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RESEARCH

Harnessing inter-subspecific genetic variability for hybrid rice improvement: analysis of genetic diversity, heterosis, and combining ability

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Abstract The primary constraint in Indian hybrid rice breeding programs is the limited genetic diversity of parental lines. To address this, pre-breeding strategies are essential for broadening the genetic base. In this study, we evaluated 106 *indica* × tropical *japonica*-derived (IJD) lines, along with their respective parental lines and standard checks (n = 150 genotypes). The evaluation focused on morphological, molecular, and grain quality traits to identify high-yielding lines with desirable grain characteristics. Morphological characterization focused on 12 yield-related traits and revealed several derived lines that significantly outperformed the standard checks across multiple attributes. Notably, the genetic distance between B (female) and R (male) lines increased substantially in the newly developed parental lines compared to previously used lines, indicating enhanced genetic divergence. Combining ability and heterosis of the newly developed inter subspecific R lines were also assessed. Molecular analysis using random simple sequence repeat (SSR) markers showed a significant positive correlation ($r = 0.30$, $P < 0.05$) between molecular genetic distance and the general combining ability (GCA) of male parents for grain yield. These findings highlight the value of selecting genetically diverse parents with high GCA to generate heterotic hybrids and improve breeding efficiency. Overall, this study developed a set of genetically diverse and agronomically superior parental lines, offering a valuable resource for future hybrid rice breeding and transgressive segregation-based improvement programs.

Keywords Intersubspecific hybridization · *Indica* and tropical *japonica* derived lines · Genetic base · Parental lines · Combining ability · Heterosis · Hybrid rice

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Introduction

In the context of climate change and increasing global food demand, hybrid rice breeding has become a key strategy to develop high-yielding cultivars with enhanced resistance to both biotic and abiotic stresses, alongside improved grain quality (Hari et al. 2013; Prasad et al. 2019). Recent innovations in this domain include the development of DRRH4 and DRRH5—the world's first aerobic and coastal salinity-tolerant rice hybrids, respectively. Under Indian field conditions, DRRH4 has demonstrated a yield advantage of 11–30%, while DRRH5 has shown a yield increase of 35–70% over standard check varieties (Senguttuvel et al. 2023; 2024). In China, hybrid rice occupies approximately 57% of the total rice cultivation area of about 16 million hectares out of 30.18 million hectares and contributes around 65% of total rice production, achieving average productivity levels of 7.5 t/ha (Qian et al. 2021; Ali et al. 2021). India has also made notable progress, with the release of 162 rice hybrids to date (ICAR-IIRR, Progress Report, AICRPR, Vol. 1 Varietal Improvement 2023). However, despite these advances, the area under hybrid rice cultivation in India remains limited. One of the primary challenges in hybrid rice breeding is to enhance the heterosis level or yield advantage of hybrids over varieties. Additionally, addressing regional grain quality preferences—particularly for medium slender (MS) or short slender (SS) grain types with cooking and eating qualities similar to BPT5204 is crucial, especially in Southern India. To overcome these challenges, harnessing diverse germplasm sources to broaden the genetic base represents a key approach for maximizing heterosis and breaking through current yield plateaus. To overcome the yield plateau associated with the predominant use of *indica* germplasm and the narrow genetic base of parental lines, hybrid rice breeding must explore alternative strategies (Peng et al. 2004). These include the deployment of two-line breeding systems and inter-subspecific hybridization, particularly between *indica* and tropical *japonica* lines. While *indica*×*japonica* hybrids often exhibit strong vegetative heterosis, they frequently suffer from reproductive sterility and poor grain quality due to high inter-subspecific divergence and segregation of undesirable traits in the hybrid progeny (Yang 1990; Khush and Aquino 1994). Partial sterility in *indica*×*japonica* hybrids is another major challenge encountered in their crosses, often limiting their effective use in hybrid breeding programs (Kato et al. 1928). To mitigate these challenges, Yuan (1991a, b) proposed the use of tropical *japonica* (*javanica*) cultivars or biased *indica*/*japonica* lines as parental sources. Building on this approach, Khush and Aquino (1994) developed tropical *japonica* lines at the International Rice Research Institute (IRRI), characterized by a novel plant type with reduced tillering, larger panicles, and fewer unproductive tillers traits associated with enhanced yield potential. Given their broader genetic diversity compared to *indica* rice (Glaszmann 1987), tropical *japonica* lines are expected to express stronger heterotic responses when crossed with *indica* lines. Therefore, the development and evaluation of *indica*×tropical *japonica*-derived parental lines and their hybrids offer a promising avenue to enhance heterosis and broaden the genetic base of hybrid rice breeding programs. In the present study, we analyzed a set of 150 genotypes, including 106 lines derived from *indica*×tropical *japonica* crosses and their parental lines. These lines were characterized using both agro-morphological traits and molecular markers (SSR) to assess genetic diversity. The selected parental lines were evaluated for their combining ability and heterotic response, with the aim of identifying superior hybrid combinations and gaining insights into the combining ability and heterotic potential of newly developed *indica* tropical *japonica* derived-R lines.

Material and methods

Plant material

A total of 150 genotypes comprising 106 *indica*×tropical *japonica*-derived (IJD) lines developed from 32 inter-subspecific crosses were evaluated for morphological characterization. The female parents in these crosses included 16 elite *indica* hybrid parental lines, and the male parents consisted of 24 tropical *japonica* lines. Of the original 24 tropical *japonica* parents, six were excluded from field evaluation due to poor germination. In addition, 10 checks were included for comparative analysis (Supplementary Table 2). To assess performance across different maturity groups, 10 high-yielding national checks were selected based on recommendations from the

All India Coordinated Research Project on Rice (AICRPR). Genotypes were categorized by flowering duration in accordance with AICRPR classification: early (81–90 days), mid-early (91–100 days), medium (101–110 days), and late (> 110 days to 50% flowering). For molecular characterization, a total of 149 genotypes were analyzed using simple sequence repeat (SSR) markers (Supplementary Table 3). This included the same 106 IJD lines, along with 16 *indica* parental lines (IP), 24 tropical *japonica* parental lines (TPJ), and three reference lines: RPHR1005 (a widely used restorer line), IR58025B (a popular maintainer line), and IRGC328 (a representative tropical *japonica* accession).

Phenotyping for yield and grain quality related traits

The experiment was conducted in wet season of 2018 using an alpha lattice design with three replications, which was adapted from the IASRI design of the resource server (www.iasri.res.in/design). A total of 150 genotypes (v = 150) were evaluated under three replications (r = 3), with 15 blocks per replication (s = 15) and a block size of 10 (k = 10). Each row contained 25 hills, with a spacing of 20 cm by 15 cm. Recommended agronomic practices were followed to ensure the healthy growth of the crop. Field data were collected on 12 yield and yield-related traits according to the descriptors prescribed by the International Rice Research Institute (IRRI) (SES, IRRI 2013) at the research farm of ICAR-Indian Institute of Rice Research (ICAR-IIRR), located in Hyderabad (17° 19′ N, 78° 29′ E) at an altitude of 549 m above mean sea level. A total of 12 agronomic traits were measured in the study, which included: days to 50% flowering (DFF), plant height (cm) (PH), panicle length (cm) (PL), number of productive tillers per plant (PT), total number of filled grains per panicle (FG), total number of unfilled grains per panicle (UFG), spikelet fertility (%) (SF), single plant yield (g) (SPY), biological yield (g) (BY), harvest index (%) (HI), 1000-grain weight (g) (TGW), and per day productivity (kg ha^{-1}) (PDP). Data were recorded using the Field Book app on Android devices (Rife and Poland 2014). In addition to the yield-related traits, six grain quality parameters were also evaluated across the 150 genotypes. These included amylose content (%) (AC), gel consistency (mm) (GC), alkali spreading value (ASV), kernel length (mm) (KL), kernel breadth (mm) (KB), and the kernel length-to-breadth ratio (L/B ratio).

Genotyping and data analysis

A set of 50 SSR markers, selected by IRRI under the Generation Challenge Programme (GCP) of CGIAR (*Gramene SSR markers*), was used for molecular diversity analysis. Seed material from 149 lines was initially germinated in Petri plates, and leaf samples were collected from 14-day-old seedlings for DNA extraction. Total genomic DNA was isolated following the protocol described by Zheng et al. (1991). The extracted DNA from all 149 lines was then used for PCR amplification. PCR was performed using a programmable thermocycler (Veriti Thermo Cycler, Applied Biosystems). The reaction mixture (prepared in a 1.5 ml microcentrifuge tube) consisted of 3 µl of nuclease-free water, 0.5 µl each of forward and reverse primers, 4 µl of EmeraldAmp GT PCR Master Mix (Takara), and 2 µl of template DNA. The thermal cycling conditions included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 50–69 °C for 30 s, and extension at 72 °C for 1 min, with a final extension step of 10 min at 72 °C. The amplified PCR products were resolved on a 3% agarose gel prepared in 1× TBE buffer and stained with ethidium bromide. The bands were visualized and documented using a gel documentation system (Bio-Rad, USA). Allele scoring was performed manually with reference to a 100 bp DNA ladder.

To identify genotypes with statistically significant superiority over duration-based varietal and hybrid checks, replicated field data were analyzed using alpha lattice design in PB Tools version 1.4. Correlation analysis was performed in R Studio using the *cor* function to examine relationships among traits (R Core Team 2020). Morphological diversity was assessed using DARwin software version 6.0.010 (Perrier and Jacquemoud-Collet 2006). For molecular diversity analysis, genetic data were processed using POWERMARKER version 3.25 (Liu and Muse 2005), and population structure was inferred using STRUCTURE software version 2.3.4 (Pritchard et al. 2000). STRUCTURE HARVESTER (Earl 2012) was used to determine the optimal number of subpopulations (K) based on the ΔK method. To assess the congruence between morphological and molecular

distance matrices, a Mantel test was conducted in R Studio using the *mantel.rtest* function from the *ade4* package.

Combining ability and heterosis

Out of a total of 106 IJD lines, fertility restorers were identified using functional markers for fertility restoration genes and further validated through test cross nursery performance (Sruthi et al. 2023). Based on the validation results, 13 restorer lines were selected and subsequently crossed with four CMS lines—IR68897A, IR79156A, APMS6A, and PUSA5A—using a line × tester mating design during the Rabi season of 2019–20. This crossing program resulted in the development of 52 hybrid combinations. The 52 hybrids, along with 17 parents (13 restorer lines and 4 maintainer lines) and 8 checks, were evaluated during the Kharif season of 2019 at the Research Farm of ICAR-IIRR, Rajendranagar, Hyderabad. The evaluation was conducted in a randomized complete block design (RCBD) with two replications. To assess hybrid performance across varying maturity durations, 8 high-yielding national checks from the AICRPR were included—comprising 4 varietal checks (BPT5204, ISM, Gontrabidhan, and NDR359) and 4 hybrid checks (US314, US312, HRI174, and DRRH3). Data were recorded for 11 yield and yield-contributing traits, which included: days to 50% flowering (DFF), plant height (cm) (PH), panicle length (cm) (PL), number of productive tillers (PT), pollen fertility (%) (PF), spikelet fertility (%) (SF), 1000-grain weight (g) (TGW), single plant yield (g) (SPY), biological yield (g) (BY), harvest index (%) (HI), and per day productivity (kg ha^{-1}) (PDP).

