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HETEROSIS AND HETEROTIC GROUPING EFFECTS ON GRAIN YIELD, HEIGHT, TILLER DENSITY, AND DAYS TO HEADING IN HYBRID RICE (*ORYZA SATIVA* L.)

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SUMMARY

Heterotic groups are necessary for high vigor in hybrid rice. However, hybrids produced from crosses between parents from different rice subspecies (i.e., *Indica* × *Japonica*) have extensive incompatibility issues exhibited by low seed sets. The study objectives were to evaluate the heterosis in grain yield and yield-related traits between hybrids produced from low and high parental genetic distances (PGDs) and demonstrate the heterotic group approach in rice. From PGDs, eight and three hybrids were assigned to the low and high PGD hybrid groups, respectively. Neighbor-joining clustering and model-based population structure analyses classified the hybrid parents into four heterotic groups, with the low and high PGD hybrid groups found consisting of intra-subpopulation and inter-subpopulation crosses, respectively. Replicated yield trials conducted at Beaumont, Texas, transpired in 2019 and 2020. The hybrids exhibited normal seed sets, with at least one of each hybrid's parents determined to possess the wide compatibility *S5n* allele necessary for normal seed sets in wide crosses. Trait and standard heterosis values estimates included the number of days to heading, plant height, tiller density, and grain yield. Higher trait values and heterosis for tiller density and grain yield occurred in the high than the low PGD hybrid group, especially in the inter-subpopulation crosses with indica rice. PGD had consistent positive correlations with heterosis for grain yield ($r = 0.41$ to 0.60) and tiller density (0.28 to 0.36) in both years. PGDs aid in determining highly heterotic cross combinations for tiller density and grain yield and in forming heterotic groups. Heterotic grouping is advisable through cluster and structure analyses of genome-wide markers instead of identifying genetically-distant crosses based on pedigree information.

Key words: Heterosis, heterotic group, hybrid, rice, yield, *Oryza sativa* L.

Key findings: Rice hybrids from wide-compatible parents in *japonica* × *indica* crosses were studied. Heterosis was higher in hybrids produced from parents with extreme genetic distances (PGDs). PGDs help form heterotic groups and in selecting highly heterotic crosses.

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INTRODUCTION

Heterosis is the basis for which hybrid plants have been applied to increase yield production in maize, cotton, peanuts, wheat, sorghum, soybeans, and rice. Heterosis observed in maize came as early as 1908, in which hybrids produced from crosses between nearly pure lines were more vigorous than either parent (Shull, 1908), and in 1926 in rice, in which hybrids were taller, had more culms and had higher yield per plant than inbred rice (Jones, 1926). Since then, the increased use of heterosis has required that parental germplasm be bred to produce genetically diverse heterotic groups, which are sets of inbred lines with high kinship coefficients, similar main characteristics, and strong general combining ability (Lu and Xu, 2010). Heterotic groups have been developed or proposed for maize (Laude and Carena, 2015), cotton (Li *et al.*, 2019; Geng *et al.*, 2021), and rice (Yingheng *et al.*, 2018). Heterotic patterns have been identified, which refer to the specific cross combinations between two heterotic groups that produce high performing hybrids (Melchinger and Gumber, 1996). Developing and utilizing genome-based heterotic patterns are necessary for sustainable long-term success in hybrid rice breeding (Beukert *et al.*, 2017; Kahani *et al.*, 2018; Islam *et al.*, 2022).

Although hybrid rice breeding and research have progressed considerably over the past 50 years, systemic studies on heterotic groups have had minor importance (Yingheng *et al.*, 2018). In addition, less information on these aspects of tropical hybrid rice breeding exists (Xie *et al.*, 2014; Beukert *et al.*, 2017). The practice for establishing heterotic groups in rice is limited (Bhati *et al.*, 2015; Thippeswamy *et al.*, 2016; Labroo *et al.*, 2021), and literature on heterotic group rice breeding is few in the United States.

In rice, the *aus* and *indica* (IND) subpopulations are grouped within the traditional *Indica* subspecies, and the temperate *japonica* (TEJ), tropical *japonica* (TRJ), and aromatic subpopulations are grouped within the *Japonica* subspecies (Garris *et al.*, 2005). Heterosis is affected by parental diversity, such that when intercrossing TRJ, TEJ, and IND rice subpopulations, heterosis for grain yield generally decreases in this order: IND/TEJ > IND/TRJ > TEJ/TRJ > IND/IND > TEJ/TEJ > TRJ/TRJ (Virmani *et al.*, 2003). There are more hybrids with higher standard heterosis in *Indica/Japonica* crosses than in crosses between two different ecotypes within the same subspecies (Jiang *et al.*, 2002). DNA markers have been applied to differentiate subspecies for use as heterotic groups, and using inter-subspecies crosses (i.e., *Indica*-inclined × *Japonica*-inclined parents) has produced and grown very high-yielding hybrids (called “super rice”) on a large scale in China (Cheng *et al.*, 2007).

Molecular-based techniques and markers’ use helped to classify lines into heterotic groups based on genetic distance (Wegary *et al.*, 2013; Li *et al.*, 2019). Studies determining heterotic groups in rice have mainly focused on *indica* germplasm (Xie *et al.*, 2014; Wang *et al.*, 2015). At the International Rice Research Institute (IRRI) in the Philippines, two heterotic groups resulted with the use of neighbor-joining cluster and model-based population structure analyses (Pritchard *et al.*, 2000) on 207 simple sequence repeat (SSR) markers and 353 single nucleotide polymorphisms (SNPs) (He *et al.*, 2012) and by evaluating inter and intra-group hybrids in yield trials (Xie *et al.*, 2014). Another study identified four heterotic groups and three heterotic patterns from 17 parents for breeding for superior yield based on marker-based grouping (using 384 SNPs),

genetic distance estimation, and cluster analyses (Wang *et al.*, 2015). Rice genotypes widely used in Chinese hybrid breeding programs were grouped into seven heterotic groups, superior heterotic patterns or crosses were identified, and relationships among parental genetic distance (PGD), yield, and yield components were estimated (Yingheng *et al.*, 2018).

Although hybrids produced from crosses between parents from different rice subspecies (i.e., *Indica* × *Japonica*) have the potential to create high levels of heterosis, these may have broad incompatibility issues that are exhibited by low seed set or low spikelet fertility in the F₁ (Liu *et al.*, 1996). In rice, the *S5* major gene located in chromosome 6 controls inter subspecies hybrid fertility. The three variants (alleles) of the *S5* gene are the *S5i* (in *Indica*), *S5j* (in *Japonica*), and the *S5n* (nonfunctional or neutral; called the wide-compatibility or *WC* gene) (Ikehashi and Araki, 1986). Hybrid sterility occurs when crossing parents containing *S5i* and *S5j*. On the other hand, crosses between parents with either *S5i* or *S5j* produce a normal seed set. Therefore, to eliminate low seed set and to produce fertile hybrids from intersubspecific crosses, the *S5n* variant must be present in at least one of the parents (Ikehashi and Araki, 1986; You-Xin *et al.*, 2012).

In the United States, private companies chiefly developed hybrids grown commercially, and the production area grown to these cultivars has increased since the early 2000s. However, limited literature exists on evaluating and applying heterotic groups or crossing genetically distant parents to produce extremely high yields in the hybrids. Therefore, studies that compare hybrids produced by TRJ in intra or inter-subpopulation crosses or genetically distant parental crosses need more execution to determine and evaluate potential heterotic patterns that create superior grain yield and yield components. This research screened hybrids produced by crossing inbred cultivars from diverse rice germplasm (Alpuerto *et al.*, 2022; Sanchez *et al.*, 2022) as study material. The research objectives sought to evaluate the heterosis in grain yield and yield-related traits in hybrids produced from

low and high genetically-distant parents and demonstrate the heterotic group approach in rice.

