

Screening of Indica Rice (*Oryza sativa* L.) Genotypes Against Low Temperature Stress

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Abstract: In this investigation, genotypes having yield level more than 400 g m⁻² were selected primarily as low temperature tolerant. There were short and long duration genotypes in those groups. The combination of yield components was quite better in Group I. The genotypes having the highest spikelet number with better yield was found in Group III and IV but they had lower thousand-grain weight. The maximum and minimum temperature around panicle initiation (PI) were 28.7°C and 10.9°C for short duration genotypes and 24.4 and 11.0°C for long duration genotypes. The genotypes encountered low temperature and low solar radiation from vegetative to reproductive phases. A few genotypes maintained better phenotypic acceptability. Most of the selected genotypes experienced spikelet degeneration symptom. The genotypes (Group I, III and IV) producing huge number of spikelets might be able to execute panicle differentiation at the initiation of panicle development. But at the subsequent stage there must have some growth impairment activities. In contrast, the genotypes (Group II and V) which having lower number of spikelet m⁻² might be affected primarily at the differentiation stage then at the reduction division stage. The genotypes having short growth duration might have the tolerance to withstand low minimum temperature and those with long growth duration are appeared to have stress avoidance mechanism. As D² between the cluster 2 and cluster 3 was the highest, breeders could have the better chance of heterosis crossing between these two groups.

Key words: Screening • *Oryza sativa* • Cold stress • Phenotypic acceptability • Yield • Tolerance

INTRODUCTION

The rice plant could normally grow between 20°C and 35°C and these critical temperatures vary with genotype, duration of critical temperature, diurnal changes and physiological status of the plant [1]. Rice might suffer from low temperature at the reproductive stage of the crop add reference here [1]. Even low temperature at the crop establishment and tiller development stage (vegetative phase) of the crop might affect the growth and development of the crop. The most sensitive stages against the stresses are agronomic panicle initiation stages (24 days before heading), reduction division stage (12-14 days before heading) and anthesis (0 days before heading) stage [2,3].

There are various types of damages due to low temperature. The vegetative phase-stress may be recoverable but make the crop experience flash flood submergence by extending the growth duration. But the unrepairable loss is occurred if the stress is imposed at

the reproductive phase. Many authors studied the causes of sterility [2-4] Sterility caused by low temperature depends on the development stage and length of the exposure of the panicle to low temperature [4,5]. On the other hand low temperature may also result in non-receptive stigma, fertilization failure of post fertilization development. These important aspects of flowering behavior in rice were studied by Nizigiyimana [6] who also reported that low temperature sterility due to degeneration of embryo sac, malformation of ovaries and deformation of young sporocytes during meiosis.

In Bangladesh, the low temperature prevails from October to early March. The temperature often reaches below 20°C. Boro (winter rice), one of the most important rice crops, might suffer from critical low temperature at the different growth stages from germination to maturity. The growing season of boro is longer, extending from November to June. Bangladesh Rice Research Institute (BRRI) recommends seeding of short and long duration varieties between 15 and 30 November and 5 and 25

November, respectively [7]. But farmers in some of the intensive boro cultivation area (haor area) do not follow the prescribed schedule due to early recession of floodwater, as they have to utilize residual floodwater for the seedling raising and initial crop establishment practices. So, the low temperature may affect the boro rice plant not only at the reproductive stage of rice but in the other stages also. Early seeding of short duration variety on seed bed (late October to early November) may experience low temperature at any stages of growth. Even a long duration, direct seeded crop may succumb to low temperature injury at its reproductive stage [8].

There are records of low temperature injury in rice [9-11]. As the frequency of sterility in boro rice is increasing, the problem is getting importance now a day. To escape flash flood, farmers have to go for early crop establishment allowing it prone to sterility problem. Grain yield reduction in rice is often associated with spikelet sterility, which in turn, usually reflects the effects of adverse growing conditions on reproductive development [12,13]. Low temperature during microspore development stages causes male sterility as result of impaired pollen development [14]. During anthesis, the second most sensitive period, low temperature can reduce the number of pollen grains intercepted by stigma [15].