Results

Morphological and grain quality characterization

The mean performance of 150 genotypes for yield and yield-related traits is presented in Supplementary Table 2, while data for grain quality traits are summarized in Supplementary Table 4. Analysis of variance revealed highly significant differences among the genotypes for all the traits studied (Sruthi et al. 2020; Supplementary Table 1). With respect to DFF, the IJD lines, IP, and TJP were classified as mid-early maturing, with flowering durations ranging from 96 to 100 days. Among the four groups, TJP exhibited the greatest average PH at 111.8 cm, followed by IJD lines (105.3 cm), IP (99.2 cm), and checks (92.26 cm). For PL, TJP again recorded the highest mean value (24.28 cm), whereas the other groups showed comparable average lengths (23 cm). In traits such as PT, FG, UFG, and SPY, TJP consistently recorded the lowest mean values, while the IJD lines, IP, and checks demonstrated similar performance. Among all groups, checks achieved the highest average FG (156) and also recorded the highest SF%, while IP had the lowest SF%.TJP showed the highest TGW at 24.14 g. The checks recorded the highest HI at 50% and PDP at 31.06 kg ha^{-1}. All four groups exhibited intermediate AC, and similarly, GC values across groups were indicative of hard consistency, measuring less than 40 mm. Grain type across the four groups generally fell under the medium slender category, although TJP lines were primarily classified as short bold. In terms of ASV, all four groups fell under the intermediate gelatinization temperature category, with ASV values ranging from 4 to 5. Trait-wise significance among the four groups was assessed using Tukey's multiple comparison test (Fig. 1). No significant differences were observed among groups for DFF, UFG, SF%, SPY, HI, and PDP. However, TJP exhibited significantly higher PH (mean: 111.82 cm) compared to the checks (92.26 cm) and IP (99.24 cm). Significant differences in PL were observed between IJD lines (23.03 cm) and TJP (24.24 cm). TJP also showed significantly lower PT compared to IJD lines and IP. For FG, IJD lines (148) significantly outperformed TJP (123). A significant difference in TGW was recorded between IP (20.54 g) and TJP (24.14 g). In terms of biological yield (BY), TJP (20 g) showed significantly lower performance compared to checks (25.34 g) and IJD lines (23.55 g).

Superior performance of IJD lines based on pairwise comparisons with checks

In the pairwise comparison analysis with checks, the best-performing check for each trait was selected from a set of 10. Among these, DRRH3 showed superior performance for several traits: PT (9), FG (259), SPY (19.83 g), BY (37.04 g), and PDP (47.99 kg/ha). Gontrabidhan performed best for UFG (14), SF% (91.89%), and HI (61%). NDR359 recorded

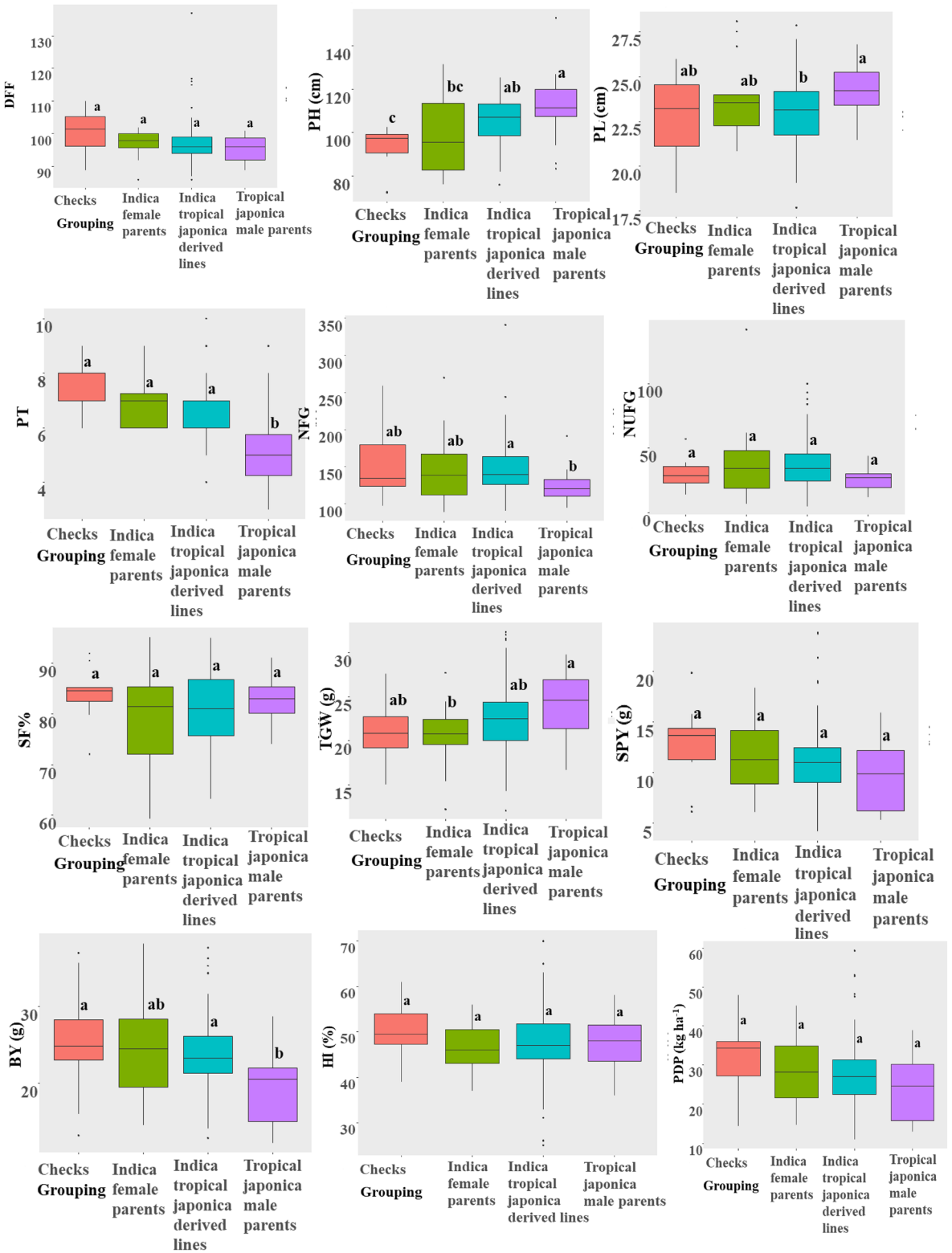


Fig. 1 Phenotypic performance of *indica* parents, tropical *japonica* parents, *indica* tropical *japonica* derived lines and checks for 12 yield attributing traits and their significance indicated through letters using tukeys multiple comparison test

the highest PL (26.01 cm) and TGW (27.63 g), followed by US312 with a PL of 25.09 cm. No genotype showed significant superiority over these two checks in PL. For PT, none of the genotypes significantly outperformed the checks. However, RP6375-68 (341) showed significant superiority in FG over all 10 checks. For SF%, no genotype surpassed Gontrabidhan (91.89%). In TGW, although none exceeded NDR359, three genotypes—RP6389-83 (32.42 g), RP6389-85 (32.34 g), and RP6389-100 (32.08 g)—were significantly superior to the other nine checks. In terms of SPY, RP6380-44 (23.84 g) and RP6378-89 (23.73 g) significantly outperformed all checks except DRRH3. For BY, HI, and PDP, no genotype showed significant improvement over the respective best-performing checks.

Duration-wise mean performance of genotypes

In the early duration category, 12 genotypes were identified, comprising 7 IJD lines, 1 IP, 2 TJP, and 2 checks (Supplementary Table 5). Among them, DR 714-1-2R (PH: 79.40 cm), RP6369-2 (PL: 25.74 cm), and two TJP genotypes—IRGC34018 (TGW: 29.81 g) and IRGC5726 (TGW: 27.49 g)—showed significant superiority in PH, PL, and TGW over US314, the best-performing check in this group. However, for PT, FG, SPY, BY, HI, and PDP, none of the early-duration genotypes surpassed the respective best checks. In the mid-early category, 106 genotypes were grouped, including 77 IJD lines, 12 IP, 15 TJP, and 2 checks (Supplementary Table 6). Gontrabidhan and US312 used as the varietal and hybrid checks, respectively. For FG, RP6375-68 (341) was significantly superior to Gontrabidhan (185). RP6380-44 also outperformed Gontrabidhan in SPY, BY, and PDP. The medium duration group included 26 genotypes: 17 IJD lines, 3 IP, 1 TJP, and 5 checks (ISM, NDR359, HRI174, JKRH3333, DRRH3). Their mean performance is presented in Supplementary Table 7. No genotype in this group showed significant superiority over the trait-wise best checks. In the late category, 6 genotypes were evaluated (5 IJD lines and 1 check—BPT5204), with mean performance summarized in Supplementary Table 8.

Table 1 provides a summary of duration-wise average trait performance. The early group recorded the highest average PH, followed by mid-early, late, and medium groups. The late group showed the highest mean values for PL (23.69 cm), SPY (12.77 g), BY (23.88 g), and HI (52.01%). However, the mid-early group exhibited the highest individual genotype values: SF% (95.19%), TGW (32.42 g), SPY (23.84 g), BY (38.25 g), HI (69.65%), and PDP (59.31 kg/ha). PT values were similar across all four duration groups. The medium group had the highest averages for FG (165) and UFG (52). For SF% (82.26%), TGW (22.97 g), and PDP (28.20 kg/ha), the mid-early group recorded the highest group means.

Correlation studies

Correlation among IJD lines for yield traits: SPY showed significant positive correlations with several traits: PH (0.17, $p<0.05$), PL (0.20, $p<0.05$), PT (0.36, $p<0.01$), TT (0.31, $p<0.01$), BY (0.90, $p<0.01$), HI (0.75, $p<0.01$), and PDP (0.99, $p<0.01$).

Correlation among hybrid yield traits: The correlation analysis among hybrid yield traits revealed that SPY was significantly and positively associated with PH (0.62, $p<0.01$), PL (0.43, $p<0.01$), PF% (0.43, $p<0.01$), SF% (0.53, $p<0.01$), BY (0.97, $p<0.01$), HI (0.79, $p<0.01$), PDP (0.94, $p<0.01$), Specific combining ability for grain yield (SCA_GY) (0.71, $p<0.01$), General combining ability of male parents for grain yield (GCAMP_GY) (0.35, $p<0.01$), and General combining ability of female parents for grain yield (GCAFP_GY) (0.61, $p<0.01$). Furthermore, the molecular genetic distance (GD) between parental lines exhibited a weak positive correlation with hybrid SPY (0.12) but showed a significant positive correlation with GCAMP_GY ($r=0.30$, $P<0.05$) (Fig. 2).

