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Morphological and biochemical markers for cold tolerance in Boro rice

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ABSTRACT

Twenty two genotypes of rice were grown in winter season nursery to study their tolerance ability against low temperature stress at seedling stage and to find out suitable morphological and biochemical parameters, which may help in screening for cold tolerance at seedling stage. Out of ten morphological and biochemical parameters, seedling vigour, reducing sugar, phenol content and soluble protein exhibited highly significant correlation with cold tolerance as well as among themselves. Path coefficient analysis revealed maximum direct effect of germination percentage, reducing sugar and phenol content on cold tolerance. These traits were found to be most efficient markers for screening of cold tolerant genotypes at seedling stage. The genotype PSRM-1-16-48-1 showed excellent cold tolerant reaction and found superior to the best check Gautam for cold tolerance.

Key words : *Boro rice, cold tolerance, morphological markers, biochemical markers, correlation, path analysis.*

Boro rice grown during winters in north eastern parts of India is often subjected to low temperature stress at seedling stage and early vegetative stage (Thakur and Mishra, 1990). West Bengal, Bihar, Odisha, U.P. and Assam have about 90% of total deep water area in India, where crop faces unpredicted flood in wet season and boro rice is the best option in this situation. Boro rice receives comparatively more sunshine hours, lower night temperature, optimum temperature at ripening stage and a growing environment partially free from insect pests and diseases as compared to rice grown in wet season. All these provide higher yielding ability to this crop if compared to wet season. But this potential is severely threatened by low temperature stress at seedling stage and early vegetative stage which is caused by low air as well as water temperatures (Nanda and Sheshu, 1979). Several workers have made efforts in the direction of inducing cold tolerance in the rice genotypes at the seedling stage (Vergara, 1982; Kaw, 1991; Barua and Medhi, 1993).

Cold tolerance in rice refers to thriving ability of the crop at daily mean temperatures of 15-20^{0}C (Chung, 1979), which is mainly the function of genotype of the crop growth stage at which the crop is susceptible, duration of critical temperature as well as morpho-physiological and biochemical status of the crop plant. Morphological and biochemical changes are very closely associated with cold tolerance (Maruyama, 1990). It is desirable to make selection in that particular stress condition, for which the tolerance is needed. Different morphological and biochemical parameters i.e. germination percentage, shoot root ratio, seedling vigour, grain yield, biological yield, carbohydrates, proteins, free amino acids and proline have been suggested as selection criteria for cold tolerance by Change *et al*. (1995) and Chaturvedi *et al*. (1993). The present investigation was therefore conducted to identify suitable morphological and biochemical parameters to aid selection for cold tolerance at seedling stage in rice.

MATERIALS AND METHODS

The experimental material consisted of twenty two genotypes of rice including check *viz*., Gautam, Prabhat, Richharia and Dhanlaxmi, which were selected on the basis of their differential ability to tolerate cold condition at seedling stage. The experiment was laid out in randomized block design with three replications at rice research farm of Rajendra Agricultural University, Bihar in winter season. Each plot consisted of four rows of 5.0m length with spacing of 20 x 10 cm. The standard package of practices were followed during the crop growth period.

The season was endowed with wide range of temperature variation and minimum temperature recorded was 4^{0}C. After one week of exposure to the low environmental temperature, cold tolerances (CT) score was recorded in the nursery and twenty random seedlings from survival ones were uprooted from each plot for biochemical analysis. CT score was recorded by simple eye observations as per standard SES procedure IRRI (1996) on 1-9 scale (1- excellent, 9- extremely poor) based on survival percentage, yellowing and chlorosis of seedlings. Observations were recorded for twelve morpho-biochemical characters at different stages of crop growth. The characters studied were germination percentage, cold tolerance, shoot-root ratio, seedling vigour, grain yield (kg ha^{-1}), biological yield, reducing sugar, total soluble sugar, non-reducing sugar, phenol content, proline soluble protein. Biochemical analysis was done for shoot and root separately on dry weight basis and was expressed as mg g^{-1}.