MATERIALS AND METHODS

Plant materials

Eleven hybrids produced by crossing 17 inbred cultivars or lines underwent field experiment evaluation. The hybrids were selected as follows:

Both parents of each hybrid were part of a diverse germplasm of released cultivars and inbred lines evaluated in a genome-wide association study (Alpuerto *et al.*, 2022; Sanchez *et al.*, 2022). DNA extraction used a standard protocol for leaf tissue with the Thermo Fisher Scientific KingFisher Flex (Thermo Fisher Scientific, Waltham, MA, USA). The DNA samples for library preparation and sequencing ensued at the Texas A&M AgriLife Genomics and Bioinformatics Service (TxGen). Sample libraries' sequencing used the Illumina HiSeq 4000. Genotyping-by-sequencing at 1X coverage was done for each accession. The reference genome used was *Oryza sativa* ssp. *Japonica* cultivar Nipponbare, International Rice Genome Sequencing Project (IRGSP) Build 5 (Kawahara *et al.*, 2013). After preliminary filtering, imputation proceeded using BEAGLE V4.0 (Browning and Browning, 2007). The 854,832 SNPs, selected from the initial 1,075,302 SNPs by filtering out low-quality SNPs (i.e., loci with >5% missing data and minor allele frequency of <5%), employed TASSEL 5.2.61 (Bradbury *et al.*, 2007) to compute genetic distance.

Calculating the genetic distance between each hybrid parent used the 1 minus Identity by State (1 – IBS) similarity method (Bradbury *et al.*, 2007). An IBS = 1.0 indicates that the DNA sequences of two rice genotypes are the same. Genetic distances between each 66 genotypes used as parents in a crossing nursery ranged from 0.019 to 0.668, with a mean of 0.301. Based on the mean PGD, selected hybrids for study are those with below or above-average PGDs and had normal seed set (at least 75% seed set per panicle), as

visually observed in an F₁ nursery conducted at Texas A&M AgriLife Research Center at Beaumont in 2018.

Among the 11 selected hybrids, eight and three were assigned to the low and high PGD groups, respectively (Table 1). Pedigree information of the parents of each crossing is also shown.

Presidio, an inbred long-grain cultivar grown in most rice hectareage in Texas from 2010 to 2019 (Wilson *et al.*, 2022), served as the check in the field experiment of this study. It has superior ratoon yield potential, high milling yield, blast resistance (McClung, 2005), and low chalkiness (Samonte *et al.*, 2022).

The wide compatibility of parents of hybrids

Parental lines of the 11 test hybrids were grown in the greenhouse at Texas A&M AgriLife Research in Beaumont, Texas, in 2019. DNA extraction from their leaf samples used a QIAGEN DNeasy Plant Mini Kit (Qiagen, Inc., Valencia, CA, USA), with extracts tested to identify the type of *S5* alleles they possess (*S5i*, *S5j*, or *S5n*) using markers designed to amplify short DNA segments located within the *S5* gene (Ikehashi and Araki, 1986; Sundaram *et al.*, 2010).

Field experiment

Field experiments conducted at the Texas A&M AgriLife Research Center in Beaumont, Texas, began in 2019 until 2020, using a randomized complete block design with two replications. In 2019, planting rice seeds in the greenhouse started on 24 May, with the seedlings transplanted to the field on 26 June. In 2020, seeding in the greenhouse occurred on 29 May, and field transplanting progressed on 16 June. Each plot had three rows, with six plants row⁻¹ in 2019 and eight plants row⁻¹ in 2020. More hybrid plants per row were planted in 2020 because extra seeds were produced in the crossing nursery before planting. Each plant was space-planted at 30 cm × 30 cm to minimize competition and allow for increased tillering. Plot sizes were 1.62 m² in 2019 and 2.16 m² in 2020. A Presidio row planted

between each entry plot served as a border. Fertilizer broadcasting in 2019 was on 7 June (30 kg ha⁻¹ N), 27 June (130 kg ha⁻¹ N), and 24 July (50 kg ha⁻¹ N), and in 2020 on 12 June (30 kg ha⁻¹ N), 19 June (90 kg ha⁻¹ N), 20 July (90 kg ha⁻¹ N), and 7 August (25 kg ha⁻¹ N). The fourth fertilization in 2020 was due to N deficiency symptoms. Permanent flood maintained in the rice field was from transplanting to harvesting.

The number of days to heading (50% of the tillers have exerted panicles) estimates for each plot proceeded. Plant height (cm) from the ground to the tip of the tallest panicle and tiller density (tillers m⁻²) were estimated from two plants in the middle row of each plot. Rice plots harvested at maturity had the grain yield (g m⁻²) estimated. Standard heterosis estimation for traits of each hybrid and heterotic group used the equation, with Presidio as a check cultivar.

$$\text{Standard Heterosis (\%)} = \frac{F1 - \text{Check cultivar}}{\text{Check cultivar}} \times 100$$

Statistical analyses

Pair-wise PGD values were used to construct an unrooted neighbor-joining tree-like figure in which the lengths of branches correspond to genetic distances (Saitou and Nei, 1987). PGD calculation and neighbor-joining tree clustering used TASSEL 5.2.61 (Bradbury *et al.*, 2007).

Applying the model-based population structure inference, which uses DNA marker data, helped identify groups based on shared patterns of SNP variation and assign genotypes to each group based on the best fit in their SNP patterns. Implementing this used the STRUCTURE 2.3.4 software (Pritchard *et al.*, 2000). A total of 6,542 unimputed SNPs without missing data and minor allele frequency >5%, filtered using TASSEL 5.2.61 (Bradbury *et al.*, 2007), were used for this analysis. Models with the number of groups or subpopulations (K) from one to five were tested in five independent replications. The parameter settings for estimating membership coefficients for accessions in each subpopulation were a burn-in length of 10,000, followed by 100,000 iterations after burn-in.

Table 1. Experimental rice hybrids and their parents' genetic distances, pedigree, rice subpopulation based on structure plot analyses and unrooted neighbor-joining tree clustering, and their possession of the wide compatibility *S5n* allele.

PGD Group	PGD	Hybrid	Female Parent				Male Parent			
			Name	Pedigree	Rice Subpop	<i>S5</i> Allele	Name	Pedigree	Rice Subpop	<i>S5</i> Allele
Low	0.019	F19A01	RU-1603126	LGRU/LCSN/CF4-85//Sierra	TRJ	<i>S5n</i>	M-204	M-201/M7/3/M7//ESD7-3/Kokuhorose	TRJ	<i>S5j</i>
Low	0.023	F19A02	Lacassine	Newbonnet/Lemont	TEJ	<i>S5n</i>	M-401	Semi-dwarf mutant derived from Terso	TEJ	<i>S5j</i>
Low	0.029	F19A03	Cheniere	Newbonnet/Katy/3/L-202/Lemont//L-202	TRJ	<i>S5n</i>	Lemont	Lebonnet//CI 9881/PI 331581	TRJ	<i>S5n</i>
Low	0.036	F19A04	Lebonnet	Bluebell//Belle Patna/Dawn	TRJ	<i>S5j</i>	RU-1403138	043752/0047277/CHEN	TRJ	<i>S5n</i>
Low	0.042	F19A06	RU-1303181	043752/0047277/CHEN	TRJ	<i>S5n</i>	Cheniere	Newbonnet/Katy/3/L-202/Lemont//L-202	TRJ	<i>S5n</i>
Low	0.043	F19A07	RU-1303181	043752/0047277/CHEN	TRJ	<i>S5n</i>	Lemont	Lebonnet//CI 9881/PI 331581	TRJ	<i>S5n</i>
Low	0.083	F19A08	Cypress	L-202/Lemont	TRJ	<i>S5n</i>	171R	Katy/Minghui63//CDR22	TRJ	<i>S5i</i>
Low	0.090	F19A09	Antonio	Cypress/Cocodrie	TRJ	<i>S5n</i>	171R	Katy/Minghui63//CDR22	TRJ	<i>S5i</i>
High	0.440	F19A13	RU-1403141	AC110DH2/AC108DH2//CHEN	TEJ	<i>S5n</i>	L-202	723761/7232278//L-201	TRJ	<i>S5n</i>
High	0.543	F19A14	RU-1403166	AC110DH2/AC108DH2//CYBT	IND	<i>S5n</i>	IR24	IR8/IRI27-2-2	TRJ	<i>S5i</i>
High	0.666	F19A32	RU-1403141	AC110DH2/AC108DH2//CHEN	TEJ	<i>S5n</i>	IR 1321-12	Jin Heung/IR 262-43-8-11//Senbon Asahi	IND	<i>S5i</i>

Abbreviations: PGD = Parental genetic distance; IND = *indica*; TEJ = temperate *japonica*; TRJ = tropical *japonica*.