Genetic relationships through phenotypic clustering

Phenotypic clustering was performed using Darwin software ver. 6.0.010 based on 12 yield traits and six grain quality traits, resulting in the formation of eight distinct clusters (Fig. 3a). Cluster IV was the largest, comprising 38 genotypes, followed by Cluster VII with 28 genotypes and Cluster I with 21 genotypes (Supplementary Table 9). The clustering pattern reflected both phenotypic similarities and pedigree (Table 2). Cluster I consisted mainly of lines derived from RPHR1005 and IBL-57, including the parent RPHR1005. ISM is an improved line of BPT5204

Table 1 Comparison of trait performance among different duration groups

Sl. No.	Trait	Early Duration (80–90 Days DFF)		Mid Early Duration (91–100 Days DFF)		Medium Duration (101–110 Days DFF)		Late Duration (Above 110 Days DFF)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
1	DFF	89	86–90	96	91–100	103	101–108	118	110–137
2	PH	106.4	79–125	105.79	75.8–152.73	99.79	72.4–119.73	100.12	72–112.67
3	PL	23.45	20.49–25.74	23.31	17.7–28.1	22.71	18.51–26.01	23.69	19.68–26.22
4	PT	6	5–9	7	3–10	7	4–9	7	6–8
5	FG	129	89–162	142	91–341	165	97–270	135	102–209
6	UFG	31	17–65	32	5–88	52	12–142	35	23–53
7	SF	80.66	64.91–89.03	82.26	65.85–95.19	76.99	56.4–90.03	78.80	69.13–87.59
8	TGW	22.41	18.24–29.81	22.97	14.86–32.42	19.49	12.06–27.63	22.38	15.06–32.34
9	SPY	10.05	4.21–14.26	11.32	4.39–23.84	11.01	5.97–19.83	12.77	6.64–23.73
10	BY	21.03	14.52–26.19	23.46	12.22–38.25	23.86	13.21–37.05	23.88	13.19–36.33
11	HI	47.13	25.64–57.4	47.62	25.23–69.65	45.70	32.93–56.34	52.01	43.87–65.33
12	PDP	26.38	11.16–36.29	28.20	11.02–59.31	26.09	14.45–47.99	27.70	14.87–52.81
13	AC	22.54	18.1–25.89	24.33	12.49–27.49	24.76	17.12–27.25	25.37	20.32–27.37
14	GC	44.75	22–64	37.83	21.67–93.67	43.17	23.67–73.33	33.78	25.33–62.67
15	ASV	5.00	3–7	4.45	3–7	4.35	3–7	4	3–5
16	KL	5.19	4.44–6.28	5.40	4.57–6.69	5.21	4.61–6.34	5.27	4.87–6.32
17	KB	2.17	1.82–2.76	2.17	1.73–2.68	1.99	1.7–2.44	2.08	1.83–2.49
18	L/B ratio	2.43	2.13–2.8	2.52	1.81–3.51	2.63	2.17–3.07	2.56	2.37–2.85

DFF Days to 50 per cent flowering; *PH* Plant height (cm); *PL* Panicle length (cm); *PT* Productive tillers plant-1; *FG* Number of filled grains; *UFG* Number of unfilled grains; *SF* Spikelet fertility (%); *TGW* Thousand grain weight (g); *SPY* Single plant yield (g); *BY* Biological yield (g); *HI* Harvest index (%); *PDP* Per day productivity (kg ha^{-1}); *AC* Amylose content (%); *GC* Gel consistency (mm); *ASV* Alkali spreading value; *KL* Kernel length (mm); *KB* Kernel breadth (mm); L/B ratio- Length/breadth ratio

with enhanced resistance to bacterial blight. Both genotypes exhibited the least phenotypic genetic distance and were grouped together in Cluster I. Cluster II was the smallest, consisting of seven distinct crosses. Cluster III included several maintainer lines and their derivatives. Cluster IV comprised derived and tropical *japonica* lines. Cluster V primarily featured lines derived from RPHR619-2, while Cluster VI contained ten derived lines and one check variety. Cluster VII included 28 genotypes such as RP6390-91 and KMR3R, and Cluster VIII comprised ten restorer-derived lines along with two hybrid checks (US312 and US314). The morphological genetic distance among genotypes ranged from 1.64 to 14.15, with a mean of 6.10. Duration-wise, medium-duration genotypes were predominant in Cluster I, while late-duration types were grouped in Cluster II. Clusters I, II, III, V, and VI had semi-dwarf PH (< 110 cm), while Clusters IV, VII, and VIII exhibited intermediate PH (110–130 cm). Cluster I recorded the highest average FG (195) and UFG (63), while Cluster VIII showed the highest average SF%. Cluster V exhibited the highest TGW (29.19 g), with genotypes predominantly having long bold (LB) grain types. Cluster VII recorded the highest averages for SPY, BY, and PDP. Grain type distribution across clusters showed that Clusters I, II, III, VI, and VIII were associated with medium slender (MS) types, Clusters IV and VII with short bold (SB) types, and Cluster V with long bold (LB) types (Supplementary Table 10).

Genotypic clustering

All 50 SSR markers used in this study were polymorphic, generating a total of 306 alleles across 149 IJD lines. The number of alleles per locus ranged from 2 (RM495) to 13 (RM536), with an average of 6.12 alleles per locus (Supplementary Fig. 3). Major allele frequency varied from 0.32 (RM316) to 0.75 (RM431), with a mean of 0.48. Gene diversity ranged from 0.41 (RM431) to 0.77 (RM316 and RM552), averaging 0.65 across markers.

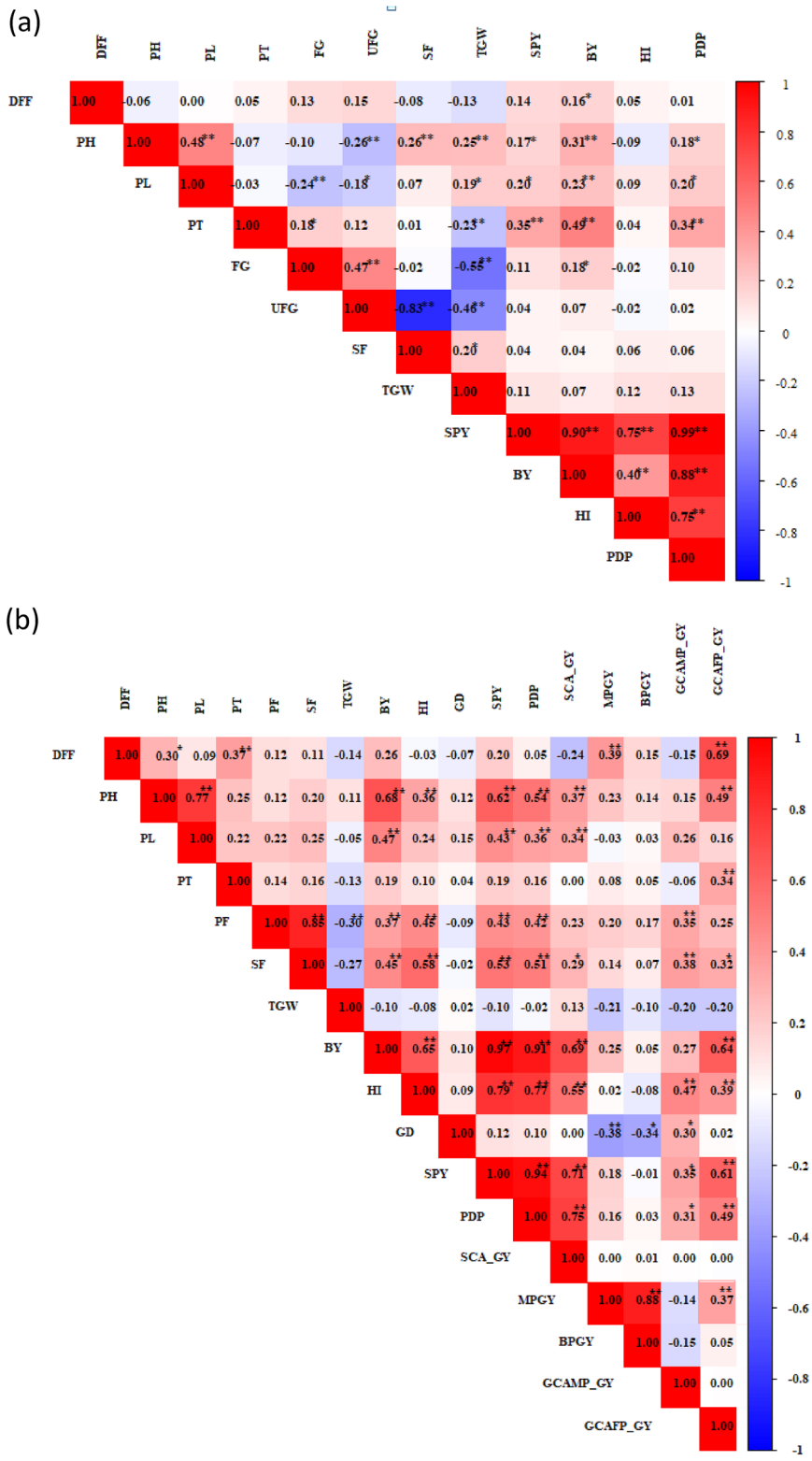


Fig. 2 Correlation among yield and its contributing traits **a** IJD lines correlation analysis, **b** hybrid data correlation analysis

Heterozygosity ranged from 0.00 (observed in markers such as RM277, RM552, RM484, RM284, RM454, RM133, RM178, RM431, RM237, RM312, RM283, and RM452) to 0.24 (RM144), with an overall mean heterozygosity of 0.042. Out of the 50 loci, 32 showed heterozygosity while 12 exhibited none. Polymorphism information content (PIC) values ranged from 0.35 (RM133) to 0.74 (RM552 and RM124), with a mean PIC value of 0.60 (Supplementary Table 11). A total of 149 genotypes were grouped into three clusters: two major (Clusters I and II) and one minor (Cluster III) (Fig. 3b; Supplementary Table 12). Cluster I (95 genotypes) consisted mainly of IJD lines, while Cluster II (48 genotypes) included both TJP and IP lines. Cluster III comprised six genotypes, all IJD. Cluster I was further divided into five subclusters (IA–IE): IA was dominated by RPHR1005-derived lines; IB included lines from 10 crosses; IC from 7 crosses; ID was primarily from Uttrirajappan-derived lines; and IE was mostly Akshyadhan-derived. Cluster II included four subclusters (IIA–IID), with more diverse lineage backgrounds. Genetic distances ranged from 0.02 to 0.85 (mean = 0.33), indicating wide genetic variability. A weak and non-significant correlation (r = −0.00928) was observed between phenotypic and genotypic distance matrices.

Population structure analysis

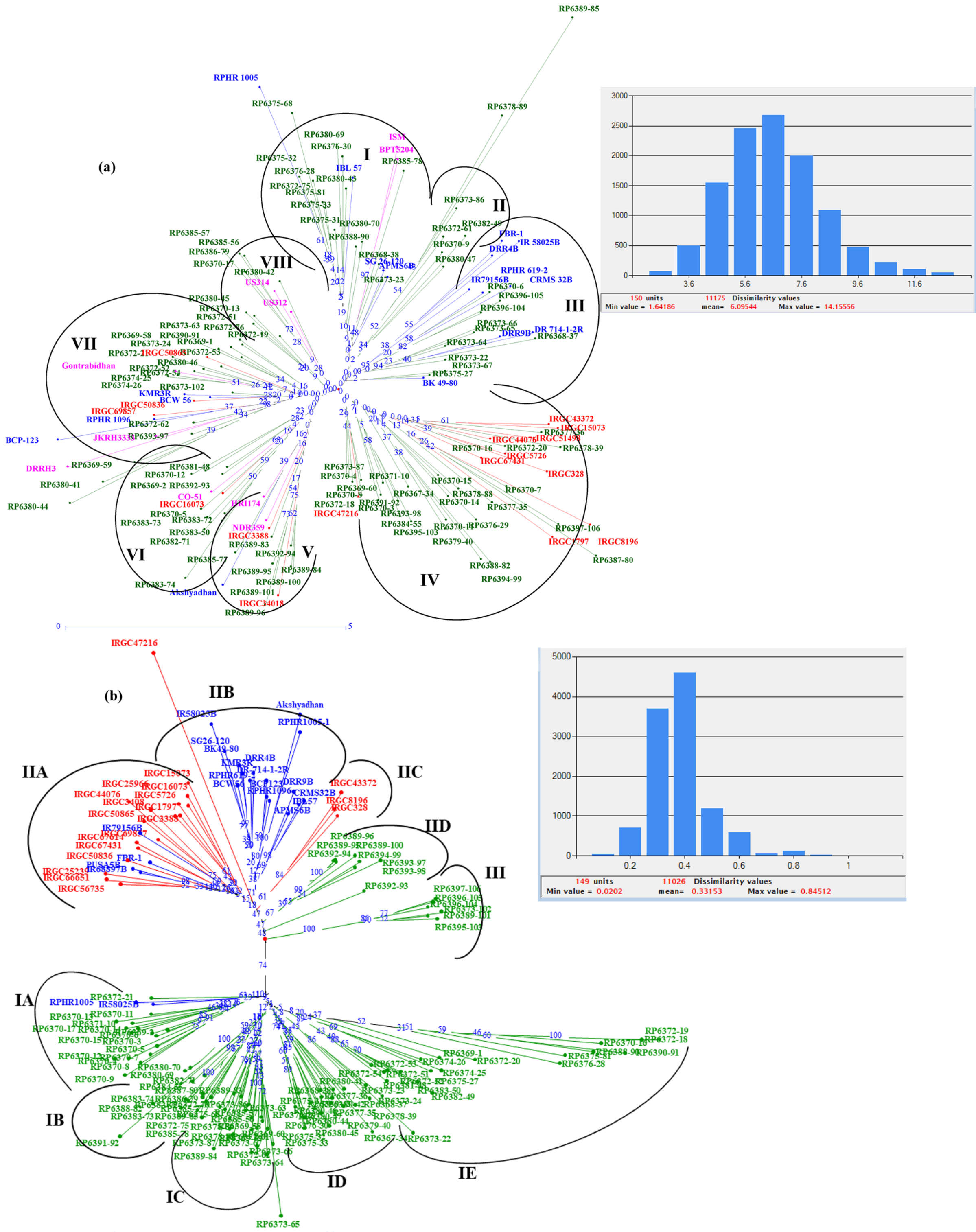
STRUCTURE analysis revealed two distinct populations (K = 2) (Fig. 4a, b). Population I contained 54 pure lines from IP and TJP, while Population II comprised 94 pure IJD genotypes, along with one admixed genotype (< 80% membership probability) (Supplementary Table 13). Further subdivision of Population I (based on ΔK peak at K = 3; Fig. 4c, d) revealed three subgroups, reflecting greater heterogeneity among IJD lines. The allele frequency divergence between clusters was 0.1481. Average genetic distances within Population I and II were 0.6596 and 0.5435, respectively. The mean alpha value was 0.0290, indicating low admixture. Fixation index (Fst) values were 0.1112 (Population I) and 0.3342 (Population II), suggesting moderate to high genetic differentiation.

