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Biochemical markers for cold tolerance in *Boro* rice

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Abstract

26 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 biochemical parameters, which may help in screening for cold tolerance at seedling stage. Out of several biochemical parameters, non- reducing sugar and phenol content were found to be most efficient markers for screening of cold tolerant genotypes at seedling stage as these traits exhibited highly significant correlation with cold tolerance as well as among themselves.

Key words : *Boro* rice, cold tolerance, biochemical markers

Introduction

Boro or dry season rice grown during winters in north eastern parts of India is often subjected to low temperature stress at seedling stage and early vegetative stage [1]. West Bengal, Bihar, Orissa, U.P. and Assam have about 90% of total deep water area in India, where crop faces unpredicted flood in *kharif* 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 *kharif* season. Besides these, it is also dependent on controlled irrigation system. All these provide higher yielding ability to this crop if compared with the *kharif* season crop. 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[2]. Several workers have made efforts in the direction of inducing cold tolerance in the rice genotypes at the seedling stage [3-5].

Cold tolerance in rice refers to thriving ability of the crop at daily mean temperatures of $15^{o}C$-$20^{o}C$ [6], 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. Biochemical changes are very closely associated with cold tolerance[7]. It is desirable to make selection in that particular stress condition, for which the tolerance is needed. Different biochemical parameters like carbohydrates, proteins, free amino acids and proline have been suggested as selection criteria for cold tolerance by different workers [5, 8-10]. The present investigation was conducted to identify suitable biochemical parameters as aid to selection for cold tolerance at seedling stage in rice.

Materials and methods

26 different rice genotypes, out of which two viz. Gautam and Pusa 2-21 were released cultivars and the others at different stages in hybridisation and mutation programme for cold tolerance were raised in nursery in R.B.D. in winter season of 1997-98 with all recommended production and protection practices. The season was endowed with wide range of temperature variation and minimum temperature was recorded as $4^{o}C$.

After one week of exposure to the low environmental temperature, cold tolerance (CT) score was recorded in the nursery and 20 random seedlings from survived ones were uprooted from each plot for biochemical analysis. CT score was recorded by simple eye observation as per standard procedure suggested by IRRI [11] on 1-9 scale [1 (excellent) - 9 (extremely poor)] based on survival percentage, yellowing and chlorosis of seedlings. Biochemical analysis was done for shoot and root separately on dry weight basis and was expressed as mg/g.

For estimation of total sugar and starch, anthrone reagent was used [12], whereas for non-reducing sugar method suggested by Malik and Singh [13] was adopted. Ninhydrin reagent was used in extraction of protein, free amino acids and proline [14-16]. Phenol content

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*Present address: Directorate of Maize Research, IARI, New Delhi 110 012 was estimated following the method by Bray and Thorpe[17]. Genetic variability for these traits in the experimental material was assessed as per standard procedures. Correlation coefficient analysis was also done to get a clear picture of association of these traits with cold tolerance in order to get a proper screening criterion.

Results and discussion

ANOVA for the design of experiment revealed that genotypes differed significantly for all the characters.

Table 1. Mean, range and genotypic coefficient of variation (GCV) for cold tolerance (CT) traits in 26 rice genotypes

Characters		Mean	Range	GCV%
Starch content	S	25.0	9.1-40.	43.21
	R	12.5	5.2-19.4	36.89
Reducing sugar	S	14.0	6.1-20.0	26.48
	R	7.1	2.8-10.8	26.76
Non reducing sugar	S	55.8	30.7-80.5	26.91
	R	25.6	18.6-34.6	20.75
Total sol. Sugar	S	69.5	38.4-97.7	24.60
	R	33.5	25.2-51.0	18.72
Water sol. Protein	S	44.3	27.0-60.6	22.87
	R	40.2	23.7-57.3	22.08
Free amino acids	S	19.7	8.3-37.4	51.57
	R	14.7	5.1-25.8	57.41
Proline content	S	3.9	2.3-6.1	34.22
	R	2.9	1.6-4.8	35.26
Penole content	S	17.6	14.2-21.2	23.97
	R	8.4	8.0-11.3	22.01
C T Score		6.5	4.0-8.5	17.70