An admixture model was applied with independent allele frequencies. Determining the ideal number of K employed a method that calculates an ad hoc (ΔK) statistic, which is estimated using the rate of change in the log probability of data between consecutive K values (Evanno *et al.*, 2005), using the program Structure Harvester (Earl and vonHoldt, 2012).

Known representatives of the IND (IR64 and Minghui 63), TRJ (Cocodrie and Wells), and TEJ (M-201 and Nipponbare) (Ali *et al.*, 2011; Kim and Tai, 2013; McCouch *et al.*, 2016) subpopulations were added to the 17 hybrid parents and check cultivar Presidio in the neighbor-joining cluster and structure analyses to serve as checks for the locations of the three rice subpopulations.

Levine's test for homogeneity of variances across years proceeded before the analyses of variance (ANOVA) of the combined 2019 and 2020 data for the number of days to heading, plant height, tiller density, and grain yield using years as a random effect. Mean comparisons of trait values and heterosis estimates of the rice genotypes and hybrid groups used the least significant difference test at the 5% level. Correlation analyses at the 5% level between PGD and heterosis in grain yield, height, heading, and tiller density followed. These statistical analyses applied SAS version 9.4 (SAS Institute Inc).

RESULTS

Genetic distance and wide compatibility

The genetic distances between parents of hybrids ranged from 0.019 to 0.090 in the low PGD hybrid group and from 0.440 to 0.666 for the high PGD group (Table 1). In addition, there was a significant difference between the mean genetic distance of the low (0.046) and high (0.550) PGD groups based on a t-test at the 5% level.

All hybrids showed to have at least one parent possessing the *S5n* allele (Table 1), making all crosses wide-compatible and confirming the normal seed set observed in the hybrids during the 2018 F₁ nursery. Among the 17 parents, 11 had the wide compatibility *S5n* allele, three had the *S5i* allele, and three had the *S5j* allele.

Heterotic grouping

The unrooted neighbor-joining tree visually illustrated the PGDs between the 17 hybrid parents used in this study, check cultivar Presidio, as well as representatives of *indica* (IR64 and Minghui 63), *tropical japonica* (Cocodrie and Wells), and *temperate japonica* (M-201 and Nipponbare) rice subpopulations (Figure 1). Group 1 in the unrooted neighbor-joining tree consisted of hybrid parents Antonio, Cheniere, Cypress, IR24, Lebonnet, Lemont, M-204, RU-1303181, RU-1403138, RU-1603126, and 171R. These genotypes were considered to belong to the *tropical Japonica* subpopulation, since rice check TRJ representatives Cocodrie and Wells were located in this cluster. Group 2 classification is the *indica* subpopulation rice cluster, as it contained IND checks representatives, Minghui 63 and IR64, and hybrid parents IR 1321-12 and RU-1403166. Classification of group 3 belonged to the *temperate japonica* rice cluster and consisted of TEJ check representatives, Nipponbare and M-201, and hybrid parents Lacassine, M-401, and RU-1403141. Group 4 consisted solely of L-202, located between the *Indica* and *Japonica* subspecies in the unrooted neighbor-joining tree.

The structure plot identified four heterotic groups based on shared SNP pattern variations (Figure 2a); the highest $\Delta K = 1955$ was attained at $K = 4$. The second highest $\Delta K = 1426$ reached $K = 2$. The mean $\text{LnP}(D)$ values over five replications for each K peaked when $K = 4$ and sharply decreased when $K = 5$ (Figure 2b). Based on this population structure

analysis, the hybrid parents assigned to each of the four groups were identical to that of the unrooted neighbor-joining tree clustering. The neighbor-joining clustering and population structure analyses helped allocate each of the 17 hybrid parents to their appropriate rice subpopulations (Table 1). The eight hybrids

with low PGD consisted of intra-subpopulation crosses, i.e., seven TRJ/TRJ and one TEJ/TEJ. In comparison, the three hybrids with high PGD consisted of inter-subpopulation crosses, i.e., one TEJ/TRJ, one IND/TRJ, and one TEJ/IND.

