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RESEARCH ARTICLE

Dissecting gene action for yield traits in rice using Line × Tester analysis

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Abstract

Rice (*Oryza sativa* L.) production is increasingly threatened by abiotic stresses, notably salinity and phosphorus (P) deficiency which constrain crop performance in vulnerable agro-ecosystems. The current study was designed to assess the combining ability of four *Saltol 1* introgressed backcross inbred lines and two *Pup1* introgressed testers through a Line × Tester mating design with the objective of identifying the nature of gene action of yield traits and superior parental genotypes and cross combinations for the genetic enhancement under stress-prone conditions. Eight F_1 hybrids along with six parental lines were assessed using a randomized complete block design with two replications during *Rabi* 2023-24. Analysis of variance revealed considerable genetic variability across all the traits studied. Combining ability analysis demonstrated that BIL 33, D5-1-3-2-1 and C16-1-2-8 were superior general combiners, particularly for grain yield and associated component traits while cross combinations such as BIL 33 × C16-1-2-8 and BIL 1102 × D5-1-3-2-1 exhibited significant specific combining ability effects reflecting the yield potential of hybrids. Variance component analysis further indicated that traits such as days to 50% flowering, plant height and grain yield were chiefly administered by additive gene action whereas traits *viz*., flag leaf length, spikelet fertility and grain number were influenced by non-additive action. These findings provide valuable insights into the genetic construction of key agronomic traits and offer a strong basis for breeding stress-resilient, high-yielding rice hybrids.

Keywords: combining ability; dominance ratio; general combining ability; specific combining ability; line x tester; rice

Introduction

Rice (*Oryza sativa* L.) is the staple food for over half the global population and constitutes a significant component of food and nutritional security, particularly in Asia (1). Despite significant advancements in rice production over recent decades, productivity remains liable to a multitude of abiotic stresses, of which salinity and phosphorus (P) deficiency are among the most pervasive (2). Globally, soil salinity adversely affects around 20% of irrigated agricultural land weakening the plant physiological processes by means of osmotic stress and ionic toxicity which in turn lower the yield (3). Concurrently, phosphorus deficiency, primarily due to the fixation of applied phosphorus in insoluble forms, represents a major constraint to rice cultivation in low-input and acidic soils, limiting root growth, tillering and ultimately grain yield (4).

The breeding of rice cultivars with improved salinity tolerance and low P tolerance stress is imperative to ensure sustainable crop productivity under resource-constrained environments. However the genetic improvement of intricate traits like salinity and phosphorus stress tolerance poses significant challenges owing to their polygenic inheritance and strong genotype x environment interactions. Recent advancements in molecular breeding have facilitated the identification and introgression of major quantitative trait loci (QTLs) related with stress tolerance. Notably, *Saltol 1* QTL confers seedling-stage salinity tolerance and has been successfully deployed in several breeding programs (5,6). Likewise, the *Pup1* locus, derived from the aus-type donor *Kasalath*, is linked with superior phosphorus uptake and utilization efficacy under P-deficient environment (7). Marker-assisted backcrossing (MABC) has enabled the precise introgression of *Saltol 1* (8) and *Pup 1* (9) QTLs into popular rice cultivars resulting in the development of backcross inbred lines (BILs) with enhanced abiotic stress resilience and the evaluation of hybrid combinations elucidate the genetic variation and decipher gene actions.

In this context, Line × Tester analysis proposed by Kempthorne (10) provides a precise biometrical approach for the assessment of combining ability of parental genotypes and the nature of gene action governing complex quantitative traits. General combining ability (GCA) effects provide an estimate of additive gene action whereas specific combining ability (SCA) effects capture dominance and epistatic interactions. The relative magnitudes of GCA and SCA variances offer insights into the efficiency of selection strategies and the potential of heterosis breeding (11).

The goal of the current study was to determine the combining ability of *Saltol 1* introgressed backcross inbred lines and *Pup1* introgressed lines for grain yield and its component traits. The specific objectives were to: (i) evaluate the GCA and SCA effects of the parents, (ii) assess the relative contributions of additive and non-additive genetic variances and (iii) identify superior parents and cross combinations for the genetic enhancement of salinity and low P stress tolerance in rice.

