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GENETICS OF COLD TOLERANCE IN RICE (*ORYZA SATIVA* L.): A CONCEPT OF MULTIPLE ALLELISM

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ABSTRACT

The gene action for cold tolerance in rice (*Oryza sativa* L.) cultivars was investigated in three sets of half diallel crosses, viz., a 5 x 5 indica x indica (I x I) set, a 5 x 5 japonica x japonica (J x J) set, and a 7 x 7 indica x japonica (I x J) set. Minimum temperature for chloroplast biogenesis (MTCB) and spikelet fertility depression coefficient (FDC) were used for evaluating cold tolerance at the vegetative and reproductive phases, respectively. Whereas in the I x I set, general combining ability (gca) variance was predominant, in the J x J set the specific combining ability (sca) variance was more prevalent. In the I x J set, both effects were of similar magnitude, indicating japonica cultivars as primary source of nonadditive effects. The covariance–variance (Wr–Vr) graph indicated that japonica cultivars had dominant genes for MTCB but recessive genes of FDC. As these cultivars were cold tolerant at both vegetative and reproductive phases, the reversal of gene action appears to be due to involvement of two different traits evaluated at two different growth phases, rather than reversal in gene action for cold tolerance per se. Persistent heterogeneity of Wr–Vr over the arrays in noninteracting I x J set for MTCB and FDC and in I x I set for FDC indicates involvement of multiple alleles in the control of cold tolerance. In view of this, an appropriate breeding strategy has been proposed.

Key words: Cold tolerance, diallel analysis, multiple alleles, chloroplast biogenesis, fertility depression.

High yielding indica rice cultivars with their streamlined production technology created impact largely in the areas with either predictable or to a certain extent controllable environment. However, high altitudes of tropics did not benefit much due to cold susceptibility of the cultivars. Development of cold tolerant–rice cultivars can render more than 7 million ha of land more productive in South and Southeast Asia alone, making it possible to increase cropping intensity by a significant margin.

Of the three principal ecogeographic races of cultivated Asian rice, the japonicas have high cold tolerance. But high spikelet sterility in F_1 and succeeding generations in indica x japonica crosses have prevented the transfer of cold tolerance from japonica to indica rice [1]. Beachell et al. [2] and Ikehashi [3] suggested the use of bridging parents like ponali-type japonicas and wide compatible varieties (WCVs).

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Breeding for cold tolerance in rice has a long history [4], although the components involved and their genetics have received very little attention. The situation is complicated by the growth phase-dependent expression of cold tolerance. Tolerance at seedling stage in terms of nonchlorosis of leaves is different from tolerance at anthesis expressed as spikelet fertility [1, 5–8]. This has yet another constraint in the selection programmes for cold tolerant rices.

With this background, the present investigation has been undertaken to characterize the nature of gene action for cold tolerance involving some indica and japonica cultivars and to identify and evaluate suitable donor parents, and assess their combining ability, particularly in indica genetic background.

MATERIALS AND METHODS

Three sets of half diallel crosses, viz., 5 x 5 I x I, 5 x 5 J x J and 7 x 7 I x J were made at the International Rice Research Institute, Los Banos, Philippines in 1987. The description of the cultivars used as parents is presented in Table 1. Among these cultivars, Bir-ze-goo has been reported to be tolerant only at vegetative phase [9].

Table 1. Description of cultivars used in the three diallel sets of crosses

Cultivar	Origin	Reaction to cold	Used as parent in the set(s)
Indica:			
CB1	India	Tolerant	I X I
Bir-ze-goo	China	Tolerant (at vegetative phase only)	I X I, I X J
China 1039	India	Tolerant	I X I, I X J
IR 8	IRRI	Susceptible	I X I, I X J
IR 64	IRRI	Susceptible	I X I, I X J
Japonica:			
Ching shi 15	China	Tolerant	J X J, I X J
Stejaree 45	USSR	Tolerant	J X J, I X J
Barkat	India	Tolerant	J X J, I X J
Chechon Chun Shun 1	China	Tolerant	J X J
H305-84	Hungary	Tolerant	J X J

I x I—indica x indica, I x J—indica x japonica and J x J—japonica x japonica crosses.

Minimum temperature for chloroplast biogenesis (MTCB) at vegetative phase and spikelet fertility depression coefficient (FDC) at reproductive phase were recorded.