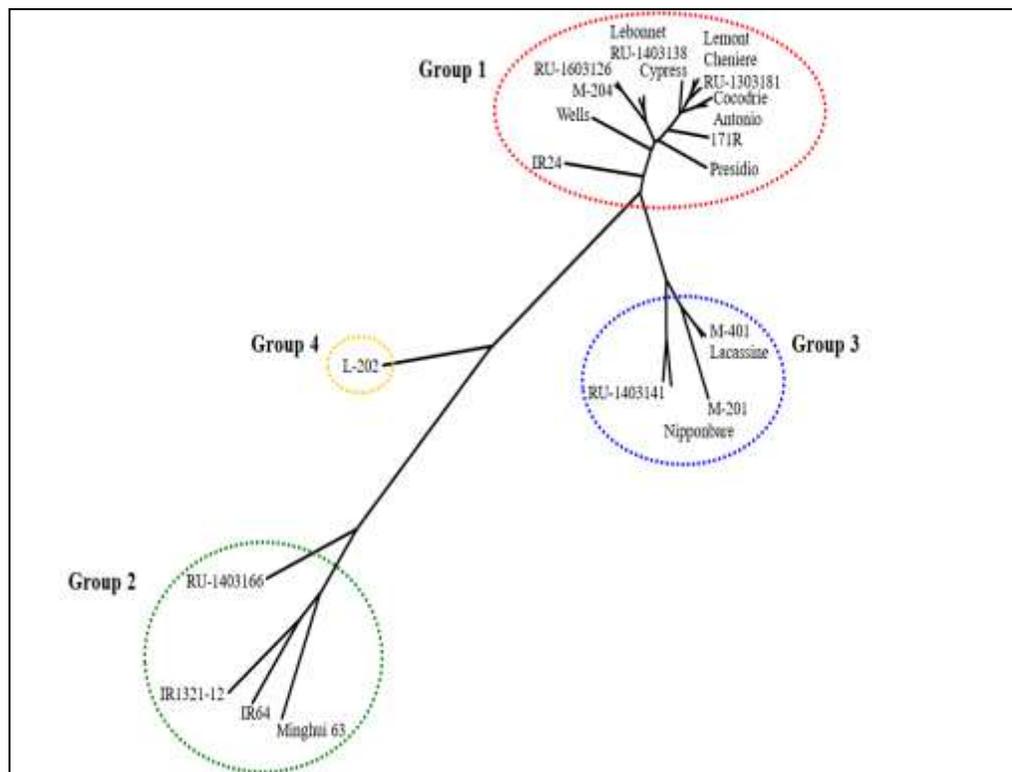


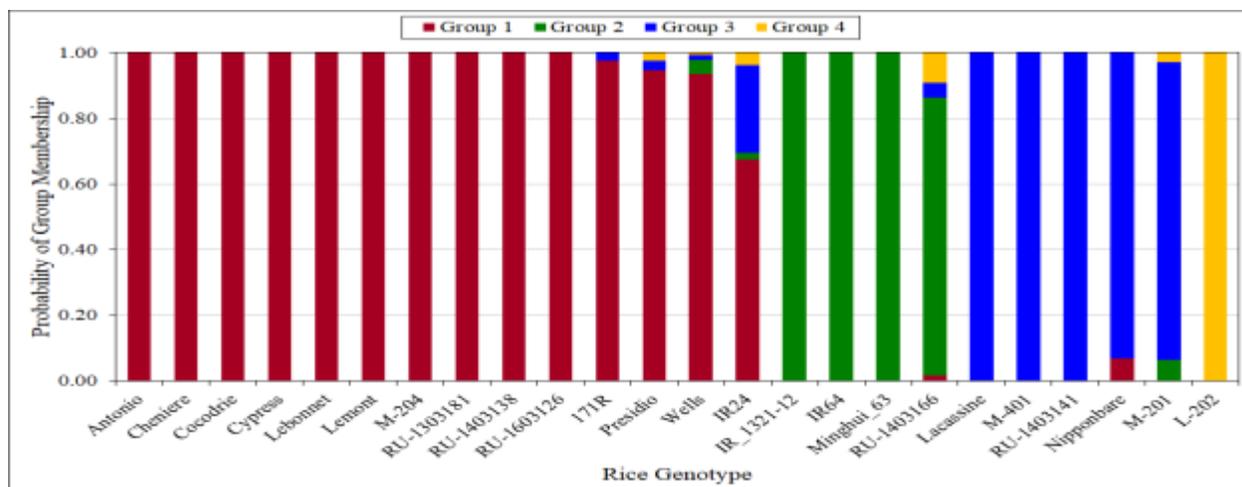
Figure 1. Unrooted neighbor-joining tree showing the heterotic grouping of 17 hybrid parents, check Presidio, and representatives of *indica* (IR64 and Minghui 63), *tropical japonica* (Cocodrie and Wells), and *temperate japonica* (M-201 and Nipponbare) rice subpopulations.

Genotype and year effects on traits

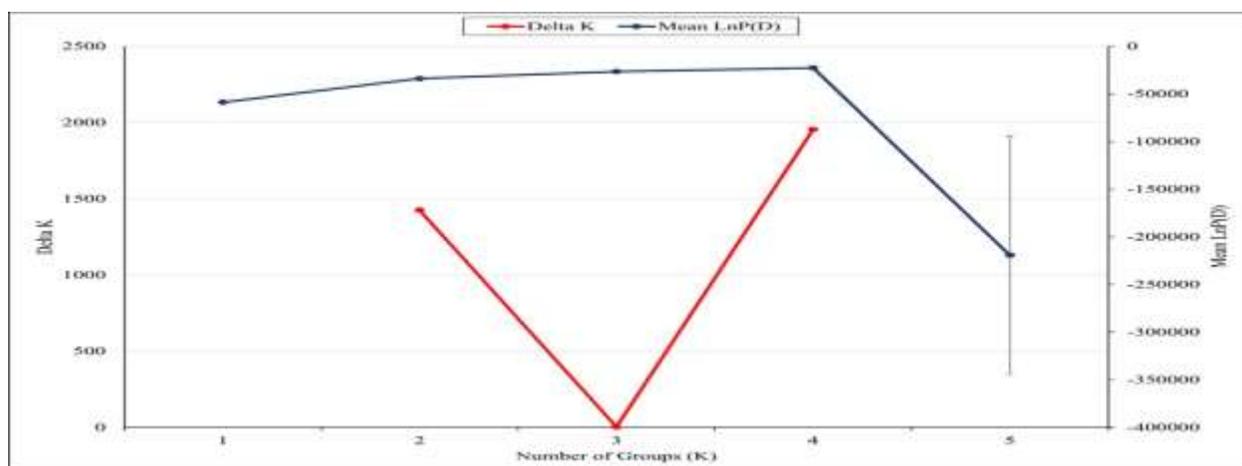
Levine's test indicated that the variances were homogeneous across years for four traits. The combined 2019–2020 ANOVA indicated that the year significantly affected days to heading, plant height, and grain yield (Table 2). Genotype significantly affected plant height, tiller density, and grain yield. Genotype × year interaction significantly affected the number of days to heading and grain yield.

Grain yield and agronomic traits of hybrids

Overall, the mean number of days to heading, plant height, tiller density, and grain yield were higher in 2019 than in 2020 when averaged across genotypes (Table 3). The number of days to heading ranged from 85.0 to 102.8 in 2019 and 78.0 to 97.0 in 2020. Hybrid F19A13 (RU-1403141/L-202) headed the earliest in 2019 and 2020, while Presidio and hybrid F19A02 (Lacassine/M-401) headed the latest in 2019 and 2020, respectively.



(a)



(b)

Figure 2. (a) Structure plot, based on shared patterns of single nucleotide polymorphism (SNP) variations, which identifies the heterotic grouping of 17 hybrid parents, check Presidio, and representatives of *indica* (IR64 and Minghui 63), *tropical japonica* (Cocodrie and Wells), and *temperate japonica* (M-201 and Nipponbare) rice subpopulations. (b) ΔK and mean $\text{LnP}(D)$ for $K = 1$ to 5, over five replicated runs in STRUCTURE. The peak values of ΔK and mean $\text{LnP}(D)$ were observed when $K = 4$, suggesting that the ideal number of groups is 4.

Plant height ranged from 94.0 to 130.2 cm in 2019 and 72.4 to 103.5 cm in 2020 (Table 3). Hybrids F19A02 (Lacassine/M-401) and F19A01 (RU-1603126/M-204) were the tallest in 2019 and 2020, respectively. The shortest crosses were F19A09 (Antonio/171R) in 2019 and F19A07 (RU-1303181/Lemont) in 2020. Among the parents of hybrids, Lebonnet was the only non-semi-dwarf rice. Given this, plant height data of hybrid F19A04 (Lebonnet/RU-1403138) were omitted from

subsequent analyses to remove the unwanted effect of a non-semi-dwarf variable affecting plant height.

Tiller densities ranged from 75.4 to 191.1 tillers m^{-2} in 2019 and 51.1 to 139.9 tillers m^{-2} in 2020 (Table 3). Hybrids F19A01 (RU-1603126/M-204) and F19A04 (Lebonnet/RU-1403138) produced the lowest tiller densities in 2019 and 2020, respectively.

Table 2. Significance of sources of variation affecting rice traits estimated from the field tests conducted at Beaumont, Texas, in 2019 and 2020.

Source of variation	Days to heading		Plant height		Tiller density		Grain yield	
	Mean Square	Prob > F	Mean Square	Prob > F	Mean Square	Prob > F	Mean Square	Prob > F
Year, Y	1651.7	0.0001**	4585.0	0.0365*	10691.0	0.1622	340431.0	0.0004**
Block (Year)	4.3	0.5018	170.8	0.0174*	2460.1	0.0387*	3317.2	0.2115
Genotype, G	78.2	0.1793	356.2	0.0002**	4081.0	0.0137*	45471.0	0.0014**
G × Y	44.2	<0.0001**	32.8	0.5092	993.6	0.1894	6338.6	0.0114*
Coefficient of Variation (%)	2.8		5.9		23.4		18.4	

*, ** Significant source of variation at the 5% and 1% levels, respectively.

Table 3. Mean comparison of agronomic traits of rice hybrids and check Presidio estimated from field tests conducted at Beaumont, Texas, in 2019 and 2020.

