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# GENETIC PARAMETERS OF COLD TOLERANCE IN RICE

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## ABSTRACT

**Direct and indirect effects of nine traits associated with cold tolerance at an early seedling, seedling and booting stages on per cent fertility were identified in rice using path coefficient analysis. Selection for increase in tolerance index 5 days after revival tolerance, based on 15 days of survival, and number of fertile spikelets, and decrease in tolerance index 10 days after revival treatment and based on final survival would be effective in increasing fertility. Tolerance index 5 days after revival treatment and based on 15 days of survival appear useful as selection criteria. As these traits were negatively correlated with fertility, a compromise would have to be made for simultaneous selection for cold tolerance at vegetative and reproductive stages in rice.**

**Key words:** Rice, path-coefficient analysis, cold tolerance.

Low temperature affects the rice plant at different stages of growth and cold tolerance at one stage does not mean tolerance at another stage [1]. Cold damage to rice plants is classified as delayed-growth type and sterile type [2]. The cool temperatures delay germination, emergence and ultimately flowering and ripening causing low yields. However, the most serious decrease in yield is due to sterility induced by low temperature at the meiotic stage [3].

While reports of cool weather damage in rice are numerous, studies on relationship of cold tolerance at different growth stages are not available. The investigation reported was, therefore, conducted to determine the genetic variability and associations among ten traits measuring cold tolerance at early seedling, seedling and booting stages in 102 $F_1$ hybrids of rice and their 23 parents, to help improve selection efficiency. The direct and indirect effects of nine variables on fertility were evaluated using path-coefficient analysis.

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## MATERIALS AND METHODS

Six high yielding indica type IRRI lines were crossed as pollinators with nine japonica and eight indica type females, having low-temperature tolerance at different growth stages, in a line x tester fashion at the International Rice Research Institute (IRRI), Los Banos, Philippines. The resultant 54 japonica x indica and 48 indica x indica hybrids along with their 23 parents were evaluated for low-temperature tolerance at the early seedling, seedling and booting stages under controlled environments.

For early seedling stage evaluation, the 102 $F_1$, 23 parents and the resistant check, Fujisaka 5, were screened in the Koitotron KG cabinets of the phytotron for low-temperature tolerance from seedling emergence to 3-leaf stage. Twelve sprouted seeds in each culture were sown in duplicate sets in soil-filled porcelain trays and transferred to the artificially lighted cold cabinets at $15 \pm 0.5^{\circ}C$ for 22 days. The cold tolerance score of the cultures [4] and seedling height were recorded on the 8th, 15th and 22nd day of treatment (only the last two reported). Survival percentages of seedlings were recorded on a 0-1 scale on the 15th and 22nd day of cold stress as per Amirshahi and Patterson [5]. A cold tolerance index (TI) that allowed rating the genotypes for relative cold stability was worked out by combining cold tolerance score based on leaf-discolouration and survival percent [6]. A TI value greater than 1.0 denotes better cold tolerance than the resistant check. Seedling stage screening was conducted by subjecting another set of 10-day-old seedlings to 12°C water for 10 days in the Plant Physiology Greenhouse at IRRI. The plants were removed after 10 days of cold water stress, scored for leaf discolouration, and were allowed to revive for 10 days. The revival score was made 5 and 10 days after the treatment on a 0-1 scale. Most of the hybrids revived quickly and appeared green. For a critical assessment of resistance or susceptibility of the cultures to cold water, they were subjected to a second cold water treatment of 10 days and were rated for survival immediately after removal from cold water. The TI was worked out again based on the final survival and cold tolerance score. Evaluation at booting stage was done by transferring the glasshouse grown plants in 5 cm x 5 cm plastic pots, one plant per pot, to a dark room chamber from 1700 h to 0700 h for 5 consecutive days at $15 \pm 1^{\circ}C$ at –5 to 0 auricle distance between auricles of the last two leaves on the main stem. The plants were allowed to flower and mature in the glasshouse at 29/21°C after the expiry of the low-temperature treatment. Fertility of the low temperature stressed plants was used as a measure of cold stability of the cultures at booting stage.