Conventional high yielding varieties have little tolerance to low temperature. It is already mentioned that the cropping season is in the process of changing. To utilize early recession of residual flood water and also to avoid flash flood at the maturity, early *boro* transplanting is a must. But the early transplanted boro has every probability to encounter low minimum temperature at the vegetative as well as reproductive phase. That is why the present study was conducted to achieve objectives to screen genotypes with good phenotypic acceptability and good emergence index, to screen genotypes having better tolerance to low or minimum temperature and to have a preliminary understanding about the nature of tolerance of genotypes with respect to low or minimum temperature.

MATERIALS AND METHODS

The study was conducted from October, 2007 to May, 2008 comprising of seed collection, growing and experimentation, data collection and compilation etc. at the Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur, Bangladesh. It is located at the center of Madhupur Tract (25°66' N latitude and 91°78' E longitude) at an elevation of 8.4 m above the sea level.

Genotypes have been collected from different sources that are given in Seedbed was prepared by October 14, 2007 and sprouted seeds were sown on October 15, 2007 following BRRI recommendation [16] so that the experimental genotypes phase low temperature at their growth stages. The experimental land was prepared as per BRRI recommendation [7]. The fertilizer N, P₂O₅, K₂O, S and Zn were used at the rate of 120, 80, 60, 10 and 4 kg per ha in the form of urea, triple superphosphate, muriate of potash, gypsum and zinc sulphate. One-third of urea and the other fertilizers were applied during final land preparation. The rest of the urea was top dressed in two splits one at active tillering and the other around the panicle initiation stage. Thirty-day-old seedlings were transplanted at spacing of 25×15-cm on November 2007. The transplanting was done a month earlier to ensure the lines have to encounter low temperature stress at their vegetative as well as reproductive stage. A single 5 meters line was considered for each breeding lines. Intercultural operations such as weeding, irrigation, pest monitoring and control with pesticide were done as and when necessary. Panicle initiation (PI): Frequent monitoring of panicle initiation was done regularly in December, January and February.

Scoring of Panicle Emergence Index: Panicle emergence index was recorded (Table 2) as per International Rice Research Institute standard evaluation system [17] during panicle exertion.

Scoring of Phenotypic Acceptability: Phenotypic acceptability was recorded (Table 3) as per International Rice Research Institute standard evaluation system [17]. Several factors interact, influencing seedling vigor and phenotypic acceptability as well. e.g. tillering ability, plant height, occurrence of disease in plants etc.

Table 1: Different sources of germplasm used in the experiment

Sources of germplasm	No. of genotypes
Observational trial (OT)	63
Preliminary yield trial (PYT)	30
International rice temperate observational nursery (IRTON)	55
International rice boro observational nursery (IRBON)	96
Total	244

Table 2: Scoring of % panicle exertion

Score (Panicle emergence index)	% Panicle Exsertion	Explanation
1	=100	Well exerted
3	95-99	Moderately well exerted
5	75-94	Just exerted
7	50-74	Partly exerted
9	<50	Enclosed

Table 3: Scoring of phenotypic acceptability

Score	Explanation
1	Extra vigorous (very fast growing; plants at 5-leaf stage have 2 or more tillers in majority of population)
3	Vigorous (fast growing; plants at 4-5 leaf stage have 1-2 tillers in majority of population)
5	Plants intermediate or normal (plants at 4-5 leaf stage)
7	Plants weak (plants somewhat stunted; 3-4 leaves; thin population; no tiller formation)
9	Plants very weak (stunted growth; yellowing of leaves)

Different morphological characters of spikelet or grain like healthy or normal, malformation or deformation of grain, presence of double lemma and palea and empty glume was observed and recorded. Yield components were estimated from the 10 randomly selected hills from a line. As the breeding lines were too many (244) 3 hills were selected from 10 hills as per Matsushima method [18]. To simplify 3 panicles were randomly selected from 3 hills. Yield and yield components like spikelet per square meter, fertile grain percentage, thousand-grain weight etc. were estimated from those three panicles.

