most studies performed under controlled temperature at the germination stage of rice were related to the identification of variability and genotype characterization. They were responsible for identifying genotypes of better performance during germination under cold temperature. In general, it is known that japonica genotypes are more cold tolerant than indica ones [8].

The objective of this study is evaluation some of rice genotypes to low temperature treatment at germination stage under controlled conditions and identification the better genotypes. Estimation the correlation between traits is the other objectives of this study.

MATERIALS AND METHODS

Rice Materials: Eighteen rice genotypes containing Iranian traditional landraces, Iranian improved genotypes and some of the genotypes from other countries were studied in this research. All of genotypes were collected from Rice Research Institute of Iran (RRII). The cold tolerance evaluation was carried out in a laboratory of the Department of Agronomy and Plant Breeding in Islamic Azad University of Rasht (Guilan Province), Iran in the spring of 2010.

Cold Tolerance Evaluation: Rice cold tolerance at the germination stage was considered by the method of Cruz and Milach [9]. Seeds of 18 rice genotypes were germinated at four different temperature conditions containing 13, 17, 21 and 25°C. 13 and 17°C treatments exerted constantly for 28 days according to delay in germination in low temperature condition. Entire treatments were performed in germinator (Iran-Khodsaz, 1KH RH). For each genotype, seeds harvested in the same cropping season, were selected based on size uniformity and absence of spots. They were sterilized with ethanol 70% for 30s and washed with sterile water. Seeds were placed on Petri dishes containing two layers of germination paper and soaked with distilled water. The experiments were conducted in a factorial design based on randomized complete block design with three replications. The shelves of germinator were as blocks and each Petri dish contained 30 seeds was used as a replication.

Evaluation of genotypes for cold tolerance in this experiment was carried out by means of the following traits. Germination rate = $n/30\times100\%$, where n is the number of germinated seeds and 30 being the total number of seeds per genotype. For germination rate calculation, only the seeds presenting both coleoptile and radicle were considered. Coleoptile length and radicle length were obtained based on all germinated seeds at the end of each mentioned treatment. The data were calculated only on the germinated seeds.

Statistical Analysis: The analysis of variance was carried out in data after collecting and the means of genotypes were compared by the least significant difference test [10]. Relationships between the measured traits were established through the Pearson's coefficient correlation. Entire analyses were performed with the Statistical Analysis System (SAS) Software.

RESULTS

Analysis of variance revealed significant differences between rice genotypes for all of three traits containing germination rate, coleoptile and radicle length. The results also showed significant effects of temperature and interaction between two fatorss for all of three traits. The effects of blocks (replication) were not significant for any of traits (Table 1).

According to significance of genotype × temperature interaction effects for all of traits, simple effects of two factors (genotypes in each level of temperature and *vice versa*) were analyzed (Table 2). The results of simple effects ANOVA for genotypes in each level of temperature indicated significant effects of genotype for all of traits. On the other hand, temperature simple effects were significant for all of traits (tables did not show).

Mean comparison were performed by least significant differences (LSD) method due to simple effects (Table 3). The results indicated significant differences between genotypes in 13°C for radicle length. The length of radicles was small in all of genotypes, but Ali-kazemi had most radicle length in this treatment. For second level of temperature (17 °C) Dolar and Garde posses the lowest and highest radicle length, respectively. Two genotypes contain Ali-kazemi and Dolar had highest and lowest radicle length in 21 °C. Overall, for this trait Garde, Sadri and Taichung were as tolerant genotypes to low temperature stress and Dolar and Hasansaraii as sensitive varieties.

Table 1: Analysis of variance for germination related traits in different temperatures for some of rice genotypes

		MS		
SOV	df	Radicle length (cm)	Coleoptile length (cm)	Germination rate (ratio)
Replication	2	0.702	0.179	0.0025
Genotype	17	3.103**	14.97**	0.2249**
Temperature	3	118.80**	51.97**	0.7004**
Genotype× Temperature	51	2.40**	2.65**	0.0980**
E	142	0.391	0.699	0.0053
CV		28.64%	30.15%	9.88%

**, $P<0.01$.

Table 2: Analysis of variance for simple effects of genotypes in each of temperature levels

		Radicle length (cm)				
		Temperature	1 (13°C)	2 (17°C)	3 (21°C)	4 (25°C)
SOV	df	MS				
R	2		0.0002	1.017	0.380	0.52
G	17		0.0029**	3.154**	2.458**	4.689**
E	34		0.0002	0.366	0.746	0.45
		Coleoptile length (cm)				
		Temperature	1 (13°C)	2 (17°C)	3 (21°C)	4 (25°C)
		MS				
R	2		0.005	1.022*	0.030	0.047
G	17		4.68**	6.379**	4.862**	7.014**
E	34		0.152	0.286	1.015	1.412
		Germination rate (ration)				
		Temperature	1 (13°C)	2 (17°C)	3 (21°C)	4 (25°C)
		MS				
R	2		0.008	0.016	0.009	0.007
G	17		0.249**	0.113**	0.087**	0.069**
E	34		0.004	0.005	0.007	0.004