Inter-sub specific derived restorer lines: genetically distinct and diverse

To evaluate the genetic diversity of newly developed inter-subspecific restorer (R) lines in comparison with existing *indica* parental lines, a separate diversity analysis was conducted using both molecular and morphological data. The molecular genetic distance (based on the Simple Matching method) among the newly developed R lines and existing *indica* parental lines ranged from 0.030 to 0.668, with an average of 0.333 (Fig. 5a). The clustering pattern clearly indicated that the new R lines formed distinct groups, separate from the existing *indica* parental lines. Within the *indica* parental lines, genetic distances ranged from 0.061 to 0.364 (mean = 0.226) (Fig. 5b), while within the newly developed R lines, distances ranged from 0.030 to 0.630, with an average of 0.311 (Fig. 5c), suggesting considerable diversity among the new lines. Morphological genetic distances between the new and existing *indica* lines ranged from 1.81 to 13.02 (mean = 6.08) (Supplementary Fig. 1a). Within *indica* lines, the range was 2.37 to 9.65 (mean = 6.14) (Supplementary Fig. 1b), while within the newly developed R lines—most of which were derived through inter-subspecific hybridization—the range was 1.87 to 11.94, averaging 6.10 (Supplementary Fig. 1c). When compared with commonly used B lines, the average molecular distance was higher for the newly developed R lines (0.37) than for the existing *indica* R lines (0.23). However, morphological distances were relatively similar, with B vs. new R lines at 6.32 and B vs. *indica* R lines at 6.53.

Heterotic potential and combining ability of inter-subspecific derived R lines

Analysis of variance revealed highly significant differences among the genotypes for all traits under study, indicating substantial genetic variability among the 69 genotypes (including parents and hybrids) (Table 3). The mean performance of hybrids, along with their parents and checks, is presented in Fig. 6. Among the eight checks, the mid-early duration hybrid US312 recorded the highest yield (24.02 g), followed by the medium duration hybrid HRI-174 (23.33 g) and the late duration hybrid DRRH3 (16.18 g). The standard heterosis for single plant yield relative to the


(a)
I
II
III
IV
V
VI
VII
VIII
RP6389-85
RPHR 1005
BCP-123
DRRH3
Gontrabidhan
150 units
11175 Dissimilarity values
Min value = 1.64186
mean= 6.09544
Max value = 14.15556
(b)
IIA
IIB
IIC
IID
III
IA
IB
IC
ID
IE
IRGC47216
Akshyadhan
RP6373-65
149 units
11026 Dissimilarity values
Min value = 0.0202
mean= 0.33153
Max value = 0.84512

◂**Fig. 3** **a** Phenotypic cluster from DARwin software based on Euclidean genetic distance, **b** Unweighted neighbor joining radial tree showing distribution of 149 *indica* tropical *japonica* derived lines based on 50 GCP SSR marker allelic data. Green colour indicates *indica* tropical *japonica* derived lines; Red colour indicates tropical *japonica* lines; Blue colour indicates *indica* female parents. (Color figure online)

best check, US312, ranged from −89.03 to 33.08%. Against HRI-174, heterosis ranged from −88.71 to 37.01%, and for DRRH3, it ranged from −83.71 to 97.62% (Supplementary Table 14). Out of 52 hybrids evaluated, 16 exhibited significant positive mid parent heterosis, while 8 showed significant superiority over the better parent. In terms of standard heterosis, none of the hybrids surpassed US312 significantly; however, one hybrid outperformed HRI-174. Additionally, four hybrids demonstrated significant superiority over DRRH3, and eleven hybrids outperformed US314. Notably, the hybrid PUSA5A × RP6370-13 showed significant superiority for grain yield over all checks except US312, against which it recorded a non-significant positive standard heterosis of 33.08%. The trait-wise minimum and maximum heterosis over the best check is illustrated in Supplementary Fig. 2.

Analysis of variance (ANOVA) further revealed significant genotypic differences across all traits studied. Variance due to parents was significant for most traits, except PH, SF%, and HI%. According to Sharma, 1988, in the present study, male sterile lines were used as testers (female parents) and test genotypes were used as lines (pollen parents). Among the parents, lines (R lines) showed significant variation for DFF, PH, PL, SPY, TGW, BY, and PDP, whereas testers (CMS lines) were significant for DFF, PL, PF%, SF%, and TGW. The mean sum of squares comparing parents and crosses was non-significant for PT, SF%, TGW, and BY. The variance due to crosses and line × tester interaction was significant for all traits. GCA variance exceeded SCA for DFF, PH, SPY, BY, and HI, suggesting a predominance of additive gene action. Conversely, SCA variances were greater than GCA for PL, PT, PF, SF, TGW, and PDP, indicating the importance of non-additive gene action. The line × tester interaction contributed substantially to variation in all traits: PH (39.13%), PL (64.08%), PT (57.93%), PF (64.04%), SF (61.02%), SPY (50.52%), TGW (59.83%), BY (50.28%), HI (47.39%), and PDP (63.65%). DFF was the only trait where testers accounted for the highest contribution (48.48%) among hybrids. Overall, while the line × tester interaction accounted for the largest share of variance in hybrid performance, testers contributed more to traits such as DFF, PH, SPY, BY, and PDP, whereas lines had a greater influence on PF, SF, TGW, and HI.

General and specific combiners for yield and related traits

Parents exhibiting significant General Combining Ability (GCA) effects for various yield-attributing traits are illustrated through a stacked bar graph (Fig. 7 and Supplementary Table 15). Among the four testers, PUSA5A emerged as the strongest general combiner for most yield and yield-related traits, except PL and TGW. IR68897A was identified as a good general combiner for DFF and PH, while IR79156A showed strong GCA for PL and PT. APMS6A was a strong general combiner specifically for PF%. Among the 13 R lines evaluated, five were identified as top general combiners for yield-related traits: RP6376-28 excelled in SPY and BY; RP6368-38 showed high GCA effects for PT, PF%, and SF%; RP6370-13 was superior for PL and TGW; RP6370-4 for TGW; and RP6376-30 for PF, SF, and BY. Based on Specific Combining Ability (SCA) effects, the following hybrids were identified as top performers, with their corresponding mean performance and GCA effects of the parents provided in Supplementary Table 16. PUSA5A × RP6372-75 showed highly significant SCA effects for SPY (12.996, $P < 0.01$), PDP (34.976, $P < 0.01$), DFF (−8.673, $P < 0.01$), and HI (18.933, $P < 0.01$). IR68897A × RP6368-38 was significant for SPY (10.38, $P < 0.01$), PDP (25.598, $P < 0.05$), PL (1.905, $P < 0.01$), and BY (14.66, $P < 0.01$). PUSA5A × RP6370-13 recorded high SCA effects for SPY (9.537, $P < 0.01$), PDP (22.026, $P < 0.05$), PL (3.803, $P < 0.01$), SF (11.287, $P < 0.01$), and BY (13.058, $P < 0.01$). APMS6A × RP6376-30 showed significant SCA effects for SPY (8.014, $P < 0.01$), BY (10.263, $P < 0.01$), and PT (4.632, $P < 0.01$). Similarly, APMS6A × RP6376-29 had significant SCA for SPY (7.406, $P < 0.05$) and BY (9.154, $P < 0.05$).

Discussion

Morphological characterization plays a crucial role in assisting breeders with the identification

