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RESEARCH ARTICLE

Parent progeny regression analysis of herbicide-tolerant rice (*Oryza sativa* L.) developed through marker-assisted selection

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Abstract

Weed infestation poses a significant challenge to the adoption of direct-seeded rice (DSR), leading to substantial yield losses. This study involved introgression of imazethapyr herbicide tolerance into ADT 55 rice variety using the ethyl methane sulfonate-induced mutant Robin HTM as a donor. Phenotypic evaluation with imazethapyr herbicide spray demonstrated the transfer of herbicide tolerance locus into the ADT 55 background. Additionally, the research focuses on assessing the inheritance of key agronomic traits in F_2 and F_3 generations following initial crosses and marker-assisted selection. The findings revealed positive intergenerational correlations and significant regression coefficients for traits such as plant height and the number of productive tillers, indicating stable inheritance of these traits. Moderate to low heritability values suggest the possible influence of non-additive gene action or environmental variance, reducing the effectiveness of early-generation selection for certain traits. The development of herbicide-tolerant rice varieties offers a promising strategy for effective weed management in DSR, reducing reliance on manual weeding and minimizing yield losses. This approach contributes to more sustainable and efficient rice production systems.

Keywords: direct-seeded rice; herbicide tolerance; imazethapyr; intergenerational correlation; marker-assisted selection; mutation breeding; weed management

Introduction

Importance of rice in global food security

Rice (*Oryza sativa* L.) is a staple food crop that plays a crucial role in global food security. It serves as a primary caloric source for a significant portion of the global population, making it essential to enhance its productivity and resilience to various biotic and abiotic stresses. However, its cultivation is labour-intensive and requires substantial water resources, posing challenges for sustainable agricultural practices. Direct-seeded rice (DSR) has emerged as an alternative to traditional transplanting methods, offering potential advantages in reducing labour and water requirements. Despite these benefits, weed infestation remains a significant limitation to the successful adoption of DSR, leading to yield losses (1).

Role of herbicide tolerance in direct-seeded rice systems

Herbicide-tolerant varieties offer a promising component of integrated weed management strategies, alongside crop rotation and mechanical methods. Employing broad spectrum herbicide tolerant rice varieties enables efficient adoption of DSR cultivation without weed menace. Imazethapyr, a broad-spectrum herbicide, has been widely used to control weed infestations in rice fields. However, for effective implementation, introgression of herbicide resistance genes into elite rice varieties is necessary to prevent herbicide induced crop damage while ensuring high productivity. Mutation breeding has been widely utilized to develop novel traits in rice, including herbicide tolerance. EMS mutagenesis introduces random mutations, creating a pool of genetic variability from which beneficial traits like herbicide tolerance can be selected through screening (2).

Objectives of the present study

In this study, the herbicide-tolerant mutant Robin HTM, developed through ethyl methane sulfonate (EMS) mutagenesis of the Nagina 22 rice cultivar, was used as a donor parent. The objective was to introgress the acetolactate synthase (ALS) gene mutation into the high-yielding, bacterial blight-resistant variety ADT 55 through marker-assisted selection (MAS). While MAS is a powerful tool, its precision depends on the tight linkage of markers and the genetic background of the parents involved (3). The present study focuses on the development of an herbicide-tolerant version of ADT 55 through MAS, while retaining its desirable agronomic traits. This research aims to develop and evaluate herbicide-tolerant lines of ADT 55 × Robin HTM across multiple generations. Progenies were developed through hybridization and selfing, followed by molecular screening and field evaluations. Agronomic traits such as plant height, flowering time, tiller number, spikelet fertility and grain yield were assessed under both herbicide-sprayed and manually weeded (control) conditions to determine the impact of herbicide tolerance on plant performance. Furthermore, heritability estimates and intergenerational correlation analyses were conducted to understand the genetic basis of key agronomic traits and their potential for stable inheritance in subsequent generations. These findings provide valuable insights into the feasibility of herbicide-tolerant rice breeding and contribute to the development of improved rice cultivars for sustainable and efficient weed management in direct-seeded rice cultivation.