For estimation of total sugar and starch anthrone reagent was used (Yem and Willis, 1954) whereas for non-reducing sugar method suggested by Malik and Singh (1980) was adopted. Ninhydrin reagent was used in extraction of protein, free amino acids and proline (Lowry *et al*. 1951; Bates *et al*. 1973). Phenol content was estimated following the method by Bray and Thorpe (1954). Genetic variability for these traits in the experimental material was assessed as per standard procedure. Heritability (broad sense) was estimated according to Hanson *et al*. (1956). Phenotypic and genotypic coefficients of variation were estimated as per Burton (1951). Genetic advance as per cent of mean, genotypic and phenotypic correlations were determined as per the method suggested by Johnson *et al*. (1955). Path coefficient analysis was done as suggested by Wright (1921) and as described by Dewey and Lu (1959).

RESULTS AND DISCUSSION

The analysis of variance (Table 1) revealed highly significant differences among the genotypes for all the morphological and biochemical characters studied, indicating the existence of considerable variation in the experimental material. Similar observation has been reported earlier by Kaw (1991). The estimates of phenotypic coefficient of variation (PCV) was higher than those of genotypic coefficient of variation (GCV)

Table 1. Analysis of variance for twelve characters in boro rice

Characters Source of variation	d.f. (%)	Germi-nation	Seedling vigour (kg/ha)	Biological yield ratio	Shoot root (mg/g)	Reducing sugar (Mg/g)	Total sugar sugar	Non-reducing (mg/g) (mg/g)	Phenol content	Proline (mg/g) (mg/g)	Soluble protein score	Cold tolerance (kg/ha)	Grain yield
Replication	2	180.54	9.51	3138176.00	0.08	0.29	1.09	1.41	1.79	0.10	0.05	3.15	668896.00
Treatment	21	201.65**	6.49**	6787261.00**	0.065**	29.89**	11.55**	537.89**	23.25**	6.17**	6.94**	3.36**	1588722.00**
Error	42	5.84	1.26	106855.60	0.005	0.10	0.17	0.41	0.12	0.03	0.009	0.73	9257.90

** Significant at P = 0.01

Table 2 Genetic parameter of variation for grain yield, its attributes and cold tolerance score in boro rice

Characters	Mean ± SE (m)	Range	CV	GCV	PCV	h^2	GA	GA (% of mean)
Germination (%)	76.90 ± 1.39	65.00-90.00	3.14	10.51	10.96	91.79	15.94	20.72
Seedling vigour	3.84 ± 0.64	1.00-6.33	29.18	34.38	45.05	58.00	2.07	53.83
Biological yield (t ha^{-1})	5.21 ± 0.18	2.07-7.75	6.27	28.57	29.21	95.74	2.70	57.57
Shoot-root ratio	1.61 ± 0.04	1.32-1.89	4.57	8.70	9.93	66.66	0.22	13.66
Reducing sugar (mg g^{-1})	13.35 ± 0.19	7.84-19.90	2.46	23.60	23.74	98.90	6.46	48.36
Total sugar (mg g^{-1})	73.45 ± 0.24	46.86-93.36	0.57	20.96	20.97	99.93	31.70	43.16
Non-reducing sugar (mg g^{-1})	59.81 ± 0.37	36.84-76.55	1.07	22.39	22.40	99.77	27.54	46.05
Phenol content (mg g^{-1})	14.05 ± 0.20	8.06-17.63	2.51	19.79	19.93	98.47	5.68	40.43
Proline (mg g^{-1})	4.57 ± 0.10	2.11-6.52	3.86	31.33	31.51	98.66	2.92	63.89
Soluble protein (mg g^{-1})	39.88 ± 0.05	38.38-43.44	0.24	3.79	3.81	99.57	3.12	7.82
Cold tolerance score	5.03 ± 0.49	3.00-6.33	17.08	18.49	25.25	54.03	1.41	28.10
Grain yield (t ha^{-1})	2.38 ± 0.05	0.98-4.32	5.02	29.77	30.23	97.67	1.31	60.93

for all the traits indicating environmental factors influencing the characters. The results are in agreement of earlier reports of Barbora and Hazarika (1998) and Kumar *et al*. (2005). The highest PCV and GCV was recorded for seedling vigour, biological yield, proline, grain yield and moderate value were found for reducing sugar, total sugar, non-reducing sugar, phenol content and cold tolerance score. Variability for seedling vigour and germination percentage in rice at low temperature has been reported by Kumari and Mishra (2001). High heritability with high genetic advance were observed for germination percentage, biological yield, reducing sugar, total sugar, non-reducing sugar, phenol content, proline, soluble protein and grain yield. However, shoot-root ratio exhibited comparatively moderate heritability. Thus, we can expect the preponderance of additive gene action in the expression of the traits under study as suggested by Panse (1957). High heritability should be accompanied by high genetic advance to arrive at more reliable conclusion about the genetic gain to be achieved (Johnson *et al*. 1955, Gandhi *et al*. 1964) which is expected to be resultant of additive gene effect. Therefore, these traits should be considered for selection of obtaining high genetic gain.

