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Effect of low temperature on seedling characters and yield of *boro* rice (*Oryza sativa* L.)

B. C. ROY AND V. CHALLA

Department of Genetics and Plant Breeding,
Uttar Banga Krishi Viswavidyalaya,
Pundibari-736 165, Cooch Behar, West Bengal, India

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ABSTRACT

Low temperature during December and January is major constrain for boro rice cultivation in northern part of West Bengal. Very low temperature (10 ± 5 ^{0}C) and low light-intensity during those months inhibit normal growth leading to stunted growth and degradation of chlorophyll. The leaves turned yellow and became albino at extreme low temperatures (<10 ^{0}C). After mid-February, temperature rises gradually and the seedlings also get full sunshine due to clear sky and the seedlings turn green in colour. In the present endeavour, 37 genotypes of rice were screened against low temperature at nursery for seedling characters (seedling height, shoot fresh weight, shoot dry weight), scoring for leaf bleaching, days to 50% flowering and yield under agro-climatic conditions of northern parts of West Bengal. The genotypes Parijat, Krishna Hamsha, IR 64, UBKVR-5, UBKVR-6, UBKVR-8, UBKVR-16, UBKVR-17, UBKVR-19, UBKVR-21, IVT-B4001, IVT-B4007, and IVT-B4008 were found to be low temperature tolerant at seedling stage based on leaf bleaching scoring (1-9 scale). Crop duration was more (varied from 20 to 35 days) for the genotypes grown in Boro-season than in Kharif. There was no notable difference in yield of the genotypes for the different dates of sowing in Boro-season.

Key words**:** *Boro*-genotypes, low temperature, seedling characters

Chilling injury occurs in many plants of tropical and subtropical origin when exposed to low non-freezing temperatures 10-15 ^{0}C (Saltveit and Morris, 1990). In Northern and North-eastern parts of India low temperature problem occurs in winter season usually during December to February and the minimum temperature remains often below 20^{0}C. Sometimes minimum temperatures are bellow 20^{0}C during March and April in some parts of the northern states of India. *Boro*-rice, particularly seedling stage encountering critical low temperature is appeared to suffer from cold injury. The extent of cold injury depends on the nature and duration of low temperature and diurnal change of low (night) and high (day) temperatures. The critical low temperatures for rice crop at agronomic panicle initiation, reduction division and anthesis are considered to be 18 ^{0}C, 19 ^{0}C and 22 ^{0}C, respectively.

Optimum temperatures for maximum photosynthesis ranged from 25 ^{0}C to 30 ^{0}C for rice. Rice often suffers from low temperature stress during the growing season, which is a major constraint to its growth and development (Levitt, 1980). There are three seasons of rice in West Bengal, viz., *Aus* (late winter), *Aman* (summer) and *Boro* (winter). The main season is the *Aman* for cultivation of rice in West Bengal, whereas the productivity is highest in *Boro* season. *Boro*-rice is cultivated during October/November to May/June. Cold tolerance at seedling stage is the primary requirement of *Boro* cultivars as seedlings are raised during the cold months of November and December. Water temperature during seedling establishment drops to about 10 ^{0}C and such low temperature significantly reduces seedling growth and establishment (Humphreys *et al*., 1996) subsequently increased the length of vegetative period, particularly the seedling stage. The common symptoms of low temperature injury in rice are poor germination of seeds, poor establishment of seedling in the field and yellowing (leaf bleaching and tissue necrosis) of leaves (Abdelkhalik *et al*., 2010). Leaf bleaching and tissue necrosis directly impair photosynthesis (Smillie *et al*., 1988) which reduced seedling growth. Synthesis of intracellular components, in particular of key proteins required for photosynthesis, is specifically susceptible to low temperature stress during development of rice leaves (Maruyama *et al*., 1990). Breeding for tolerance against low temperature to avoid or adaptation plant is widely recognized as effective way to overcome the limitations to production in low temperature environment (Bodapati *et al*., 2005; Cruz *et al*., 2006; Jiang *et al*., 2008). The present study was carried out to screen some working collections of rice for reaction to low temperature at seedling stage for identifying the tolerant lines, to find out the effect of different dates of sowing during *Boro* season on seedling characters, on crop duration and seed yield as compared to *Kharif* rice.