Cluster and Discriminant Function Analysis (DFA) was performed using the analytical package SPSS v. 16.1 [19]. In the first step, only two parameters “phenotypic acceptability” and “panicle emergence index” were considered to select 48 breeding lines. Irrespective of yield status, these lines were good in phenotypic acceptability and panicle emergence index. In the second step considering the yield and yield components these 48 genotypes were subjected to another cluster analysis to select genotypes with better yielding ability.

RESULTS AND DISCUSSION

The experiment was aimed to screen out rice genotypes able to tolerate low temperature. The number of genotypes was quite large (244). Therefore, considering phenotypic acceptability and panicle emergence index as the preliminary attributes to tolerate low temperature at the reproductive stage, the cluster and discriminant function analysis were performed. Twenty-two genotypes from cluster 1 having phenotypic acceptability of 3.0

(very good) and emergence index of around 5.0 (good) and 25 genotypes from cluster 2 (phenotypic acceptability: 4 and emergence index: 7) were considered for further consideration with other yield and yield contributing attributes.

The cluster and DFA analysis is employed to make homogenous grouping of large number of germplasm based on the variables under consideration. DFA is assigned to indicate the eligibility of a particular set of variables in the previously designated groups. In addition the precision level of the clustering can also be ascertained through DFA. The discriminatory functions (e.g. the principal components in the analysis) play the vital role in identifying the set of variables contributing in clustering a large number of germplasm into the homogenous groups. Through stepwise procedures of DFA, Chi-square test of discriminatory functions structure matrix of variables, test of equality of group means were done. For grouping of large number of genotypes similar analysis were followed by many authors [20-23]. The contribution of canonical discriminant function (obtain from cluster analysis) to explain the variance with corresponding Eigen values and Canonical Correlation Coefficient were considered. Vincent *et al.*, [24] adopted canonical analysis in relation to the traits of aluminum sensitivity of rice. Singh *et al.*, [25] showed the contribution of different principal components in grouping of rice genotypes, in their studies. Gonzales [26] Kumari and Rangasamy [27] showed that a set of variables corresponded the different principal components (function) under different studies in grouping genotypes.

Table 4: Pairwise Mahalanobis (D²) between 5 clusters of 48 rice genotypes

Average Linkage (Between Groups)		1	2	3	4
2	e.g. D ²	133.6300			
	Sig.	0.0001			
3	e.g. D ²	76.4700	283.9400		
	Sig.	0.0001	0.0001		
4	e.g. D ²	7.2200	62.6400	11.9600	
	Sig.	0.0100	0.0001	0.0010	
5	e.g. D ²	15.3500	41.7600	132.6100	22.8700
	Sig.	0.0001	0.0001	0.0001	0.0001

Table 5: Selected 48 Genotypes under group and their estimated yield, yield components, PACP, panicle emergence index score and PI date

