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Heterosis in Traits Related to Low Temperature Tolerance in Rice

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The manifestation of heterosis for 17 traits related to low-temperature tolerance and productivity in rice in 102 F_1 hybrids (42 for germination coefficient) at 5 growth stages is reported. Average heterosis of 163.0% for cold tolerance index at final survival, 61.24% for germination coefficient, 50.62% and 39.92% for cold tolerance indices based on revival 10 days and 5 days after treatment at the seedling stage, and 69.32% and 54.59% for cold tolerance indices at 22 and 15 days survival, at the early seedling stage was recorded over the mid-parent. Heterosis for cold hardiness was greater for the *japonica/indica* group of hybrids at the early seedling stage and for the *indica/indica* group at the seedling stage. Number of fertile spikelets and fertility showed negative average heterosis at booting and flowering stages of low-temperature treatment. They hybrids were intermediate in blooming and showed no advantage for seedling height on the 15th and 22nd days of cold stress.

Intercharacter correlations among hybrids indicated that the parental averages for tolerance indices at revival 10 days and 5 days after treatment were most highly associated with percent fertility at both booting and flowering stages of cold stren in low temperature tolerance evaluation studies. Correlations between the mid-parent mean and hybrid performance were highly significant for 13 of the 17 traits. The coefficients for cold tolerance indices at 15 days survival, revival 10 days and 5 days after treatment, at 22 days survival and at final survival, and flowering duration were of sufficient magnitude to indicate that mid-parent values would be highly effective indicators of hybrid performance.

Key words: hybrid vigor, heterobeltiosis, cold tolerance index, germination coefficient, emergence coefficient.

For further improvement in the yielding ability of rice, utilization of heterosis may set up new levels of varietal productivity. F_1 rice hybrids in China have registered 20-30% yield advantage over nonhybrid rice varieties and are cultivated in about six million hectares (Virmani et al, 1981). The hybrids possess wider adaptability, vigorous vegetative growth, better tillering and panicle size, besides better adaptation to day length and temperature variations (Lin and Yuan, 1980). Jennings (1967) suggested that heterosis may be of greater significance in temperature rice improvement where further increases in yield may be difficult to achieve.

Heterotic manifestations reported in rice (Virmani et al, 1981) include earlier blooming, greater height, greater tillering and more grains per panicle, heavier seeds, and higher yields of grain. The superiority of F_1 hybrids with regard to physiological traits such as photosynthetic area, chlorophyll content per unit area, photosynthetic efficiency, mitochondrial activity, root activity and lower photorespiration intensity has also been reported in China (Lin and Yuan, 1980). While some hybrid combinations are reportedly adaptable from 18°N to 34°N latitude in China and from elevations ranging from 500 to 1,200 m in the mountainous regions, information does not seem to be available regarding manifestation of heterosis in traits associated with adaptability in the cooler climates.

The research reported here was designed to elucidate information regarding heterotic response of the traits associated with low temperature adaptability and tolerance in rice in evaluation studies at different stages of plant growth.

MATERIALS AND METHODS

Seventeen rice cultivars and elite lines having low temperature tolerance at different growth stages were crossed as female parents to six high yielding and/or blast-resistant elite lines from IRRI's on-going breeding program as male parents, to generate 102 hybrid populations. The female parents (9 *japonica* and 8 *indica*) were selected on the basis of ecogeographic diversity. They originated from India (Kashmir and Assam), Korea, China, Japan, Indonesia, and USSR. A total of 299,500 spikelets were crossed during February-April, 1984, resulting in a total seed set of 165,000 (overall seed set of 55%), averaging 1,617 seeds per cross-combination within a range of 747 to 2,934 seeds produced for different hybrids.

The female parents used in the crossing program were the *japonicas* Suweon 235 (Sangpungbyeo), SR-5204-91-4-1, SR3044-78-3, Barkat (K78-13), K332, K84, Shimokita, Stejaree 45, and Anna; and the *indicas* Suweon 287 (Taebaegbyeo), Samgangbyeo, China 988, K39-96-3-1-1-2, Leng Kwang, Shoa-Nan-Tsan, Silewah and ARC 6000. The six pollinators used as testers to cross with each of the females (lines) in a line x tester fashion were indica type elite IRRI lines IR8866-30-1-4, IR8455-K2, IR15889-32-1, IR7167-33-2-3, IR29506-60-3-3-2, and IR9202-10-2-1-5-1. The 102 hybrids and the 23 parents were evaluated for low temperature tolerance in the controlled environment at 5 different stages of growth, viz., germination, early seedling, seedling, booting and flowering, as well as in the cold water screening nurseries in the field at Chuncheon, Korea, and Swat, Pakistan. However, the data pertaining to only the controlled environment are presented. Heterosis for the F_1 hybrids was worked out as F_1 mean minus mid-parent or high parent mean, divided by either of the 2 latter values, multiplied by 100 (Weber et al, 1970). The screening procedures relevant to this study were as follows:

Experiment I – Germinability at low temperature

Because of space limitation, only 42 *japonica/indica* hybrids involving seven female parents from the *japonica* group other than Anna and K84 and six IRRI lines used as testers, as well as 13 parents were evaluated for germination ability at low temperature (15°C) in the Koitotron KG cabinets of the phytotron at IRRI. The rice seeds were carefully selected for uniformity of size and shape, surface sterilized with 0.2% mercuric chloride solution for 1 min, and then thoroughly washed with distilled water. Replicated lots of 15 seeds per line or F_1 were placed, with embryos down, on a double layer of Whatman no. 1 filter paper in a 9-cm-diameter sterilized glass petri dish. The paper was moistened with 10 mL sterile distilled water and the dish was covered. Three replications were adopted and the dishes were randomized within the growth cabinets. The measurements on vigor of germination (germination percentage) and speed of germination (the average number of days to germination) were started the 3rd day onwards and were made at 24-hour intervals on seed germinated in darkness for 15 days. For consistency, parental seeds with hulls removed by hand were used and a seed was considered as having germinated when it had developed a radicle approximately 2 mm in length. Any seed which had not germinated by the 15th day was scored as inviable. The low temperature germinability (LTG) was represented by germination coefficient which was calculated by dividing germination percentage by average number of days required for germination (Sasaki, 1983).