MTCB. Patterson et al. [10] described this technique for screening chilling resistant genotypes of *Lycopersicon hirsutum*. The dark grown seedlings containing a small amount of protochlorophyllide–protein complex have served as a convenient system for studying chloroplast biogenesis [11]. Hence, different hybrids along with their respective parents in the three diallel sets were germinated in dark at 30°C. Seven-day-old etiolated seedlings were transferred to controlled temperature cabinets in the phytotron. After 24 h at the

designated temperature for equilibration in dark, the seedlings were subjected to an alternate cycle of 12 h light (20 klux) and 12 h darkness. Green coloration was evident between 16°–17°C in the cold tolerant japonica cultivars and between 19°–20°C in the susceptible indicas. None of the cultivars used in the different diallel sets had the ability for greening below 16°C. In order to observe the heterotic effects, if any, the hybrids and parents of different diallel sets were also simultaneously evaluated under six temperature regimes with 1°C interval from 15°–20°C. Each temperature treatment consisted of three replications of five seedlings of each hybrid and parent. The minimum temperature and the average number of days required at the corresponding temperature range for greening by each experimental material were recorded. As none of the hybrids developed green colour at 15°C, this base value was subtracted from the average temperature range and multiplied with the respective average number of days to obtain the value for degree – days (days x temperature).

FDC. Fertility depression coefficient measuring changes in spikelet fertility under different temperature conditions was computed according to Morishima et al. [12].

$$\text{FDC} = \frac{\text{Fertility (\%) at 29°/21°C–Fertility (\%) at 18°C}}{\text{Fertility (\%) at 29°/21°C+Fertility (\%) at 18°C}}$$

The hybrid and the parents were grown in pots in the phytotron at 29°/21°C (day/night) in two separate sets with three replications in each. A replication was represented by three pots, each with two plants. At heading, one set was transferred to KG-cabinets of the phytotron (18°C day/night, 50 klux, 70% RH). After 7 days of treatment at this temperature, this set was returned to 29°/21°C [7,13]. To facilitate development of uniform single-culm plants, so that a difference in tillering between parents and hybrids does not confound the results, all side tillers were removed from three weeks after seeding to about a week before heading. At maturity three plants per replication were randomly chosen in each set and the panicles were bagged individually for counting the fertile and sterile spikelets.

For both the aforementioned traits, the lower values indicate better tolerance to low temperature.

The gca and sca were investigated according to Griffing's model 1, method 2 [14]. The data were also subjected to Wr–Vr graph analysis following Jinks and Hayman [15] and Hayman [16, 17]. Analysis of parental mean [Yr], parental order of dominance (Wr + Vr) as standardized deviation graph was done following Johnson and Aksel [18]. In addition to a t^2 test for the overall assumptions of the diallel analysis, (Wr–Vr) ANOVA was carried out following Allard [19] to determine the adequacy of the model with respect to nonallelic interactions.

RESULTS AND DISCUSSION

Highly significant differences among parents and hybrids indicated the presence of genotypic variability for MTCB and FDC in each set of diallel crosses (Table 2). A detailed analysis of combining ability and gene action was, therefore, appropriate.

Table 2. Mean values of MTCB and FDC in indica x indica (5 x 5), japonica x japonica (5 x 5) and indica x japonica (7 x 7) diallel crosses

Female parent	Parameter	Male parents					Array total
A. I X I cross:		CB 1	Bir-ze-goo	China 1039	IR 8	IR 64	
CBI	MTCB	19.25	16.92	18.67	20.25	18.67	93.76
	FDC	0.47	0.43	0.34	0.79	0.66	2.69
Bir-ze-goo	MTCB		15.17	14.58	18.67	16.33	81.67
	FDC		0.63	0.38	0.97	0.92	3.33
China	MTCB			20.42	21.00	19.83	94.50
1039	FDC			0.40	0.57	0.49	2.18
IR 8	MTCB				27.75	26.25	113.92
	FDC				1.00	0.99	4.32
IR 64	MTCB					21.75	102.83
	FDC					0.99	4.05
B. J X J cross:		Ching shi 15	Stejaree 45	Barkat	Chechon Chun Shun 1	H305-84	
Ching shi 15	MTCB	6.50	5.50	8.50	5.50	6.50	32.50
	FDC	0.02	0.03	0.32	-0.15	0.14	0.36
Stejaree 45	MTCB		7.75	8.75	6.00	7.25	35.25
	FDC		0.02	0.32	0.22	0.15	0.74
Barkat	MTCB			8.00	5.25	5.25	35.75
	FDC			0.32	0.31	0.21	1.48
Chechon	MTCB				5.75	4.75	27.25
Chun Shun 1	FDC				0.03	0.02	0.43
H305-84	MTCB					6.25	30.00
	FDC					0.05	0.57