**, $P<0.01$.

Table 3: Mean of germination related traits in different temperatures for some of rice genotypes

		Radicle length (cm)					Coleoptile length (cm)					Germination rate (ration)				
Genotype	Origin	1	2	3	4	LSD5%	1	2	3	4	LSD5%	1	2	3	4	LSD5%
1(IR36)	IRRI	0.009	1.06	3.17	2.60	0.81	0.12	0.87	1.54	2.61	0.42	0.03	0.31	0.44	0.97	0.11
2(Khazar)	Iran	0.008	2.72	3.03	3.68	1.14	2.82	5.39	4.66	5.40	1.44	0.86	0.96	0.89	0.94	0.15
3(Binam)	Iran	0.01	2.08	4.12	2.15	0.70	1.50	4.39	3.83	3.11	1.01	0.70	0.78	0.94	0.73	0.15
4(Anbarbo)	Iran	0.011	1.27	3.78	1.41	1.07	2.75	1.53	2.20	2.34	1.09	0.93	0.88	0.88	0.88	0.07
5(Taichong)	Thailand	0.011	2.41	2.54	6.82	1.16	4.04	4.32	4.28	4.94	1.23	0.97	0.97	1.00	1.00	0.06
6(Dolar)	USA	0.02	0.87	1.83	2.43	0.17	0.81	1.12	1.82	1.88	0.57	0.38	0.46	0.44	0.96	0.05
7(Kanto-51)	Japan	0.009	4.01	3.05	3.15	0.81	2.23	3.28	2.64	5.59	1.42	0.48	0.96	0.93	0.94	0.08
8(Hashemi)	Iran	0.01	3.01	2.74	2.73	1.91	0.18	1.86	3.43	4.42	1.62	0.12	0.73	0.98	0.88	0.11
9(Domsiah)	Iran	0.006	1.84	2.11	2.34	1.28	1.42	1.29	1.64	2.23	1.38	0.40	0.90	0.92	0.88	0.14
10(Hasansaraii)	Iran	0.01	1.87	1.68	2.35	0.54	0.47	1.81	0.91	3.48	0.79	0.32	0.94	0.88	0.82	0.09
11(Hasani)	Iran	0.009	2.25	5.13	3.81	1.38	2.54	2.25	4.86	3.33	0.53	0.92	0.74	0.84	0.87	0.18
12(Neamat)	Iran	0.011	2.51	3.52	1.98	1.81	0.15	1.41	1.66	1.81	0.49	0.47	0.67	0.87	0.88	0.27
13(Ali-kazemi)	Iran	0.10	2.15	4.64	1.87	0.68	1.87	1.97	3.68	2.33	2.44	0.35	0.91	0.87	0.82	0.11
14(Tarom)	Iran	0.01	1.50	3.26	4.38	1.54	2.11	2.21	2.80	4.86	0.73	0.53	0.51	0.48	0.57	0.11
15(Sadri)	Iran	0.008	3.69	2.78	4.29	0.90	3.62	6.08	5.12	5.47	1.30	0.57	0.72	0.78	0.80	0.13
16(Garde)	Iran	0.012	4.57	3.93	3.09	1.24	0.44	1.90	3.44	5.77	3.58	0.73	0.91	0.90	0.83	0.29
17(Saleh)	Iran	0.021	2.09	2.89	3.13	1.40	0.56	2.98	2.19	4.33	2.27	0.54	0.71	0.79	0.76	0.23
18(Bahar1)	Iran	0.011	3.56	3.27	3.69	1.22	0.40	3.03	3.80	6.60	1.66	0.85	0.87	0.88	0.87	0.12
LSD5%		0.021	1.00	1.43	1.11		0.65	0.89	1.67	1.97		0.11	0.12	0.14	0.11	

Table 4: Correlation coefficients between germination rate (GP); coleoptile length (PL) and radicule length (RL) evaluated in rice genotypes

Germination rate (ration)	Coleoptile length (cm)	Radicle length (cm)	Traits
Radicle length (cm)	1	0.539**	0.411**
Coleoptile length (cm)		1	0.412**
Germination rate (ration)			1

**, *P*<0.01.

Taichung had the highest coleoptile length at 13°C treatment. In two other treatments (17°C and 21°C) Sadri had the highest coleoptile length. IR36 had the lowest coleoptile length in 13°C and 17°C and Hasansaraii in 21°C. Sadri and Taichung were as best and IR36, Dolar and Hasansaraii as worse genotypes due to coleoptile length in these three temperature treatments.

The third trait that studied, were germination rate. Taichung and IR36 had highest and lowest germination rate in two temperature treatments (13°C and 17°C), respectively. IR36 and Dolar had the lowest germination rate in 21°C. In this temperature treatment, many of varieties have not significant differences with 25°C.