Table 2 Pedigree details of 106 *indica* tropical *japonica* derived lines

S. No	Genotype	Cross Combination
1	RP6369-1	APMS-6B × IRGC3388
2	RP6369-2	APMS-6B × IRGC3388
3	RP6370-3	RPHR-1005 × IRGC43372
4	RP6370-4	RPHR-1005 × IRGC43372
5	RP6370-5	RPHR-1005 × IRGC43372
6	RP6370-6	RPHR-1005 × IRGC43372
7	RP6370-7	RPHR-1005 × IRGC43372
8	RP6370-8	RPHR-1005 × IRGC43372
9	RP6370-9	RPHR-1005 × IRGC43372
10	RP6371-10	RPHR-1005 × IRGC47216
11	RP6370-11	RPHR-1005 × IRGC43372
12	RP6370-12	RPHR-1005 × IRGC43372
13	RP6370-13	RPHR-1005 × IRGC43372
14	RP6370-14	RPHR-1005 × IRGC43372
15	RP6370-15	RPHR-1005 × IRGC43372
16	RP6370-16	RPHR-1005 × IRGC43372
17	RP6370-17	RPHR-1005 × IRGC43372
18	RP6372-18	Akshayadhan × IRGC50836
19	RP6372-19	Akshayadhan × IRGC50836
20	RP6372-20	Akshayadhan × IRGC50836
21	RP6372-21	Akshayadhan XIRGC50836
22	RP6373-22	APMS-6B × IR 79156B
23	RP6373-23	APMS-6B × IR 79156B
24	RP6373-24	APMS-6B × IR 79156B
25	RP6374-25	RPHR-1096 × IRGC1797
26	RP6374-26	RPHR-1096 × IRGC1797
27	RP6375-27	RPHR-1005 × IRGC67614
28	RP6376-28	RPHR-1005 × IRGC56735
29	RP6376-29	RPHR-1005 × IRGC56735
30	RP6376-30	RPHR-1005 × IRGC56735
31	RP6375-31	RPHR-1005 × IRGC67614
32	RP6375-32	RPHR-1005 × IRGC67614
33	RP6375-33	RPHR-1005 × IRGC67614
34	RP6367-34	RPHR-1096 × IRGC66755
35	RP6377-35	RPHR-1096 × IRGC66891
36	RP6377-36	RPHR-1096 × IRGC66891
37	RP6368-37	IBL-57 × IRGC66651
38	RP6368-38	IBL-57 × IRGC66651
39	RP6378-39	IBL-57 × IRGC67431
40	RP6379-40	BCP-123 × IRGC63102
41	RP6380-41	Uttri Rajappan × IRGC69857
42	RP6380-42	Uttri Rajappan × IRGC69857
43	RP6380-43	Uttri Rajappan × IRGC69857
44	RP6380-44	Uttri Rajappan × IRGC69857
45	RP6380-45	Uttri Rajappan × IRGC69857
46	RP6380-46	Uttri Rajappan × IRGC69857
47	RP6380-47	Uttri Rajappan × IRGC69857
48	RP6381-48	RPHR-1096 × IRGC15073
49	RP6382-49	RPHR-1096 × IRGC16073
50	RP6383-50	RPHR-1005 × FBR-1
51	RP6372-51	Akshayadhan × IRGC50836
52	RP6372-52	Akshayadhan × IRGC50836
53	RP6372-53	Akshayadhan × IRGC50836
54	RP6372-54	Akshayadhan × IRGC50836
55	RP6384-55	DR714-1-2R × IRGC8196
56	RP6385-56	RPHR-1096 XIRGC328
57	RP6385-57	RPHR-1096 × IRGC328
58	RP6369-58	APMS-6B × IRGC3388
59	RP6369-59	APMS-6B × IRGC3388
60	RP6369-60	APMS-6B × IRGC3388
61	RP6372-61	Akshayadhan × IRGC50836
62	RP6372-62	Akshayadhan × IRGC50836
63	RP6373-63	APMS-6B × IR 79156B
64	RP6373-64	APMS-6B × IR 79156B
65	RP6373-65	APMS-6B × IR 79156B
66	RP6373-66	APMS-6B × IR 79156B
67	RP6373-67	APMS-6B × IR 79156B
68	RP6375-68	RPHR-1005 × IRGC67614
69	RP6380-69	Uttri Rajappan × IRGC69857
70	RP6380-70	Uttri Rajappan × IRGC69857
71	RP6382-71	RPHR-1096 × IRGC16073
72	RP6383-72	RPHR-1005 × FBR-1
73	RP6383-73	RPHR-1005 × FBR-1
74	RP6383-74	RPHR-1005 × FBR-1
75	RP6372-75	Akshayadhan × IRGC50836
76	RP6372-76	Akshayadhan × IRGC50836
77	RP6385-77	RPHR-1096 × IRGC328
78	RP6385-78	RPHR-1096 × IRGC328
79	RP6386-79	RPHR-1005 × IRGC34018
80	RP6387-80	DRR-4B × IRGC51498
81	RP6375-81	RPHR-1005 × IRGC67614
82	RP6388-82	DRR-9B × IRGC25966
83	RP6389-83	RPHR-619-2 × IRGC328
84	RP6389-84	RPHR-619-2 × IRGC328
85	RP6389-85	RPHR-619-2 × IRGC328
86	RP6373-86	APMS-6B × IR 79156B
87	RP6373-87	APMS-6B × IR 79156B
88	RP6378-88	IBL-57 × IRGC67431
89	RP6378-89	IBL-57 × IRGC67431
90	RP6388-90	DRR-9B × IRGC25966
91	RP6390-91	KMR-3 × IRGC25239

Table 2 (continued)

S. No	Genotype	Cross Combination
92	RP6391-92	SG 26-120 × IRGC56704
93	RP6392-93	BK 49-80 × IRGC66756
94	RP6392-94	BK 49-80 × IRGC66756
95	RP6389-95	RPHR-619-2 × IRGC328
96	RP6389-96	RPHR-619-2 × IRGC328
97	RP6393-97	IR 58025B × IRGC44076
98	RP6393-98	IR 58025B × IRGC44076
99	RP6394-99	BCW-56 × IRGC44076
100	RP6389-100	RPHR-619–2 × IRGC328
101	RP6389-101	RPHR-619–2 × IRGC328
102	RP6373-102	APMS-6B × IR 79156B
103	RP6395-103	L2-182 × IRGC24528
104	RP6396-104	DR 714–1-2R × IRGC50865
105	RP6396-105	DR 714–1-2R × IRGC50865
106	RP6397-106	CRMS-32B × IRGC5726

and selection of high-performing genotypes. The results showed that TJP exhibited higher PH, PL, and TGW, but fewer PT, while IJD lines showed medium PH and high TGW. Three groups (IP, IJD, and Checks) had medium slender grains, while TJP displayed short bold grains, consistent with Jyothi et al. (2018). A negative correlation between PL and grain number was observed, likely due to panicle branching patterns, aligning with Xu et al. (2005). Although environmental factors influenced phenotypic traits, the phenotypic clustering pattern primarily reflected overall genetic lineage rather than strict subspecies differentiation.

High polymorphism (100%) was recorded with the SSR primers deployed in 150 genotypes with 88% of primers showing PIC values above 0.5, demonstrating a high level of genetic diversity within the studied genotypes. This corroborates with the earlier studies by Garris et al. (2005), Roy et al. (2016) and Ali et al. (2011). These markers have been widely used in diversity analyses (Ali et al. 2011; Yadav et al. 2013; Roy et al. 2016; Kumar et al. 2019; Sruthi et al. 2019), consistently demonstrating high polymorphism across various materials. Clustering analysis using DARwin software effectively separated the *indica* and tropical *japonica* subgroups, consistent with previous findings (Ali et al. 2011; Thomson et al. 2007).

Heterosis and combining ability of inter-subspecific derived R lines

Expanding the genetic base of parental lines is crucial for enhancing heterosis, as supported by recent studies emphasizing the role of tropical *japonica* germplasm and derived NPT lines in heterosis breeding (Shidenur et al. 2019, 2020; Singh et al. 2022). The genetic distance analysis revealed a significant broadening of the genetic base in the new parental lines developed by crossing *indica* lines with bulu, with notable increases in genetic distance between newly developed restorers (R lines) and existing maintainers (B lines). To understand the genetic potential of newly developed restorers, 52 hybrids, 17 parental lines, and 8 checks were evaluated. Male parents showed considerable variation for most traits, but significant line vs tester variance was observed only for DFF, BY, and PF%. No significant parent vs. hybrid differences were found for PT, SF%, TGW, and BY, indicating limited average heterosis. Interaction effects were high for all traits except DFF, where 48.48% of hybrid variance was contributed by the female parent. Female parents influenced DFF (48.48%) and PH (35.01%) more, this observation contradicts to Gramaje et al. (2020) observed male parents more influential except for PF%. Additive gene action (higher GCA variance) predominated for DFF, PH, SPY, BY, and HI; non-additive gene action (higher SCA variance) governed PT, PL, PF, SF, TGW, and PDP. The findings align with previous reports: Sravan Raju et al. (2017) and Parimala et al. (2018) for DFF, PT, PL, SF, and TGW; Gramaje et al. (2020) for SPY; Kulkarni et al. (2022) for the predominance of non-additive gene action in most yield traits and Anusha et al. (2021) for TGW.

Correlation among genetic distance, hybrid yield, combining ability, and other yield-attributing traits

In both IJD lines and hybrids, SPY showed significant positive correlations with PH, PL, BY, HI, and PDP, with stronger correlations between SPY and PH/PL in hybrids than in parental lines. This observation aligns with the findings of Wang et al. (2023). Additionally, PF%, SF%, GCA_MP, GCA_FP, and SCA were significantly associated with hybrid SPY. Parental performance for mid parent grain yield (MPGY) and better parent grain yield (BPGY) did not directly

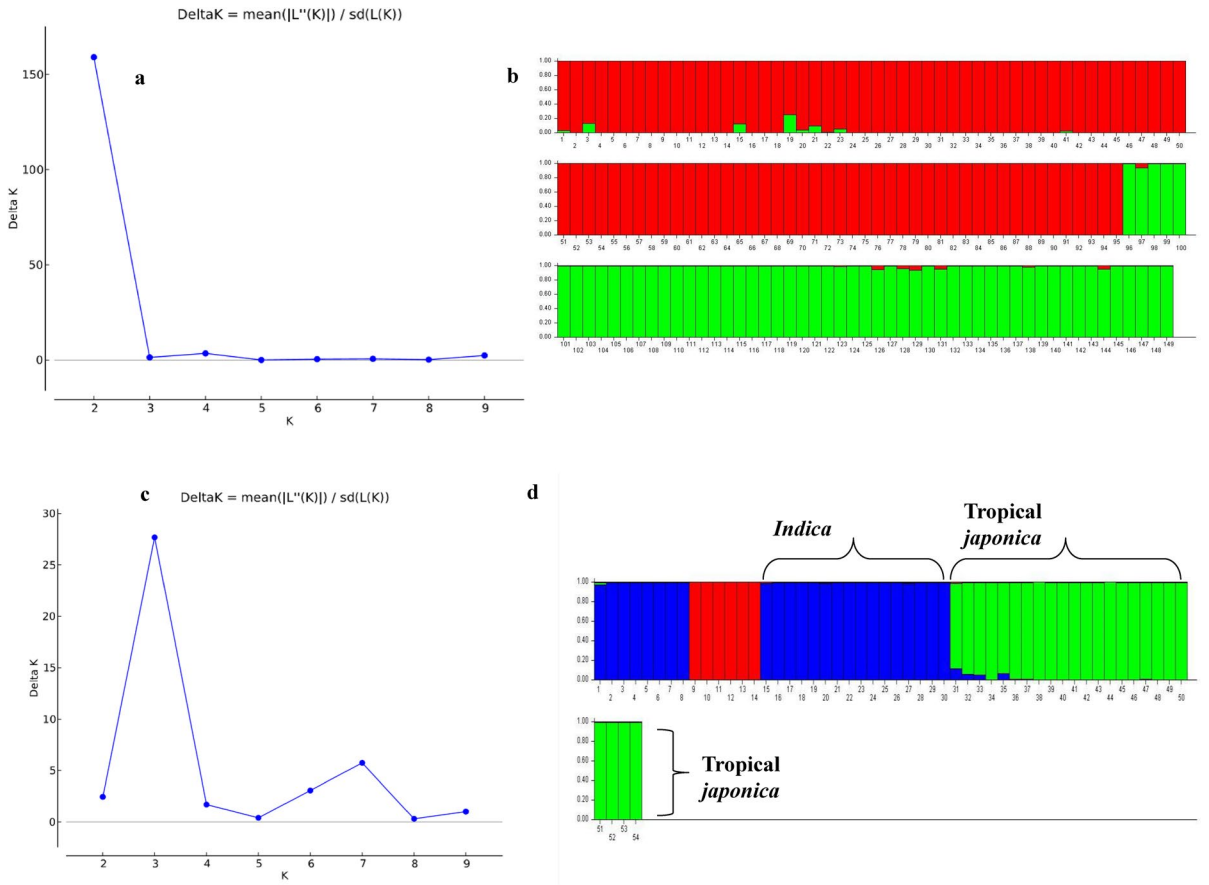


Fig. 4 **a** Estimation of population using LnP(D) derived delta K for determining optimum number of subpopulations. The maximum of adhoc measure ΔK determined by structure harvester was found to be K = 2 which showing peak at K = 2; **b** Population structure of 149 genotypes with K = 2; **c** Estimation of sub groups in POP1 using LnP(D) derived delta K. The maximum of adhoc measure ΔK determined by structure harvester was found to be K = 3; **d** Population structure showing the sub groups of POP1 with K = 3

influence hybrid SPY but was linked to the female parent's GCA for grain yield. Gupta et al. (2020) and Wang et al. (2015) also reported significant relationships between parental and hybrid grain yields. This highlights the importance of selecting female parents with good combining ability along with male parents to enhance grain yield. A weak positive correlation (0.12) was observed between GD of parental lines and hybrid SPY, with no correlation with SCA. The molecular genetic distance (GD) showed a statistically significant correlation with GCA_MP for grain yield ($r = 0.30$, $p < 0.05$). However, the strength of this correlation is relatively weak. This indicates that GD may assist in identifying male parents with favorable GCA. However, its predictive utility remains limited and is currently being validated through field-based evaluation of test crosses for combining ability, using parents with high genetic divergence. This observation is consistent with findings by Liu and Wu (1998), who reported a significant correlation ($r = 0.571$, $p < 0.01$) between GCA and hybrid yield. The relationship between GD and GCA can vary depending on the genetic background of the materials used (Maroof et al. 1997), and positive associations are generally observed only within an optimal range of genetic divergence (Würschum et al. 2023). Poor or negative correlations between GD and hybrid yield or SCA have been reported in various crops (Xu et al. 2002; Singh et al. 2011; Xie et al. 2013; Wang et al. 2015; Yingheng et al. 2018; Gupta et al. 2020;