C. I X J cross:		Ching shi 15	Stejaree 45	Barkat	Bir-ze-goo	China 1039	IR 8	IR 64	Array total
Ching shi 15	MTCB	6.50	5.48	8.50	6.50	16.92	9.00	5.50	58.40
	FDC	0.02	0.03	0.32	0.19	0.25	0.95	0.54	2.30
Stejaree 45	MTCB		7.75	8.75	6.25	7.75	6.50	6.25	48.73
	FDC		0.02	0.32	0.17	0.81	0.87	0.55	2.77
Barkat	MTCB			8.00	7.25	14.58	10.25	6.25	63.58
	FDC			0.32	0.27	0.07	0.57	0.37	2.24
Bir-ze-goo	MTCB				15.10	14.58	18.67	16.33	84.68
	FDC				0.63	0.38	0.97	0.92	3.53
China	MTCB					20.42	21.75	19.83	115.83
1039	FDC					0.40	0.57	0.49	2.97
IR 8	MTCB						27.75	26.25	120.17
	FDC						1.00	0.99	5.92
IR 64	MTCB							21.75	102.16
	FDC							0.99	4.85

Griffing's approach. Highly significant differences were observed for gca and sca for both the traits (Table 3). In I x J combinations, the ratios of gca : sca variances were 0.98:1 and 1:1 for MTCB and FDC, respectively, suggesting both dominant and additive effects to be equally important in expression of these traits. The contribution of individual parents to hybrid tolerance was evaluated by comparing the gca effects (Table 4). The tolerant japonica parents, viz., Ching shi 15, Stejaree 45 and Barkat, had desirable negative gca effects for

Table 3. Analysis of variance for gca and sca for MTCB and FDC in indica x indica (I X I), japonica X japonica (J X J), and indica X japonica (I X J) crosses

Source	d.f.			M.S.					
				MTCB			FDC		
	I x I	J x J	I x J	I x I	J x J	I x J	I x I	J x J	I X J
gca	4	4	6	38.78**	2.93**	150.28**	0.20**	0.05**	0.35**
sca	10	10	21	2.85**	1.08**	17.09**	0.01**	0.01**	0.04**
Error	28	28	54	0.32	0.05	0.23	0.00003	0.0001	0.00004
Estimated gca variances				5.49	0.41	16.67	0.03	0.007	0.04
Estimated sca variances				2.53	1.03	16.86	0.01	0.009	0.04

** Significant at p=0.01.

both traits. The tolerant indica cultivar, China 1039, also exhibited significant negative gca effect for FDC, while susceptible indica cultivars IR 8 and IR 64 had significant positive values for both the traits. For MTCB, all the three cold tolerant japonica cultivars showed

Table 4. Estimates of gca (in bold) and sca for MTCB and FDC in indica x japonica (7 x 7) half diallel crosses

Variety	Parameter	Ching shi 15	Stejaree 45	Barkat	Bir-ze-goo	China 1039	IR 8	IR 64
Ching shi 15	MTCB	**-3.91**	1.73	3.07	-2.06	4.30	-4.91	-5.75
	FDC	**-0.18**	-0.15	0.17	-0.14	0.01	0.31	0.02
Stejaree 45	MTCB		**-4.85**	4.25	-1.38	-3.93	-6.48	-4.06
	FDC		**-0.15**	0.11	-0.21	0.51	0.18	-0.02
Barkat	MTCB			**-3.17**	-2.06	1.23	-4.40	-5.74
	FDC			**-0.16**	-0.09	-0.20	-0.09	-0.17
Bir-ze-goo	MTCB				**-0.04**	-1.91	0.88	1.21
	FDC				**0.02**	-0.07	0.12	0.20
China 1039	MTCB					**4.01**	-0.09	0.67
	FDC					**-0.07**	-0.18	-0.15
IR 8	MTCB						**5.31**	5.78
	FDC						**0.33**	-0.03
IR 64	MTCB							**2.64**
	FDC							**0.20**
SE(gca):	MTCB	0.14		SE(sca):	MTCB	0.36		
	FDC	0.002			FDC	0.005		

significant negative effects in combination with susceptible indica cultivars (Table 4). For FDC, however, the situation varied in the crosses Ching shi 15 x IR 8. Ching shi 15 x IR 64, and Stejaree 45 x IR 8.

The sca involves both dominance and epistasis, whereas gca is based on additive and additive x additive type of genetic effects [14]. Hence, both additive and nonadditive gene actions were equally involved in controlling the cold tolerance at vegetative as well as reproductive stages.

In I x I crosses (Table 3), both traits were predominantly controlled by additive effects (ratios 2.16:1 for MTCB and 3:1 for FDC), whereas in J x J crosses, the controlling factor was predominantly nonadditive (ratios 0.39:1 for MTCB and 0.77:1 for FDC). Thus, the significant nonadditive gene action for cold tolerance in I x J crosses was primarily due to the involvement of japonica cultivars.