Experiment II – Cold hardiness at early seedling stage

A total of 125 entries comprising 102 F_1 hybrids and 23 parents were evaluated for low temperature tolerance from seedling emergence to 3-leaf stage in the Koitotron KG cabinets of the phytotron. The seeds were surface sterilized and soaked as in Experiment I and incubated for 24 h at 30°C. Pre-sprouted seeds were sown in soil-filled porcelain trays with adequate supply of nutrients and water, then transferred to and kept in artificially lighted cold cabinets at 15°C for 3 weeks. Twelve pre-sprouted seeds were sown for each culture in each of two replications. Each tray contained 10 test cultures and the constant resistant check variety Fujisaka 5 allocated at random.

Emergence data were recorded each day up to 12 days. The seedlings were scored as having emerged when the shoot tip was visible on the soil surface. Based on speed of emergence and vigor of emergence, an emergence coefficient was worked out. Cold tolerance scores of the cultures and seedling height were recorded on the 8th, 15th and 22nd day of treatment (only the latter two are reported). Survival percentage of the emerged seedlings were also recorded on the 15th and 22nd day of treatment. To obtain the survival score, a plant was scored 0 when dead, 0.5 when severely damaged but living, and 1 when it showed little or no damage (Amirshahi and Patterson, 1956). The sum of these scores on about 12 plants was converted to a percentage of the total possible score. The length and the percentage of the surviving seedlings from seeds planted were calculated as percent of the check Fujisaka 5, which was included in each tray. Combining cold tolerance score based on leaf discoloration and survival percent, a cold tolerance index was worked out (Kaw and Khush, 1985a) which allowed quantifying the data on cold tolerance and also rating the genotypes for relative cold stability. Emergence coefficient, seedling height and tolerance indices were the criteria for evaluating genotypes for low temperature tolerance at the early seedling stage.

Experiment III – Cold hardiness at seedling stage

Pre-sprouted seeds of 102 F_1 hybrids and 23 parents were sown in porcelain trays filled with 5 kg of Maahas clay soil fertilized with 5 g ammonium sulfate in the screenhouse at 30°C temperature. Each tray contained a single row of about 12 plants of each of the 10 test cultures and the resistant check Fujisaka 5. Each set of 125 entries was accommodated in 13 trays forming one replication. The 10-day-old seedlings in the trays were assigned to 150 x 150 x 40 cm steel tanks filled with cold water held at 12°C by a double acting thermostat. Three such tanks were used each representing a replication and housing one set of 13 trays at a water depth of 3-4 cm from the soil surface. Percent establishment was recorded for each entry before the cold water treatment.

The plants were removed after 10 days of cold water stress in the tanks, scored for cold tolerance as per Standard Evaluation System (IRRI, 1980), and allowed to revive in the glasshouse for 10 days. The revival score was made twice, at 5 and 10 days after treatment on a 0-1 scale as in Experiment II. Most of the F_1 hybrids were observed to revive fast and appear green. Therefore, to get a critical assessment of the resistance or susceptibility of the cultures to cold water, two of the three replications, because of space limitation, were subjected to a second cold-water treatment and rated for survival after 10 days in the same manner as for revival, immediately after removal from cold water stress. The tolerance index was worked out based on revival/survival of plants, and the cold tolerance score as described earlier.

Experiment IV – Low temperature stress at booting stage

About 45-50 naked seeds of each of the 102 F_1 hybrids sterilized, soaked, and incubated for 24 hours, were sown in 5- x 5-cm Rapid Generation Advance (RGA) plastic pots, one seed per pot in the screenhouse. The 23 parental seeds were incubated for 48 h before sowing to maintain parity in sprouting as the naked F_1 seeds sprouted 24 h earlier. The RGA pots were filled up to demarcation with Maahas clay soil fertilized with 1 g ammonium sulfate, 0.5 g solofos, and 0.5 g muriate of potash per kg of soil. One-month-old normal looking and healthy plants (30 plants per culture) were transferred to the glasshouse chamber of the phytotron under a day/night temperature regime of 29°C/12°C, relative humidity of 70/70° ± 1 and natural daylength conditions. Out of 30 plants, 10 plants per entry composed a set. Two such sets represented two replications, to be subjected to low temperature treatment. The remaining 10 plants represented the control. These two sets were placed in large steel trays at random, four trays housing one replication. To facilitate development of uniform single-culm plants for the evaluation studies, so that the differences in tillering between parents and hybrids would not confound the results, all side tillers were removed from 3 weeks after sowing to about a week before heading. Plants earmarked for treatment were kept in the dark room from 1700 hours to 0700 hours for 5 days at a temperature of 15°C ± 1°C, at -5 to 0 auricle distance between auricles of the last two leaves. Auricle 0 means overlapping auricles; auricle distance -5 means that the auricle of the flag leaf is located 5 cm below the auricle of the penultimate leaf. At the end of the treatment period, the test cultures were allowed to flower and mature in the glasshouse at 29°C/21°C. The plants were tagged for time of flowering two times a day. Panicle length was measured at harvest and the panicles were bagged for count of fertile and sterile spikelets in each panicle for computation of fertility percent. For each genotype, the rate of depression caused by low temperature stress in number of fertile spikelets and percent fertility was computed as the difference between control and treated plants and transposed to a percentage of the control. Variance analyses for percentage heterosis were calculated. Correlation coefficients between hybrid and mid-parent values were calculated from the original means.

Experiment V. Low temperature stress at flowering stage

The sowing method and the management practices were the same as those in Experiment IV. The cultures were grown in the glasshouse under natural daylength and 25°C/21°C temperature regime, except during cold treatment. At first indication of panicle exsertion, the plants were subjected to cold treatment in the 3-SAL naturally lighted cabinets in the phytotron at 20°C/15°C from 0600-1800/1800-0600 hours at 70/70° + 1% R.H. After the treatment, the plants were allowed to mature in the glasshouse for data collection at maturity. All computations were made as above.