Materials and Methods

Plant materials used

ADT 55, a high yielding medium slender bacterial blight resistant rice variety, which matures in 115 days, is used as a recipient parent in the present study. Robin HTM, which is an EMS induced mutant developed from Nagina 22 rice cultivar, resistant to imazethapyr herbicide, is used as donor parent. The seeds were procured from Centre of Plant Molecular Biology and Biotechnology, TNAU, Coimbatore.

Generation of progenies harboring herbicide tolerant allele

The parents ADT 55 and HTM were raised under glasshouse conditions and the donor parent Robin HTM served as the pollen source for hybridization with vacuum emasculated female recurrent parent. The F_1 seeds are then harvested, raised under glasshouse conditions and DNA was isolated by modified CTAB method (4) which was further quantified using a nanodrop spectrophotometer (ND-1000 Spectrophotometer, NanoDrop Techonologies, USA). The quantified DNA is then diluted to 50 ng/µL and used for further molecular analysis. Standard Polymerase Chain Reaction and Agarose gel electrophoresis protocol were followed. The PCR reaction mixture was kept in a thermal cycler (DNA Engine, Bio-Rad Laboratories, Inc., USA) with denaturation temperature of 95 °C, annealing temperature of 58.4 °C and extension temperature of 72 °C. The molecular marker RM 6844 (Chromosome 2: 18063725-18063917 bp), which is linked with the HTM allele is used as a foreground marker to identify the true F_1s (5). True F_1s were selfed to generate F_2 seeds.

The F_2 generation was raised in the field during Summer 2024 at Paddy Breeding Station, Department of Rice, Tamil Nadu Agricultural University, Coimbatore and foreground genotyping was done with RM 6844 to identify the homozygous and heterozygous positive plants. Plants with superior agronomic performance having high single plant yield and homozygous for the HTM allele were forwarded to F_3 generation as distinct families (Fig. 1).

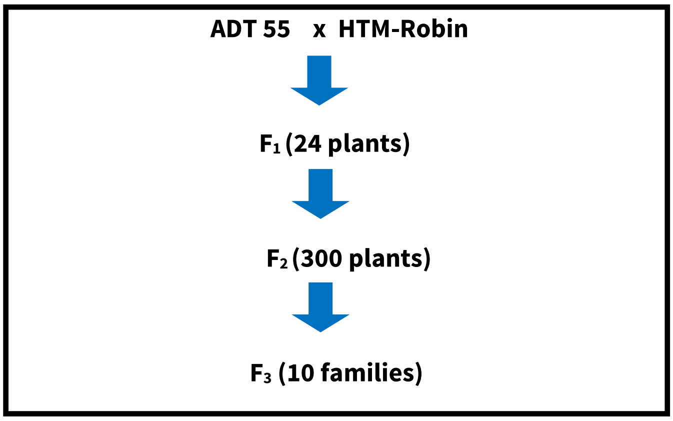

Fig. 1. Flow of work followed for marker assisted selection scheme followed for ADT 55 × HTM in the study.

Phenotypic evaluation of herbicide tolerant lines developed through MAS

The F_3 families were raised by wet direct seeding method with spacing of 20 × 20 cm during Samba 2024, at Paddy Breeding Station, Department of Rice, Tamil Nadu Agricultural University, Coimbatore under two conditions *viz*., one with imazethapyr spray at the rate of 2 mL/L after 21 days of sowing and other one as a control with manual weeding. Agronomic traits from both the sprayed and unsprayed plots *viz*., plant height, days to first flowering, days to 50 % flowering, number of productive tillers, panicle length, flag leaf length, flag leaf width, spikelet fertility and single plant yield were recorded from three random plants.

Statistical analysis

Student's t-test was carried out to check the statistical significance of the agronomic traits between sprayed and unsprayed plots $p \leq 0.05$.