MATERIALS AND METHODS

The study material comprised of 37 rice genotypes from working collection of diverse origin of which 10 notified varieties, 20 advanced lines developed at Uttar Banga Krishi Viswavidyalaya (UBKV) and 7 advanced lined of IVT-*Boro*-2011 trial of Directorate of Rice Research, Hyderabad, India (Table 3). The experiment was conducted at University Farm, UBKV, Pundibari, Cooch Behar, West Bengal using a randomized complete block design with two replications. Seeds were sprouted and sown in the nursery beds on two different dates during

Email:bcroy10@yahoo.com

Boro (winter) season of 2010-11 (E1 and E2) as against timely sown during *Kharif* (summer) 2011 (E3). First and second dates were done on 28.12.2010 (E1) and 13.01.2011 (E2), respectively. Standard agronomic practices compatible to the humid tropic of Terai zone were followed to obtain good seedling stand. Weather parameters were recorded on maximum temperature and minimum temperature (weekly average) from the date of sowing to seedling uprooting for each date of sowing (Table 1).

Table 1: Weekly average maximum and minimum temperatures at seedling stage of rice

WAS	Temperature					
	E1		E2		E3	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
1	23.871	10.585	23.957	14.000	33.385	27.400
2	24.985	12.657	27.014	12.471	32.714	27.728
3	24.257	14.371	28.628	14.242	32.928	27.285
4	25.700	12.471	29.485	16.000	32.928	27.300
5	28.371	13.557	30.571	19.457	-	-
6	29.314	15.300	30.214	20.385	-	-
7	30.457	18.600	-	-	-	-
8	30.071	20.300	-	-	-	-
DOS	28.12.2010		13.01.2011		20.06.211	
Observation	56 DAS		36 DAS		28 DAS	

WAS: Week after sowing; E1: Boro (winter) sown on 28.12.2010; E2: Boro (winter) sown on 13.01.2011, E3: Kharif (summer); DOS: Date of sowing; DAS: Days after sowing

Observations were taken on low temperature tolerance ability (1-9 scale), seedling length, seedling fresh weight, seedling dry weight, days to 50% flowering and yield in kg per square meter. Data on seedling parameters were recorded on just two days before seedling uprooting for transplanting in main field (Table 1). The recording of low temperature tolerance was done following the Standard Evaluation System for Rice as out lined by International Rice Research Institute (IRRI, 2002). The scale for seedling cold tolerance ranged from 1-9 scale as- score 1: seedling dark green, score 3: seedling light green, score 5: seedling yellow, score 7: seedling brown/leaf bleaching (white), score 9: seedling dead. All the genotypes were rated for their response to low temperature tolerance on the basis of their colour and assigned scores accordingly. Low temperature tolerance scoring was not recorded for the *Kharif* crop as the seedlings were grown under normal temperature.

Seedling length was measured from the base of the seedling to the tip of longest leaf. Seedling fresh weight was weighed as the weight of whole plant, while for the dry weight, the seedlings were dried in an oven dryer for 48 hours at 80 ^{0}C.

The experiment was conducted using randomized complete block design with two replications. The data were subjected to analysis of variance and significant treatment differences selected by LSD (Gomez and Gomez, 1998).