RESULTS AND DISCUSSION

A portion of the analyses of variance pertinent to the heterosis considerations, together with indicated levels of significance for the F tests, is presented in Table 1. Highly significant differences among parents and hybrids were indicated for all the characters. Parents vs. hybrids mean square were highly significant for most of the characters, except for seedling height and cold tolerance indices based on 22 days survival at early seedling stage and at revivals 5 and 10 days after treatment at the seedling stage. Mean square for parents vs. hybrids and average heterosis may serve as a measure of nonadditive gene effects (Kirby and Atkins, 1968). The nonsignificance of F-tests for the parents vs. hybrids mean square for the above characters appears to signify that the expression of these traits is governed to a large extent by the additive effects of genes. Indications of appreciable nonadditive effects of genes were obtained for germination coefficient, tolerance index based on 15 days survival at early seedling stage, tolerance index at final survival at seedling stage and panicle length, number of fertile spikelets and percent fertility at both the booting and flowering stages, where high heterosis values were observed, besides highly significant comparison of parents vs. hybrids. Significance for the parents vs. hybrids mean square was also obtained for emergence coefficient and flowering duration. For these traits, however, the heterosis values were smaller and differences between the parental and hybrid means would seem of limited consequence.

The tabulations of the levels of heterosis on an individual cross basis being too voluminous to be presented herein, a summary of salient features for 17 characters related to cold tolerance and productivity is shown in Table 2. Since japonica type cultivars reportedly are more cold hardy at the vegetative stage than the indica type (Chang, 1970; Heu and Bae, 1972; Kaneda and Beachell, 1974; Chuong and Omura, 1982; and Tseng and Teng, 1982), an attempt was made to classify the various hybrids in groups of japonica/indica and indica/indica crosses to ascertain the inherent differences in heterosis between the two groups for various traits. Average heterosis to the extent of 163.0% for tolerance index at final survival, 50.62% and 39.32% for tolerance indices based on revival 10 days and 5 days after treatment at the seedling stage were recorded over the mid-parent value in the indica/indica group of hybrids (Table 2). In the japonica/indica group also, the highest mid-parent hybrid advantage was shown for tolerance index on final survival (113.30%), followed by tolerance index on 22 days survival (69.32%), germination coefficient (61.24%), tolerance index on 15 days survival (54.59%), tolerance index at revival 10 days after treatment, panicle length at flowering stage (20.36), etc. The japonica/indica group of hybrids demonstrated greater heterosis for cold tolerance indices at the early seedling stage and the indica/indica group at the seedling stage. Number of fertile spikelets and percent fertility exhibited negative average heterosis, suggesting the presence of dominance in the negative direction as one cause of the manifestation of heterosis. The hybruds were intermediate in flowering and showed no advantage for seedling height on the 15th and 22nd day of low-temperature treatment

Table 1. Analysis of variance and indicated levels of significance for 17 traits measured on 102 hybrids (42≠) and 23 parents (13≠) during cold tolerance evaluation at different stages.

Trait[a]	Source of variation				
	Treatments	Parents	Hybrids	Parents vs. hybrids	Error mean square
A. Germination stage (df)	54	12	41	1	108
1. Germination coefficient	**	**	**	**	8.41
B. Early seedling stage (df)	124	22	101	1	124
2. Emergence coefficient	**	**	**	**	3.97
3. Seedling ht. on 15th day	**	**	**	NS	0.53
4. Seedling ht. on 22nd day	**	**	**	NS	1.85
5. T. I. on 15 days survival	**	**	**	**	0.01
6. T. I. on 22 days survival	**	**	**	NS	0.11
C. Seedling stage (df)	124	22	101	1	248
7. T.I. on revival 5 DAT	**	**	**	NS	0.01
8. T.I. on revival 10 DAT	**	**	**	NS	0.01
(df)	124	22	101	1	124
9. T.I. on final survival	**	**	**	**	0.07
D. Booting stage (df)	124	22	101	1	124
10. Flowering duration	**	**	**	**	0.37
11. Panicle length	**	**	**	**	0.25
12. Number of fertile spikelets	**	**	**	**	8.83
13. % Fertility	**	**	**	**	3.21
12a. Number of fertile spikelets (depression rate)	**	**	**	**	29.49
13a. % Fertility (depression rate)	**	**	**	**	15.54
E. Flowering stage (df)	124	22	101	1	124
14. Flowering duration	**	**	**	**	0.43
15. Panicle length	**	**	**	**	0.40
16. Number of fertile spikelets	**	**	**	**	4.44
17. % Fertility	**	**	**	**	1.61
16a. Number of fertile spikelets (depression rate)	**	**	**	**	13.53
17a. % Fertility (depression rate)	**	**	**	**	4.89

**Significant at the 1% level of probability. NS = not significant. [a] T.I. - Tolerance index , DAT = days after treatment.

≠ for germination coefficient

Table 2. Hybrid performance of F_1 rice hybrids for traits related to cold tolerance at different stages of growth.

Character[b]	Group[c]	Range of heterosis (%)[a]		Mean heterosis (%)		% F_1s< MP	MP≤ % F_1s≤ HP	% F_1s> HP	% F_1s with sig. negative heterosis		% F_1s with sig. positive heterosis	
		MP	HP	MP	HP				MP	HP	MP	HP
A. Germination stage:												
1. Germination coefficient	JxI	-7.4-130.7	-15.1-106.3	61.24	42.39	4.76	4.76	90.48	0.00	0.00	85.71	73.81
B. Early seedling stage:												
2. Emergence coefficient	JxI IxI	-22.6-68.7 -33.8-61.0	-30.0-49.0 -43.1-56.8	9.51 6.07	- 0.30 - 5.44	39.6 42.6	14.6 22.2	45.8 35.2	14.58 20.37	31.25 35.18	37.50 29.63	22.92 12.96
3. Seedling ht. on 15th day	JxI IxI	-38.4-59.1 -42.4-23.1	-40.6-41.3 -51.9-17.8	16.64 - 9.28	4.63 -19.09	18.7 68.5	25.0 16.7	56.3 14.8	4.17 44.44	14.58 55.55	56.25 18.52	29.17 1.85
4. Seedling ht. on 22nd day	JxI IXI	-11.6-43.9 -33.2-151.3	-21.6-38.4 -36.5-19.9	15.54 - 8.43	5.35 -16.69	10.4 72.2	25.0 16.7	64.6 11.1	0.00 25.92	6.25 44.44	39.58 3.70	12.50 1.85
5. T.I. on 15 days survival	JxI IxI	-46.1-131.6 -64.4-151.3	-67.1-50.0 -71.3-128.6	54.59 - 4.00	- 2.43 -23.10	8.3 64.8	54.2 24.1	37.5 11.1	2.08 0.00	14.58 3.70	77.08 24.07	14.58 1.85
6. T.I. on 22 days survival	JxI IxI	-48.3-239.7 -84.9-228.3	-70.4-101.9 -86.4-186.0	69.32 1.30	1.14 -22.41	18.8 66.7	35.4 14.8	45.8 18.5	0.00 0.00	0.00 0.00	29.17 5.55	4.17 0.00
C. Seedling Stage:												
7. T.I. on revival 5 DAT	JxI IxI	-10.5-59.0 - 6.2-143.0	-42.8 to-6.4 -43.4-100.3	20.07 39.32	-25.67 9.53	6.2 7.4	93.8 57.4	0.0 35.2	0.00 0.00	83.33 20.37	45.83 51.85	0.00 9.26
8. T.I. on revival 10 DAT	JxI IxI	-0.6 -71.2 -9.3-181.6	-40.2to-1.3 -42.3-111.3	24.95 50.62	-24.12 4.84	2.1 1.8	97.9 53.7	0.0 44.5	0.00 0.00	75.00 51.85	52.08 20.37	0.00 7.41
9. T.I. on final survival	JxI IxI	7.8-413.7 -65.0-635.0	-43.5-204.1 -78.5-473.4	113.30 163.0	18.57 93.5	0.0 20.4	47.9 9.2	52.1 70.4	0.00 0.00	6.25 0.00	72.9 14.81	16.67 3.70