Narrow sense heritability was calculated using intergenerational correlation and regression coefficient (6) with the help of agronomic data collected from F_2 and F_3 progenies, using Microsoft Excel and R software for data processing and graphing respectively.

Results

Generation of progenies harboring herbicide tolerant allele

The initial hybridization of ADT 55 and HTM parents resulted in 24 F_1 plants. Molecular analysis with the SSR marker RM 6844 revealed 12 positives for HTM allele (Fig. 2). The 12 plants were forwarded to F_2 generation under field conditions, which comprised 300 plants in total. Genotyping with RM 6844 marker analysis showed that 32 plants were homozygous positive for HTM allele whereas 82 plants were heterozygous positive for HTM allele (Fig. 3). To avoid segregation in the F_3 generation, the homozygous positive plants were considered for further advancement. Ten plants with superior agronomic performance, homozygous for HTM allele were selected and forwarded to F_3 generation (Fig. 1).

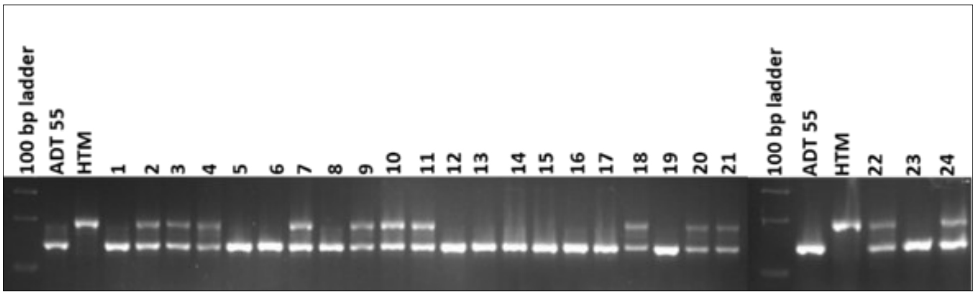

Fig. 2. Foreground marker genotyping of F_1 generation of ADT 55 × HTM cross.

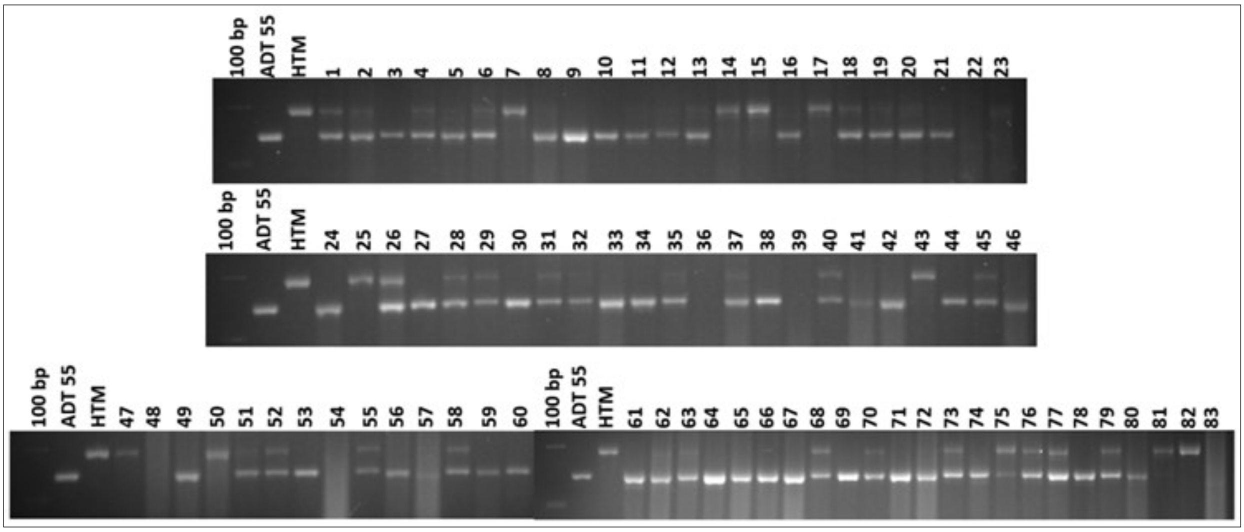

Fig. 3. Foreground marker genotyping of F_2 generation of ADT 55 × HTM cross.