Genotypic and phenotypic variance, coefficient of variation (GCV, PCV) and heritability were computed as per Burton [7]. Phenotypic and genotypic correlation coefficients for all possible comparisons were calculated from the variance and covariance components. Path-coefficient analysis involving a nine-variable model for fertility was computed according to Dewey and Lu [8].

## RESULTS AND DISCUSSION

Highly significant differences (>0.01) among genotypes for all variables were observed (Table 1), indicating the presence of sufficient genetic variability in the material.

**Table 1. Analysis of variance for characters associated with cold tolerance in rice**

| Source | d.f. | Mean squares | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | early seedling stage | | | | seedling stage | | | booting stage | | |
| | | seedling height on | | tolerance index on | | tolerance index on | | | flowering duration | fertile spikelets | fertility |
| | | 15th day | 22nd day | 15-day survival | 22-day survival | 5 DAT revival | 10 DAT revival | final survival | | | |
| Replications | 1 | 1.14 | 1.82 | 0.08** | 0.08 | 0.10** | 0.18** | 5.19** | 0.0 | 13.1 | 4.5 |
| Treatments | 124 | 8.66** | 14.11** | 0.26** | 0.34** | 0.14** | 0.14** | 0.34** | 232.6** | 968.4** | 1113.3** |
| Error | 124 | 0.50 | 1.86 | 0.01 | 0.10 | 0.01 | 0.01 | 0.05 | 0.41 | 8.8 | 3.2 |

**Significant at P = 0.01; DAT—days after treatment.

The mean, range, phenotypic and genotypic variances, GCV and PCV for ten traits, together with their heritability values, are presented in Table 2. Early seedling stage evaluation revealed TI based on 22 days of survival to have lower mean but higher range and GCV than TI based on 15-day survival, suggesting this attribute to be more critical in evaluating genotypes and reflecting better chances for selection because of the wider range. The TI based on 15-day survival was, however, more heritable and hence a more reliable attribute. In seedling stage evaluation, TI based on final survival had lower mean, wider range, higher GCV and equally high heritability, compared to TI based on revival 5 days after treatment (5 DAT) and 10 days after treatment (10 DAT), suggesting selection to be more effective for TI on final survival. However, TI on 10 DAT revival with its smaller coefficient of variation (cv) and higher heritability appeared to be a more reliable measure of seedling cold tolerance. Appreciably higher heritability of flowering duration, fertile spikelet number, and fertility was obtained in the reproductive stage evaluation, indicating that selection for these traits should be very effective. Sawada and Takahashi [9] have also reported high (83.5%) heritability for reproductive stage cold tolerance. Fertile spikelet number with its lower mean, wider range, higher genotypic variation and heritability would appear to be the best attribute to selection for reproductive stage cold tolerance, closely followed by fertility.

The genotypic and phenotypic correlation coefficients for all possible comparisons (Table 3) indicate that phenotypic correlations were significant and similar in sign with their genotypic correlations in all cases. Genotypic coefficients were, however, higher in magnitude. The traits associated with early seedling stage and seedling stage cold tolerance

**Table 2. Mean, variability and heritability of characters associated with cold tolerance in rice**

| Character | General mean ± SE | Range | Genotypic variance | GCV (%) | Phenotypic variance | PCV (%) | Heritability |
|---|---|---|---|---|---|---|---|
| **Early seedling stage:** | | | | | | | |
| Seedling height on 15th day (cm) | 9.2 ± 0.7 | 5.16 – 13.2 | 4.08 | 22.0 | 4.58 | 23.3 | 89.1 |
| Seedling height on 22nd day (cm) | 13.3 ± 1.4 | 7.26 – 20.20 | 6.12 | 18.5 | 7.98 | 21.2 | 76.7 |
| TI on 15-day survival | 0.6 ± 0.1 | 0.10 – 1.0 | 0.12 | 61.3 | 0.14 | 64.8 | 89.2 |
| TI on 22-day survival | 0.5 ± 0.3 | 0.03 – 1.37 | 0.12 | 64.0 | 0.22 | 87.6 | 53.1 |
| **Seedling stage:** | | | | | | | |
| TI on 5 DAT revival | 0.7 ± 0.1 | 0.08 – 1.4 | 0.06 | 34.3 | 0.07 | 37.2 | 85.1 |
| TI on 10 DAT revival | 0.7 ± 0.1 | 0.04 – 1.4 | –2.50 | 35.6 | –2.50 | 38.7 | 99.9 |
| TI on final survival | 0.6 ± 0.2 | 0.03 –1.5 | –6.42 | 64.7 | –6.49 | 75.8 | 98.9 |
| **Booting stage:** | | | | | | | |
| Flowering duration (days) | 72.3 ± 0.6 | 50.6 – 109.8 | –1.95 | 14.9 | –1.97 | 14.9 | 99.4 |
| Fertile spikelet No. | 44.8 ± 3.0 | 2.2 – 96.8 | 26.04 | 48.8 | 26.08 | 49.3 | 99.9 |
| Fertility (%) | 51.2 ± 1.8 | 2.7 – 95.8 | 118.95 | 46.0 | 119.33 | 46.1 | 99.7 |