Table 2 continued.

Character [b]	Group[c]	Range of heterosis(%)[a] MP	Range of heterosis(%)[a] HP	Mean heterosis (%) MP	Mean heterosis (%) HP	% $F_1s <$ MP	$MP \leq$ % $F_1s \leq$ HP	% $F_1s >$ HP	% F_1s with sig. negative heterosis MP	% F_1s with sig. negative heterosis HP	% F_{1s} with sig. positive heterosis MP	% F_{1s} with sig. positive heterosis HP
D. Booting stage												
10. Flowering duration	JxI	-11.4-18.8	-30.2-3.7	2.36	-7.48	41.7	41.7	16.6	33.33	79.17	47.92	8.33
	IXI	- 8.6-43.9	-19.0-24.8	2.72	-2.05	42.6	18.5	38.9	40.74	59.26	48.15	31.48
11. Panicle length	JxI	- 9.4-43.6	-22.5-16.8	12.95	-2.07	8.3	56.3	35.4	2.08	37.50	79.17	16.67
	IxI	-13.8-31.6	-18.0-18.5	5.35	0.90	25.9	25.9	48.2	16.67	25.92	51.85	29.63
12. Number of fertile spikelets	JxI	-95.5-35.5	-96.0-31.4	-46.05	-53.00	91.7	2.1	6.2	83.33	91.67	6.25	4.17
	IxI	-68.2-74.4	-70.6-51.9	7.84	- 2.84	33.3	16.7	50.0	24.07	40.74	44.44	35.18
13. % Fertility	JxI	-96.5to-27.8	-96.5to-37.3	-61.64	-65.35	100.0	0.0	0.0	100.0	100.00	0.00	0.00
	IxI	-75.3-44.9	-79.6-44.0	- 3.42	- 9.61	44.4	20.4	35.2	37.04	50.00	46.30	27.78
E. Flowering stages												
Flowering duration	JxI	-10.7-21.7	-30.6to-0.5	3.97	-13.49	27.1	70.8	2 .1	20.83	93.75	64.58	0.00
	IxI	-14.9-20.9	-17.4-12.8	0.69	- 4.54	53.7	20.4	25.9	44.44	66.67	38.89	18.52
Panicle length	JxI	-4.0-44.6	-11.1-40.8	20.36	14.51	4.2	10.4	85.4	0.00	4.17	87.50	72.92
	IxI	-23.7-55.2	-28.6-49.6	9.01	- 0.90	33.3	22.2	44.4	16.67	40.74	50.00	31.48
Number of fertile spikelets	JxI	-99.4-43.6	-99.5-30.3	-65.76	-71.17	89.6	8.3	2.1	87.50	93.75	8.33	2.08
	IxI	-97.2-80.2	-98.0-31.0	-11.45	-32.36	63.0	18.5	18.5	42.59	77.78	22.22	7.41
% Fertility	JxI	-99.6to-15.8	-99.7to-34.2	-78.05	-81.36	100.0	0.0	0.0	100.00	100.00	0.00	0.00
	IxI	-96.9-31.5	-97.6-9.04	-22.90	-31.44	61.1	18.5	20.4	57.41	74.07	31.48	55.55

[a] MP = mid-parent, HP = high-parent. [b] T.I. = tolerance index, DAT = days after treatments. [c] J = japonica, I = indica

at early seedling stage.

In germination coefficient, 90.48% of the hybrids surpassed the high parent, 73.81% of them significantly so (overdominance). Averaged over all crosses, 50.98% of hybrids expressed significant heterobeltiosis for main panicle length at flowering stage and 23.53% at booting stage evaluation, 20.59% for number of fertile spikelets and 14.70% for percent fertility at booting, 17.65% for emergence coefficient, 14.70% for seedling height on the 15th day, 9.80% for tolerance index based on final survival and others. Compared to the late parent, 20.59% of the hybrids had significantly delayed flowering at booting stage of screening and 9.80% at the flowering stage. Interestingly, all hybrids showed hybrid advantage for cold hardiness at vegetative stage as no hybrid had significantly lower cold tolerance than the mid-parent, except for 0.98% (1 out of 102 hybrids) for tolerance index at 15 days survival. On an overall basis, 50 percent of the hybrids, however, revealed significant negative heterosis (83.33% for japonica/indica group) compared to the high parent for tolerance index based on revival 5 days after treatment, 46.08% (75.0% for japonica/indica) for tolerance index based on revival 10 days after treatment, and 2.94% (6.25% for japonica/indica) for tolerance index based on final survival at the seedling stage. At early seedling stage, while no hybrid was significantly inferior to the high parent in tolerance at 22 days survival, only 8.82% (14.58% for japonica/indica group) were so for tolerance at 15 days survival. Compared to the mid-parent, 49.02% hybrids showed cold tolerance advantage (77.08% in japonica/indica group) by being significantly superior in tolerance index on 15 days survival and 16.67% (29.17% in japonica/indica group) in tolerance index on 22 days survival. At seedling stage, 49.02% of the hybrids for tolerance indices based on revival 5 days after treatment, 51.96% on revival 10 days after treatment and 42.15% at final survival had significantly superior cold hardiness than the mid-parent (dominance). For percent fertility, an attribute having a direct bearing on productivity, 86.27% of the hybrids (100% for japonica/indica group) at flowering stage screening and 73.53% (100% for japonica/indica group) at booting stage of screening demonstrated significant decrease over the high parent. A similar trend was also revealed for number of fertile spikelets, an attribute being primarily responsible for yield advantage of hybrids (Virmani et al, 1982). For number of fertile spikelets, however, the hybrids exhibited an overall range of -99.45 to 30.97% over the high parent at the flowering stage and -96.06 to 51.86% at the booting stage of low-temperature treatment. This suggests that selection among hybrids for heterotic response could be practiced effectively. Although only 20.59% hybrids in booting stage and 4.9% in flowering stage had significantly more spikelets than the higher parent, all were intermediate in earliness and flowered several days earlier than the high parent. This advantage for the hybrids is likely to be magnified in the temperate regions where the limited growing season poses a ceiling on the cultivation of highly productive, cold-susceptible, and late-maturing indica cultivars.