Jinks-Hayman approach. To test the validity of the major assumptions postulated for diallel analysis [16], Wr–Vr homogeneity test was performed (Table 5). Except in the JxJ combinations for MTCB, all other t^2 values were nonsignificant. A further verification of the assumption comes from the Wr–Vr graph (Fig. 1). If the assumptions are valid, the points on the Wr–Vr graph are expected to fall on a line of unit slope and the regression coefficient 'b' is to be significantly different from zero but not from one [16]. Deviation from this was observed only in the J x J crosses. For MTCB in these combinations, 'b' value was significantly different from unity, while for FDC, 'b' value itself was nonsignificant (Table 5), suggesting the presence of nonallelic interaction. The ratio further supports this conclusion.

Table 5. Estimates of uniformity and regression of Wr on Vr in indica x indica (I x I), japonica x japonica (J x J) and indica x japonica (I x J) crosses

Parameter	I x I		I x J		J x J	
	t^2	b	t^2	b	t^2	b
MTCB	2.77	0.8648	0.0320	0.9494	21.06*	0.4500[1]
FDC	3.00	0.8333	0.7121	1.176	0.75	0.497[2]

* Significant at P = 0.05.

[1] Significantly different from 1.0.

[2] Not significantly different from 0 as well as from 1.0.

In the presence of nonallelic interactions, the deductions from the Wr–Vr graph are likely to be erroneous. Hence, the interacting arrays from the J x J crosses, viz., Barkat for MTCB and Chechon Chun Shun 1 for FDC, were eliminated to obtain a satisfactory fit for the additive–dominance model (Fig. 1, E, F). In the new set, the regression of Wr on Vr was not significantly different from unity and t^2 value was also nonsignificant. The Wr–Vr graphs revealed the following: a) The regression line of the Wr–Vr intersected Wr axis above the origin for MTCB in I x I and I x J crosses indicating partial dominance (Fig. 1, A, G). For MTCB in J x J crosses, however, the regression line intersected Wr axis below the origin, suggesting the presence of overdominance (Fig. 1, E). For FDC, in I x I and J x J crosses dominance was partial (Fig. 1, B, F), whereas in