Materials and methods

Plant materials

The research material consist of six genotypes inclusive of four lines and two testers. The four backcross inbred lines (BILs), BIL 33 (L1), BIL 752 (L2), BIL 1094 (L3) and BIL 1102 (L4) were developed through marker-assisted backcrossing (MABC) by incorporating the *Saltol 1* QTL associated with salinity tolerance into the parental lines CR 1009 Sub1 and ADT37. The two testers *viz.*, D5-1-3-2-1 (T1) and C16-1-2-8 (T2) were *Pup1* introgressed lines of CR1009 *Sub1* developed for enhanced phosphorus uptake efficiency.

Hybrid development and experimental design

A Line × Tester mating design proposed by Kempthorne (10) was employed wherein the four lines were hybridized with two testers to generate eight F_1 hybrids. The resulting crosses together with their six parents were assessed in a randomized complete block design (RCBD) with two replications. Each genotype was planted in a single 3-meter-long row, maintaining a spacing of 20 cm between rows and 15 cm between individual plants. Recommended agronomic practices were applied all over the crop growth period to ensure optimal plant development (Fig 1.).

Experimental site

The experiment was conducted at the Research Farm of Department of Plant Breeding and Genetics, Agricultural college and Research Institute, Tamil Nadu Agricultural University, Madurai during *Rabi*, 2023- 24. The experimental site is situated 115 meters above mean sea level at latitude 10°09' N and longitude 78°47' E. The climate is semi-arid tropic with red and red lateritic soil type and 892 mm of annual rainfall.

Data collection

Data were recorded on twelve agro-morphological traits from five randomly selected plants per plot. The traits measured included: days to 50% flowering (DFF), height of the plant (PH), number of productive tillers per plant (NPTP), length of the panicle (PL), length of the flag leaf (FLL), width of the flag leaf (FLW), flag leaf area (FLA), number of filled grains per panicle (NFGPP), total number of grains per panicle (NGPP), spikelet fertility percentage (SF), 100-seed weight (HSW) and grain yield per plant (GY).

$$\text{Spikelet fertility percentage} = \frac{\text{Number of filled grains per panicle}}{\text{Total Number of grains per panicle}} \text{ X } 100$$

Statistical analysis

The recorded data were analysed using analysis of variance (ANOVA) based on the Line × Tester design described by Kempthorne (10). The mean square values for lines, testers and their interactions were utilized to assess the contribution of additive and non-additive genetic components.

The GCA and SCA variances were computed using the following formulae:

$$\sigma^2\text{GCA} = (\text{MS Lines/Testers} - \text{MS Error}) / k \cdot r$$

$$\sigma^2\text{SCA} = (\text{MS Line} \times \text{Tester} - \text{MS Error}) / r$$

Fig. 1. Field view of F_1 evaluation.

Where, MS represents the mean square, *k* is the number of testers (for lines) or lines (for testers), *r* is the number of replications.

Subsequently, the additive genetic variance (σ^2A) and dominance variance (σ^2D) were estimated using:

$\sigma^2 A = 2\,\sigma^2$GCA

$\sigma^2 D = 2 \cdot \sigma^2$SCA

The dominance ratio and predictability ratio were also calculated to infer the nature of gene action:

Dominance Ratio = $\sqrt{(\sigma^2 D / \sigma^2 A)}$

Predictability Ratio = $[\sigma^2 A / (\sigma^2 A + \sigma^2 D)]$

All statistical analyses were carried out with Microsoft Excel and R software (version 4.1.3) with the genetic evaluations conducted through the use of the 'metan' and 'agricolae' packages.

Results and Discussions

Analysis of variance

The analysis of variance (ANOVA) following the Line × Tester mating design (Supplementary Table 1) exhibited that the genotypes varied significantly for all the traits evaluated. The significance of treatment effects for these traits indicates the occurrence of substantial genetic variability in the experimental material which is essential for the success of genetic improvement programs (12). Replication effects were insignificant across the traits assessed, with the exception of NPTP and GY suggesting that environmental variation had negligible impact on most traits. Significant variation between parental lines was detected for the majority of traits excluding NPTP and FLW indicating substantial genetic diversity among the parents. This observation underscores the potential of the selected parents to serve as valuable sources of alleles for the enhancement of various agronomical traits. Moreover, Parents *vs*. Crosses exhibited extremely significant mean squares for the majority of traits which may result in heterotic cross combination. Such heterotic effects are critical in hybrid rice breeding programs aimed at maximizing yield potential and other desirable characteristics in stress prone environment.