There was a considerable reduction in germination rate in rice genotypes at 13°C and 17°C, compared to the other two treatments. Treatment seeds with low temperature cause delaying in germination rate and reducing in seedling traits such as radicle and coleoptile length. The results showed also the decrease in radicle length was more pronounced compared to that in coleoptile length in low temperature treatments. The results of this study indicate high temperature treatments increase germination rate and seedling growth.

The germination response of genotypes under different temperature treatments showed considerable differences. Taichung, Anbarbo and Hasani showed good germination response at 13°C among all the studied genotypes. In this treatment, more reduction of germination rate was observed in IR36. The results also showed a significant reduction in germination rate in genotypes at 17°C in comparison to 21°C and 27°C. The normal and healthy rice seeds began to germinate within 36 h after imbibition and completed on the 7th day at standard temperature (25°C). However, the germination of seeds that treated with lower temperatures was delayed and the deferment of germination was related to the level of temperature reduction. The results of this experiment revealed at 13°C, retardation of germination was more than the other treatments.

In all of genotypes, radicle length was near to zero at 13°C, this means that 13°C is a critical temperature for germination of rice genotypes. Ali-kazemi has the highest radicle length, whereas the greatest inhibition in radicle length was recorded in IR36. Early and rapid elongation of roots is important for indicating resistance to abiotic stresses such as drought and cold. Ability of continued elongation of radicle under the situation of cold stress was remarkable in some of the genotypes. Some of the genotypes, such as Kanto-51 and Garde exhibited more than 4 cm long radicles whereas the radicle lengths of other genotypes were shorter than 4 cm at 17°C. Also other genotypes such as Bahar1 (introduced hybrid genotype in the Rice Research Institute of Iran), showed the lowest reduction in radicle length in this temperature treatment. It was interesting to note that the cultivated genotype with lowest reduction in radicle length established rapidly at early growth season and could be utilized in rice cold tolerance breeding program as parents for crosses.

Intermediate positive significant correlation was indicated between three traits. This means some of genotypes were tolerant based on a bigger coleoptile length, were also considered as tolerance due to the other two traits and *vice versa*. It is thus worthy discussing whether the positive correlation between traits indicates either they are related to similar aspects of cold tolerance or not.

DISCUSSION

Balanced growth was observed in cold resistant genotypes. Cold resistant genotypes (for example Khazar, Binam, Sadri and Taichong,) were noted with longer than 4 cm coleoptiles under 17°C, whereas IR36 recorded the coleoptile lengths shorter than 1 cm and were as sensitive genotype to cold stress. Drastic reduction in coleoptile and radicle growth was observed with decreasing temperature.

Evaluation of rice seed germination rate and new plantlet trait's under more than one temperature treatments are necessary to distinguish cold tolerance genotypes from characteristics related to vigor [11]. Coleoptile length, radicle length and germination rate seem to be the most adequate traits for evaluate cold tolerance during the seed germination period in rice [9]. Correlation between them was positive, proving that a greater coleoptile length reduction was accompanied by a greater reduction in radicle length and germination rate due to cold temperature stress.

This study revealed that traits at the early growth stage of the investigated rice genotypes were significantly influenced by low temperature treatments. However, the responses of these genotypes to low temperature were different. Among the 18 rice genotypes used in this study, Taichung showed significantly higher germination rate under temperature treatments and its coleoptile length was significantly higher than other genotypes. It probably possesses genetic tolerance to low temperature stress and might be exploited as a source of cold tolerant genotype for rice breeding programs. Taichung is a japonica genotype with high milling rice rate and grain yield and it is also known as a semi-dwarf, low amylose content and early maturing genotype. Hashemi, which is widely used in north of Iran, is a genotype with intermediate tolerance to low temperature.

The variability among the rice genotypes was expected because of their different origins. The availability of low temperature sources is interesting for breeding cold tolerant rice, since it allows them to be used as genitors in crosses, as well as the transfer of undesirable characters to adapted genotypes. The negative effects of low temperature that indicated in this study, agreed to the results of previous researchers, which explained the occurrence of low temperature stress below 15°C at the germination stage usually results in poor seedling establishment and increasing the seedling mortality [2, 6].

Temperature has a profound influence on seed germination by affecting the activation stage and post-germination growth. Low temperatures can affect the rice plant's developmental processes and impair photosynthesis. Low temperature at the seedling stage can reduce rice yield due to reduction in establishment of plantlets and subsequently plant population. In the breeding process for cold tolerance at the germination period, evaluation of reduction in some of traits such as germination rate, coleoptile length and radicle length, which indicates for identification of cold tolerant genotypes, could be used for recognition of genitors. Some of genotypes such as Dolar and IR36 can be crosses with Taichong and Sadri and used from their offspring's for breeding programs such as QTL mapping, diallel analysis and generation mean analysis.

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