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance showed highly significant differences among the genotypes, environments and interactions between genotypes × environment for shoot length, shoot fresh weight, shoot dry weight, days to 50% flowering and yield (Table 2). Presence of variability within the breeding materials is very essential to pick better donor for improvement of rice for low temperature tolerance. The breeding materials used in this experiment showed significant differences for all the characters. Similar results were obtained by Abdelkalik *et al*. (2010) while analyzed G × E interactions of rice under different temperature conditions. The mean performance for the 37 rice genotypes under three different environments were presented in table 3. There are significant differences among the genotypes for seedling height, seedling fresh weight, seedling dry weight and days to 50% flowering between the E1 and E2, E1 and E3, E2 and E3 (Table 4). The seedlings grown under environment E1 and E2 were outstandingly short as compared to the environment E3 as the seedlings under E1 and E2 experienced very low temperature in early seedling phase (Table 1) and took longer time to recover. This result coroborate with the findings of Tiwari *et al*. (2009), Satya *et al*. (2010) and Abdelkhalik *et al*. (2010). The higher seedling height was obtained for UBKVR-6, UBKVR-2 and UBKVR-1 during *Boro*-season for both E1 and E2 (Table 3). The seedling height as affected by the low temperature during seedling stage directly influenced the seedling fresh and dry weights accordingly. High mortality of rice seedlings under low temperature condition is recurrent feature during *Boro*-season as compared to the *Kharif* season (Seema and Roy, 2007).

Table 2: ANOVA for the effect of three different temperatures regimes on seedling characters and yield of rice genotypes

Source	df	Mean sum of squares				
		Seedling height	Shoot fresh weight	Shoot dry weight	Days to 50% flowering	Yield
Total	221	256.832	6951.062	1018.931	364.699	0.012
Replication	1	0.128	4252.281	17.935	1.626	0.011
Treatment	110	515.916**	9361.865**	2038.341**	730.757**	0.020**
Error	110	0.082	4564.793	8.620	1.880	0.004
Environment (E)	2	14995.713**	18598.965*	20876.243**	29976.581**	0.061**
Genotpe (G)	36	239.375**	11153.617**	1789.263**	434.066**	0.046**
E × G	72	251.970**	8209.402**	1639.605**	66.7199**	0.006**
Error	110	0.082	4567.793	8.620	1.881	0.004

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

Classification of genotypes based on leaf bleaching

Recording of low temperature tolerance was done following the Standard Evaluation System for Rice as outlined by IRRI. All the genotypes were rated for their response to cold tolerance on the basis of their seedling colour (chlorophyll bleaching) and assigned score accordingly. Based on the rating, the genotypes were classified into four groups (Table 5), viz. tolerant, moderately tolerant, susceptible and highly susceptible.

Low temperature injury seems to be damage in the intersystem that blocks electron transfer from PSII to PSI that leads to the subsequent over reduction in PSII, a possible trigger for the visible injury, only in the light as suggested by Suzuki *et al*. (2008). Although the over-reduction of PSII seems harmful enough to induce the disintegration of the Mn cluster (Higuchi *et al*., 2003) and the production of reactive oxygen species (Anderson *et al*., 1998; Nishiyama *et al*., 2006; Murata *et al*., 2007), it is unlikely that the visible symptoms were only caused by degradation of PSII the complex. Both PSII and PSI complexes must be destroyed, as the first visible symptom was the bleaching of the leaves in which chlorophyll was almost lost. Based on the extent of leaf bleaching, the genotypes were classified into four classes (Table 5) following the Standard Evaluation System for Rice out lined by International Rice Research Institute (IRRI 2002). Among the thirty seven genotypes, 13 were classified as tolerant genotypes (Parijat, Krishna Hamsha, IR 64, UBKVR-5, UBKVR-6, UBKVR-8, UBKVR-16, UBKVR-17, UBKVR-19, UBKVR-21, IVT-B4001, IVT-B4007, IVT-B4008), 5 were as moderately tolerant (Annada, UBKVR-20, IVT-B4002, IVT-B4004, IVT-B4005), 7 were susceptible (Lalat, MTU 1010, BR-28, IR 58025A, UBKVR-4, UBKVR-10, UBKVR-13) and 12 were highly susceptible (Sadabahar, Pusa Basmati-1, UBKVR-1, UBKVR-2, UBKVR-3, UBKVR-9, UBKVR-11, UBKVR-15, UBKVR-22, UBKVR-23, UBKVR-24, IVT-B4006). The genotypes belonging to the tolerant group are suitable as donors for low temperature tolerance in crop improvement work for *Boro* rice. The entries in showing cold tolerance score 3-4 indicated moderately tolerant to low temperature and those are also suitable as donors for cold tolerance as well as high seed yield. Similar suggestions also made by Tiwari *et al*. (2009) for selection of donors for *Boro*-rice improvement. Annada (scored 3), a popular variety grown in this region during *Boro* season, whose productivity is comparatively high and the crop takes about 135 days during this season. Tiwari *et al*. (2009) also categorized Annada as moderately low temperature tolerant rice cultivar.