Groups	Variety/Line	Estimated grain yield (g m ⁻²)	PACP	Panicle emergence index	% fertile grain	1000-grain weight (g)	PI Date (2008)
I	IR77496-31-2-1-3-1	711.6	3	5	48.8	24.9	Jan 15
	IR77504-36-3-3-1	740.1	3	7	59.4	19.4	Jan 16
	BR7528-2R-20-1	557.2	5	3	37.2	24.8	Jan 19
	IR73689-19-1	560.0	5	7	57.5	17.4	Jan 30
	PSB RC 68	637.0	3	5	68.6	32.8	Jan 30
	IR62266-42-6-2	743.9	3	5	51.4	21.0	Jan 30
II	IR77496-31-2-1-3-2	411.0	3	5	47.1	24.7	Jan 21
	RCPL3-2	253.5	3	7	39.4	20.2	Jan 15
	SIM2 SUMADEL	261.2	3	7	36.9	21.3	Jan 18
	IR78878-65-1-1-1	490.3	3	3	59.0	23.6	Jan 18
	BR4828-54-4-1-4-9-AC60	369.1	3	5	50.3	26.2	Jan 30
	BR4828-54-4-1-4-9-AC81	391.3	3	5	58.4	23.2	Jan 30
	BR4828-54-4-1-4-9-AC82	401.5	3	5	38.5	27.0	Jan 30
	BR4828-54-4-1-4-9	381.0	5	7	47.4	25.4	Jan 30
	IR 79262-42-2-2-3	360.6	3	5	52.1	24.9	Jan 30
	IR 79262-109-1-3-3	143.5	3	5	22.2	27.3	Jan 30
	IR 79262-86-3-2-2	166.4	3	5	22.6	29.2	Jan 30
	IR 79262-29-3-2-2	337.5	3	7	38.1	24.8	Jan 30
	IR 79262-24-3-2-3	289.7	3	5	29.1	26.3	Jan 30
	IR24637-38-2-2-1	83.5	5	7	25.8	18.1	Jan 30
	B7003D-MR-3-1-3	270.4	5	7	46.7	21.4	Jan 30
	IR 79269-21-2-2-3	468.0	5	5	67.9	21.6	Jan 30
	IR 47554-3B-4-2B-1-2	118.6	5	5	17.3	22.2	Jan 30
	IR60080-46A	280.7	3	5	60.8	25.3	Jan 30
	IR73689-76-2	414.3	3	5	87.9	18.9	Jan 30
	IR60059-4B-4-1-1-2-1	472.0	3	7	89.2	24.1	Jan 30
	PSB RC 70	923.6	3	7	85.2	26.2	Jan 30
	BR4828-54-4-1-4-9	585.3	5	5	60.4	25.4	Jan 30
	HOA VANG THAI BINH	726.1	3	7	81.1	23.1	Jan 30
	IR 78910-187-B-2-3	727.8	3	3	89.8	22.9	Feb 28
	SABITA (NC492)	553.0	3	5	52.6	30.0	Feb 28
	NAMSAGUI 19	358.0	3	5	56.9	27.0	Jan 30
	PSB RC 46	532.0	3	5	63.8	23.1	Jan 30
	IR68333-R-R-B-19	327.3	5	7	61.0	15.2	Dec 17a
	CT6743-33-3-2-M-1-M	189.6	3	7	34.7	23.0	Dec 17a
	IR73689-19-1	280.7	3	9	76.8	17.4	Dec 17a
	DOURADAC	436.9	3	7	71.9	34.3	Dec 17a
	IR73305-14-2-2	401.9	5	7	82.5	19.5	Dec 17a
IR73689-31-1	193.8	3	5	35.4	17.8	Dec 17a	
III	BR358-1	771.3	3	5	52.1	13.4	Jan 15
	HbjB IV	0.0	7	5	0.0	0.0	Jan 13
IV	BG358-3	648.0	5	7	61.3	13.5	Jan 16
V	IR77504-36-3-3-2	636.3	3	7	55.2	22.0	Jan 16
	BR6460-298-2-2-689-1-1-4	282.5	5	7	27.6	22.7	Jan 30
	BR802-78-1-1-AC84	179.0	5	7	17.8	21.5	Jan 30
	IR 79269-45-1-2	340.7	5	5	37.5	18.8	Feb 28
	IR 79262-130-1-3-2	328.4	3	5	28.7	25.9	Jan 30
	IR42	596.5	3	5	67.5	18.5	Jan 30

The lines with bold fonts are long duration type, rest lines are of short duration;^aDates of the year 2007

In the Table 4 the D² value is highest between Group-II and Group-III. It indicates that crossing between these two groups will create more heterosis i.e. more tolerancy to low temperature.