Rice genotypes	Days to heading (d)		Plant height (cm)		Tiller density (no m ⁻²)		Grain yield (g m ⁻²)	
	2019	2020	2019	2020	2019	2020	2019	2020
F19A01	93.5bcd†	90.3b	124.0ab	103.5a	83.4cd	137.2ab	322.6cd	210.1abc
F19A02	98.5abc	97.0a	130.2a	97.6ab	110.3bcd	88.8abcd	58.0f	42.6e
F19A03	97.0abc	81.0de	106.2cde	83.7bcd	88.8cd	70.0cd	262.6de	161.9bcd
F19A04	101.5ab	79.7ef	¶	¶	86.1cd	51.1d	306.6d	53.2de
F19A06	93.0bcd	80.2e	102.6de	81.3cd	129.2abcd	70.0cd	325.7cd	144.3bcde
F19A07	97.7abc	83.7c	106.4cde	80.4cd	110.3bcd	61.9cd	302.1de	99.5cde
F19A08	85.2d	79.4ef	105.7cde	84.7bcd	169.5ab	134.6ab	476.8ab	218.1ab
F19A09	92.0cd	78.3f	94.0e	83.6bcd	145.3abc	139.9a	393.5bc	233.9ab
F19A13	85.0d	78.0f	102.2de	82.0cd	137.2abcd	86.1bcd	275.6de	98.2cde
F19A14	95.8abc	83.3c	111.7bcd	97.9ab	164.2ab	107.6abc	553.6a	313.8a
F19A32	99.3abc	82.7cd	115.9bc	95.4abc	191.1a	129.2ab	527.5a	229.0ab
Presidio	102.8a	81.1de	96.8e	72.4d	75.4d	53.8d	218.9e	76.9de
Mean ‡	95.1a	82.9b	108.7a	87.5b	124.2a	94.2a	335.3a	156.8b
Coefficient of Variation (%)	3.7	1.1	4.6	7.9	22.1	25.0	10.4	32.6

† Trait means of hybrids within a column followed by at least one same letter are not significantly different from each other based on the Least Significant Difference Test at the 5% level.

‡ Mean comparison between years for each trait was based on the F-test (5% level) of the analyses of variance combined for years.

¶ Plant height data of the F19A04 (Lebonnet x RU-1403138) hybrid was omitted because Lebonnet was the only non-semi-dwarf among the parents of all hybrids.

Meanwhile, F19A32 (RU-1403141/IR 1321-12) and F19A09 (Antonio/171R) produced the highest tiller densities in 2019 and 2020, respectively.

Grain yields ranged from 58.0 to 553.6 g m⁻² in 2019 and 42.6 to 313.8 g m⁻² in 2020 (Table 3). In both years, hybrid F19A02 (Lacassine/M-401) produced the lowest yields, and hybrid F19A14 (RU-1403166/IR24) had the highest grain yields.

Trait means in low vs. high parental genetic distance hybrid groups

There was no consistent pattern across years in the number of days to heading, as both the low and high PGD hybrid groups had significantly fewer days to heading than Presidio in 2019 but more days in 2020 (Table 4). However, the low PGD hybrids had more days to heading than the high PGD in both years, and the difference in 2020 was significant. There was a distinct pattern in that the plant heights of both the low and high PGD hybrid groups were significantly taller than Presidio in both years. However, no significant difference in plant heights between the low and high PGD hybrid groups appeared. In both years, the high PGD hybrid group > low PGD group > Presidio in tiller density and grain yield, with the differences between low and high PGD hybrid groups being significant in 2019.

Heterosis in low vs. high parental genetic distance hybrid groups

Standard heterosis values for the number of days to heading were higher in the low PGD hybrid group than in the high PGD group in both years, but these values were negative in 2019 and positive in 2020 (Table 5). Heterosis values for plant height were positive in the low and high PGD hybrid groups, but the former had higher values in 2019, and the latter had higher values in 2020. Standard heterosis values in tiller density and grain yield were consistently higher in the high PGD hybrid group than in the low PGD group in both years, with these differences being significant in 2019. For tiller density, heterosis in the high

PGD group was greater than the low PGD hybrid group by 64% and 33% in 2019 and 2020, respectively. For grain yield, heterosis in the high PGD group was better than in the low PGD hybrid group by 69% and 110% in 2019 and 2020, respectively.

Correlation between parental genetic distance and heterosis

The parental genetic distance of hybrids had consistent positive correlations with standard heterosis in tiller density and grain yield in 2019 and 2020 (Table 6). The correlation $r = 0.60$ between heterosis and grain yield was significant in 2019, while the correlation $r = 0.41$ in 2020 had a p -value = 0.0591. Positive significant correlations consistent in both years occurred between heterosis in grain yield and tiller density and between heterosis in days to heading and plant height. Negative correlations consistent in both years showed between heterosis in grain yield and days to heading.

DISCUSSION

Genetic distance and wide compatibility

The statistically different genetic distance means between the low and high PGD hybrid groups indicated the successful selection of parental combinations to produce hybrids for this study. This distinctness allowed precise comparison between the low and high PGD hybrid groups in yield and yield-related traits.

As expected, the intra-subpopulation cross hybrids consisting of seven TRJ/TRJ and one TEJ/TEJ cross belonged to the low PGD group, and inter-subpopulation cross hybrids with one each of TEJ/TRJ, IND/TRJ, and TEJ/IND crosses belonged to the high PGD group. The pedigree of each hybrid's female and male parents shows that most are not single crosses but complex crosses among diverse cultivars and lines of different geographical origins or rice subpopulations (Table 1). Therefore, there is difficulty, potential inaccuracy, and uncertainty in using the genotypes' pedigree information alone to determine their rice subpopulation or in

Table 4. Mean comparison of agronomic traits of Presidio and hybrids grouped by parental genetic distances. Agronomic traits were estimated from field tests conducted at Beaumont, Texas, in 2019 and 2020.

Genotype groups	Days to heading (d)		Plant height (cm)		Tiller density (no. m ⁻²)		Grain yield (g m ⁻²)	
	2019	2020	2019	2020	2019	2020	2019	2020
Low PGD cross	94.8b†	83.7a	109.9a	87.8a	115.4b	94.2a	306.0b	145.5ab
High PGD cross	93.4b	81.3b	109.9a	91.8a	164.2a	107.6a	452.2a	213.7a
Presidio (check)	102.8a	81.1b	96.8b	72.4b	75.4b	53.8b	218.9c	76.9b
<i>Coefficient of Variation (%)</i>	3.7	1.1	4.6	7.9	22.1	25.0	10.4	32.6

Abbreviations: PGD = parental genetic distance

† Means within a column followed by at least one same letter are not significantly different based on the Least Significant Difference test at the 5% level.

Table 5. Mean comparison of standard heterosis of agronomic traits of hybrid groups that were produced from low and high parental genetic distances. Presidio was used as check cultivar in the estimation of heterosis. Agronomic traits were estimated from field tests conducted at Beaumont, Texas, in 2019 and 2020.

Hybrid groups	Standard Heterosis (%)							
	Days to heading		Plant height		Tiller density		Grain yield	
	2019	2020	2019	2020	2019	2020	2019	2020
Low PGD	-7.7a†	3.2a	14.8a	21.4a	80.0b	75.5a	38.6b	94.5a
High PGD	-9.0a	0.3b	13.6a	27.3a	144.4a	108.3a	107.8a	204.8a
<i>Coefficient of Variation (%)</i>	-34.6	38.3	37.1	46.8	53.4	67.2	29.2	89.3

Abbreviations: PGD = parental genetic distance

† Means within a column followed by the same letter are not significantly different based on the analyses of variance F-test at the 5% level.