For exploitation of heterosis in crop improvement, only the heterosis in excess of the better parent is of significance. Average heterosis over the high parent, expressed by the hybrids involving a female parent in an array of crosses with the six common IRRI lines as pollinators, is presented in Table 3 for the 17 traits of each of the japonica and indica types. Examination of the actual means of the parents and their hybrids reveals a tendency for a relatively high percentage heterosis to be expressed by hybrids whose parents are comparatively low performing for the trait under consideration and vice versa. The tendency has not, however, been universal. The percentage heterosis appeared to be associated with differences in the "base" performance of the parental varieties *per se* as reported for sorghum (Kirby and Atkins, 1968) and cotton (Miller and Lee, 1964), because of the diversity of parents crossed. In general, hybrids of japonica females have revealed high magnitude of negative heterosis in cold tolerance indices, flowering duration, number of fertile spikelets, and percent fertility as compared with hybrids of indica females. This could be expected as the japonica parents in general flower earlier, have high vegetative stage cold hardiness, and produce high sterility in crosses with indica parents. The hybrids with indica females have on the other hand resulted in high negative heterosis for seedling height at 15 and 22 days of cold stress at the early seedling stage, because of their slow rate of growth under low temperatures.

Table 4 lists the highly fertile top 10 hybrids in each of the indica/indica and japonica/indica groups in the booting and flowering stage evaluations, as well as their cold hardiness indices at the vegetative stage. The indica/indica hybrids have demonstrated high fertilities as compared to the japonica/indica hybrids. In contrast, the japonica/indica hybrids have shown high vegetative stage cold tolerance as against the indica/indica hybrids (a score of 1 refers to cold tolerance equal to that of the resistant check) which agrees with the earlier reports. The only exception appears to be ARC 6000/IR7167 which has evidenced a moderately high level of vegetative stage cold tolerance, coupled with high fertility. This hybrid also happens to have maximum heterobeltiosis at the booting stage. At flowering stage evaluation, maximum high parent heterosis was shown by K39-96/IR15889, which, however, does not figure in the 10 highly fertile hybrids. In the japonica/indica group, the best high parent heterotic cross combination was K332/IR9202 at the booting stage screening and SR3044-78-3/IR9202 at the flowering stage screening. Both hybrids also showed comparatively high percent fertility. These results would suggest that both additive and nonadditive factors govern the expression of heterosis for fertility. Singh and Shrivastava (1982) have reported spikelet sterility to be conditioned by nonadditive gene action while Acharya and Sharma (1983) have emphasized the importance of both additive and dominance gene effects for spikelet fertility and other characters. The hybrids Stejaree 45/IR9202, SR3044-78-3/IR9202, Suweon 235/IR9202, K332/IR9202, Stejaree 45/IR29506, and SR5204-91-4-1/IR9202 could be listed besides ARC 6000/IR7167 as having potential of yielding cold tolerant and fertile lines after selection in later generations. Stejaree 45/IR9202 and

Table 3. High-parent heterosis of hybrids grouped as lines from japonica/indica and indica/ indica crosses, averaged over six common male parents for 17 traits in rice.

Female parents	Germination coefficient[a] 1	Early seedling stage 2	3	4	5	6	Seedling stage 7	8	9	Booting stage 10	11	12	13	Flowering stage 14	15	16	17
Japonica group																	
Suweon 235	51.3	9.4	24.4	15.3	- 6.8	- 5.6	-29.7	-29.7	44.6	-8.8	-5.3	-46.3	-63.4	-1.2	4.5	-73.3	-79.9
SR5204-91-4-1	45.5	-8.2	-8.9	-14.6	-12.2	-32.8	-20.9	-20.9	119.6	-6.8	0.4	-42.2	-53.8	-8.4	11.9	-62.7	-75.4
SR3044-78-3	48.7	13.6	17.1	7.2	- 0.5	-24.6	-31.8	-31.5	-27.0	-8.0	-2.8	-54.8	-64.0	-11.4	12.3	-67.3	-75.0
Barkat	37.9	-5.1	-11.0	- 3.3	- 7.1	-5.3	-21.4	-17.1	-11.7	-12.7	1.4	-61.5	-70.6	-16.4	16.8	-72.8	-82.3
K 332	44.2	11.5	10.7	11.8	0.6	18.7	-25.0	-24.0	24.5	-21.0	2.2	-60.4	-72.8	-21.7	20.0	-76.8	-86.7
Shimokita	44.6	-12.1	-13.3	2.52	6.1	-14.4	-32.5	-23.5	-11.1	-15.2	-3.3	-53.4	-63.9	-16.8	13.5	-72.5	-79.5
Stejaree 45	24.5	6.7	25.5	20.4	43.3	62.8	-23.8	-24.4	17.0	- 5.2	1.7	-39.5	-63.0	-10.1	26.7	-59.5	-82.1
Anna	-	-18.1	- 7.5	3.5	-42.8	10.5	-20.2	-21.8	- 7.4	- 8.1	-10.9	-66.0	-71.2	-10.8	10.4	-84.4	-89.9
K84	-	-12.6	-8.4	3.4	-6.0	39.7	-30.8	-30.8	39.2	-[illegible]5.3	4.2	-24.0	- 50.9	-13.5	17.1	-51.6	-73.5
Indica group																	
Suweon 287	-	-2.3	-19.9	-28.0	-36.2	-48.4	- 3.3	14.3	-67.9	-6.5	-5.5	5.6	10.0	-3.8	-3.4	-3.6	0.2
Samgangbyeo	-	20.0	-18.1	-24.6	-27.9	-45.5	11.6	18.0	-1.4	-6.0	-0.5	-2.8	-7.3	-6 .0	-6.9	-14.3	-6.2
China 988	-	-2.7	-18.0	-13.3	-41.4	-35.7	8.5	16.5	168.4	4.2	-1.6	0.2	0.5	-1.4	-17.8	-34.0	-14.0
K39-96	-	-2.8	-31.2	-20.1	-34.3	-25.7	72.5	77.1	261.4	10.3	-1.8	15.9	5.5	0.4	-13.6	-10.1	- 3.3
Leng Kwang	-	-15.8	-40.7	-29.1	-57.9	-46.3	-15.6	-15.1	38.6	-7.4	2.1	-3.7	-5.3	-1.4	7.4	-35.4	-37.3
Shoa-Nan-Tsan	-	-23.7	-34.3	-22.8	-39.0	-40.6	-15.5	-11.1	141.2	4.5	-9.0	12.6	-0.7	-5.1	-6.0	-31.0	-21.6
Silewah	-	0.2	-1.0	-11.5	- 3.9	-26.3	- 8.0	- 8.4	16.6	-5.8	8.5	- 6.8	-26.5	-6.7	19.7	-43.8	-65.3
ARC 6000	-	-9.2	-0.1	- 4.3	38.8	27.2	-14.3	-16.8	245.3	-6.3	4.5	-22.5	-11.9	-3.2	- 4.5	-67.4	-62.0