The genotypes which had their panicle initiation (PI) more or less within 15 January they were considered here as short duration and the others are long duration type. Some of the genotypes from Group-II showed their

PI within a month after transplanting (in December) and they were different from others and deserve special observation.

Out of five groups only the genotypes having yield level more than 400 g m⁻² were selected primarily as low temperature tolerant genotypes as it contribute to higher yield per unit area inspite of having lower air temperature (Table 5). We have short and long duration genotypes in

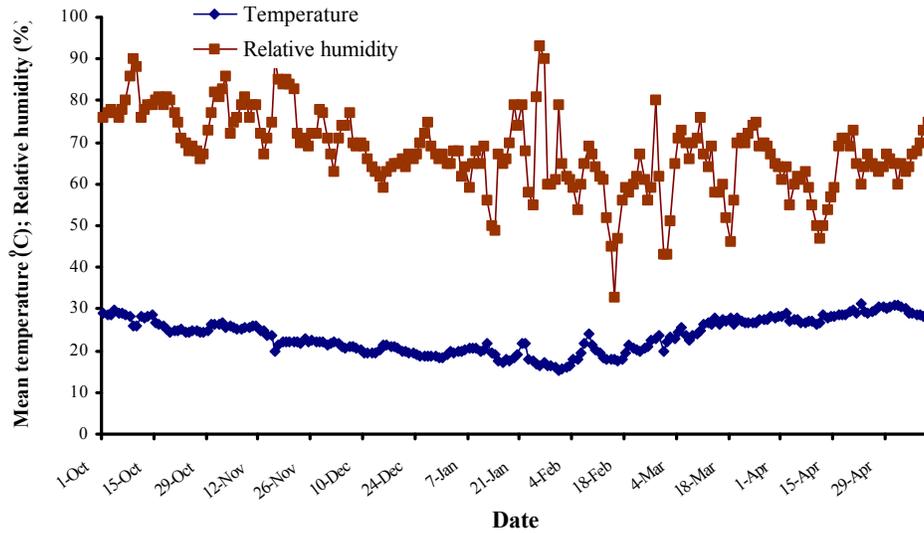


Fig. 1: Average temperature and humidity of this region during the study period

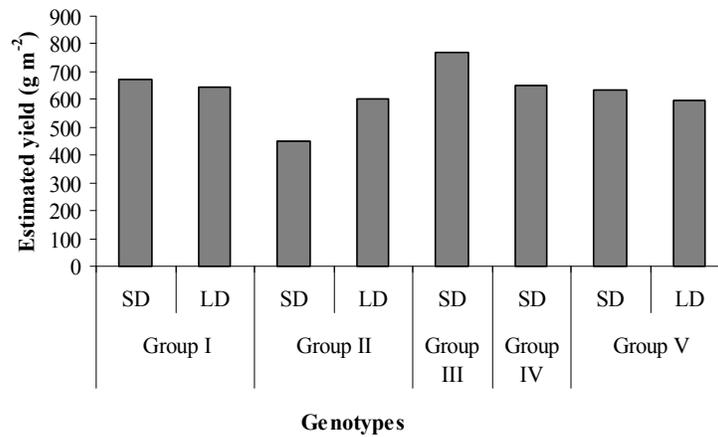


Fig. 2: Estimated yield of short and long duration genotypes of the respective groups. (Average over the selected long and short duration genotypes) SD= Short duration, LD= Long duration