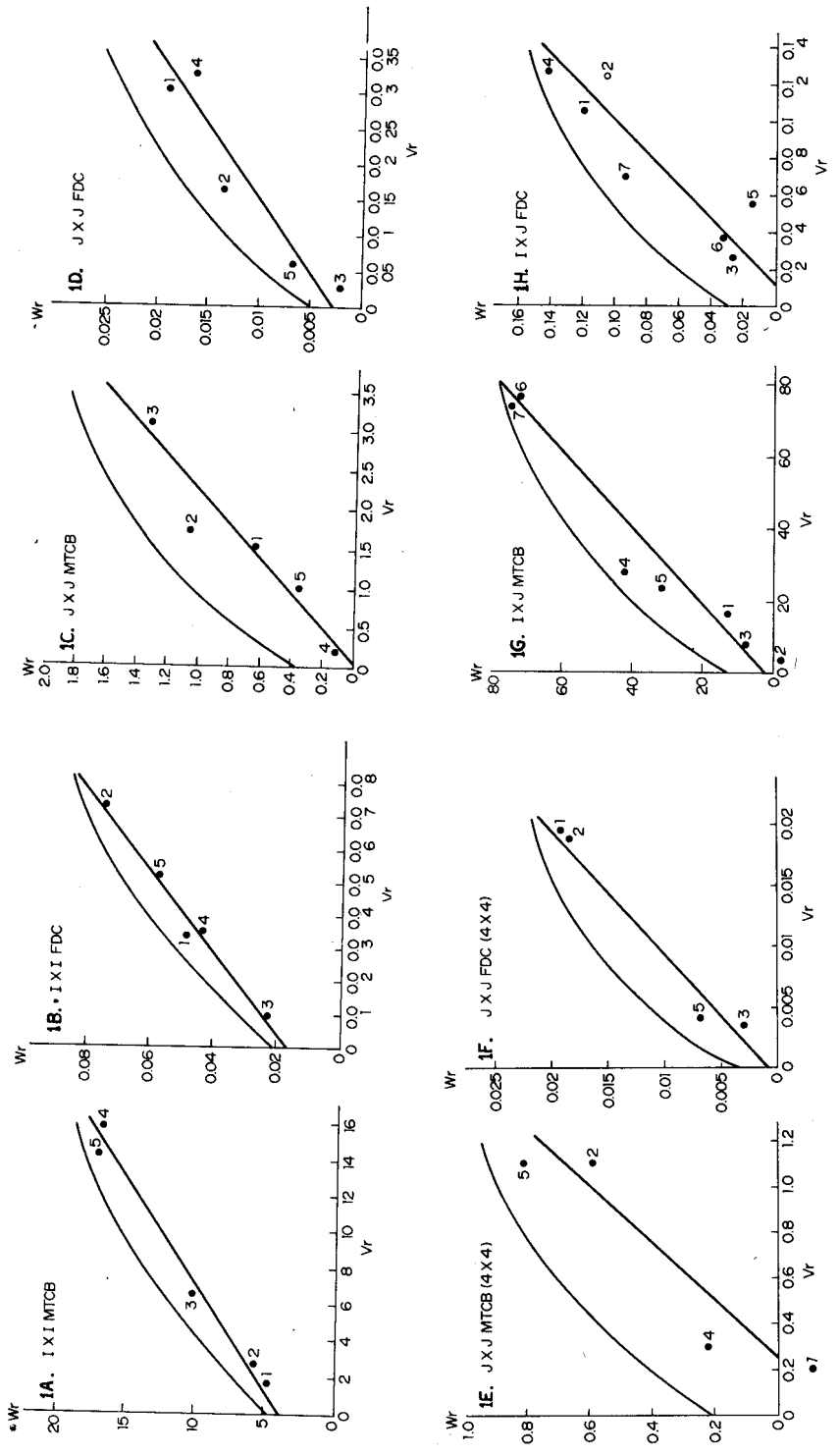

Fig. 1. Wr–Vr graphs for MTCB (A,C,E and G) and FDC (B, D, F and H) in 5 x 5 I x I; 4 x 4 J x J, and 7 x 7 I x J dialel sets. Array numbers: In I x I: CBI(1); Bir-ze-goo (2); China 1039 (3); IR 8(4), and IR 64(5). In J x J: Ching shi 15 (1); Stejaree 45 (2); Barkat (3); Chechon Chun Shun 1 (4), and H305-84 (5). In I x J: Ching shi 15 (1); Stejaree 45 (2); Barkat (3); Bir-ze-goo (4); China 1039 (5); IR 8 (6), and IR 64 (7).

I x J crosses it was in the overdominance range (Fig. 1, H). b) The relative values of Wr and Vr showed that the cold tolerant cultivars CB1 and Bir-ze-goo had more dominant genes and the cold susceptible cultivars IR 8 and IR 64 had more recessive genes for MTCB in I x I crosses. Variety China 1039, on the other hand, had intermediate position between these two groups. Similarly, varieties Ching shi 15 and Chechon Chun Shun 1 had more dominant genes than Stejaree 45 and H 305-84 in J x J crosses, whereas, all the three japonica cultivars, Stejaree 45, Barkat and Ching shi 15, possessed more dominant genes relative to both the tolerant and susceptible indica cultivars in I x J crosses.

For FDC, variety China 1039 had more dominant genes and Bir-ze-goo had excess of recessive genes in I x I crosses. In J x J crosses, var. Barkat expressed excess of dominant genes, followed by var. H305-84. Varieties Ching shi 15 and Stejaree 45 had excess of recessive genes. In the I x J crosses, the tolerant japonica cultivar Barkat, tolerant indica cultivar China 1039 as well as the most cold susceptible indica cultivar IR 8 had excess of dominant genes. c) Correlation coefficient (r) between Yr and Wr + Vr revealed that at vegetative phase, cold tolerance (lower expression of MTCB) was controlled by dominant genes irrespective of cross combinations (r = 0.87,0.90, and 0.45 for I x I, I x J, and J x J crosses, respectively). The gene action at reproductive phase in I x I and I x J crosses, however, was ambidirectional as the correlation values were very low (r = 0.33 and –0.30, respectively). In JxJ crosses, on the other hand, the value was higher and negative (r = –0.71), indicating the excess of recessive genes in the tolerant parents. Thus, the dominance relationship in Wr–Vr graph as well as correlations seem to indicate a change in gene action for cold tolerance from vegetative to reproductive phase.

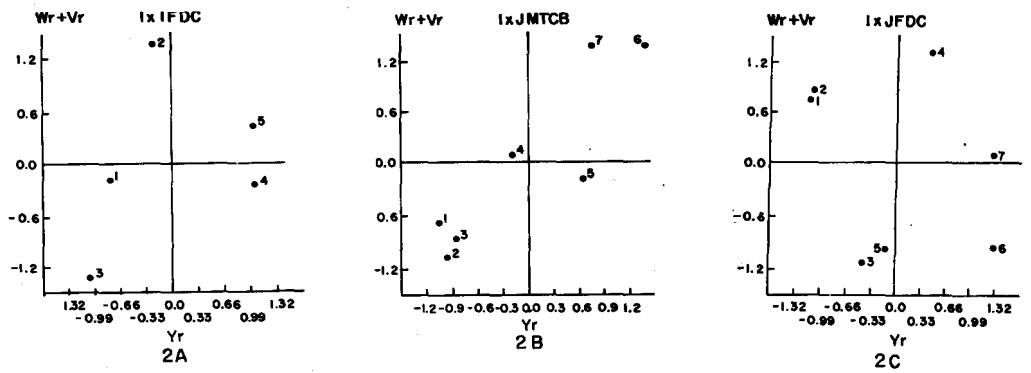

Fig. 2. Yr, Wr + Vr standardized deviation graphs for FDC (A and C) and MTCB (B) in 5 x 5 I x I; and 7 x 7 I x J diallel sets. Array numbers as in Fig. 1.