[a] Traits 1 to 17 the same as in Table 1.

Table 4. Top 10 ranking F_1 hybrids in % fertility at booting and flowering stress stages with the corresponding values for cold tolerance indices at early seedling and seedling stages.

Booting stage		Tolerance indices[a] at					Flowering stage		Tolerance indices[a] at				
Fertility (%)	Hybrids	5	6	7	8	9	Fertility (%)	Hybrids	5	6	7	8	9
Indica/indica crosses													
86.36	China 988/IR8866	0.170	0.101	0.553	0.520	0.248	79.41	Shoa-Nan-Tsan/IR7167	0.129	0.100	0.553	0.553	0.076
84.77	Shoa-Nan-Tsan/IR8866	0.108	0.109	0.669	0.631	0.398	75.41	China 988/IR9202	0.136	0.151	0.446	0.416	0.158
82.44	Leng Kwang/IR8866	0.104	0.122	0.692	0.675	0.482	74.87	China 988/IR7167	0.188	0.144	0.441	0.414	0.190
81.97	K39-96/IR8866	0.159	0.109	0.537	0.460	0.282	74.67	Leng Kwang/IR29506	0.237	0.098	0.662	0.662	0.249
81.49	Leng Kwang/IR29506	0.237	0.098	0.662	0.662	0.249	74.21	ARC 6000/IR7167	0.587	0.560	0.675	0.655	0.324
80.87	China 988/IR29506	0.167	0.103	0.599	0.545	0.195	72.07	Samgangbyeo/IR7167	0.265	0.147	0.353	0.312	0.047
80.20	K39-96/IR29506	0.194	0.206	0.605	0.598	0.318	71.44	Shoa-Nan-Tsan /IR29506	0.159	0.044	0.361	0.345	0.092
79.76	ARC 6000/IR7167	0.587	0.560	0.675	0.655	0.324	70.96	Suweon 287/IR7167	0.196	0.069	0.425	0.414	0.155
78.99	Shoa-Nan-Tsan/IR15889	0.182	0.116	0.587	0.606	0.085	70.27	Samgangbyeo/IR15889	0.147	0.099	0.359	0.334	0.041
78.74	K39-96/IR15889	0.122	0.118	0.598	0.580	0.334	69.88	Samgangbyeo/IR29506	0.141	0.083	0.255	0.242	0.096
Japonica/indica crosses													
57.70	Stejaree 45/IR9202	0.916	1.127	0.756	0.747	0.631	51.55	SR3044-78-3/IR9202	1.000	1.341	0.787	0.787	0.805
51.10	Suweon 235/IR9202	0.833	0.821	0.722	0.699	0.907	43.91	SR5204-91-4-1/IR9202	0.854	0.780	0.719	0.719	0.777
47.61	K332/IR9202	0.979	1.084	0.719	0.719	0.788	43.07	Stejaree 45/IR9202	0.916	1.127	0.756	0.747	0.631
46.40	Stejaree 45/IR29506	1.000	1.131	0.862	0.862	1.036	42.77	Suweon 235/IR9202	0.833	0.821	0.722	0.699	0.907
44.63	Stejaree 45/IR15889	1.000	0.954	0.753	0.753	0.879	42.37	Shimokita/IR9202	0.341	0.391	0.646	0.658	0.697
44.48	SR5204-91-4-1/IR29506	0.875	0.596	0.731	0.742	1.005	36.09	K332/IR9202	0.979	1.084	0.719	0.719	0.788
44.31	Shimokita/IR9202	0.341	0.391	0.646	0.658	0.697	30.82	Barkat/IR9202	0.854	1.134	0.813	0.813	1.065
43.24	SR5204-91-4-1/IR9202	0.854	0.780	0.719	0.719	0.777	30.02	SR3044-78-3/IR29506	0.956	0.604	0.821	0.798	0.913
42.68	Barkat/IR9202	0.854	1.134	0.813	0.813	1.065	27.80	Suweon 235/IR29506	0.608	0.560	0.725	0.719	0.892
40.32	Shimokita/IR29506	0.581	0.517	0.821	0.821	0.919	27.78	Shimokita/IR29506	0.581	0.517	0.821	0.821	0.919

[a] Traits 5 to 9 as in Table 1.

SR3044-78-3/IR9202 have earlier demonstrated least depression and good stability of performance under cold stress (Kaw and Khush, 1985b).