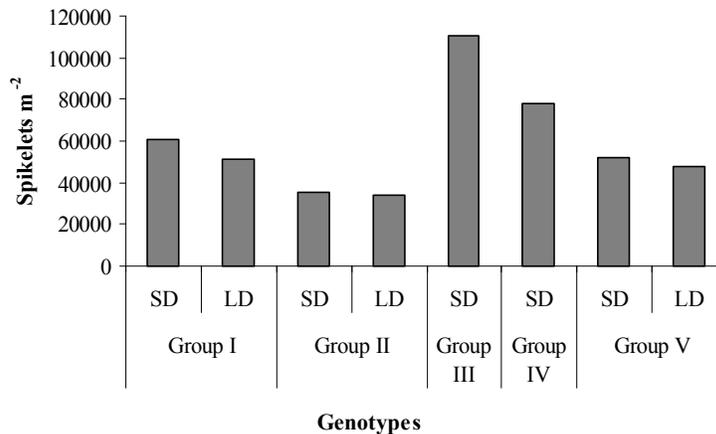


Fig. 3: Spikelets per m² of short and long duration genotypes of the respective groups. (Average over the selected long and short duration genotypes) SD= Short duration, LD= Long duration

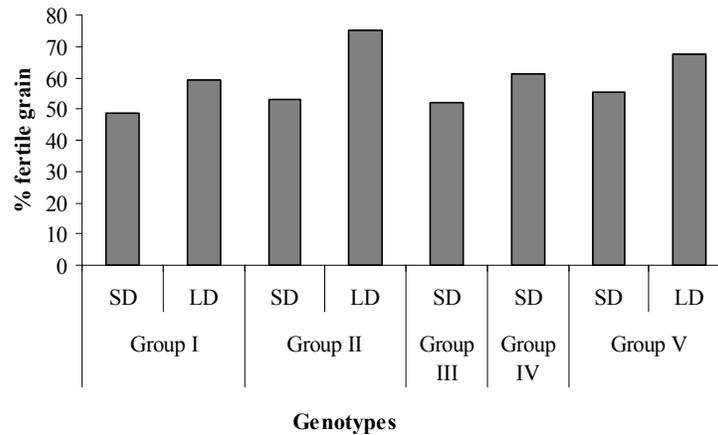


Fig. 4: Percent fertile grain of short and long duration genotypes of the respective groups. (Average over the selected long and short duration genotypes) SD= Short duration, LD= Long duration

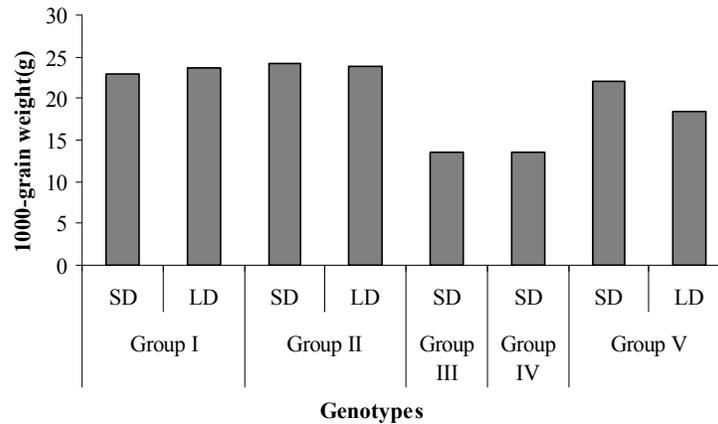


Fig. 5: 1000-grain weight of short and long duration genotypes of the respective groups. (Average over the selected long and short duration genotypes) SD= Short duration, LD= Long duration

these groups. The average yield and yield components of selected long and short duration genotypes (genotypes having the yield more than 400 g m⁻²) have presented here (Fig. 2-5).

The plants showed different cold injury symptoms. Some of them showed yellowing, irregular maturity, incomplete panicle exertion, spikelet degeneration, spikelet sterility. Banik [10] stated about some cold injury symptoms which were somewhat similar to our findings. Lee [28] Farrell *et al.* [29], Hamdani [30] all reported that cold stress significantly reduced panicle emergence which is one of the most important criterion of cold tolerance. Malformed or deformed spikelet and grain like deformation in the shape of kernels, presence of double lemmas, paleae and empty glumes etc. were also found in the study. These symptoms are similar to the findings of Terao *et al.* [31] and Shimizu and Kumo [32].