Selection for one trait may lead to correlated response in other traits. This may help in indirect selection for cold tolerance via other traits. The genotypic correlation coefficients were higher than the corresponding phenotypic correlation coefficients (Table 3), thus indicating that low phenotypic correlation might be due to the masking modifying effect of environment in genetic association between characters (Johnson *et al*. 1955 and Meenakshi *et al*. 1999).Grain yield had strong significant positive correlation with biological yield. One things needs special mention here is that negative correlation of a trait with CT score reflects positive association with CT score reflects positive association with cold tolerance, because lower the score better is the reaction. Thus, it is evident from the table that reducing sugar and proline content have significant and positive association with cold tolerance. It is evident from above facts that breeding for high reducing sugar and phenol content may be helpful in improvement of cold tolerance. These findings are in agreement with earlier reports of Saruyama and Tanida (1995) and Jha (1999). They also reported the role of enzymes for cold tolerance and suggested that at low temperature cold tolerant genotypes exhibited higher activities of catalases and ascorbic peroxidase, which are germination and seedling vigour enzymes.

Total soluble sugar always gets decreased at lower temperature, but this decrease is more pronounced in susceptible genotypes than in cold tolerant genotypes (Patel and Vora, 1987). Sucrose which is a non-reducing sugar is less susceptible to low temperature stress and reserves of this may act as

Table 3 Estimates of correlation coefficients among different morphological and biochemical pairs in boro rice

Characters		Seedling vigour	Biological yield (kg ha^{-1})	Shoot-root ratio	Reducing sugar (mg g^{-1})	Total sugar (mg g^{-1})	Non-reducing sugar (mg g^{-1})	Phenol content (mg g^{-1})	Proline (mg g^{-1})	Soluble protein (mg g^{-1})	Cold tolerance score	Grain yield (kg ha^{-1})
Germination (%)	G	-0.790	-0.170	-0.313	0.470	0.166	0.005	0.438	0.568	-0.087	-0.622	-0.138
	P	-0.607**	-0.151	-0.251	0.445*	0.160	0.009	0.407	0.536**	-0.079	-0.406	-0.133
Seedling vigour	G		-0.213	0.030	-0.639	-0.363	-0.226	-0.731	-0.162	0.232	0.903	-0.271
	P		-0.151	0.013	-0.498*	-0.273	-0.175	-0.581**	-0.452*	0.177	0.505*	-0.210
Biological yield (kg ha^{-1})	G			0.44	-0.026	0.254	0.304	0.231	0.264	-0.160	-0.124	0.984
	P			0.007	-0.028	0.249	0.297	0.218	0.257	-0.158	-0.095	0.958**
Shoot-root ratio	G				-0.281	-0.211	-0.167	-0.222	-0.379	0.286	0.215	0.122
	P				-0.231	-0.193	-0.155	-0.190	-0.322	0.252	0.151	0.110
Reducing sugar (mg g^{-1})	G					0.694	0.613	0.576	0.652	-0504	-0.711	-0.031
	P					0.689**	0.609*	0.572**	0.643**	-0.501*	-0.536**	-0.032
Total sugar (mg g^{-1})	G						0.973	0.505	0.453	-0.446	-0.534	0.256
	P						0.972**	0.500*	0.449*	-0.445*	-0.394	0.253
Non-reducing sugar	G							0.430	0.350	-0.418	-0.463	0.294
(mg g^{-1})	P							0.427*	0.347	-0.416	-0.339	0.292
Phenol content (mg g^{-1})	G								0.727	-0.391	-0.687	0.256
	P								0.713**	-0.389	-0.534*	0.255
Proline (mg g^{-1})	G									-0.270	-0.362	0.242
	P									-0.268	-0.249	0.242
Soluble protein (mg g^{-1})	G										0.643	-0.178
	P										0.497*	-0.177
Cold tolerance score	G											-0.189
	P											-0.139

