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Identification of cold tolerant rice genotypes and associated traits at seedling stage

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Abstract
Rice (*Oryza sativa* L.) is the most important C3 crop and it is the staple food crop in the world. Rice is susceptible towards cold, hence an attempt was made to identify cold tolerance genotypes. The experiment was laid out in a completely randomized design with 3 replications in 40 rice genotypes. Rice genotype K 32and K107 showed higher mean values for seven physiological characters *viz.,* germination %, total seedling length, seedling vigour index, shoot length, root length, root dry weight and total seed dry weight. Association pattern, direct and indirect effect was analyzed for identifying character related to cold stress, which revealed that the characters *viz.,* germination percent, total seedling length and root length as reliable indicators. Hence selection for these characters could aid in identifying cold tolerant genotypes at seedling stage.

Keywords: Cold stress, Rice, Seedling vigour index, Association analysis, path analysis

Introduction
Rice (*oryza sativa* L.) is one of the pivotal crop among cereals which feeds 3/4 of the world population. It is a tropical and a subtropical plant which requires a fairly high temperature ranging from 20˚c to 40˚C (Sridevi and Chellamuthu, 2015) [7]. The standard temperature for rice seed germination is considered to be approximately 30˚c and anything below 20˚c results in gradual decrease of germination rate (Ueno and Miyoshi, 2015) [8]. Moreover, Yoshida (1981) [9] considered 10˚C as the minimum critical temperature of rice germination. Coldstress in rice delays germination and emergence, soil temperature of below 10˚C can result in complete failure of germination.
Cold temperature slows down the diffusion rate resulting in disrupted imbibition process and escape of solutes from the seeds. However, the successive stages of germination (*i.e.*, growth of coleoptiles and radical) are the most vulnerable phases to cold spell (Yoshida, 1981) [9]. The physiological effects include retarded cell division and cell elongation in plants because of unbalanced metabolic activities at such low temperature (Lyons, 1973) [4]. Considering the above facts a laboratory study was conducted to identify best performing rice genotypes under cold stress at seedling stage as high seed and seedling vigor are good indicators for a successful crop.

Materials and Methods
To determine seed and seedling vigour index under cold stress, forty rice genotypes were taken for laboratory studies. The experiments were laid out in completely randomized design with three replications, each containing 25 seeds. The seeds were kept in petri dishes containing germination paper moistened with sterile distilled water and germination was allowedto proceed at 11ºC for 30 days in growth chamber. After 30 days the germinated seedlings were taken and measured the seedling vigour traits.
Observations were recorded on nine physiological indices *viz.,* germination percentage (%), first leaf area (cm^2), total seedling length (cm), shoot length (cm), root length (cm), shoot dry weight, root dry weight (g), total seedling dry weight (g) and seedling vigour index.
Seed vigour (SVI) = germination percentage × seedling length
The protocol followed for recording data were from International Seed Testing Agency (ISTA 1996). The observed data were subjected to analysis of variance, *per se* performance and correlation studies using TNAUSTAT software.

Results and Discussion
The analysis of variance revealed significant variation between the fourty rice genotypes for all the seedling characters under cold stress, hence selection of desirable genotype was possible from the lot. Perry (1972) [6] reported that seed vigour is determined by the genotype

and is modified by the environment. The population was highly heterogeneous for the traitsfirst leaf area, shoot length, root length, total seedling length, root dry weight, shoot dry weight and total dry weight whereas, it was homogenous for germination percent (Table 1).

Assessment of *perse* performance revealed that the genotypes K32 and K107 were tolerant towards cold stress as they showed highest significant mean values for seven characters *viz.*, germination percentage, shoot length, root length, total seedling length, root dry weight, total dry weight and seedling vigour index. The genotype K40 showed the least mean values for all the characters thereby deemed as susceptible towards cold stress (Table 2).

Character association studies were conducted to elucidate the traits related with cold tolerance during germination and early seedling growth stages (Table 3 & 4). Under normal conditions the total seedling length showed highest significant positive correlation towards seedling vigour index followed by root length and total dry weight. Similar observations were made in rice genotypes by Cui *et al.*, (2002) [2]. For cold stress the trait germination percentage was in positive correlation of higher magnitude with seedling vigour index which was followed by traits *viz.*, total seedling length, root length and shoot length. Similar observations were made by Asch *et al.* (1999) [1] and Mazaredo and Vergara (1982) [5]. The path analysis revealed that the trait total seedling lengthwas higher direct effect with seedling vigour followed by shoot length and the least direct effect was shown by the trait first leaf area.