The weather data from November to April has presented (Table 6). It can be observed that the genotypes encounter low temperature stress throughout the growth duration from vegetative to reproductive phase. In seedling stage yellowing of plants and further the disease infestation of the cold effected plants are the proofs. But under that stress condition some genotypes were green and it can be said they are cold tolerant at their vegetative stage. The short duration genotypes should have their growth duration similar to that of BRRI dhan28 and the long duration have similar even more than that of BRRI dhan29 [7]. The short duration types encounter their panicle initiation early (near middle of the month January) and the long duration types had their panicle initiation at or near the end of the month January (Fig. 3). There are some genotypes in group II (appear to be of temperate origin) which have their panicle ignition and in some

Table 6: Weather elements (maximum temperature, minimum temperature, sun shine hours, solar radiation: average over 10 days) around the growth stages

Period	Range of Max. Temp.(°C)	Mean Max. Temp. (°C)	Range of Min. Temp.(°C)	Mean Min. Temp.(°C)	Mean Temp. (°C)	Range of Sun shine hours/day	Sunshine hours/ day	Range of Solar radiation (Cal cm ⁻²)	Solar radiation (Cal cm ⁻²)
Nov 1-10, 2007	30.2-32.5	31.22	21.5-24.7	22.47	26.84	3.1-8.1	6.60	200.48-343.69	289.99
Nov 11-20, 07	21.5-32	27.83	17.3-21.5	18.96	23.39	Nil to 9.4	6.53	121.20-361.59	288.20
Nov 21-30, 07	28.4-29.6	29.17	16.2-18.1	17.11	23.14	6.3-9.1	8.12	282.31-353.92	328.86
Dec 1-10, 2007	26.5-28.6	27.12	13.2-19.9	16.19	21.65	3.8-8.4	6.89	201.06-338.66	274.94
Dec 11-20, 2007	25.4-27.5	26.34	11.6-14.1	13.05	19.69	6.4-8.3	7.64	263.23-308.66	292.88
Dec 21-31, 2007	23.2-26.2	24.90	10.2-14.4	12.39	16.84	0.8-6.2	4.94	129.32-256.05	228.32
Jan 1-10, 2008	25.6-28.2	26.80	12.9-14.7	13.70	20.30	5.7-8.1	7.30	256.79-316.32	297.4
Jan 11-20, 2008	23.4-28.7	25.50	10.9-17.2	12.90	19.20	2.0-7.5	4.80	157.56-301.44	235.2
Jan 21-31, 2008	17.7-24.4	21.80	11.0-18.7	13.70	17.70	Nil and 7.7	3.00	115.4-306.4	191.5
Feb 1-11, 2008	22.2-28.1	25.10	10.9-19.5	14.80	20.00	0.1-8.8	5.30	140.22-385.70	287.5
Feb 11-20, 2008	23.0-27.5	25.00	9.7-17.9	12.00	18.50	1.4-9.9	6.60	176.90-391.34	324.7
Feb 21-28, 2008	26.1-29.7	27.80	14.2-23.5	17.00	22.40	3.1-9.6	8.10	224.86-408.27	365.9
Mar 1-10, 2008	26.2-31.4	29.22	16.2-20.5	18.50	23.86	0.6-8.5	5.15	182.06-433.05	326.62
Mar 11-20, 2008	31.4-33.5	32.29	20.1-23.7	21.90	27.09	2.9-9.2	7.24	255.13-455.29	393.02
Mar 21-31, 2008	29.5-34.2	31.82	20.3-25.0	22.43	27.12	0.4-9.2	6.90	175.70-458.47	382.22
Apr 1-10, 2008	27.2-34.9	32.31	19.5-23.3	21.37	26.84	5.7-10.4	7.98	296.82-514.54	435.90
Apr 11-20, 2008	32.7-35.7	34.31	21.4-27.7	24.35	29.33	5.9-9.4	7.62	316.32-482.04	424.20
Apr 21-30, 2008	34.6-35.9	35.48	25.2-26.9	26.21	30.84	8.5-10.5	9.71	452.80-456.05	492.12

cases flowering just a month later of transplanting. Those varieties might have experienced their PI at seed bed. However, their flowering tiller died and had tiller produced anew to yield again. PI of the genotypes was checked almost in time. But there were difficulties of checking the flowering dates. The flowering was so irregular and confusion arose in recording the flowering time. Ultimately the idea of recording the flowering dates was kept off.