S-Content in shoot, R - Content in root

Mean value, range and GCV estimates reflect presence of ample exploitable variability among the genotypes for all the characters under study. Similar observation has been reported earlier by Kaw [4]. Noticeable variability was also observed in differential response of leaf tissue and root tissue in case of phenol content. Such finding for protein content has been reported earlier by Houde et al [18]. This gives confirmation to the concept of association between the capacity of different tissues to develop cold tolerance.

Selection for one trait may lead to correlated response in other traits. This may help in indirect selection for cold tolerance via other traits. Correlation coefficients between all possible character combinations have been presented in Table 2. One things needs special mention here is that negative correlation of a trait 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 all the characters have significant and positive association with cold tolerance.

Total soluble sugar always gets decreased at lower temperature, but this decrease is more pronounced in susceptible genotypes than in tolerant genotypes [5, 6, 21]. Sucrose which is a non-reducing sugar is less susceptible to low temperature stress and reserves of this may act as protectant against low temperature [22, 23]. Protein is the molecule, which mainly functions in form of enzymes and is very much susceptible to degradation at adverse temperatures. In cold tolerant genotypes, mechanism for reformation of protective enzymes is found. Accumulation of free amino acids and proline in cold tolerant genotypes has been reported earlier by several workers [5, 24]. Path analysis [25]

Table 2. Estimates of phenotypic correlation coefficients among different biochemical character-pairs

Characters		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Starch content	(S)	0.91**	0.73**	0.82**	0.73**	0.68**	0.76**	0.64**	0.27	0.30	0.85**	0.82**	0.87**	0.78**	0.84**	0.80**	−0.81*
2. Starch content	(R)		0.73**	0.82**	0.73**	0.54**	0.76**	0.63**	0.47**	0.48*	0.80**	0.72**	0.86**	0.78**	0.88**	0.80**	−0.81**
3. Reducing sugar	(S)			0.84**	0.80**	0.52**	0.78**	0.64**	0.61**	0.42*	0.73**	0.76**	0.74**	0.78**	0.71**	0.74**	−0.70**
4. Reducing sugar	(R)				0.78**	0.62**	0.54**	0.62**	0.28	0.46*	0.24	0.76**	0.56**	0.76**	0.58**	0.72**	0.62**
5. Non reducing sugar	(S)					0.64**	0.82**	0.82**	0.36	0.42*	0.62**	0.62**	0.64**	0.68**	0.74**	0.75**	0.62**
6. Non reducing sugar	(R)						0.38	−0.12	0.42*	0.36	0.64**	0.64**	0.62**	0.50**	0.61**	0.56**	−0.42*
7. Total sol. Sugar	(S)							0.86**	0.36	0.42*	0.74**	0.62**	0.69**	0.72**	0.86**	0.52**	−0.78**
8. Total sol. Sugar	(R)								0.50**	0.36	0.65**	0.62	0.68**	0.69**	0.68*	−0.50*	−0.58**
9. Water sol. Protein	(S)									0.50**	0.10	0.18	0.16	0.38	0.20	0.42*	−0.54**
10. Water sol. Protien	(R)											0.28	0.22	0.22	0.14	0.42*	0.31
11. Free amino acids	(S)											0.48*	0.86**	0.40*	0.78*	0.54**	−0.68*
12. Free amino acids	(R)												0.62**	0.80**	0.72**	0.72**	−0.68**
13. Proline content	(S)													0.80**	0.80**	0.26	−0.72**
14. Proline content	(R)														0.80**	0.82**	−0.64**
15. Phenol content	(S)															0.52**	−0.68**
16. Phenol content	(R)																−0.52**
17. C T Score																	1.00

S-Content in shoot, R-Content in root *,**Significant at $p = 0.05$ & 0.01 respectively