Since vegetative stage cold hardiness and high fertility are the attributes of primary importance in the adaptability of a cultivar under cool climates, the relationship between these characters may be of value in determining useful avenues for indirect selection. To this end, intercharacter associations among hybrids and parents were worked out between some important traits for cold tolerance at the early seedling and seedling stages and number of fertile spikelets and percent fertility at both booting and flowering stages of cold stress, based on original data and the depression data. The correlations are given in Table 5. As mentioned, the difference between values obtained for number of spikelets and fertility under normal and low temperatures, expressed as percentage of the normal, was called the depression rate and uses as a measure of the cold stability of the character within a genotype.

within a genotype.

Tolerance index based on final survival and tolerance index based on 15 days survival were the characters most highly and negatively associated with number of fertile spikelets and percent fertility. Seedling heights on the 15th and 22nd day also revealed highly significant negative relationship with fertile spikelets and fertility. These results seem to suggest that any increase in number of fertile seeds and percent fertility would be associated with a corresponding decrease in the level of cold tolerance at the vegetative stage. The depression in number of fertile seeds and percent fertility were also highly correlated with tolerance indices based on final survival and on 15 days survival at both booting and flowering stages, and with seedling heights on the 15th and 22nd day and tolerance indices for revival at 10 and 5 days after treatment at the flowering stage. It appears, therefore, that high level of cold tolerance at the vegetative stage is likely to accompany relatively large amounts of depression due to cold stress in the characters number of fertile seeds and percent fertility. Figures 1 and 2 represent the relationships of tolerance index based on final survival at the seedling stage and tolerance index based on 15 days survival at the early seedling stage with depression in percent fertility. The scatter diagrams would reveal two definite constellations of test cultures even though the r values of 0.391** and 0.327** were not of a high magnitude. The group of entries with a low seedling cold tolerance index (up to 0.5), representing mostly indica parents and indica/indica hybrids, had the least depression in percent fertility (0 to 30%), indicating their higher cold stability for fertility. On the other hand, entries with a high seedling cold tolerance index (> 0.8), representing mostly japonica parents and japonica/indica hybrids, had a high range of depression in fertility (-4 to 58%), suggesting that the higher the seedling cold tolerance, the lower the cold

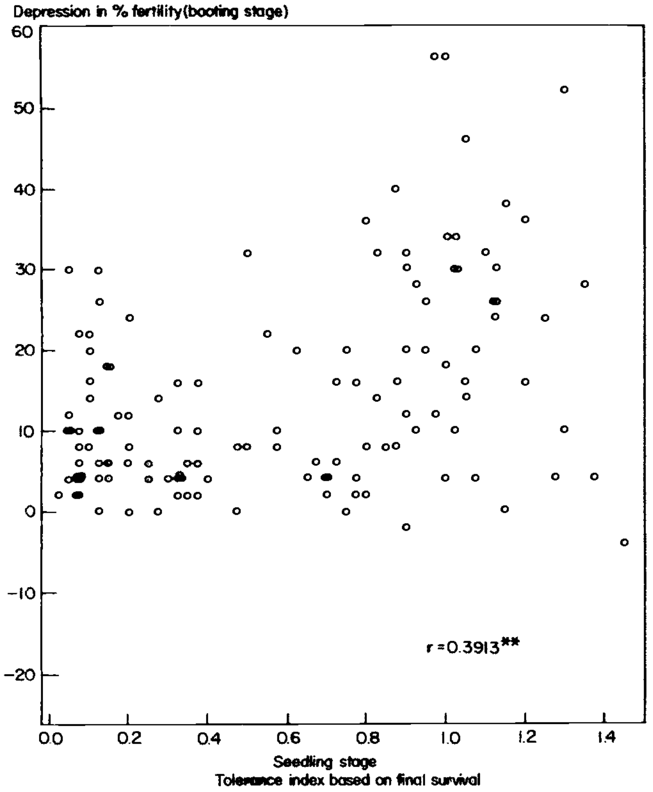

Fig. 1. Relationship between tolerance index on final survival (seedling stage) and depression in percent fertility due to low-temperature treatment at the booting stage.

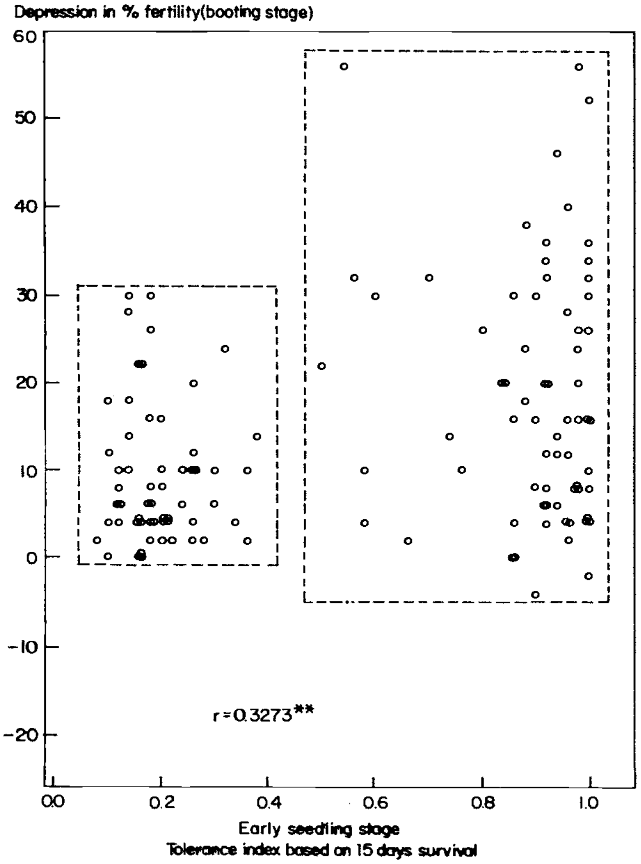

Fig. 2. Relationship between tolerance index on 15 days survival (early seedling stage) and depression in percent fertility due to low-temperature treatment at the booting stage.

Table 5. Correlation of traits related to cold tolerance at early seedling and seedling stages with number of fertile spikelets and percent fertility at booting and flowering stages.