The crosses exhibited significant mean squares for all traits, signifying ample genetic differences among the hybrid combinations. This justifies a further partitioning of hybrid variation into GCA and SCA effects. Amongst the components, lines exhibited highly significant variation for DFF, PH, PL and GY, suggested the prevalence of variability among the lines. Similarly, testers demonstrated significant variation for NPTP, PL, FLA, NFGPP, NGPP, SF and GY, highlighting their genetic divergence. Importantly, the Line × Tester interaction effects were found to be highly significant for PH, NPTP, PL, FLL, FLA, NFGPP, NGPP and GY, indicating the differential performance of crosses. The significance of the interaction effects also implies that certain specific cross combinations performed better than others, emphasizing the need to identify and exploit such superior combinations in hybrid development (11).

Combining ability variances and genetic components

The combining ability variances and associated genetic components for the studied traits provided important insights into the nature of gene action governing their inheritance in rice (Supplementary Table 2). The relative magnitude of general combining ability (GCA) and specific combining ability (SCA) variances along with additive (σ^2A) and dominance (σ^2D) genetic variances as well as the derived genetic parameters such as dominance ratio ($\sqrt{(\sigma^2 D/\sigma^2 A)}$) and predictability ratio [σ^2A / (σ^2A + σ^2D)], offer a complete understanding of the inheritance patterns of key traits. For most traits, GCA variances exceeded SCA variances, signifying the preponderance of additive gene action. This trend was particularly evident in traits such as DFF, PH, PL, FLW, FLA and GY where the predictability ratios were notably high, ranged from 0.6554 in PL to 0.984 in DFF. The high predictability ratio for DFF (0.984) and PH (0.8689) strongly suggests that these traits are primarily administered by additive gene action and thus, selection based on phenotype would likely be effective in early generations. Similarly, grain yield showed a relatively high predictability ratio (0.8595) indicating a significant additive genetic component contributing to this complex trait. Traits such as FLL, NFGPP, NGPP, SF and HSW exhibited relatively higher SCA variances and dominance variances accompanied by lower predictability ratios. For instance, FLL showed a high dominance ratio (1.4477) and a low predictability ratio (0.323), proposed the predominance of non-additive gene action. Similarly, SF, NFGPP and NGPP had dominance ratios exceeding unity, further representing the involvement of over-dominance or epistasis in their inheritance. These traits may therefore benefit more from hybrid breeding approaches rather than from simple selection. Interestingly, the trait 100-seed weight exhibited a dominance ratio of approximately 1.0, suggested equal contributions of additive and dominance variance. However, the low absolute magnitude of variances for this trait indicates that it may be more susceptible to environmental fluctuations and careful selection strategies would be required to make genetic gains. In summary, the overall results indicate that traits such as DFF, PL, FLW, FLA and GY are predominantly administered by additive genetic effects and can be effectively enhanced through selection in early generations. In contrast, traits like FLL, NFGPP, NGPP and SF are regulated primarily by non-additive gene action, emphasizing the importance of heterosis breeding to exploit dominance and epistatic interactions. Maring and Aruna (13,14) observed that SCA variances exceeded GCA variances for most studied traits, indicating the important role of non-additive gene action. Similarly, Manivelan (15) found that SCA variance was higher than GCA variance for all traits except alkali spreading value.