* and ** significant at P = 0.05 and 0.01 level, respectively. a - genotype, p - phenotype

protectant against low temperature (Pollock, 1986). Water soluble protein had a positive and significant correlation with cold tolerance are agreement with the results of Moll and Steinback (1986). Cold stress stimulated the phosphorylation of protein in the cold sensitive rice genotypes, whereas in the cold tolerant rice genotypes, water soluble protein was already phosphorylated (Komatsu and Kato, 1997). Phenol content showed a negative and significant correlation with cold tolerance score. The phenolic substances have been identified to impart resistance to plants against low temperature stress. The accumulation of higher quantities of total phenol in certain genotypes clearly indicated their cold tolerant status, whereas low concentration may lead to susceptible nature of genotypes. As phenols are usually produced in secondary metabolic reactions, enhancement in such reaction due to cold stress can be conjunctured as the underlying cause of increased phenol contents in cold resistant genotypes (Guy, 1990).

Path analysis revealed maximum direct effect of germination percentage, reducing sugar and phenol content on cold tolerance (Table 4). The degree of correlation was also high and significant for these traits with cold tolerance, this gives an indication that we will get maximum selection response for cold tolerance, if selection is imposed on these traits and so selection for these traits may be rewarding in development of cold tolerant varieties. The path analysis for grain yield indicated a greater contribution of biological yield to grain yield (Table 5). So, the selection for biological yield will be beneficial for yield improvement in boro season.

Table 4 Path analysis showing direct and indirect effects of morphological and biochemical characters on cold tolerance in rice

Characters	Germination (%)	Seedling vigour	Biological yield (kg ha^{-1})	Shoot root ratio	Reducing sugar (mg g^{-1})	Total sugar (mg g^{-1})	Non-reducing sugar (mg g^{-1})	Phenol content (mg g^{-1})	Proline (mg g^{-1})	Soluble protein (mg g^{-1})	Correlation with Cold tolerance score
Germination (%)	-1.557	0.928	0.147	0.064	-0.611	0.058	-0.001	-0.535	0.895	-0.012	-0.622**
Seedling vigour	1.231	-1.174	0.184	-0.006	0.831	-0.128	0.001	0.893	-0.964	0.034	0.903**
Biological yield (kg ha^{-1})	0.265	0.250	-0.865	-0.009	0.037	0.090	-0.002	-0.282	0.415	-0.023	-0.124
Shoot-root ratio	0.487	-0.036	-0.038	-0.207	0.366	-0.074	0.001	0.272	-0.597	0.042	0.215
Reducing sugar (mg g^{-1})	-0.732	0.750	0.024	0.058	-1.301	0.245	-0.004	-0.704	1.026	-0.074	-0.711**
Total sugar (mg g^{-1})	-0.259	0.426	-0.220	0.043	-0.902	0.353	-0.006	-0.616	0.713	-0.065	-0.534*
Non-reducing sugar(mgg^{-1})	-0.009	0.265	-0.263	0.034	-0.797	0.343	-0.006	-0.526	0.551	-0.061	-0.463*
Phenol content (mg g^{-1})	-0.683	0.859	-0.200	0.046	-0.750	0.178	-0.002	-1.221	1.144	-0.057	-0.687**
Proline (mg g^{-1})	-0.886	0.719	-0.228	0.078	-0.848	0.160	-0.002	-0.888	1.573	-0.039	-0.362
Soluble protein (mg g^{-1})	0.135	-0.273	0.138	-0.059	0.656	-0.157	0.002	0.477	-0.425	0.147	0.643**

Residual effect = 0.276
* and ** Significant at P = 0.05 and 0.01 level, respectively.