Phenotypic evaluation of herbicide tolerant lines developed through MAS

The F_3 families were raised under two plots, the control and the treatment. Imazethapyr spray was given in the treatment plot and the surviving ability of seedlings was monitored to check the presence of herbicide resistance allele in the progenies of ADT 55 × HTM cross. All the progenies survived, showing 100 % resistance against Imazethapyr herbicide thus confirming the presence of the HTM allele (Fig. 4). Agronomic traits were recorded from three random plants in each family from both control and treatment plots (Table 1). Student's t-test was performed to test the significance between the imazethapyr sprayed and unsprayed plots ($p \leq 0.05$). Plant height and number of productive tillers expressed significant difference indicating a possible influence of the herbicide, though micro-environmental factors and random variation may also contribute. The remaining traits, *viz*., days to first flowering, days to 50 % flowering, panicle length, flag leaf length, flag leaf width, spikelet fertility, single plant yield did not exhibit significant difference between the two plots.

Assessment of narrow sense heritability using parent progeny regression

The F_2 and F_3 data were used to calculate intergenerational correlation and regression coefficient, from which narrow sense heritability was worked out for the traits (Table 2). All the traits had significant regression coefficients ($p < 0.01$) indicating that there is significant association between F_2 and F_3 generation. Some traits like number of tillers (0.08) and single plant yield (0.03) showed low predictive power despite significance, likely due to sample size or environmental noise. Plant height had highest regression coefficient (0.51), followed by days to first flowering (0.42), days to 50 % flowering (0.39), panicle length (0.33), spikelet fertility (0.33), flag leaf length (0.16), flag leaf width (0.16), number of tillers (0.08) and single plant yield (0.03).

Intergenerational correlation studies aid in predicting the extent to which the genetic potential of a character is passed on to the next generations. In this study, highly significant intergenerational correlation coefficient was observed for plant height (0.80), days to first flowering (0.74) and days to 50 % flowering (0.73). Moderate intergenerational

Fig. 4. Field image taken after five days of imazethapyr application.

Table 1. Performance of yield and its associated traits in F_3 progenies

Sprayed	DF	DFP	PH	NPT	PL	FLL	FLW	SF	SPY
2-47	69.00	73.00	101.67	29.00	19.37	23.50	1.13	52.51	12.00
3-82	70.00	73.00	85.00	25.33	19.10	22.53	1.00	57.72	13.33
3-87	69.00	72.00	106.67	28.00	22.63	32.70	1.33	68.15	13.33
4-90	72.00	77.00	97.67	49.33	22.60	25.80	1.23	61.85	28.33
4-92	72.00	78.00	123.33	24.67	25.20	31.27	1.27	60.69	13.33
7-112	72.00	78.00	107.33	15.00	19.83	34.23	1.20	9.22	5.00
18-193	70.00	76.00	85.00	33.67	18.37	21.03	0.87	53.58	15.33
21-251	68.00	72.00	103.33	37.67	20.13	27.83	1.10	69.29	21.67
21-256	69.00	74.00	100.00	35.00	19.60	24.30	1.10	72.41	23.33
24-296	71.00	75.00	95.00	31.00	19.37	21.40	1.00	48.57	16.00
HTM	**60.00**	**65.00**	**100.67**	**18.33**	**16.90**	**24.13**	**1.00**	**45.73**	**17.00**
ADT 55	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Unsprayed									
2-47	72.00	75.00	75.67	15.00	21.30	23.43	1.13	51.36	16.67
3-82	72.00	76.00	68.00	22.00	17.33	24.63	1.23	79.54	27.67
3-87	72.00	75.00	73.33	13.67	21.70	31.83	1.00	81.93	15.67
4-90	75.00	78.00	42.33	8.67	15.77	20.50	1.00	57.55	2.33
4-92	74.00	78.00	114.33	16.00	24.10	28.00	0.87	72.56	13.33
7-112	75.00	79.00	77.00	8.33	19.07	31.60	1.10	13.64	3.00
18-193	72.00	77.00	71.00	18.33	21.57	25.43	1.13	65.87	16.67
21-251	71.00	74.00	90.00	15.00	22.67	29.47	1.13	67.00	13.67
21-256	71.00	75.00	85.00	12.33	21.60	25.67	1.00	63.30	10.33
24-296	75.00	79.00	80.00	9.67	19.50	26.63	1.13	73.19	11.00
HTM	**64.00**	**68.00**	**87.67**	**8.67**	**15.37**	**20.60**	**0.97**	**40.02**	**9.67**
ADT 55	**76.00**	**79.00**	**96.67**	**14.00**	**23.27**	**26.63**	**1.17**	**79.91**	**21.00**