It is a general observation in the cold tolerance breeding programmes of rice that cold tolerance at vegetative phase does not always correlate with tolerance at reproductive phase [1, 5–8]. The present analysis indicates that gene actions of opposite nature were involved in controlling cold tolerance in japonica cultivars. The investigation also reveals that the cold tolerance mechanism in indica cultivars is somewhat different from that of japonica cultivars.

All the japonica cultivars used in the present investigation were cold tolerant both at vegetative and reproductive phases. Hence, a pertinent question arises regarding the change in gene action for cold tolerance in them. Probably, it is due to the fact that two different traits (MTCB and FDC) have been evaluated at two different growth phases and considered as single trait, i.e. cold tolerance. Further, even after eliminating the interacting arrays in J x J crosses, the gene action for MTCB was in the overdominance range (Fig. 1, E). In I x J crosses also, the gene action was in the overdominance range for FDC. Jinks [20] suggested that apparent overdominance may be partially due to epistasis. Thus, after analyzing the data of the present study through Wr–Vr graph in the usual way, when t^2 value was nonsignificant and the regression of Wr on Vr was not significantly different from unity [16], a renewed search for the reasons of failure of the different assumptions underlying diallel analysis was undertaken.

Jinks [20] and Hayman [16] have listed certain assumptions that must be fulfilled for fitting the additive–dominance model. The assumptions of homozygosity and diploid segregation are usual and, in all probability, apply to rice–a diploid and predominantly self-pollinated species. No reciprocal differences were observed for MTCB and FDC in some representative crosses in the present investigation (unpublished). In a large number of crosses, Ikehashi and Araki [21] also did not observe reciprocal differences for spikelet fertility in rice. Heterogeneity of Wr–Vr is a good indicator for validity of the assumptions about lack of epistasis, correlated gene distribution, and multiple alleles at each locus [16]. Two tests for heterogeneity of Wr–Vr are available. One of these is t^2 test based on Wr–Vr graph. Significant t^2 value indicates the failure of the hypothesis. The second test is to analyze the Wr–Vr variance due to array and block differences when Wr and Vr are computed replicationwise. A significant array effect also indicates failure of the hypothesis.

In J x J crosses, both t^2 and variances due to arrays in Wr–Vr ANOVA were nonsignificant after eliminating the interacting arrays. In I x J crosses, though t^2 was nonsignificant, variances due to Wr–Vr of arrays were significant for MTCB as well as FDC. A similar situation was observed for FDC in I x I crosses (Table 6).

Jinks [20] and Hayman [16] have shown that regression of Wr on Vr will be a line of unit slope if two assumptions hold true: a) genes at different loci are randomly distributed

Table 6. Analysis of variance of Wr–Vr estimates for 5 x 5 indica x indica (I x I), 5 x 5 japonica x japonica (J x J), and 7 x 7 indica x japonica (I x J) crosses

Source	d.f.			MTCB			FDC		
	I x I	J x J	I x J	I x I	J x J	I x J	I x I	J x J	I x J
Wr–Vr array	4	6	(4)	5.91	148.32**	(1.12)**	0.00008**	0.002**	(0.0002)**
differences			3			0.19			0.000003
Wr–Vr block	10	14	(10)	4.29	5.81	(0.07)	0.00001	0.00004	(0.00001)
differences			8			0.17			0.000001

Figures in parentheses refer to interacting sets.
**Significant at P = 0.01.

among the parents, and b) there is no interaction between the genes at different loci. Failure of either of these assumptions leads to departure from the rectilinear relation of Wr and Vr. Caughtrey and Mather [22] and Jana [23], on the other hand, pointed out that with additive x additive type interaction alone or with dominance x dominance type of interaction alone, as well as in the presence of gene association, the array points scatter along the regression line of unit slope when p=q=0.5. A line of unit slope is, therefore, not a completely unequivocal indication of the absence of nonallelic interactions. Hence, for detection of epistasis in the three diallel sets (MTCB and FDC) in I x J; and FDC in I x I, where array variances of Wr–Vr were significant, arrays were eliminated one by one and the data reanalyzed. Nevertheless, the variances of Wr–Vr due to arrays remained significant. Even after removal of the most scattered arrays based on the Wr–Vr graph and subsequent removal of each of the remaining arrays, in turn, as suggested by Hayman [16], the situation did not improve. However in I x J crosses, for FDC, when var. Stejaree 45 was removed, the degree of dominance changed from overdominance to partial dominance. In contrast, for MTCB, when var. Bir-ze-goo was removed, the degree of dominance changed from partial to mild overdominance. In both situations, however, array differences of Wr–Vr still remained significant. Thus, the apparent overdominance observed in some crosses may not be attributable to epistasis, rather, it may be a situation of pseudo-overdominance due to linkage [24], as in japonica cultivars.