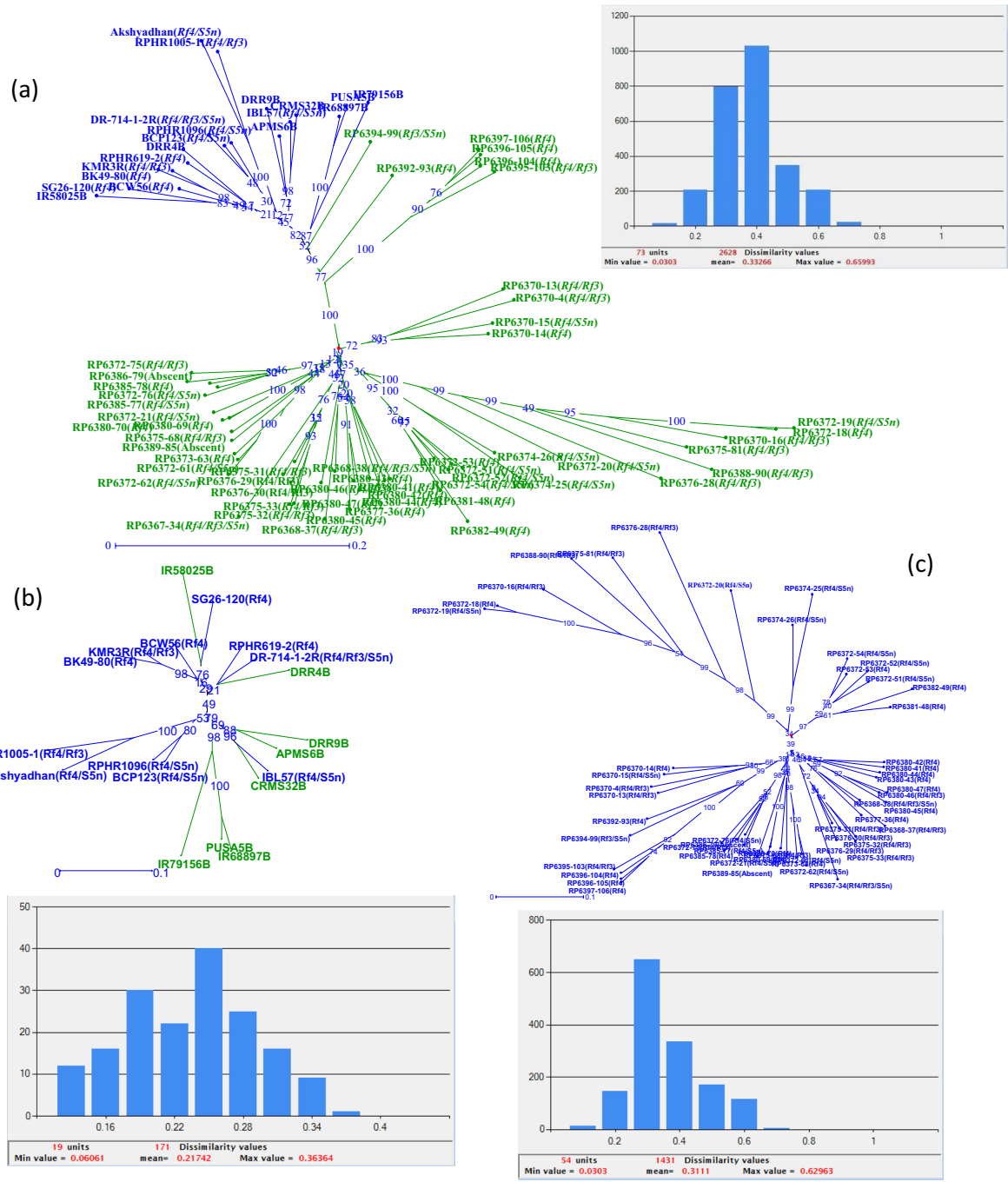


Fig. 5 **a** Molecular clustering pattern of *indica* hybrid rice parental lines (Blue) and newly developed restorers (Green) from inter subspecific hybridization between *indica* and tropical *japonica* germplasm; **b** clustering pattern and GD between *indica* parental lines **c** clustering pattern and GD between newly developed parental lines. (Color figure online)

Kumar et al. 2020; Labroo et al. 2021). GCA_MP_GY was not associated with MPGY, while GCA_FP_GY correlated significantly with parental MPGY, emphasizing the need to select parents with both high GCA and good per se performance to maximize heterosis and hybrid breeding efficiency (Liu and Wu

Table 3 Analysis of variance for combining ability for yield and its component traits in rice (*Oryza sativa* L.)

Source of variation	df	DFF	PH (cm)	PL (cm)	PT	PF (%)	SF (%)	SPY (g)	TGW (g)	BY (g)	HI (%)	PDP (kg ha^{-1})
Replicates	1	687.42**	1.784**	4.31*	22.34*	75.30*	2.632	21.865	14.544	118.11*	8.333	6.396
Treatments	68	224.80**	200.91**	4.87**	19.36**	835.28**	232.67**	79.52**	41.25**	139.12**	182.26**	531.38**
Parents	16	408.99**	346.75**	4.84**	5.825	34.77*	37.545	32.43*	60.24**	85.06**	70.857	455.18*
Parents (Line)	12	421.34**	458.91**	5.67**	4.743	22.665	27.211	32.59*	61.18**	77.66**	87.452	571.13**
Parents (Testers)	3	116.83**	10.94	2.91*	6.938	69.24**	90.05*	21.632	53.97*	61.2	27.272	137.096
Parents (L vs. T)	1	1137.28**	8.363	0.668	15.474	76.68*	4.007	62.935	67.83	245.38**	2.477	18.084
Parents vs. Crosses	1	5186.6**	840.10**	29.28**	0	3433.11**	211.417	144.03**	24.398	45.404	625.42**	1390.67*
Crosses	51	79.98**	142.62**	4.40**	24.00**	1035.50**	294.30**	93.02**	35.62**	157.92**	208.53**	538.44**
Line Effect	12	52.25	156.717	3.648	15.944	1288.14	354.12	48.685	46.635	60.814	319.55*	275.01
Tester Effect	3	659.24**	848.94**	12.29*	107.77**	1176.91	533.433	587.62**	56.71	1091.55**	586.54*	2226.55**
Line * Tester Eff	36	40.95**	79.06**	3.99**	19.69**	939.50**	254.44**	66.594**	30.19*	112.49**	140.02*	485.58**
Error	68	20.08	24.516	0.995	5.025	16.689	31.725	17.014	17.348	26.438	87.826	214.233
Total	137	130.1	111.903	2.944	12.268	423.429	131.252	48.075	29.192	83.04	134.122	370.136
Genetic components												
σ^2gca		39.49	56.27	0.82	6.69	143.04	48.48	35.43	4.04	64.68	42.97	121.95
σ^2sca		10.43	27.27	1.5	7.33	461.4	111.36	24.79	6.42	43.03	26.1	135.68
σ^2gca/ σ^2sca		3.79	2.06	0.55	0.91	0.31	0.44	1.43	0.63	1.5	1.65	0.9
Nature of gene action		Additive	Additive	Non additive	Non additive	Non additive	Non additive	Additive	Non additive	Additive	Additive	Non additive
Degree of Dominance (σ^2 D/σ^2A)$^{1/2}$		0.51	0.84	1.35	1.05	1.80	1.52	0.84	1.26	0.82	0.78	1.06
Contribution of Line, Tester and Line × Tester Interaction												
Line		15.37	25.85	19.49	15.63	29.27	28.31	12.31	30.8	9.06	36.05	12.01
Tester		48.48	35.01	16.42	26.42	6.68	10.66	37.15	9.36	40.65	16.54	24.32
Line × Tester		36.14	16.42	64.08	57.93	64.04	61.02	50.52	59.83	50.28	47.39	63.65

*Significant at 5 per cent level, **Significant at 1 per cent level

**DFF* Days to maturity; *PH* Plant height (cm); *PL* Panicle length (cm); *PT* Productive tillers; *PF* Pollen fertility (%); *SF* Spikelet fertility (%); *SPY* Single plant yield (g); *TGW* Thousand grain weight (g); *BY* Biological yield (g); *HI* Harvest index (%); *PDP* Per day productivity (kg ha^{-1}); Tester-Female Parents; Lines-Male Parents

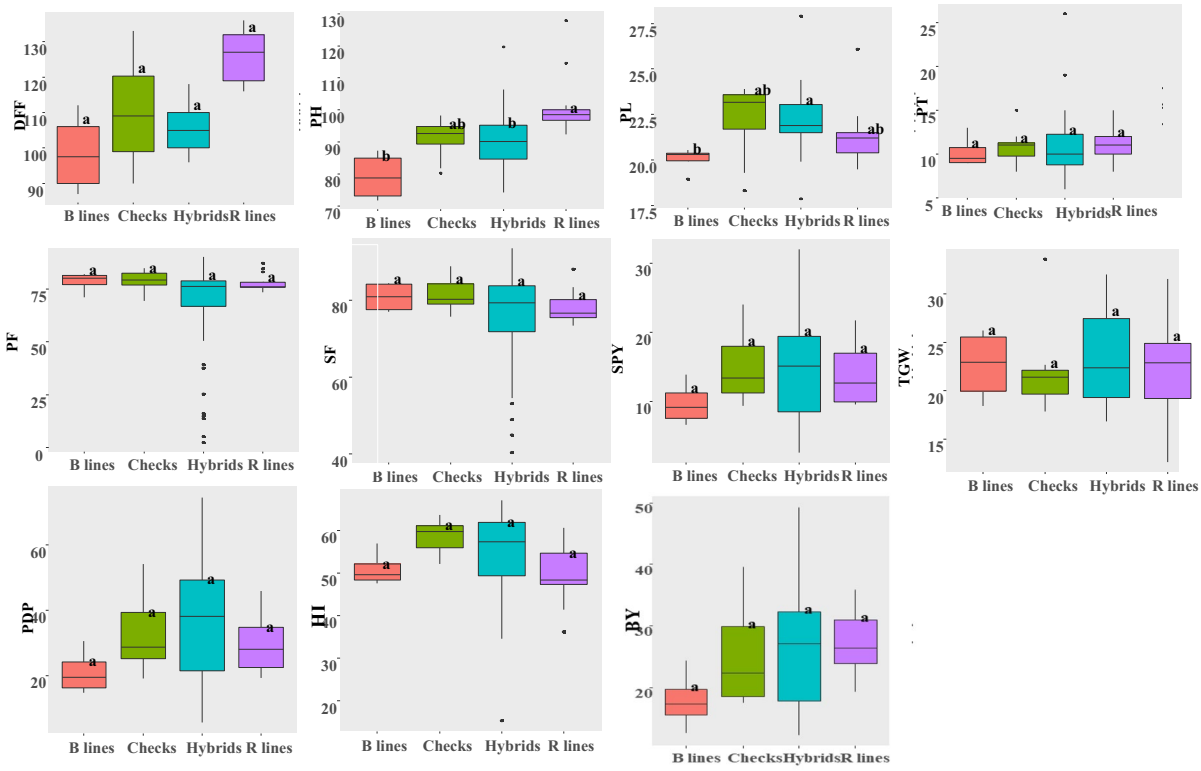


Fig. 6 Mean performance of hybrids, parents and checks for different yield attributing traits