df: days to first flowering; dfp: days to 50 % flowering; ph: plant height; npt: number of productive tillers per plant; pl: panicle length; fll: flag leaf length; flw: flag leaf width; sf: spikelet fertility percentage; spy: single plant yield.

Table 2. Intergenerational correlation, regression coefficient and heritability values for yield and yield contributing traits in F_2 and F_3 progenies of ADT 55 × HTM cross

Traits	Intergenerational Correlation	Regression (b)	Heritability (h^2) in %
Days to first flowering	0.74	0.42	28.35
Days to 50 % flowering	0.73	0.39	26.89
Plant height	0.80	0.51	31.99
Number of productive tillers	0.14	0.08	29.51
Panicle length	0.55	0.33	29.67
Flag leaf length	0.44	0.16	18.67
Flag leaf width	0.40	0.16	20.25
Spikelet fertility	0.47	0.33	34.68
Single plant yield	0.07	0.03	24.53

correlation coefficient was observed for the characters such as panicle length (0.55), spikelet fertility (0.47), flag leaf length (0.44) and flag leaf width (0.40). Low intergenerational correlation coefficient was observed for number of productive tillers (0.14) and single plant yield (0.07).

Narrow sense heritability was calculated by using the intergenerational correlation coefficient and regression coefficient (Table 2 and Fig. 5). Moderate heritability was observed for spikelet fertility (34.68 %) and plant height (31.99 %). Low heritability was observed for characters such as panicle length (29.67 %), number of productive tillers per plant (29.51 %), days to first flowering (28.35 %), days to 50 % flowering (26.89 %), single plant yield (24.53 %), flag leaf width (20.25 %) and flag leaf length (18.67 %).

Discussion

Rice is one of the labor and water intensive crops which remains a serious limitation in some of the marginal farmers' households. Adopting direct seeded rice in place of traditional puddled transplanted rice is a promising approach to save water and labor resources. However, weeds pose a serious threat to realizing the full yield potential of direct-seeded rice. This challenge can be addressed using broad-spectrum herbicides that act against almost all classes of weeds. Imazethapyr is one such herbicide and can be effectively used in rice crop which is containing a resistant version of mutated ALS gene. Robin HTM, an EMS induced rice mutant resistant to imazethapyr, was used as a donor parent in this study for introgressing the mutated version of the ALS gene into ADT 55 rice variety.