According to Jana [23], in a small diallel cross (as in the present study, i.e. 7 x 7 and 5 x 5), nonrandom distribution of genes among the parents is normally expected to cause serious disturbances in the Wr–Vr graph. Jinks [25] also emphasised the Wr–Vr graph while eliminating the confounding effects between correlated gene distribution and linkage. The insignificant departure of regression coefficients from unity in the present investigation, therefore, also rules out the presence of correlated gene distribution among the parents. Hence, the variations in Wr–Vr due to arrays in all the three aforementioned nonepistatic sets (I x J cross for MTCB and FDC and I x I cross for FDC) can only be accounted for by multiple allelism in polygenic system [17].

The genetic situation in the present study now, can be better understood first by considering an example of a monogenic model.

Here, the cold tolerance genes (T) have been designated separately from those for MTCB (C) and FDC (S) genes. With regard to chloroplast biogenesis, no variation is expected among the cultivars as all of them carry the dominant allele C (for normal chloroplasts). The recessive allele C is expected to exist in different forms of albinos [26]. Thus, the variation among cultivars at vegetative phase in the present study was controlled primarily by cold tolerant genes. The delineation of the Wr–Vr graph as well as the standardized deviation graph (Yr, Wr + Vr) in I x J crosses (Fig. 1, G and 2, B) exhibited three distinct groups for MTCB. The cold tolerant japonica cultivars Stejaree 45, Barkat and Ching shi 15 formed a cluster near the origin. The cold tolerant indica cultivars China 1039 and Bir-ze-goo formed another cluster in the middle, whereas cold susceptible indica cultivars

IR 8 and IR 64, forming a separate group, were farthest from the origin. Thus, the situation in different allelic forms can be represented to tolerant japonica: T'T', tolerant indica: TT, and susceptible indica: tt; and in combination with the genes for chloroplast biogenesis as: T'T' CC, TTCC, and ttCC, respectively. The gene action, however, was not in complete dominance but in partial dominance range.

This model now can be extended to the polygenic level. To explain the variability within a specific tolerance group, it may be assumed that in cold tolerant japonicas both T' and T alleles are present and similarly the cold tolerant indicas carry both T and t alleles. The level of tolerance of a particular cultivar in a specific group depends on the proportion of these dominant alleles at various loci controlling the cold tolerance mechanism. It is argued that cold tolerant japonica cultivars evolved from the tropical indica race [27]. It appears that change from cold susceptible indica types to cold tolerant indica types and then to more cold tolerant japonica types could have been due to dominant mutation(s) at t locus, and this mutation facilitated the spread of rice cultivars from their original habitat of hot humid tropics to higher and cool elevations of mountainous subtropics and ultimately two temperate regions. Thus, this assumption bears an evolutionary significance. Based on this, it can be concluded for the present study that for MTCB only T and t alleles were present in I x I crosses and only T' and T alleles in J x J crosses. On the other hand, all the three allelic forms, viz., T', T', and t, were present in I x J crosses, and hence a significant variation in Wr–Vr array differences was observed due to multiple alleles in polygenic system.

The genetic situation at reproductive phase was quite different due to involvement of a separate trait, i.e., FDC. Generally, the spikelet sterility is an important trait at reproductive phase for cold tolerance breeding programme in rice [8, 28].

Over the past decades, the genetic sterility in distant crosses of rice has been mainly ascribed to genic differences [29]. While investigating the compatibility relationship among indica, japonica and javanica rices, Ikehashi and Araki [21] reported a set of multiple alleles at S locus for spikelet fertility. Javanica and indica cultivars have been classified into several compatible groups with regard to this allelic relationship. In general, for the S locus, the indica cultivars have been designated as S^iS^i, the japonica cultivars as S^jS^j and the intermediate ones, which are equally cross compatible with indicas and japonicas, as S^nS^n.