were highly and positively correlated with each other and negatively associated with reproductive stage cold tolerance. Similar results were reported earlier [10]. The more productive types, therefore, tended to be less cold hardy at the vegetative stage. Although high negative correlations existed between the traits associated with vegetative and reproductive stage cold tolerance, these associations were not strong enough to use in selection for low-temperature tolerance simultaneously for both the stages.

The TI on 22-day survival was very closely associated with TI on 15-day (0.99) and with TI on final (0.99) survival, and TI on 5 DAT revival with TI on 10 DAT revival (0.99). The other strongly associated traits were TI on 22-day survival with TI on 5-DAT and 10-DAT revival, and seedling height on 15th and 22nd days. All intercharacter relationships between TIs and seedling height were highly positively correlated. The low-temperature tolerance at the early seedling and seedling stages, therefore, appears to be controlled by the same or functionally allied genes in this material. Fertility and number of fertile spikelets were also highly correlated, indicating a high predictive value. These results support the concept of developing location-specific cold tolerant varieties.

The path-coefficient analysis was used to understand the direct and indirect causes of association and for partitioning of a correlation coefficient into components of direct and

**Table 3. Phenotypic and genotypic correlation coefficients among ten traits of rice**

| Trait | Early seedling stage | | | Seedling stage | | | Booting stage | | |
|---|---|---|---|---|---|---|---|---|---|
| | seedling height on 22nd day | TI on 15-day survival | TI on 22-day survival | TI on 5 DAT revival | TI on 10 DAT revival | TI on final survival | flowering duration | fertile spikelet number | fertility |
| Seedling height on 15th day | 0.85** | 0.82** | 0.70** | 0.62** | 0.63** | 0.62** | –0.25** | –0.47** | –0.49** |
| | 0.91 | 0.87 | 0.86 | 0.73 | 0.74 | 0.81 | –0.26 | –0.50 | –0.51 |
| Seedling height on 22nd day | | 0.73** | 0.69** | 0.58** | 0.59** | 0.55** | –0.20* | –0.39** | –0.44** |
| | | 0.82 | 0.83 | 0.73 | 0.74 | 0.75 | –0.22 | –0.44 | –0.50 |
| TI on 15-day survival | | | 0.82** | 0.70** | 0.70** | 0.77** | –0.40** | –0.62** | –0.57** |
| | | | 0.99 | 0.79 | 0.80 | 0.95 | –0.43 | –0.66 | –0.60 |
| TI on 22-day survival | | | | 0.56** | 0.57** | 0.64** | –0.39** | –0.55** | –0.47** |
| | | | | 0.89 | 0.89 | 0.99 | –0.53 | –0.74 | –0.64 |
| TI on 5 DAT revival | | | | | 0.98** | 0.68** | –0.27** | –0.39** | –0.31** |
| | | | | | 0.99 | 0.81 | –0.29 | –0.43 | 0.33 |
| TI on 10 DAT revival | | | | | | 0.67** | –0.27** | –0.40** | –0.32** |
| | | | | | | 0.82 | –0.29 | –0.44 | –0.35 |
| TI on final survival | | | | | | | –0.41** | –0.69** | –0.62** |
| | | | | | | | –0.49 | –0.80 | –0.72 |
| Flowering duration | | | | | | | | 0.58** | 0.38** |
| | | | | | | | | 0.58 | 0.38 |
| Fertile spikelet number | | | | | | | | | 0.81** |
| | | | | | | | | | 0.82 |