The present study established the genotypes K56 and K89 as cold tolerant based on mean performances. Morphological traits such as germination percentage and total seedling length were reliable indicators for indirect selection of genotypes under cold stress.

Table 1: Analysis of variance of 40 rice genotypes for seedling vigour traits

Source of variation	Genotypes	Error
df	39	78
Germination per cent	367.027**	0.028
First leaf area	0.408**	0.004
Shoot length	48.604**	0.027
Root length	64.635**	0.027
Total seedling length	127.477**	0.107
Shoot dry weight	17.582**	0.027
Root dry weight	6.328**	0.027
Total seedling dry weight	37.761**	0.107
Seedling vigour index	148.997**	0.097

*,**significant at 5% and 1% level respectively

Table 2: Mean performance of 40 rice genotypes for ninephysiological traits

Genotypes	Germination %	First Leaf Area (cm^2)	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Root dry weight (mg)	Shoot dry weight (mg)	Total seedling dry weight (mg)	Seedling vigour index
K1	70.54	0.47	10	19.50**	29.50**	4.92	1.68	6.6	26.22**
K2	86.95**	1.99**	15.13**	19.93**	35.07**	5.6	1.99	7.6	34.97**
K7	66.37	0.4	14.53**	6.53	30.77**	2.2	0.77	11.89**	9.7
K11	87.51**	1.20**	19.03**	4.03	32.13**	9.71	4.19**	13.89**	32.07**
K19	87.57**	0.31	13.63	8.73	22.37	7.56**	0.72	8.29	22.32
K32	87.61**	3.59	15.07	12.97**	24.03	8.75**	3.38**	13.94**	35.99**
K34	87.15**	0.99**	12.83	18.63**	31.47**	7.56**	4.11**	11.68**	31.38**
K38	63.09	0.5	9.77	5.17	14.93	5.87	0.55	6.41	11.91
K41	87.31**	0.4	13.87**	14.37**	28.23**	7.27**	2.43	9.69**	28.18**
K45	62.95	0.79**	11.63	9.23	20.87	3.98	0.69	4.68	16.63
K49	87.57**	0.39	11.13	13.23**	24.37	5.93	2.37	8.31	24.34**
K53	86.78**	0.59	18.93**	3.97	22.93	3.72	0.78	4.49	22.86
K58	87.29**	0.4	13.5	11.4	24.9	3.93	1.12	5.05	24.84**
K61	63.22	1.00**	7.43	3.43	10.87	4.68	0.56	5.25	8.67
K66	63.36	0.61	15.50**	13.70**	29.20**	4.5	1.25	5.75	23.34
K69	63.39	0.45	10.1	17.60**	27.70**	3.34	1.18	4.52	22.1
K73	87.93**	0.6	13.5	16.00**	29.50**	7.50**	2.53	10.03**	29.46**
K76	67	0.90**	19.43**	9.53	28.97**	9.53**	0.95	10.49**	24.54**
K79	63.02	0.90**	12.3	13.30**	25.6	3.74	0.86	4.6	20.41
K80	63.39	0.5	19.63**	17.30**	36.67**	6.53	3.32	9.86**	29.30**
K82	63.29	0.99**	10.57	18.27**	28.83**	5.03	1.81	6.83	22.99
K87	63.15	0.71**	11.27	11.17	22.43	6.45	3.19**	9.63**	17.84
K94	87.16**	0.4	12.67	15.47**	28.13**	6.80**	3.25**	10.04**	28.07**
K102	67.1	1.44**	17.83**	8.63	26.47**	7.06**	0.88	7.95	22.43
K107	93.88	2.84**	20.27	15.37	35.63	8.37	3.49	11.85	38.59
K126	86.78**	1.09**	20.97**	14.97**	35.93**	7.26**	3.42**	10.67**	35.83**
K133	88.16**	0.89**	14.47**	14.97**	29.43**	6.80**	3.53**	10.32**	29.40**
K138	88.48**	1.21**	11	13.00**	24	6.14	3.08**	9.22	23.99**
K149	87.67**	0.3	10.73	13.33**	24.07	6.36	3.15**	9.52**	24.03**
K152	86.50**	0.29	16.53**	16.03**	32.57**	6.36	3.31**	9.68**	32.45**
K182	87.57**	1.21**	10.53	13.63**	24.17	9.21**	4.78	14.00**	24.12**
K229	87.61**	0.79**	12.37	14.37**	26.73**	8.97**	4.68**	13.64**	26.69**
K238	84.72	0.23	8.27	13.77**	22.03	1.98	0.71	2.68	18.02
K260	86.78**	1.09**	21.77**	11.97**	33.73**	9.47**	4.22**	13.68**	33.63**
K273	87.67**	1.30**	20.53**	16.13**	36.67**	9.32**	4.99**	14.32**	36.61**
Swarna	76.92	1.45**	16.33**	4.17	20.53	5.11	0.53	5.63	19.49
CSR 10	66.2	1.24**	15.63**	4.63	20.27	4.64	0.34	4.99	16.99
CSR 27	70.29	1.64**	23.03**	9.23	32.27**	9.40**	1.09	10.50**	28.63**
CSR 23	67.94	1.44**	30.20**	9.4	39.60**	11.36**	1.68	13.04**	34.03**
TRY 2	68.83	1.58**	17.53**	8.83	26.37**	8.74**	1.15	9.90**	22.89