Table 6. Correlation among parental genetic distances (PGDs) of rice hybrids and heterosis in grain yield and yield-related traits based on field tests conducted at Beaumont, Texas, in 2019 (above the diagonal) and 2020 (below the diagonal). Presidio was used as a check cultivar in the estimation of heterosis.

Traits	Correlation and their Significance				
	Days to heading	Plant height	Tiller density	Grain yield	PGD
Days to Heading		0.55**	-0.65**	-0.25	0.05
Plant Height	0.61**		-0.44	-0.42	-0.09
Tiller Density	0.07	0.61**		0.57*	0.36
Grain Yield	-0.11	0.57**	0.72**		0.60**
PGD	-0.20	0.22	0.28	0.41†	

*, ** Significant correlation at the 5% and 1% levels, respectively.

† p-value = 0.0591

estimating whether two parents of a hybrid have low or high genetic distance. In breeding programs, elite inbred, male-sterile, maintainer, or restorer lines may have resulted from intercrossing *tropical japonica*, *temperate japonica*, or *indica* parents, making them admixed genotypes. Estimating genetic distance using genome-wide DNA markers provides a better alternative for an accurate and straightforward approach to selecting parents for use in wide or narrow crosses.

Hybrids produced from crossing *japonica* and *indica* rice, which are expected to have high PGDs, require the wide compatibility *S5n* allele from at least one of its parents for normal seed set to occur (Ikehashi and Araki, 1986). In hybrid breeding programs, the selection for wide compatibility is necessary to increase the potential frequency that these selections can be used as parents in crosses between *japonica* and *indica* rice (Kallugudi *et al.*, 2022). Determining whether a parent has the *S5n* allele through marker-assisted selection improves breeding efficiency by possibly eliminating the need for one season to grow the hybrids in a testcross nursery to determine wide compatibility issues, such as low seed set. In this study, wide compatibility between parents of each hybrid was evident in the normal seed set in panicles of hybrids in the 2018 testcross nursery and the 2019 and 2020 field tests, and through the marker-assisted selection that verified that at least one parent possessed the wide compatibility gene.

Heterotic grouping

The unrooted neighbor-joining tree used in this study provides a visual representation wherein the length of the branches corresponds to genetic distances between parents (Saitou and Nei, 1987) and allows for the identification of potentially narrow or wide crosses. In addition, applying the model-based population structure inference demonstrates how a diverse group of genotypes was divided into subgroups of similar patterns in SNP variation (Pritchard *et al.*, 2000). The ideal number of heterotic groups determined through testing groupings

from one to five used an ad hoc statistic ΔK and the log-likelihood of the observed genotype distribution in *K* subpopulation $\text{LnP}(D)$. It was identified when ΔK and $\text{LnP}(D)$ were at their maximum values when $K = 4$. The four heterotic groups were appropriate since the parents of each hybrid in the low PGD hybrid group belonged to the same heterotic group, and the parents of each hybrid in the high PGD hybrid group belonged to different heterotic groups. Cluster and structural analyses have been used in recent studies for heterotic grouping (Sruthi *et al.*, 2020; Kallugudi *et al.*, 2022). These methodologies can be applied to numerous potential parents (male-sterile and restorer lines) in hybrid rice breeding programs to identify or develop heterotic groups and high PGD crosses or hybrids expected to be highly heterotic for yield or yield components.

Among the four heterotic groups identified from the 24 rice genotypes (17 parents and seven checks), L-202, a variety developed in California, was the sole component of heterotic group 4. L-202 was located between the TRJ, TEJ, and IND groups, according to the unrooted neighbor-joining tree. Based on the literature, L-202 was classified as *tropical japonica* using structure (Ali *et al.*, 2011) and fast structure (McCouch *et al.*, 2016) analyses. However, its pedigree of 723761/7232278//L-201, is an example of a rice cultivar developed from crosses between different subpopulations. Rice line 723761 is a long-grain introduction from IRRI, Philippines. Rice line 7232278 has unknown parentage but has the semi-dwarf gene from either IR-8 or Taichung Native 1, while L-201 was developed in California with a pedigree of CI 9701/3/R134-1/R48-257//RS0-11. In hybrid breeding programs, a significant percentage of potential hybrid parents are admixed genotypes inclined toward being *Japonica* or *Indica* (Cheng *et al.*, 2007). In these cases, heterotic grouping through cluster and structure analyses of genome-wide markers is practical and advisable instead of identifying genetically-distant crosses based on pedigree information.

Genotype and year effects on traits

With the significance of year affecting three of the four traits and the genotype \times year impacting heading and grain yield, subsequent analyses and interpretations were conducted separately for each year. Furthermore, the separate analysis allows for identifying trait patterns affected by the type of cross that may be consistent across years. Genotype was a crucial factor affecting height, tiller density, and grain yield, indicating that the high variation among hybrids needed for this study was attained. In 2020, five days of rain (11.2 cm rainfall) within seven days after the first field fertilization appeared to have caused N leaching, which may have lowered trait values compared to 2019. Additional N fertilizer application occurred upon notice of chlorosis, making the total N applied to the field higher in 2020 (205 kg ha⁻¹ N) compared to 2019 (180 kg ha⁻¹ N), but the N stress may have already caused the trait value decreases.

Traits of hybrids produced from low vs. high parental genetic distance

Although the number of days to heading positively affected directly rice grain yield (Samonte *et al.*, 2006), the maturity preferred in commercial production is early maturing and should not exceed acceptable levels. In the southern United States, early-maturing cultivars allow for producing a ratoon crop. Concerning this study, the high PGD hybrid group would be preferred due to its mean lower number of days to heading than the low PGD ones.

Plant height is an essential trait as it has been significantly and positively correlated with leaf area and biomass at heading, number of spikelets per panicle, number of primary branches in panicle, panicle node number, and panicle weight (Samonte *et al.*, 1998); it also has a positive direct effect on grain yield (Samonte *et al.*, 2006). In this study, compared with Presidio, 11 hybrids were taller in 2019, with all 12 taller in 2020. In both years, two of the three (67%) high PGD hybrids were above-average in height compared with only two out of seven (28%)

low PGD hybrids. However, tall rice plants are prone to lodging and are phenotypically unacceptable to farmers. The Lacassine/M-401 and RU-1603126/M-204 hybrids, measuring 130 and 124 cm tall in 2019, respectively, are products of low PGD crosses and would be too tall for selection in a breeding program.

Tiller density significantly correlates with leaf area index and biomass at heading, plant height, and grain yield (Samonte *et al.*, 1998). In addition, tiller density is highly correlated with panicle density, positively influencing the grain yield directly (Samonte *et al.*, 1998; Wang *et al.*, 2015). The proportions of high PGD hybrids that produced above-average tiller densities were 100% and 67% in 2019 and 2020, respectively, with only 37.5% of the low PGD hybrids producing above-average tiller densities in both years. The lowest three tiller densities in 2019 and 2020 resulted from low PGD hybrids and were all TRJ/TRJ crosses. In commercial farms in the United States, hybrids are planted at lower seeding rates, i.e., 30% to 45% of the amount sowed in inbred rice farms. With the lower seeding and eventual plant densities, the hybrid cultivars must be high tillering to achieve higher grain yields than inbred rice cultivars. Although most rice grown in the southern United States are *tropical japonica*, this study suggests that US hybrid rice breeders avoid the use of low PGD crosses or intra-subpopulation TRJ/TRJ crosses and focus more on using crosses between *japonica* and *indica* rice to produce high-tillering hybrids. With the increase in tiller densities, grain yield is expected to increase due to their positive correlation.