As per earlier studies it is evident that cold tolerance is a complex character which is highly influenced by environmental fluctuations. Hence direct selection for cold tolerance and grain yield may not be effective. Selection indices worked out to illustrates a clear cut picture of the reaction of different genotypes at low temperature stress (Table 6). For cold tolerance, regression coefficient of three traits viz., germination percentage, phenol content and proline were found significant. These are the important traits for

Table 5 Path analysis showing direct and indirect effects of different morphological and biochemical characters on grain yield in rice

Characters	Germination (%)	Seedling vigour	Biological yield (kg ha^{-1})	Shoot-root ratio	Reducing sugar (mg g^{-1}	Total sugar (mg g^{-1})	Non-reducing sugar (mg g^{-1})	Phenol content (mg g^{-1})	Proline (mg g^{-1})	Soluble protein (mg g^{-1})	Cold tolerance score	Correlation due to grain yield
Germination (%)	0.612	-0.403	-0.217	-0.062	0.175	-0.019	0.0001	0.195	-0.290	0.005	-0.133	-0.138
Seedling vigour	-0.484	0.509	-0.271	0.006	-0.238	0.043	-0.002	-0.326	0.313	-0.013	0.194	-0.271
Biological yield (kg/ha)	-0.104	-0.108	1.274	0.008	-0.010	-0.030	0.003	0.103	-0.135	0.009	-0.026	0.984**
Shoot-root ratio	-0.192	0.015	0.056	0.199	-0.105	0.025	-0.002	-0.099	0.194	-0.016	0.046	0.122
Reducing sugar (mg/g)	0.288	-0.325	-0.036	-0.056	0.373	-0.082	0.007	0.257	-0.333	0.029	-0.152	-0.031
Total sugar(mg/g)	0.101	-0.185	0.324	-0.042	0.259	-0.119	0.012	0.225	-0.231	0.025	-0.114	0.256
Non-reducing sugar(mg/g)	0.003	-0.115	0.388	-0.033	0.228	-0.116	0.001	0.192	-0.179	0.024	-0.099	0.294
Phenol content (mg/g)	0.268	-0.372	0.295	-0.044	0.215	-0.060	0.005	0.445	-0.371	0.022	-0.147	0.256
Proline (mg/g)	0.348	-0.312	0.336	-0.075	0.243	-0.054	0.004	0.324	-0.510	0.015	-0.077	0.242
Soluble protein (mg/g)	-0.053	0.118	-0.204	0.057	0.188	0.053	-0.005	-0.174	0.138	-0.058	0.138	-0.178
Cold tolerance score	-0.381	0.460	-0.159	0.043	-0.265	0.063	-0.005	-0.306	0.185	-0.037	0.214	-0.189

Residual effect = 0.149
** Significant at P = 0.01

Table 6 Ranking of genotypes for cold tolerance on two different criteria

Genotypes	Index value based on all characters	Score (A)	Index value based on three characters	Score (B)
RAU 869-8-24-1-1	6163.74	13	87.30	7
PSRM 1-16-48-1	9025.25	4	89.28	4
Boro 3-2B-8-1	6581.74	10	82.99	9
IR 61608-213-20-22	5480.94	19	83.98	8
IR 56383-77-1-1-1	8457.76	8	73.29	12
RAU 1400-3-7-2B-2	5725.29	16	71.92	14
PSRM 5	8973.50	6	70.66	16
Boro 3-1B-3-2-5-1-1-1	5754.82	15	68.52	20
ES 21-3-1	5420.27	20	75.66	10
ES 29-3-3-1	8694.78	7	73.66	11
RAU 1346-4-1	6200.55	12	88.94	5
IR 55275-88-1-3	5182.21	21	69.20	19
CN 869-5-14-1-1	5690.32	17	69.86	17
CH 815-KGR-8-8-2-53	5590.03	18	71.93	13
RP 2199-14-2-6-1	5110.39	22	69.64	18
RP 2233-283-30	6113.74	14	67.27	21
RP 2240-86-84	6353.45	11	66.83	22
RP 2240-59-54	7015.12	9	71.26	15
Prabhat	9379.61	3	92.47	3
Richharia	9011.49	5	87.93	6
Dhanlaxmi	10566.22	2	93.16	2
Gautam	12494.89	1	93.52	1

Rank correlation coefficient (r) between A & B = 0.60**
** Significant at P = 0.01

formulating the selection criteria. On the basis of index value, only one entry PSRM 1-16-48-1 was found superior to the best check Gautam for cold tolerance.

The present study revealed that almost all the morphological and biochemical traits studied have wide range of variability. But the characters viz., seedling vigour, reducing sugar, phenol content and soluble protein have significant correlation with cold tolerance. The germination percentage, reducing sugar and phenol content have high direct effect on cold tolerance score hence can be used as markers for cold tolerance.

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