General combining ability and specific combining ability effects

The analysis of combining ability effects, encompassing both GCA and SCA, provided insights into the genetic architecture governing the expression of key agronomic and yield-related traits in rice (Supplementary Table 3). Significant GCA effects were noticed for the lines and testers specified the importance of additive gene action while the substantial SCA effects observed in several hybrid combinations suggested the role of non-additive genetic interactions such as dominance and epistasis. Among the lines evaluated, L1 emerged as the most promising general combiner. It displayed significantly negative GCA effect for DFF (-2.812), suggested its potential utility in evolving early-maturing genotypes. Furthermore, significant positive GCA effects were evident for PH (20.729), PL (2.138), FLL (4.343), FLW (0.134), FLA (5.747), NFGPP (9.500), NGPP (13.426) and GY (10.890) and thereby indicating its broad utility in enhancing both physiological and reproductive vigour. This profile makes L1 particularly suitable for hybridization programs targeting improvements in yield and related physiological traits. L2 also showed notable GCA effects for traits such as PH (23.083) and PL (2.365) as well as for flag leaf characteristics. L4 showed a significantly positive effect for spikelet fertility (2.326) indicating that it may be useful for improving reproductive efficiency in specific combinations. Regarding testers, T1 displayed significantly positive GCA effects for GY (12.563) and traits such as NPTP (9.250) indicating its strong combining ability and suitability as a female parent in hybrid combinations.

The SCA effects provided further clarity on the specific performance of cross combinations. Notably, the hybrid L1 × T2 recorded highly significant positive SCA effects for GY (3.423), PH (5.239) and other panicle-related traits suggested high heterotic potential in this combination. Similarly, L4 × T1 exhibited substantial positive SCA effects for GY (6.729) and other key traits such as FLA and NGPP making it another promising hybrid for yield enhancement. A less degree of favourable SCA effects for several traits was also demonstrated by other combinations such as L3 × T1 and L2 × T2. The contrasting nature of GCA and SCA effects across different genotypes and combinations underscores the complex genetic control of the traits studied. The occurrence of significant SCA effects in crosses when at least one parent had weak GCA indicates the presence of complementary gene interactions which can be efficiently leveraged in hybrid production. The study identified L1 and T1 as superior general combiners for grain yield and several related traits while cross combinations such as L1 × T2 and L4 × T1 demonstrated high specific combining ability, making them suitable candidates for hybrid breeding programs. These findings are consistent with those of (16) who observed the role of both additive and dominant gene actions in rice, with several parents recognized as good general combiners for yield-related traits. Crosses like Basmati 370 × Pusa Basmati 1509 and CSR 30 × Ranbir Basmati exhibited high SCA effects, indicating potential for heterosis breeding. Awad Allah (17) reported significant GCA and SCA effects across most traits representing the prevalence of additive gene action in rice with some exceptions. Crosses like Norti × Giza 178 and Gz 5121-5-2 × Dular showed high heterosis and specific combining ability, suggesting their utility in hybrid rice breeding programs. Modaressi (18) reported additive gene action for plant height and non-additive gene action for panicle length, number of panicles per plant, heading date, hundred- grain weight, number of grains per panicle and grain yield through significant effect of GCA and SCA. Similar results were reported by Ghidan WF *et al*., (19).

The comprehensive understanding of genetic architecture provided by these parameters serves as a valuable guide for the formulation of effective selection strategies and the progress of high-yielding rice hybrids and varieties.

Conclusion

The present investigation through Line × Tester analysis provided comprehensive insights into the genetic control of yield and associated traits in rice. Traits such as days to 50% flowering, plant height, panicle length and grain yield were predominantly governed by additive gene action indicating the efficacy of early-generation selection for their improvement. In contrast, non-additive gene effects played a major role in the inheritance of traits like flag leaf length, spikelet fertility and grain number, highlighting the potential for exploiting heterosis in hybrid breeding programs. The identification of BIL 33 (L1) and D5-1-3-2-1 (T1) as superior general combiners along with promising cross combinations such as BIL 33 (L1) × C16-1-2-8 (T2) and BIL 1102 (L4) × D5-1-3-2-1 (T1), provides a valuable genetic base for developing high-yielding, stress-tolerant rice hybrids suited to challenging agro-ecosystems.

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Authors' contributions

The collaborative research effort involved a diverse team with distinct roles., RCG generated the experimental material, initiated the study's concept and data collection while NA and MG designed the research and experimental framework, provided project supervision and manuscript review. RPG, SMP, PSP and RA provided predominant research guidance and final manuscript approval, collectively ensuring a comprehensive and rigorous scientific investigation.

Compliance with ethical standards

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Declaration of generative AI and AI-assisted technologies in the writing process

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