In I x I crosses (Fig. 1, B and 2, A) the five cultivars can be classified into three groups for FDC, viz., a) China 1039 wih $S^{it}S^{it}$ (indica tolerant) alleles, b) CBI, IR 8, and IR 64 with S^iS^i alleles, and c) Bir-ze-goo with S^nS^n alleles. This was clear in I x J crosses (Fig. 1, H and 2, C). Out of the three japonica cultivars, Ching shi 15 and Stejaree 45 formed a separate group (S^jS^j). The other japonica cultivar Barkat was very close to indica tolerant cultivar China 1039. Both these cultivars are adapted to north Indian hilly areas, and though Barkat is a japonica cultivar by its morphological attributes, it is a derivative of an indica x japonica cross [6]. Thus, it may be assumed that in relation to cold tolerance, var. Barkat carries the japonica tolerant allele (T'), but in relation to spikelet fertility it

appears to be close to the indica tolerant cultivar China 1039, and hence harbouring S^{it} allele. Variety Bir-ze-goo formed a separate group in I x J crosses. It is worth mentioning in this connection that var. Bir-ze-goo is cold tolerant only at vegetative phase but susceptible at reproductive phase [9]. This variation in response to cold stress has been explained in terms of differences in metabolism of polyunsaturated fatty acids in polar lipids by esterase 2 isozyme modifier at the two different growth phases [30]. IR 8 and IR 64 are cold susceptible indica cultivars. They exhibited more or less similar performance for mean FDC (Table 2), but differ in their degree of cold susceptibility. This variation also has been explained by Majumder et al. [30] by differences in esterase 2 isozyme modifiers. Thus, it is expected that var. IR 8 and IR 64 carry the same alleles for spikelet fertility. Therefore, the response of all the fertility alleles to cold stress in the present study according to their dominance relationships, as depicted by the Wr–Vr graph (Fig. 1, H), can be represented as follows :

$S^{it}S^{it}$ (China 1039, Barkat)	>	S^{i} (IR 8, IR 64)	>	S^{j} (Ching shi 15, Stejaree 45)	>	S^{n} (Bir-ze-goo)

with the cold tolerance allele the situation would be $TTS^{it}S^{it}$, $ttS^{i}S^{i}$, $TTS^{j}S^{j}$, and $TTS^{n}S^{n}$, respectively.

For FDC, three alleles for spikelet fertility were present in I x I crosses, and thus revealed a significant variation in Wr–Vr array differences. A more complex situation was observed in I x J crosses for FDC, where multiple alleles were present both for cold tolerance and spikelet fertility, and hence a significant variation in Wr–Vr array difference was recorded. Thus, all the three situations, i.e., I x J crosses for MTCB and FDC, and I x I crosses for FDC, can be explained well considering the phenomenon of multiple allelism.

It can also be seen that though a cold tolerant japonica cultivar (e.g. Stejaree 45) carries dominant alleles for cold tolerance, it has recessive alleles for spikelet fertility. Thus, the F_1 population of a cross involving cold tolerant japonica and a cold susceptible indica would exhibit tolerance at vegetative phase, but the manifestation of S^{i} and S^{j} alleles at reproductive phase causes spikelet sterility. Probably, that is why most of the genetic studies for cold tolerance in rice have concluded that the genetic mechanism for cold tolerance at vegetative phase is independent from that at reproductive phase. The present investigation, on the other hand, suggests that the genetic mechanism for cold tolerance is similar both at vegetative and reproductive phases. However, a more clear picture of this problem can be obtained if a trait like level of unsaturated fatty acids in polar lipids, which can be scored at both growth phases, is taken into consideration during such genetic analysis. It has been recently shown that cold tolerance in rice is highly positively correlated with polyunsaturated fatty acid level in polar lipids, particularly with linolenic acid content [28, 30]. Graef et al. [31] identified four alleles at a single locus that controlled stearic acid (precursor of unsaturated fatty acid) content in soybean seeds.

A BREEDING STRATEGY

From the above discussion an important strategy emerges with regard to cold tolerance breeding in rice. The partial F_1 sterility in indica x japonica crosses is considered to be due to abortion of gametes [29, 32]. Accordingly, and also from the results of the present investigation, direct transfer of cold tolerance from japonica into indica genetic background appears to be a difficult task. Past studies have also shown that some cultivars give good F_1 fertility in their crosses with indicas as well as with japonicas [2, 33, 34]. Ikehashi [3] designated these cultivars as WCVs and suggested their use as bridge parents in indica-japonica hybridization to overcome the reproductive barrier. The present investigation also supports the view of Beachell et al. [2] and Ikehashi and Araki [21] about the need of WCVs as bridge parents for success in cold tolerance breeding involving indica and japonica cultivars. A schematic representation of this breeding strategy is as follows:

Cold tolerant japonica x WCV

↓

F_1 x High yielding indica

↓

Selection for cold tolerant segregants using two-tier screening as in Majumder et al. [30].

↓

Selfing and pedigree handling of succeeding generations.

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