The top-yielding high PGD hybrid in both years (F19A14; RU-1403166/IR24) was an IND/TRJ cross and had yield advantages of 16% and 34% over the top-yielding low PGD hybrids, F19A08 (Cypress/171R) and F19A09 (Antonio/171R), in 2019 and 2020, respectively, which were TRJ/TRJ crosses. Furthermore, two-thirds of the high PGD hybrids had above-average hybrid grain yields in both years, compared with only two-eighths and three-eighths of the low PGD hybrids in 2019 and 2020, respectively. Lastly, the lowest-yielding hybrid in both years (F19A02;

Lacassine/M-401) was an intra-subpopulation TEJ/TEJ cross. These indicated that although hybrids had higher grain yields than the check Presidio, US rice breeders should prioritize making high PGD or inter-subpopulation (especially crosses between IND and TRJ or TEJ) hybrids instead of low PGD (TRJ/TRJ or TEJ/TEJ) hybrids, to achieve higher grain yield potential. In addition, the lone inter-subpopulation TEJ/TRJ hybrid (F19A13; RU-1403141/L-202) in the high PGD group produced below-average grain yields in both years, suggesting that crosses between these two subpopulations be avoided.

The consistent higher means in plant height, tiller density, and grain yield in the high PGD hybrid group over the low PGD group and Presidio in both years confirm the expectations of higher grain yield due to wide parental genetic distances (Wang *et al.*, 2015; Yingheng *et al.*, 2018; Hussain *et al.*, 2022). It also supports selecting parents with high genetic distances to improve yield-related traits.

The estimation and use of genetic distance to select parents to cross are based on quantitative values, unlike the approximation of whether a potential hybrid parent is TRJ, TEJ, or IND based on its pedigree. The latter method may be complex, especially if inter-subpopulation crosses were made to produce the potential hybrid parent.

Heterosis in low vs. high parental genetic distance hybrid groups

Using standard heterosis to compare the hybrid groups against the check cultivar Presidio in their agronomic and yield performance provides a different perspective than when comparing means. The general trends observed in this study were that hybrids produced from high PGD parents had higher standard heterosis values for tiller density and grain yield. The high PGD hybrid group showing lower heterosis for days to heading in both years may be valuable information as breeders prefer to select for early-maturing lines. These consistent patterns suggest that breeders should consider genomically sequencing their elite male-sterile and restorer lines and produce high PGD hybrids for

testcross or yield evaluation. Standard heterosis for plant height by the low and high PGD hybrid groups were 15% and 14%, respectively, in 2019 and 21% and 27%, in 2020. Excessive heterosis in plant height may make the hybrid rice unacceptably tall for commercial production. Among the high PGD hybrids, those with an indica parent had higher tiller densities, probably due to the higher tillering ability of indica compared with japonica rice. Compared with heterotic grouping approaches that are not genetically based, this high PGD hybrid approach would improve breeding efficiency for grain yield by identifying the potentially desirable crosses and reducing the number of test crosses that need to be evaluated.

Correlation between parental genetic distance and heterosis

In some studies, parental genetic distance or diversity negatively correlated with seed set and grain yield (Liu *et al.*, 2015; Yingheng *et al.*, 2018). In another study, PGD is positively correlated with yield heterosis but negatively correlated with heterosis for seed set (Zhang *et al.*, 2010). Contrastingly, in this study, the selection and use of parents with the wide compatibility gene removed the unwanted factor of low seed set in high PGD hybrids, which may have caused the lower yields in other studies. Furthermore, this study verified through DNA marker analysis that at least one parent of each hybrid studied possessed the wide compatibility *S5n* allele and that a normal seed set (>75%) was observed in all hybrids. Having the factor of inter-subspecies incompatibility eliminated with the use of at least one wide-compatible parent in producing the hybrids, study results show a positive correlation between PGD and heterosis for grain yield.

The consistent positive correlations among PGD and heterosis in tiller density and grain yield in this study are suitable relationships that hybrid rice breeders should take advantage of. Selecting cross combinations with high PGDs will improve selection efficiency for high tiller density and grain yield.

CONCLUSIONS

Establishing and applying heterotic groups to produce high yields are necessary for hybrid rice breeding programs where tropical japonica rice is predominantly grown. Inter-subpopulation cross-hybrids used in this study produced a normal seed set, verified to have at least one parent that possessed the *WC* gene. The high PGD hybrid group had higher trait values and standard heterosis for tiller density and grain yield than the low PGD hybrid group. The parental genetic distance of hybrids had positive correlations with tiller density and grain yield. Heterotic grouping is advisable using cluster and structure analyses of genome-wide markers instead of identifying genetically-distant crosses based on pedigree information. The approach demonstrated in this study can be considered for application in extensive hybrid rice breeding programs.

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REFERENCES

- Ali ML, McClung AM, Jia MH, Kimball JA, McCouch SR, Eizenga GC (2011). A rice diversity panel evaluated for genetic and agro-morphological diversity between subpopulations and its geographic distribution. *Crop Sci.* 51(5): 2021-2035. <https://doi.org/10.2135/cropsci2010.11.0641>.
- Alpuerto JBB, Samonte SOPB, Sanchez DL, Croaker PA, Wang Y-J, Wilson LT, Christensen EF, Tabien RE, Yan Z, Thomson MJ (2022). Genomic association mapping of apparent amylose and protein concentration in milled rice. *Agron.* 12(4): 857. <https://doi.org/10.3390/agronomy12040857>.
- Beukert U, Li Z, Liu G, Zhao Y, Ramachandra N, Mirdita V, Pita F, Pillen K, Reif JC (2017). Genome-based identification of heterotic patterns in rice. *Rice* 10(1): 22. <https://doi.org/10.1186/s12284-017-0163-4>.
- Bhati PK, Singh SK, Singh R, Sharma A, Dhurai SY (2015). Estimation of heterosis for yield and yield related traits in rice (*Oryza sativa* L.). *SABRAO J. Breed. Genet.* 47(4): 467-474.
- Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23(19): 2633-2635. <https://doi.org/10.1093/bioinformatics/btm308>.
- Browning SR, Browning BL (2007). Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. *Am. J. Hum. Genet.* 81(5): 1084-1097. <https://doi.org/10.1086/521987>.
- Cheng SH, Zhuang JY, Fan YY, Du JH, Cao LY (2007). Progress in research and development on hybrid rice: A super-domesticated in China. *Ann. Bot.* 100(5): 959-966. <https://doi.org/10.1093/aob/mcm121>.
- Earl DA, vonHoldt BM (2012). Structure harvester: A website and program for visualizing structure output and implementing the Evanno method *Conserv. Genet. Resour.* 4(2): 359-361. <https://doi.org/10.1007/s12686-011-9548-7>.
- Evanno G, Regnaut S, Goudet J (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Mol. Ecol.* 14(8): 2611-2620. <https://doi.org/10.1111/j.1365-294x.2005.02553.x>.
- Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch S (2005). Genetic structure and diversity in *Oryza sativa* L. *Genet.* 169(3): 1631-1638. <https://doi.org/10.1534/genetics.104.035642>.
- Geng X, Qu Y, Jia Y, He S, Pan Z, Wang L, Du X (2021). Assessment of heterosis based on parental genetic distance estimated with SSR and SNP markers in upland cotton (*Gossypium hirsutum* L.). *BMC Genom.* 22(1): 123. <https://doi.org/10.1186/s12864-021-07431-6>.
- He Z-Z, Xie F-M, Chen L-Y, Dela Paz MA (2012). Genetic diversity of tropical hybrid rice germplasm measured by molecular markers. *Rice Sci.* 19(3): 193-201. [https://doi.org/10.1016/s1672-6308\(12\)60040-7](https://doi.org/10.1016/s1672-6308(12)60040-7).
- Hussain I, Ali S, Liu W, Awais M, Li J, Liao Y, Zhu M, Fu C, Liu D, Wang F (2022). Identification of heterotic groups and patterns based on genotypic and phenotypic characteristics among rice accessions of diverse origins. *Front Genet* 13: 811124. <https://doi.org/10.3389/fgene.2022.811124>.