Correlated traits	Fertile spikelets (no.)				Fertility (%)			
	Original data		Depression data		Original data		Depression data	
	Booting	Flowering	Booting	Flowering	Booting	Flowering	Booting	Flowering
Early seedling stage								
Emergence coefficient	0.0044	-0.1010	-0.0250	0.0589	-0.1036	-0.0985	0.0331	0.1022
Seedling height on 15th day	-0.4713**	-0.4759**	0.1710	0.3877**	-0.4833**	-0.4719**	0.2071*	0.3918**
Seedling height on 22nd day	-0.4139**	-0.4536**	0.1311	0.3680**	-0.4667**	-0.4853**	0.1613	0.4137**
Tolerance index on 15 days survival	-0.6415**	-0.5914**	0.3077**	0.4335**	-0.5874**	-0.5623**	0.3273**	0.4501**
Tolerance index on 22 days survival	-0.6226**	-0.5121**	0.2925**	0.3616**	-0.5554**	-0.4950**	0.2885**	0.3935**
Seedling stage								
Tolerance index based on revival 5 DAT	-0.4162**	-0.3581**	0.0669	0.2259*	-0.3204**	-0.3423**	0.1527	0.2840**
Tolerance index based on revival 10 DAT	-0.4336**	-0.3882**	0.0864	0.2547**	-0.3466**	-0.3746**	0.1686	0.3160**
Tolerance index based on final survival	-0.7260**	-0.6497**	0.3561**	0.5126**	-0.6563**	-0.6373**	0.3913 **	0.5482**

*, ** Significant at 5% and 1% levels of probability.

Table 6. **Correlation** coefficients between performance of mid-parents and their hybrids.

Mid-parent value for trait[a]	Correlation coefficient			
	Same character in hybrid	Mid-parent heterosis (%)	Fertility (%) of hybrid	
			BS	FS
Germination coefficient	-0.217	-0.692**	-0.388*	-0.131
Emergence coefficient (ESS)	-0.126	-0.767**	-0.151	0.198*
Seedling height on 15th day	0.373**	-0.160	0.155	0.425**
Seedling height on 22nd day	0.552**	-0.021	-0.257**	-0.118
T.I. on 15 days survival	0.893**	0.608**	0.396**	0.177
T.I. on 22 days survival	0.773**	0.310**	0.233*	0.116
T.I. on revival 5 DAT (SS)	0.860**	-0.511**	0.426**	0.434**
T.I. on revival 10 DAT	0.878**	-0.630**	0.432**	0.404**
T.I. on final survival	0.763**	-0.363**	0.388**	0.169
Flowering duration (BS)	0.729**	-0.035	-0.071	0.185
Panicle length	0.508**	-0.384**	-0.520**	-0.142
No. fertile spikelets	0.562**	0.253*	-0.172	0.420**
% Fertility	-0.277**	-0.446**		
Flowering duration (FS)	0.765**	-0.289**	-0.229*	0.027
Panicle length	0.203	-0.624**	-0.036	-0.116
No. fertile spikelets	0.422**	0.123	0.085	0.371**
% Fertility	-0.125	-0.299**		

[a] T.I. = tolerance index, DAT = days after treatment, ESS = early seedling stage, SS = seedling stage, BS = booting stage, FS = flowering stage.

* P = 0.05, ** P = 0.01

stability for fertility. These results and the ones reported earlier tend to suggest that could tolerance at vegetative stage and at reproductive stage appear to be controlled by different genetic mechanisms in the material used in this study.

Correlation coefficients between the mid-parent value and hybrid performance, and between mid-parent value and heterosis for each character, and between the mid-parent means and percent fertility of the hybrids at both booting and flowering stages of evaluation are shown in Table 6. Significance at 1% level of probability was indicated for 13 of the 17 attributes tested between mid-parent means and performance of the hybrids for the same character, indicating that a close relationship exists between the parental performance *per se* and the performance of their hybrids. The coefficients for cold tolerance indices at 15 days survival, at revival 10 days and 5 days after treatment, at 22 days survival, at final survival, and flowering duration were the largest observed, and prediction of hybrid performance from parental values should prove effective for these traits. Virmani et al (1982) suggested that a high correlation between hybrid performance and mid-parental value could generally be expected when the hybrid vigor expression is predominantly contributed by additive and additive x additive gene effects. Mid-parent means showed moderate values as indicators of hybrid performance for number of fertile spikelets, seedling height at 22 days of cold stress and panicle length at booting stage treatment.

Correlation coefficients between the mid-parent value and heterosis for the same character were mostly negative and showed significance in 10 out of 17 attributes, suggesting that the percent increase over the mid-parent of the hybrids tends to decrease as the average contribution of the mid-parent increases. The largest negative coefficients were observed for emergence coefficient, germination coefficient, tolerance index at revival 10 days after treatment, panicle length at flowering, and tolerance index at revival 5 days after treatment. A significant positive relationship between the mid-parent value and heterosis was shown for tolerance indices at 15 days and 22 days survival and number of fertile spikelets at booting stage evaluation. It appears that a high level of cold tolerance of F_1 hybrids at these stages and a high number of fertile spikelets at booting are likely to accompany a relatively large amount of heterosis.

Correlations of the mid-parent means for the various attributes with percent fertility of the hybrids reveal that the best indicators for high fertility were the parental averages for tolerance indices at revival 10 days after treatment and 5 days after treatment at both booting and flowering stage stresses, seedling height on the 15th day of cold stress at the early seedling stage for the flowering stage evaluation, and tolerance indices based on 15 days survival and on final survival for the booting stage evaluation. Although these coefficients were highly significant, the values were not high enought (r = 0.434** to 0.388**) to transpose to high coefficients of determination to be of significant predictive value and to suggest that indirect selection for fertility would have been effective.

The highly significant correlation coefficients between cold tolerance indices of mid-parent and hybrids is suggestive of the fact that parental values could be effectively used as criteria for the prediction of hybrid performance. It appears that little is to be gained from testing hybrids in which one or more of the parents are clearly inferior to the standard check. Of course, potential parents must perform at acceptable levels for other important economic traits like earliness, seed number and percent fertility if the hybrids are to be commercially accepted. This would permit the elimination of a large amount of undesirable materials and thus avoid the necessity of testing numerous cross combinations. The results presented also indicate the importance of non-additive gene action in the expression of certain characters. Obviously, the performance of the parental varieties *per se* might not be sufficient to ensure the production of superior hybrids. This needs to be supplemented by a thorough evaluation of individual cross combinations for specific combining ability. Frequently, two cultivars in a particular combination may nick especially well to result in an exceptionally high performing hybrid.

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