Initial crosses were made and genotyping was performed with an SSR marker reported to be linked to the resistant gene *viz.*, RM 6844. The positive F_1 plants were forwarded to the F_2 generation, where 32 plants were homozygous positive and 82 plants were heterozygous for the herbicide tolerant allele among the 300 plants. The mendelian segregation ratio of 1:2:1 could not be obtained, which may be attributed to a decrease in seedling survival rate due to several factors such as segregation distortion, environmental effects, or sampling error. Agronomically superior plants carrying the homozygous positive allele were selected and forwarded to F_3 generation as families. Ten F_3 families were raised under two conditions *viz.*, Herbicide sprayed and unsprayed plots (control), to check the presence of herbicide tolerant loci in the progenies of ADT 55 × HTM cross. The progenies showed 100 % survival rate, indicating the introgression of HTM allele into ADT 55 background. Similar introgression was done in the Pusa Basmati variety "PB 1121" through molecular marker assisted backcross breeding method and screening with Imazethapyr was done in the BC_4F_4 generation (7). Significant differences between two plots were tested by student's t-test. Plant height and number of productive tillers showed significance whereas the rest of the traits did not. In the unsprayed plots, both traits were observed to be lower than the sprayed plot, indicating the suppression of growth of rice plants caused by surrounding weeds (Table 1). Similarly, previous study (8), observed significant differences for traits such as plant height, number of productive tillers and also for number of