Recovery after low temperature phase

Rice often suffers low temperature stress during growing season, which is major constraint to its growth and development. Development rate is not constant, but fluctuates as a function of air and water temperature and of photoperiod; these factors are especially important during the vegetative phase. Low temperature during this phase slows down rice development and lengthens growth duration. Most plants stop growth at low temperatures, in many cases, plant development is slowed after chilling and the plants may remain visually stunted for prolonged period (Roy and Basu, 2009). The seedling in E1 were exposed longest period of 56 days to low temperature to get suitable size for transplanting (Table 1). The minimum temperature was below 15 ^{0}C or just above 15 ^{0}C for the first six weeks after sowing. The seedlings in E2 were exposed to temperature below 15 ^{0}C for four weeks only. The seedlings in E2 took 36 days to attain transplanting size. The temperature below 15 ^{0}C caused cold injury leading to leaf bleaching and stunted growth of the seedling.

Table 3: Performance of rice genotypes under three different environments

Genotypes	Seedling height (cm)			Shoot fresh weight (mg)			Shoot dry weight (mg)			Days to 50% flowering			Yield (kg m^{-2})		
	E1	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**
Annada	6.2	6.3	21.3	119.0	100.0	107.9	38.5	31.5	21.7	122.0	99.5	75.5	0.80	0.79	0.61
IR 64	5.8	5.7	25.4	117.5	98.5	178.1	38.0	36.5	97.9	120.5	97.5	79.0	0.61	0.60	0.53
Parijat	4.6	4.6	40.1	107.5	89.5	140.1	39.0	34.5	106.6	114.5	89.0	75.0	0.71	0.70	0.53
Sadabahar	6.6	6.3	26.8	49.5	47.5	91.6	31.0	27.0	38.7	119.0	70.5	84.0	0.66	0.67	0.55
MTU 1010	5.6	5.5	21.0	124.0	102.5	60.2	28.0	28.5	24.5	123.5	99.0	84.0	0.60	0.59	0.57
Pusabasmati 1	6.6	6.2	23.8	116.5	101.5	51.2	34.0	31.0	23.0	113.0	86.0	82.0	0.45	0.44	0.46
IR58025B	5.2	5.0	18.5	137.0	117.0	32.7	37.5	37.0	18.1	115.0	90.5	82.5	0.41	0.40	0.36
Krishna Hamsha	6.2	5.8	25.3	64.0	56.0	113.1	34.0	29.5	50.3	136.0	111.5	89.5	0.62	0.61	0.61
BR-28	6.1	5.9	23.5	141.0	121.0	88.3	36.5	31.5	44.1	131.0	107.0	83.5	0.61	0.61	0.52
Lalat	6.4	6.3	44.6	71.5	66.5	284.1	31.5	26.5	104.6	124.0	100.5	86.0	0.55	0.54	0.47
UBKVR-7	6.3	6.1	28.1	69.0	60.5	127.1	46.5	36.5	22.8	105.5	71.0	84.0	0.50	0.48	0.36
UBKVR-13	6.2	5.9	27.2	121.0	106.0	67.3	51.0	44.5	50.9	106.0	80.0	84.5	0.45	0.46	0.44