Spikelet m⁻² played the most important role in grouping the genotypes. This is the most important yield contributing attributes (75%) also [1]. The combination of yield components was quite better in Group I. The genotypes with the highest spikelet number with better yield were found in Group III and Group IV (Table 4 and Fig. 3). But these were the genotypes with lower number of thousand-grain weight due to lower percentage of fertile grain. The maximum and minimum temperature around PI was 28.7°C and 10.9°C for short duration genotypes and 24.4 and 11.0°C for long duration genotypes (Table 6).

Solar radiation during those periods were 235.2 and 191.5 Cal cm⁻², significantly low to impair photosynthesis. The optimum solar radiation for photosynthesis is 300-400 Cal cm⁻² [1]. The critical low temperature for agronomic PI (24 days to flowering) and reduction division are 18 and 19°C [1] respectively. In

reality, in the present study the occurrence of low temperature was far below than those of the mentioned critical marks. The genotypes encountered not only low temperature but also low solar radiation from vegetative to reproductive phases.

PACP (phenotypic acceptability) and emergence index of some genotypes was very poor (7). Panicle could not emerge completely. A few genotypes like IR77496-31-2-1-3-1 from group I and IR77496-31-2-1-3-2 from group II maintained better phenotypic acceptability. Even these genotypes had moderate panicle exertion or delayed panicle exertion. Most of the selected genotypes experienced spikelet degeneration symptom. The genotypes of Group I, Group III and Group IV produced huge number of spikelets, which might have been due to the execution of panicle differentiation at the initiation of panicle development. But at the subsequent stage (most probably at the reduction division stage) there must had some growth impairment activities. In contrast, the genotypes of Group II and Group V had lower number of spikelets m⁻² which might have been affected primarily at the differentiation stage rather than at the reduction division stage. The varieties able to maintain their greenness might have the ability to maintain their photosynthetic ability. Some of the genotypes having an exciting level of yield indicated their all-round tolerance to the cold.

The mechanism of tolerance to low temperature appears to vary with respect to the growth duration or something else. Despite of delayed panicle exertion or heading some of the genotypes had their panicle initiation during the low temperature period (around 10°C). In contrast some genotypes had their PI when low temperature was several degrees higher (13°C). Two reasons might be behind this phenomenon. First, the genotypes did not initiate its PI due to certain level of low temperature. That means they avoided the critical cold spell. The second reason might be the genotype was really long duration one. However, in both the cases the genotypes were adapting avoidance strategy. But in case of the genotypes showing some extent earliness in such a condition must have some tolerance to the direct shock. The tolerance could be of two types. The genotypes reserved (long duration) their PI for the time being might have the avoidance mechanism. And the genotypes which have PI under stress (short duration) might have tolerance to the cold stress.

CONCLUSION

The genotypes having short growth duration might have the tolerance to withstand low minimum temperature and those with long growth duration are appeared to have stress avoidance mechanism. The genotypes IR77496-31-2-1-3, IR77504-36-3-3-1, BR7528-2R-20-1, IR73689-19-1, IR62266-42-6-2 and PSBRC68 from group I; BG358-1 from group III; another genotype BG358-3 from group IV could be selected as high yielding and better performing genotypes. The genotypes selected through this study have significant tolerance to critical low minimum temperature. As D² between the cluster 2 and cluster 3 is the highest, breeder could have better chance of heterosis crossing between these two groups.

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