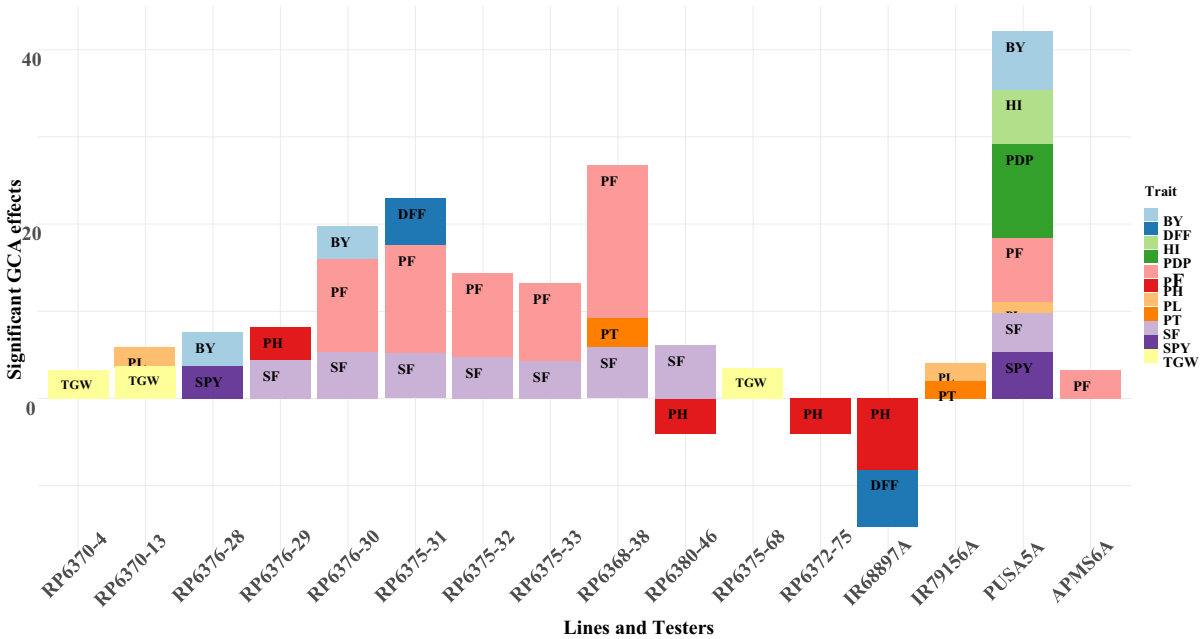


Fig. 7 Significant GCA effects of Lines and Testers for various yield component traits

1998; Gowda et al. 2012). Ultimately, parental mean performance remains crucial, as seed yield is derived from parents (Falconer and Macay 1983).

In this study, four female parents belonged to different duration groups, while all male parents were late duration. Most hybrids were medium duration (25), followed by mid-early (15) and late (12). Crosses between early duration A line and late R lines yielded mid-early to medium duration hybrids. Mid-early A line crossed with late R lines mostly produced mid-early to late duration hybrids, with most being medium duration. Medium duration A lines crossed with late R lines resulted in medium to late duration hybrids. Late × late crosses also produced medium and late duration hybrids, mostly medium duration. All parents and hybrids were semi-dwarf (< 110 cm), except one hybrid (PUSA5A × RP6370-13) and two R lines (RP6370-4 at 127.9 cm, RP6370-13 at 114.6 cm). The tallest hybrid, PUSA5A × RP6370-13, recorded the highest mean values for panicle length (PL: 27.9 cm), spikelet fertility (SF%: 93.37%), single plant yield (SPY: 31.97 g), and biomass yield (BY: 49.15 g), significantly outperforming all checks except US312 in terms of SPY (Table 4). SPY showed a significant positive correlation with plant height (PH) ($r = 0.62$, $p < 0.01$), indicating the potential role of PH in enhancing hybrid grain yield. This finding aligns with the results of Li et al. (2019), reported a strong positive association between PH and yield in *indica* hybrids, noting that this relationship may vary across rice ecotypes. Furthermore, a recent review by Li et al. (2025) emphasized the critical role of PH in contributing to increased grain yield in rice. For TGW, the IJD-derived R lines performed best. This observation is consistent with the findings of Jyothi et al. (2018), who reported that tropical *japonica* accessions commonly exhibit bold grain types, with some lines having a thousand grain weight (TGW) exceeding 30 g.

Mean performance and combining ability

To evaluate the genetic potential of parental lines for hybrid development, combining ability analysis is essential in hybrid breeding programs. It helps to determine the nature of gene action involved in trait expression and informs breeding strategies. General Combining Ability (GCA) reflects additive gene action, indicating a parent's average performance across multiple crosses. A high GCA means the parent consistently contributes desirable alleles to its progeny (Fasahat et al. 2016). Specific Combining Ability (SCA), on the other hand, highlights the performance of a particular hybrid cross relative to the average, indicating non-additive gene action (Sharma JR, 1988). Interestingly, no consistent correlation was found between mean performance and GCA. For example, although APMS6B had the highest mean performance across traits, PUSA5A was identified as a good general combiner for PT, PF%, SF%, SPY, BY, HI, and PDP (Table 5), as also reported by Saidaiah et al. (2010) for SPY and PDP. A similar observation was made among male parents.

In the present study, high per se performance and high GCA did not always lead to the best hybrids. RP6376-30, which had the highest mean yield (21.69 g), showed no significant GCA, while RP6376-28 exhibited significant positive GCA (SPY = 9.58 g) but did not contribute to the top three hybrids by SCA effects. Notably, the fourth-best hybrid was derived from RP6376-30. These results confirm earlier findings (Allard 1960; Shukla and Pandey 2008; Kenga et al. 2004; Anusha et al. 2021) that parents with high mean performance do not always produce heterotic hybrids, likely due to the predominance of non-additive gene action for most traits except DFF.

Also, good general combiners did not consistently contribute to top specific combiners, as observed by Gramaje et al. (2020) across most traits and Mohanty et al. (2025). While PUSA5A and RP6376-28 were good general combiners among A and R lines respectively, they did not produce the best specific combiner for yield, though the resulting hybrid ranked third by mean performance (26.4 g). Moreover, the best specific combiners did not always show the highest per se performance. For TGW, only one hybrid with significant SCA had a TGW of 28.8 g, while five other hybrids had TGW > 28.8 g with non-significant SCA. Similar patterns were observed across other traits. In analysing specific combiners, at least one parent typically showed good GCA for that trait. For example, IR68897A, the earliest flowering line, was a good general combiner for DFF and contributed to the best specific combiner, suggesting additive gene action for DFF. For PH, the tallest parents, APMS6A and RP6370-4, contributed to the best specific combiner for reduced height, indicating complementary gene action.

Table 4 Best hybrids identified for grain yield and their mean performance for SPY, combining ability effects and standard heterosis over varietal and hybrid checks

Identified Best Hybrid	Mean Performance		SCA effects	GCA effects		GCA Status	Standard heterosis							
	SPY(g)	Duration		Female parent	Male parent		SH1 (BPT5204)	SH2 (ISM)	SH3 (Gontrabidhan)	SH4 (NDR359)	SH5 (US314)	SH6 (US312)	SH7 (HRI174)	SH8 (DRRH3)
PUSA5A × IJD13	31.97	Late	9.537**	5.30**	2.04	High × Low	237.18**	164.83**	241.87**	118.04**	171.12**	33.08	37.01*	97.62**
PUSA5A × IJD75	29.90	Medium	12.996**	5.30**	− 3.48*	High × Low	215.40**	147.72**	219.79**	103.96**	153.60**	24.48	28.16	84.85**
PUSA5A × IJD28	26.4	Medium	2.255	5.30**	3.75*	High × High	178.48**	118.72**	182.35**	80.08**	123.92**	9.91	13.16	63.21*
APMS6A × IJD30	26.07	Medium	8.014**	1.22	1.74	Low × Low	174.95**	115.95**	178.77**	77.80**	121.08**	8.51	11.72	61.1*
APMS6A × IJD29	23.93	Medium	7.406*	1.22	0.22	Low × Low	152.43**	98.26**	155.94**	63.23*	102.97**	− 0.37	2.57	47.94
IR68897A × IJD38	20.25	Mid early	10.383**	− 6.17**	0.94	Low × Low	113.55*	67.73	116.52*	38.1	71.71*	− 15.72	− 13.22	25.16
APMS6A × IJD28	20	Medium	− 0.067	1.22	3.75*	Low × High	110.92*	65.66	113.85*	36.39	69.59	− 16.76	− 14.29	23.62

* Significant at 5 per cent level

** Significant at 1 per cent level

Table 5 Summary of trait wise hybrid performance data, including the highest-performing female and male parents, top-yielding hybrids, and the best general and specific combiners

Trait	Female parent with highest value	Male parent with highest value	Hybrid Combination with Highest Value	Average Hybrid Combination	Best General Combiner		Best Specific Combiner
					Female	Male	
DFF	87 (IR68897B)	116 (RP6367-34)	87 × 118 = 96 **IR68897A × RP6370-4**	99 × 126 = 105	IR68897A	IJD31	IR68897A × RP6380-46
PH (cm)	71.78 (IR79156B) 87.1 (APMS6B)	92.3(RP6375-31) 127.9(RP6370-4)	73.47 × 97 = 74.2 **IR68897A × RP6376-29** 84.1 × 114.6 = 119.66	79.13 × 100.86 = 90	IR68897A	RP6367-34, RP6380-46, RP6372-75, RP6376-29	APMS6A × RP6370-4(83.63)
PL (cm)	20.54 (IR68897B)	26.1 (RP6370-4)	20.3 × 22.4 = 27.9 **PUSA5A × RP6370-13**	20.02 × 21.46 = 22.19	IR79156A	RP6370-13	PUSA5A × RP6370-13(27.9) PUSA5A × RP6370-4(24.3)
PT	13 (PUSA5B)	15 (RP6368-38)	9 × 8 = 26 **IR79156A × RP6367-34** 13 × 10 = 19 **PUSA5A × RP6375-33**	10 × 11 = 11	IR79156A PUSA5A	RP6368-38	IR79156A × RP6367-34(26) PUSA5A × RP6375-33(19) APMS6A × RP6376-30(12)
PF (%)	82 (IR79156B)	87.17(RP6368-38)	71.16 × 87.17 = 90.1	78.31 × 78.12 = 66.59	PUSA5A	RP6368-38, RP6375-31, RP6376-30, IJD32, RP6375-33, RP6380-46	IR68897A × RP6370-4 (78.76)
SF (%)	84.37 (APMS6B)	88.01 (RP6375-68)	76.96X 83.27 = 93.37 **PUSA5A × RP6370-13**	80.73 × 78.12 = 75.86	PUSA5A	RP6368-38, RP6376-30, RP6375-31, IJD32, RP6375-33, RP6376-29	IR68897A × RP6370-4 (76.89)
SPY (g)	13.91 (APMS6B)	21.69 (RP6376-30)	10.37 × 12.93 = 31.97 **PUSA5A × RP6370-13**	9.69 × 13.65 = 15.09	PUSA5A	RP6376-28	PUSA5A × RP6372-75(29.9) PUSA5A × RP6370-13(31.97) APMS6A × RP6376-30(26.07)
TGW (g)	26.2 (IR68897A)	31.55 (RP6367-34)	20.5 × 21.7 = 32 **IR79156A × RP6375-68**	22.61 × 22.01 = 23.13		RP6370-13, RP6375-68, RP6370-4	IR68897A × RP6375-33(28.8)