UBKVR-11	6.8	6.5	24.2	143.5	123.5	90.2	46.0	42.0	32.8	112.0	77.5	84.5	0.72	0.71	0.68
UBKVR-24	5.1	4.8	29.4	81.0	78.0	170.4	31.0	29.5	81.6	114.5	92.0	84.0	0.43	0.42	0.56
UBKVR-15	6.1	5.9	22.6	106.0	96.0	72.8	46.0	39.5	27.6	129.5	102.5	82.0	0.66	0.65	0.58
UBKVR-16	6.6	6.3	27.3	158.0	133.0	75.4	54.0	48.5	35.0	120.0	93.5	79.0	0.82	0.81	0.68
UBKVR-5	7.1	6.9	21.5	179.0	159.0	133.7	47.0	41.0	86.1	139.0	116.0	90.5	0.62	0.65	0.64
UBKVR-6	7.7	7.3	30.7	191.0	166.0	143.6	49.0	46.5	81.9	133.5	106.0	86.0	0.59	0.58	0.51
UBKVR-1	7.5	7.1	24.7	141.0	132.0	98.4	37.0	32.5	32.8	136.0	113.0	92.5	0.79	0.78	0.73
UBKVR-9	7.4	7.3	28.3	151.0	139.5	251.3	60.0	57.5	118.3	134.0	110.5	83.0	0.56	0.58	0.63
UBKVR-4	6.3	6.1	29.7	200.5	171.5	183.5	59.5	54.5	50.9	131.0	112.0	91.5	0.62	0.60	0.69
UBKVR-3	7.8	7.3	30.9	179.0	144.0	140.7	65.0	59.5	41.4	126.0	102.5	93.5	0.68	0.67	0.77
UBKVR-8	8.2	7.7	29.5	156.5	131.0	150.3	42.0	36.5	78.3	135.5	117.5	90.5	0.59	0.59	0.71
UBKVR-23	6.7	6.2	25.5	181.5	159.0	96.8	47.5	40.5	40.2	137.0	119.0	89.5	0.59	0.62	0.62
UBKVR-10	6.9	6.6	29.3	142.0	125.5	176.8	37.5	31.0	71.9	128.5	100.5	85.5	0.51	0.49	0.63
26. UBKVR-2	7.7	7.4	30.7	112.5	101.0	139.5	54.5	49.0	87.7	124.5	97.0	86.5	0.70	0.67	0.66
UBKVR-19	5.2	5.1	27.7	166.5	149.0	140.0	41.0	38.5	85.4	132.0	109.0	91.0	0.73	0.71	0.51
UBKVR-20	6.2	6.1	28.9	142.5	121.0	143.8	46.0	42.0	72.0	136.0	113.5	92.5	0.65	0.66	0.61
UBKVR-21	4.4	4.1	23.9	90.0	80.5	95.3	34.0	27.0	35.0	145.0	123.0	96.0	0.61	0.57	0.52
UBKVR-22	5.1	5.2	27.3	90.5	86.0	97.2	27.0	24.0	49.0	131.0	102.5	92.5	0.61	0.63	0.53
IVT-B 4001	5.2	6.3	35.7	132.0	100.0	183.9	35.0	31.5	56.0	126.0	99.5	85.5	0.71	0.79	0.54
IVT-B 4002	4.1	5.7	21.9	70.0	98.5	116.0	33.5	36.5	78.1	138.5	97.5	89.0	0.52	0.60	0.47
IVT-B 4004	5.7	4.6	29.5	121.5	89.5	172.6	33.5	34.5	57.6	133.5	89.0	92.0	0.68	0.70	0.59
IVT-B 4005	5.6	6.3	30.5	118.0	47.5	133.9	33.5	27.0	56.1	117.5	70.5	82.0	0.59	0.67	0.48
IVT-B 4006	5.1	5.5	22.6	101.5	102.5	172.8	28.0	28.5	27.6	129.5	99.0	90.5	0.64	0.59	0.58
IVT-B 4007	6.3	6.2	36.2	150.0	101.5	146.0	53.0	31.0	37.8	118.0	86.0	81.5	0.54	0.44	0.43
IVT-B 4008	6.1	5.0	40.9	138.0	117.0	290.5	42.0	37.0	119.8	121.0	90.5	88.5	0.52	0.40	0.46
Mean	6.2	5.9	30.7	126.5	110.3	142.4	41.3	36.4	57.92	125.9	100.5	86.2	0.61	0.61	0.56
LSD (0.01) E		**0.123**			**29.116**			**1.265**			**0.591**			**0.025**	
V		**0.433**			**102.252**			**4.443**			**2.075**			**0.090**	
E× V		**0.752**			**177.106**			**7.696**			**3.594**			**0.156**	