Table 3. Non reducing sugar and phenol content (mg/g on dry weight basis) in 26 genotypes of rice

Genotype	Origin	CT score	Relative rank	Non reducing sugar		Phenol	
				shoot	root	shoot	root
RAU 1345-2	RAU, Pusa	3.67	1	76.90	30.70	21.67	11.50
RAU 448-46-47-1	RAU, Pusa	4.33	2	76.70	30.90	21.33	11.08
PSRM 1-15-3B-15	RAU, Pusa	5.00	3	80.50	32.30	21.33	11.33
GAUTAM (check)	RAU, Pusa	5.00	3	77.87	31.97	20.67	10.17
IR 53970-100-3-3-2	IRRI, Philippines	5.67	4	57.70	23.50	19.56	9.73
IR 59471-2B-20-2-1	IRRI, Philippines	5.67	4	71.97	19.30	19.33	9.50
PSRM 1-15-3B-13	RAU, Pusa	5.67	4	72.57	26.10	19.67	9.83
PSRM 2-1-4B-15	RAU, Pusa	5.67	4	75.70	26.50	18.17	10.17
PSRM 3-1-4B-7	RAU, Pusa	5.67	4	65.33	27.30	19.33	10.33
PUSA 835-203-2-13-102	DRR, Hyderabad	5.67	4	57.47	27.10	19.67	9.50
CN 915-KGR-38-2-5-2	DRR, Hyderabad	5.67	4	54.47	28.27	19.17	9.33
CN 869-5-14-1-1	DRR, Hyderabad	5.67	4	56.03	30.60	18.50	10.17
PUSA 2-21	RAU, Pusa	6.33	5	46.13	21.10	15.80	8.33
RAU 441-65-88-1	RAU, Pusa	7.00	6	47.97	20.27	16.67	8.33
RAU 1344-4-1	RAU, Pusa	7.00	6	48.43	20.70	16.83	9.17
IR 53964-39-1-2-3-3	IRRI, Philippines	7.67	7	33.90	23.10	14.17	8.00
IR 55275-B-8-1-1-1-3	IRRI, Philippines	7.67	7	36.67	20.00	15.50	9.17
RAU 494-62-4	RAU, Pusa	7.67	7	46.70	20.33	14.67	9.50
RAU 461-55-1	RAU, Pusa	7.67	7	48.13	20.90	17.33	9.00
RAU 520-34-8	RAU, Pusa	7.67	7	44.63	20.23	15.33	8.00
RAU 1344-3-2	RAU, Pusa	7.67	7	53.80	18.67	17.17	9.50
RAU 1346-4-1	RAU, Pusa	7.67	7	43.20	18.60	15.83	8.17
RAU 150	DRR, Hyderabad	7.67	7	66.47	20.27	15.33	8.33
CN 881-5-12	DRR, Hyderabad	8.33	8	30.70	21.33	14.17	8.17
KAU 8772	DRR, Hyderabad	8.33	8	36.20	23.97	14.33	8.33
C.D. (at 5%)		0.60		5.33	4.46	1.36	0.92

revealed maximum direct effect of non-reducing sugar and phenol content on cold tolerance. This gives an indication that we will get maximum selection response for cold tolerance, if selection is imposed on these traits.

None of the entries in the present investigation showed excellent cold tolerant reaction. Only one entry exhibited fairly tolerant reaction (CT Score - 3.67), whereas eleven other entries including check were found to be moderately tolerant (CT Score - 4.33-5.67). Rest of the entries exhibited either poor or very poor tolerance mechanism against cold tolerance. Table 3 illustrates a clearer picture of the reaction of different genotypes at low temperature stress. It also indicates the level of non-reducing sugar and phenol in different conditions.

The present study revealed that almost all the biochemical traits studied have wide range of variability and also have significant correlation with cold tolerance. But the characters viz., non-reducing sugar content in shoot and root and phenol content in shoot have high direct effect on CT score [25], hence can be used as markers for screening for cold tolerance.

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