Table 5 (continued)

Trait	Female parent with highest value	Male parent with highest value	Hybrid Combination with Highest Value	Average Hybrid Combination	Best General Combiner		Best Specific Combiner
					Female	Male	
BY (g)	24.42 (APMS6B)	35.88 (RP6368-38)	18.15 × 27.72 = 49.15	17.93 × 27.14 = 26.03	PUSA5A	RP6376-28, RP6376-30	PUSA5A × RP6372-75(46.56)
			PUSA5A × RP6370-13				IR68897A × RP6368-38(33.79)
							PUSA5A × RP6370-13(49.15)
HI (%)	56.93 (APMS6B)	60.61(RP6376-30)	56.93 × 41.39 = 67.1	50.97 × 49.72 = 54.96	PUSA5A	–	PUSA5A × RP6372-75(64.15)
			APMS6A × RP6376-28				
PDP (kg/ha)	30.61 (APMS6B)	45.9(RP6376-30)	22.1 × 25.1 = 74.34	21.1 × 29.09 = 36.79	PUSA5A	–	PUSA5A × RP6372-75(74.34)
			PUSA5A × RP6372-75				IR68897A × RP6368-38(52.35)
							PUSA5A × RP6370-13(73.77)

In the present study, the *indica* × tropical *japonica*-derived restorer (R) lines were observed to exhibit several desirable traits, including good pollen shedding capacity, strong culm, tall plant stature, elongated panicles, and a wider angle between the panicle and flag leaf. These traits are known to contribute to efficient pollen dispersal in hybrid seed production plots, as supported by earlier findings: pollen shedding capacity (Chakrabarty et al. 2023), taller plant stature (Xu et al. 2002), elongated panicles (Virmani and Edwards 1983; Rutger and Carnahan 1981), and panicle–flag leaf angle (Jiang et al. 2022).

Genetic diversity, heterosis and combining ability

The study clearly demonstrates that the introgression of the tropical *japonica* genome into *indica* parental lines has effectively broadened the genetic base of the newly developed restorer (R) lines, particularly in relation to existing maintainer (B) lines. Grain yield heterosis estimates varied widely across hybrids. Average heterosis ranged from −80.15% (APMS6A × RP6372-75) to 175.86% (PUSA5A × RP6376-28), heterobeltiosis ranged from −81.05 to 129.88%, and standard heterosis over the best check (US312) ranged from −89.03 to 33.08%, with the highest standard heterosis recorded in PUSA5A × RP6370-13. Data from a single location showed that standard heterosis can reach up to 30% over the best-performing hybrid check, underscoring the potential of these new lines. This strategy aligns with China's super hybrid rice breeding approach, which integrates ideotype breeding with intersubspecific heterosis (Cheng et al. 2007). Yuan (1991a, b) advocated the use of *javanica* cultivars, alongside *indica*-inclined and *japonica*-inclined lines, to enhance yield potential. Supporting evidence from IRRI (Virmani 1994; IRRI 1995) indicated that *indica* × tropical *japonica* hybrids showed higher heterosis and yields than either *indica* × *indica* or tropical *japonica* × tropical *japonica* hybrids.

As part of parental improvement programs, Satyanarayana et al. (2005) developed 44 potential restorers and 20 maintainers using *indica* × tropical *japonica* crosses and reported a mean heterosis of 38.3%. Similarly, Shidenur et al. (2020) documented

a maximum standard heterosis of 26.89% across three locations. Notably, the recently released Indian public sector rice hybrid DRRH6**,** developed from IR68897A × RP6380-46 (Government of India, Gazette Notification S.O. 4388(E), 8 October 2024), incorporates an *indica* × tropical *japonica*-derived restorer line. Based on three-year averages from 54 AICRPR trial locations, it demonstrated 18 and 34% yield superiority over the national hybrid (US314) and varietal (CO-51) checks, respectively. The *indica* tropical *japonica* derived R lines carries approximately 50–55% tropical *japonica* genome segments in the *indica* background (Ayeella et al. 2021). Further breeding cycles may improve heterosis levels beyond 30% by enabling more precise recombination to retain favorable tropical *japonica* alleles while eliminating undesirable ones. Beyond genetic diversity alone, the key to realizing high heterosis lies in optimizing the balance between favorable and unfavorable allelic interactions within each parent, and maximizing positive inter-parental (heterogenic) interactions (Liu and Wu 1998). As successive cycles of selection and recombination advance, both the per se performance and combining ability of the parental lines can be enhanced, ultimately leading to the development of highly heterotic and agronomically superior rice hybrids. Although standard heterosis reached up to 30% over the best hybrid check at a single location, multilocation trials are essential to accurately assess the heterotic potential and to account for genotype × environment interactions influencing yield heterosis and stability. The present findings lay a strong foundation for future research. Promising heterotic combinations identified in this study can be further validated through multilocation trials. Superior inter-subspecific derived R lines will be integrated into the existing parental pool and subjected to high-throughput genotyping, enabling the application of genomic selection strategies and predictive modeling to enhance the precision of hybrid development.

Best specific combiners/best identified hybrids

Based on mean performance, SCA, and GCA effects, the top seven hybrids identified (Fig. 8) were: PUSA5A × RP6370-13, PUSA5A × RP6372-75, PUSA5A × RP6376-28, APMS6A × RP6376-30, APMS6A × RP6376-29, IR68897A × RP6368-38, and APMS6A × RP6376-28. Notably, PUSA5A × RP6376-28 involved two good general combiners. The top three hybrids—PUSA5A × RP6370-13, PUSA5A × RP6372-75, and PUSA5A × RP6376-28—each had at least one parent with significant GCA. The top three hybrids were derived from female parents that exhibit high GCA for grain yield. This female parent effectively combined low-GCA and high-GCA parents to achieve high yields. This highlights the strategic importance of selecting female parents with both high mean performance and good GCA to improve the likelihood of producing high-yielding hybrids and enhancing breeding efficiency as emphasized by Liu and Wu (1998), Gowda et al. (2012), Huang et al. (2015) and Casco et al. (2021).

Conclusion

The present study revealed considerable genetic diversity among *indica* × tropical *japonica*-derived (IJD) lines and newly developed restorer (R) lines, reflecting the successful integration of tropical *japonica* and *indica* genetic backgrounds. Preliminary results on heterosis and combining ability are promising, with the best-performing hybrids exhibiting up to 30% standard heterosis over the best check, which has a yield potential of 6.0 t/ha—equivalent to an estimated yield gain of 1.8 t/ha. While these findings highlight the potential of inter-subspecific crosses in enhancing hybrid rice performance, the results are currently based on evaluations conducted under a single environment. Therefore, multi-location trials are necessary to validate the stability, adaptability, and economic viability of the identified best hybrids.

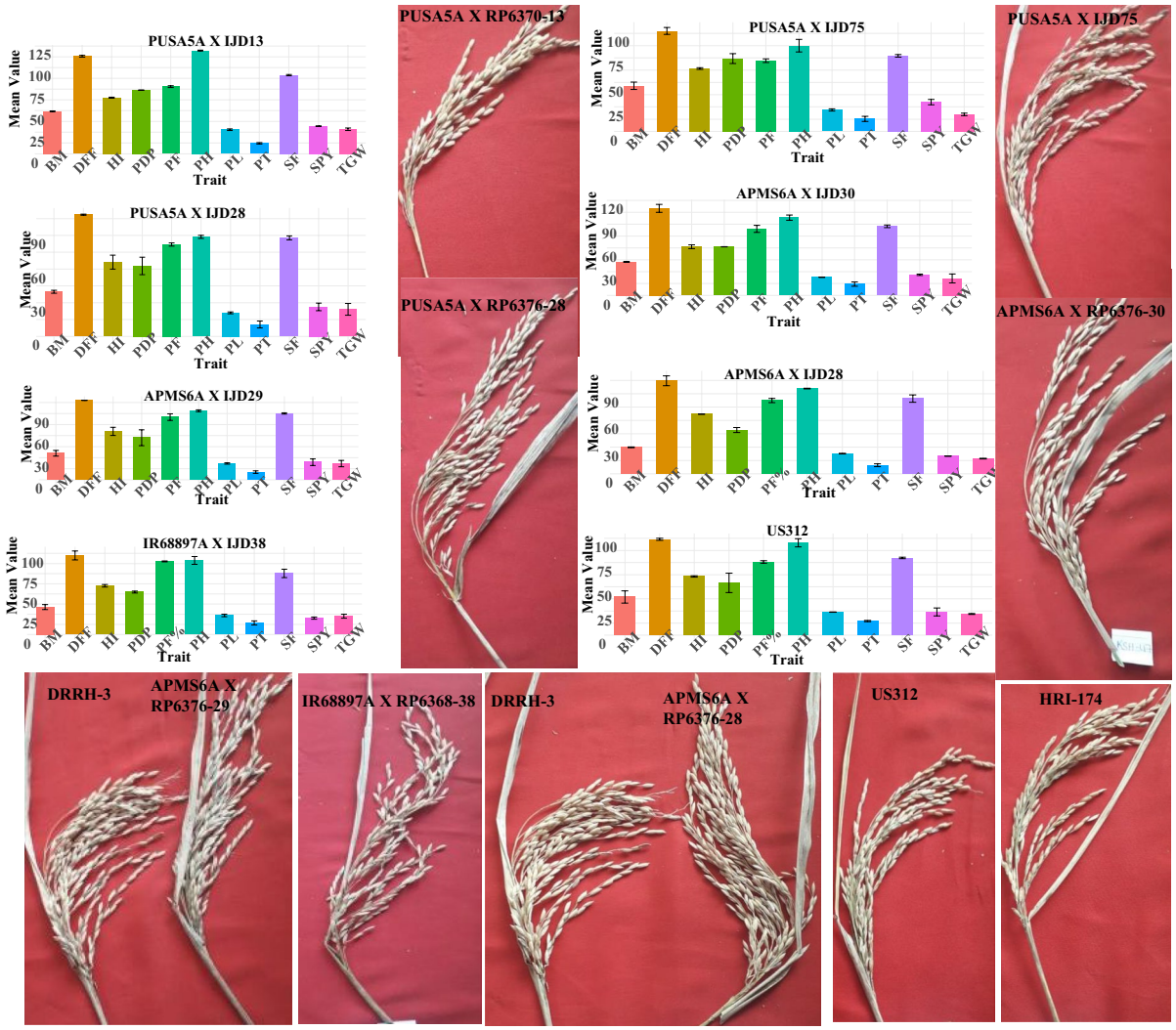


Fig. 8 Trait wise performance and panicle type of identified best hybrids from the present study

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Authors' contributions ASH, KBE, and KS conceptualized the study. ASH, PK, SA, PS, PR, and KBK developed the breeding materials. KS conducted the laboratory and field experiments, with data collection carried out by KS, MBSS, and KSL.Data analysis was performed by KS and AD. KS drafted the initial version of the manuscript. The manuscript was revised by ASH, RMS, DB, AD, KBE, ChDR, MSM, ChSR, and MBK. ASH secured the funding for the study.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of Interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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