E1: *Boro* (winter) sown on 28.12.2010; E2: *Boro* (winter) sown on 13.01.2011, E3: Kharif (summer)

Table 4: Mean values of rice genotypes of each environment

Environment	Seedling height	Shoot fresh weight	Shoot dry weight	Days to 50% flowering	Yield
Environment 1	6.162 b	126.500 ab	41.297 b	125.918 c	0.611 a
Environment 2	5.898 c	110.351 b	36.432 c	100.513 b	0.612 a
Environment 3	30.685 a	142.057 a	67.650 a	86.175 a	0.562 b

*Values marked by common letter are not significantly different at P=0.05 of LSD

Whereas, the seedling in the E3 attained transplanting size just in four weeks as the seedlings were grown under optimum temperature required for the normal growth of rice. Immediately after raise in temperature, the seedling recovery was very rapid. Highly bleached leaves started to dry and new leaves emerged with normal chlorophyll content. Gradually the seedling in the seed beds turned green leaving all the symptoms of low temperature injury. Seedling growth also returned to normal. All the genotypes in both E1 and E2 took two weeks after cold shock to attain transplanting size. Seema and Roy (2007) also reported rapid recovery of rice seedlings after low temperature shock at seed bed.

Crop duration and yield

There are significant differences in between E1 and E2, E1 and E3; E2 and E3 days to 50% flowering and yield (Table 4). Cold stress caused delayed heading in rice (Andaya and Mackill, 2003a, b) resulting in significant difference in days to 50% flowering. Whereas, the yield difference between crops under E1 and E2 was insignificant. Seedling sown in nursery for E1 took more days to attain transplanting size as compared to E2 due to low temperature which caused leaf bleaching leading to stunted growth, thus there was no aging of the seedlings. Yang *et al*. (2001) also reported reduction in chlorophyll content with increase in period of low temperature. Transplanting for E1 and E2 was done on the same day. Both the seedlings of E1 and E2 were grown under similar weather conditions after transplanting. Yang *et al*. (2001) also reported that after transplanted to the paddy field, the top dry weight, leaf age, the number of stem and the number of roots increased and recovered to the control level. Recovery after cold injury was also similar seedling of E1 and E2. The recovery after transplanting in the paddy field was similar irrespective of low temperature treatment at nursery (Yang *et al*., 2001; 2002). Thus, the yield of the corresponding genotypes under E1 and E2 are insignificant.

The actual aging of seedling started only after transplanting. Thus, the respective genotypes of E1 and E2 took almost same days to 50% flowering starting from transplanting, whereas, days to 50% flowering starting from sowing is significantly different (Table 4). These observable facts also led insignificant differences in yield between E1 and E2. Significant differences in between E1 and E3; E2 and E3 for yield were due to the seasonal effect on the crop. In general, the productivity in *Boro* (winter) is higher than the *Kharif* (summer). In nutshell, low temperature (10 ± 5 ^{0}C) during nursery stage caused leaf bleaching leading to stunted growth of rice seedling. Ultimately the low temperature at seedling stage increased crop duration without much effect on final yield of the crop.

Table 5: Classification of rice genotypes based on tolerance against low temperature tolerance

Score	Class	Genotype	No. of genotypes
1-2	Tolerant	Parijat, Krishna Hamsha, IR 64, UBKVR-5, UBKVR-6, UBKVR-8, UBKVR-16, UBKVR-17, UBKVR-19, UBKVR-21, IVT-B4001, IVT-B4007, IVT-B4008	13
3-4	Moderately tolerant	Annada, UBKVR-20, IVT-B4002, IVT-B4004, IVT-B4005	5
5-6	Susceptible	Lalat, MTU 1010, BR-28, IR 58025A, UBKVR-4, UBKVR-10, UBKVR-13	7
> 7	Highly susceptible	Sadabahar, Pusa Basmati-1, UBKVR-1, UBKVR-2, UBKVR-3, UBKVR-9, UBKVR-11, UBKVR-15, UBKVR-22, UBKVR-23, UBKVR-24, IVT-B4006	12

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