Note: Upper and lower values represent phenotypic and genotypic coefficients, respectively.
TI—tolerance index, DAT—days after treatment.

indirect effects [8]. The direct and indirect effects of nine traits (casual variables) on fertility (resultant variable) are presented in Table 4. The variation in fertility that was accounted for by the model was determined as $1\text{-}p^2_x$, where $P_x$ is the path coefficient of the residual factors. The genotypic model showed that the nine traits accounted for 62.8% of the genetic variation in fertility, the genotypic value of $P_x$ being 0.6096. It appears that some character(s) other than those included in our analysis play an important role in determining variability in fertility.

The partitioning of genotypic correlations revealed a relatively large and positive direct effect of TI on 5 DAT revival, 15-day survival, No. of fertile spikelets, and large negative direct effects of TI on 10 DAT revival, final survival, and fertility. This indicates that selection for these traits would result in increased fertility of high reproductive stage cold tolerant cultures. The indirect effect through TI on 5 DAT revival was consistently sizeable and positive, and through TI on 10 DAT revival sizeable and negative. TI also exerted high

**Table 4. Genotypic direct and indirect effects of nine traits on fertility**

| Trait | Pathway of association: seedling height on 15th day | seedling height on 22nd day | tolerance index on 15-day survival | tolerance index on 22-day survival | tolerance index on 5 DAT revival | tolerance index on 10 DAT revival | tolerance index on final survival | Flowering duration | Fertile spikelet number | Correlation with fertility |
|---|---|---|---|---|---|---|---|---|---|---|
| **Early seedling stage:** | | | | | | | | | | |
| Seedling height on 15th day | **−0.04** | −0.26 | 0.71 | −0.34 | 2.45 | −2.14 | −0.66 | 0.03 | −0.26 | −0.51 |
| Seedling height on 22nd day | −0.04 | **−0.29** | 0.67 | −0.33 | 2.44 | −2.15 | −0.60 | 0.03 | −0.23 | −0.50 |
| TI on 15-day survival | −0.03 | −0.24 | **0.82** | −0.42 | 2.65 | −2.33 | −0.76 | 0.05 | −0.34 | −0.60 |
| TI on 22-day survival | −0.03 | −0.24 | 0.85 | **−0.40** | 2.96 | −2.58 | −0.88 | 0.06 | −0.38 | −0.64 |
| **Seedling Stage:** | | | | | | | | | | |
| TI on 5 DAT revival | −0.03 | −0.21 | 0.65 | −0.35 | **3.34** | −2.89 | −0.65 | 0.03 | −0.22 | −0.33 |
| TI on 10 DAT revival | −0.03 | −0.21 | 0.66 | −0.36 | 3.34 | **−2.89** | −0.66 | 0.03 | −0.23 | −0.35 |
| TI on final survival | −0.03 | −0.21 | 0.77 | −0.43 | 2.71 | −2.37 | **−0.81** | 0.06 | −0.41 | −0.72 |
| **Booting stage:** | | | | | | | | | | |
| Flowering duration | 0.01 | 0.07 | −0.35 | 0.21 | −0.97 | 0.84 | 0.39 | **−0.12** | 0.30 | 0.38 |
| Fertile spikelet No. | 0.02 | 0.13 | −0.54 | 0.30 | −1.44 | 1.28 | 0.64 | −0.07 | **0.50** | 0.82 |

Note: Values in bold denote direct effects; TI—tolerance index, DAT—days after treatment.

positive and negative indirect effects on 15-day and final survival, respectively. These traits were important components in every correlation that involved fertility. The net effect in this system of opposing influences was counterbalancing of negative and positive effects, making the overall correlation between TIs and fertility essentially negative. Since a favourable direct and indirect relationship existed between TI on 5 DAT revival and fertility as well as between TI on 15-day survival and fertility, and since plants having tolerance at the vegetative stage have an added advantage, greater emphasis needs to be placed on these traits in a breeding programme for cold tolerance.

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