Table 3: Genotypic correlation among morphological traits under normal condition

	Germination Percent	First leaf area	Shoot length	Root length	Total seedling length	Shoot dry weight	Root dry weight	Total seedling dry weight	Seedling vigour index
Germination Percent	1								
First leaf area	0.04	1							
Shoot length	0.266**	0.382**	1						
Root length	0.348**	0.006	0.127	1					
Total seedling length	0.412**	0.240**	0.708**	0.790**	1				
Shoot dry weight	0.416**	0.283**	0.591**	0.286**	0.569*	1			
Root dry weight	0.553**	0.081	0.260**	0.641**	0,617**	0.652**	1		
Total seedling dry weight	0.511**	0.227**	0.511**	0.459**	0.643**	0.951**	0.856**	1	
Seedling vigour index	0.661**	0.207**	0.674**	0.754**	0.953**	0.600**	0.683**	0.691**	1

Table 4: Genotypic correlation among morphological traits under cold condition

	Germination percent	First leaf area	Shoot length	Root length	Total seedling length	Shoot dry weigh	Root dry weight	Total seedling dry weight	Seedling vigour index
Germination	1								
First leaf area	0.731**	1							
Shoot length	0.829**	0.811**	1						
Root length	0.848**	0.811**	0.923**	1					
Total seedling length	0.855**	0.827**	0.983**	0.978**	1				
Shoot dry weight	0.394**	0.328**	0.454**	0.466**	0.469**	1			
Root dry weight	0.378**	0.289**	0.399**	0.426**	0.420**	0.887**	1		
Total seedling dry weight	0.398**	0.324**	0.450**	0.465**	0.466**	0.994**	0.933**	1	
Seedling vigour index	0.912**	0.749**	0.880**	0.888**	0.901**	0.268**	0.284**	0.061	1

Table 5: Path analysis for physiological indices under cold stress

	Germination %	First leaf area	Shoot length	Root length	Total seedling length	Shoot dry weight	Root dry weight	Total dry weight	Seedling vigour
Germination %	0.5321	-0.0408	17.3928	15.909	-32.6232	-1.2062	-0.3151	1.4484	0.912
First leaf area	0.3891	-0.0557	17.0033	15.2146	-31.562	-1.0028	-0.2407	1.1804	0.749
Shoot length	0.4412	-0.0452	20.9761	17.3084	-37.5089	-1.3885	-0.3325	1.6373	0.88
Root length	0.4512	-0.0452	19.3506	18.7624	-37.334	-1.4263	-0.3545	1.6946	0.888
Total seedling length	0.4548	-0.0461	20.6124	18.3511	-38.1708	-1.4345	-0.3497	1.6976	0.901
Shoot dry weight	0.2097	-0.0183	9.5168	8.7442	-17.8908	-3.0605	-0.7393	3.6196	0.282
Root dry weight	0.2012	-0.0161	8.3713	7.9841	-16.0235	-2.716	-0.8331	3.3964	0.268
Total dry weight	0.2117	-0.0181	9.4309	8.731	-17.7937	-3.042	-0.777	3.6416	0.284

Residual effect = 0.3508

Diagonal values (Bold) indicate direct effects

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