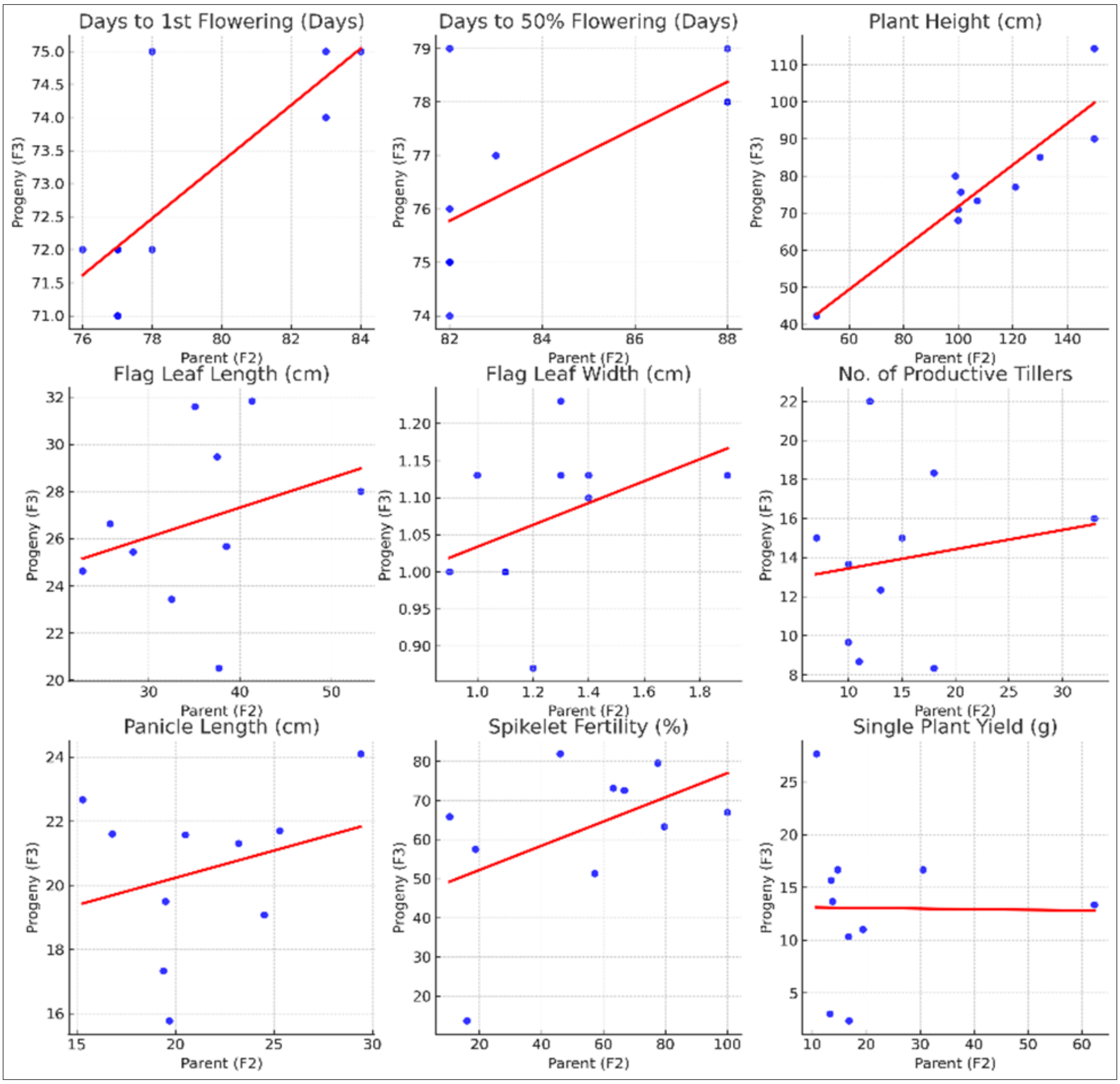

Fig. 5. Parent progeny regression plot for some of the critical traits.

filled grains per panicle, yield per plant and straw yield per plant in the control and the imazethapyr sprayed plot of the herbicide tolerant mutant Robin-HTM.

The association between the F_2 and F_3 generations was studied to determine the extent to which traits are inherited from the next generation, which in turn will aid in the efficient selection of highly heritable traits. Positive intergenerational correlation and significant regression coefficient values were observed for all the traits studied. Similarly, another study (9), reported highly significant intergenerational correlation and regression coefficients for all the traits in their study *viz.*, number of productive tillers per plant, number of chaffy grains per panicle, single plant yield, number of filled grains per panicle, days to fifty percent flowering, flag leaf length, hundred seed weight and spikelet fertility. A similar study observed highly significant intergenerational and regression values for characters like plant height and single plant yield (10). Another study also observed similar findings for plant height, number of filled grains per panicle, panicle length and hundred grain weight (11). These studies indicate that these traits are less influenced by the environment and selection based on phenotype could be effective. High correlation across generations suggests some genetic consistency, though environmental effects likely still contribute to trait variation.

However, additive genetic variance acts as a deciding factor on whether a trait can be fixed or not in the next generation, which is a key tool for selection programs. Narrow sense heritability is a measure of how much a trait is governed by additive gene action. In this study, low heritability values indicate limited additive variance, possibly due to non-additive effects, environmental variation, or both. A study reported moderate narrow sense heritability for days to fifty percent flowering, plant height, number of productive tillers per plant, flag leaf length, number of filled grains per panicle, spikelet fertility and single plant yield (9). A similar study reported moderate narrow sense heritability for all the traits except for plant height (12). Similar results were demonstrated in the previous studies (13, 14), where days to 50 % flowering, number of productive tillers per plant, number of filled grains per panicle and single plant yield showed moderate narrow sense heritability. Similar study involved introgression of herbicide tolerance trait in to ADT 43 rice variety through marker assisted backcross breeding (15).

Conclusion

This study effectively introgressed imazethapyr herbicide tolerance into the ADT 55 rice variety using Robin HTM as a donor, demonstrating the feasibility of developing herbicide-resistant direct-seeded rice (DSR) varieties. The F_3 progenies exhibited 100 % survival under herbicide application, confirming the effective transfer of the resistance gene. Agronomic trait evaluation revealed significant differences in plant height and the number of productive tillers between herbicide-treated and control plots, highlighting the impact of weed competition. Intergenerational analyses indicated stable inheritance of key traits, although low/moderate heritability suggests limited additive variance and is not sufficient to conclude gene action type. These findings provide a foundation for developing high-yielding, herbicide-tolerant rice varieties, contributing to sustainable and efficient weed management in DSR cultivation.

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Authors' contributions

RM, MS and SM aided in planning the outline of this study. RA carried out the research and drafted the manuscript. BA reviewed and corrected the manuscript. RM, MS, SM, RS, PR, SKG and SA overviewed the research and finalised the manuscript. All the authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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