- Ikehashi H, Araki H (1986). Genetics of F1 sterility in remote crosses of rice. In: *Rice Genetics I*. World Scientific. pp. 119-130.
- Islam MZ, Galib MAA, Akand MM, Lipi LF, Akter A, Matin MQI, Ivy NA (2022). Combining ability and heterotic studies in aromatic rice through line by tester analysis. *SABRAO J. Breed. Genet.* 54(2): 221-235. <http://doi.org/10.54910/sabrao2022.54.2.2>.
- Jiang T, Li R, Sun C, Wang X (2002). Utilization of diverse rice ecotypes in heterosis breeding. *Breed. Sci.* 52(2): 107-113. <https://doi.org/10.1270/jsbbs.52.107>.
- Jones JW (1926). Hybrid vigor in rice. *Agron. J.* 18(5): 423-428. <https://doi.org/10.2134/agronj1926.00021962001800050010x>.
- Kahani F, Hittalmani S, Erfani R, Haradari C (2018). Heterotic effects and combining ability for yield traits in rice developed for semi-dry aerobic cultivation. *SABRAO J. Breed. Genet.* 50(1): 46-61.
- Kallugudi J, Singh VJ, Vinod KK, Krishnan SG, Nandakumar S, Dixit BK, Ellur RK, Bollinedi H, Nagarajan M, Kumar A, Chakraborti M, Seth RK, Mondal TK, Bhowmick PK, Singh AK (2022). Population dynamics of wide compatibility system and evaluation of intersubspecific hybrids by indica-japonica hybridization in rice. *Plants* 11(15): 1930. <https://doi.org/10.3390/plants11151930>.
- Kawahara Y, De La Bastide M, Hamilton JP, Kanamori H, McCombie WR, Ouyang S, Schwartz DC, Tanaka T, Wu J, Zhou S, Childs KL, Davidson RM, Lin H, Quesada-Ocampo L, Vaillancourt B, Sakai H, Lee SS, Kim J, Numa H, Itoh T, Buell CR, Matsumoto T (2013). Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. *Rice* 6(1): 4. <https://doi.org/10.1186/1939-8433-6-4>.
- Kim S-I, Tai TH (2013). Identification of SNPs in closely related temperate japonica rice cultivars using restriction enzyme-phased sequencing. *PLoS ONE* 8(3): e60176. <https://doi.org/10.1371/journal.pone.0060176>.
- Labroo MR, Studer AJ, Rutkoski JE (2021). Heterosis and hybrid crop breeding: A multidisciplinary review. *Front. Genet.* 12. <https://doi.org/10.3389/fgene.2021.643761>.
- Laude TP, Carena MJ (2015). Genetic diversity and heterotic grouping of tropical and temperate maize populations adapted to the northern U.S. corn belt. *Euphytica* 204(3): 661-677. <https://doi.org/10.1007/s10681-015-1365-8>.
- Li X, Shahzad K, Guo L, Qi T, Zhang X, Wang H, Tang H, Qiao X, Zhang J, Wu J, Xing C (2019). Using yield quantitative trait locus targeted SSR markers to study the relationship between genetic distance and yield heterosis in upland cotton (*Gossypium hirsutum*). *Plant Breed.* 138(1): 105-113. <https://doi.org/10.1111/pbr.12668>.
- Liu KD, Zhou ZQ, Xu CG, Zhang Q, Saghai Maroof MA (1996). An analysis of hybrid sterility in rice using a diallel cross of 21 parents involving indica, japonica, and wide compatibility varieties. *Euphytica* 90(3): 275-280. <https://doi.org/10.1007/bf00027476>.
- Liu P, Dan Z, Wang Z, Li S, Li N, Yan H, Cai X, Lu B-R (2015). Predicting hybrid fertility from marker-based genetic divergence index of parental varieties: Implications for utilizing inter-subspecies heterosis in hybrid rice breeding. *Euphytica* 203(1): 47-57. <https://doi.org/10.1007/s10681-014-1249-3>.
- Lu Z-M, Xu B-Q (2010). On significance of heterotic group theory in hybrid rice breeding. *Rice Sci.* 17(2): 94-98. [https://doi.org/10.1016/s1672-6308\(08\)60110-9](https://doi.org/10.1016/s1672-6308(08)60110-9).
- McClung AM (2005). 'Presidio' rice - A new long grain rice with improved ratoon crop potential and milling yield. *Texas Rice Special Section*, pp. 11. Retrieved from https://beaumont.tamu.edu/eLibrary/Newsletter/2005_Highlights_in_Research.pdf.
- McCouch SR, Wright MH, Tung C-W, Maron LG, McNally KL, Fitzgerald M, Singh N, Declercq G, Agosto-Perez F, Korniliev P, Greenberg AJ, Naredo MEB, Mercado SMQ, Harrington SE, Shi Y, Branchini DA, Kuser-Falcão PR, Leung H, Ebana K, Yano M, Eizenga G, McClung A, Mezey J (2016). Open access resources for genome-wide association mapping in rice. *Nat. Commun.* 7(1): 10532. <https://doi.org/10.1038/ncomms10532>.
- Melchinger AE, Gumber RK (1996). Overview of heterosis and heterotic groups in agronomic crops. In: *Concepts and breeding of heterosis in crop plants*. CSSA Special Publication no. 25. pp. 29-44.
- Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. *Genet.* 155(2): 945-959. <https://doi.org/10.1093/genetics/155.2.945>.
- Saitou N, Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4(4): 406-425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>.
- Samonte SOPB, Wilson LT, McClung AM (1998). Path analyses of yield and yield-related traits of

- fifteen diverse rice genotypes. *Crop Sci.* 38(5): 1130-1136. <https://doi.org/10.2135/cropsci1998.0011183x003800050004x>.
- Samonte SOPB, Wilson LT, Tabien RE (2006). Maximum node production rate and main culm node number contributions to yield and yield-related traits in rice. *Field Crops Res.* 96(2): 313-319. <https://doi.org/10.1016/j.fcr.2005.07.014>.
- Samonte SOPB, Wilson LT, Tabien RE, Harper CL, Zhou XG (2022). Registration of 'Trinity' long-grain rice cultivar. *J. Plant Regist.* <https://doi.org/10.1002/plr2.20238>.
- Sanchez DL, Samonte SOPB, Alpuerto JBB, Croaker PA, Morales KY, Yang Y, Wilson LT, Tabien RE, Yan Z, Thomson MJ, Septiningsih EM (2022). Phenotypic variation and genome-wide association studies of main culm panicle node number, maximum node production rate, and degree-days to heading in rice. *BMC Genom.* 23(1): 390. <https://doi.org/10.1186/s12864-022-08629-y>.
- Shull GH (1908). The composition of a field of maize. *Am. Breed. Assoc. Rep.* 4: 296-301.
- Sruthi K, Divya B, Senguttuvel P, Revathi P, Kemparaju KB, Koteswararao P, Sundaram RM, Singh VJ, Ranjith Kumar E, Bhowmick PK, Vinod KK, Gopala Krishnan S, Singh AK, Hari Prasad AS (2020). Evaluation of genetic diversity of parental lines for development of heterotic groups in hybrid rice (*Oryza sativa* L.). *J. Plant Biochem. Biotechnol.* 29(2): 236-252. <https://doi.org/10.1007/s13562-019-00529-9>.
- Sundaram RM, Sakthivel K, Hariprasad AS, Ramesha MS, Viraktamath BC, Neeraja CN, Balachandran SM, Shobha Rani N, Revathi P, Sandhya P, Hari Y (2010). Development and validation of a PCR-based functional marker system for the major wide-compatible gene locus *s5* in rice. *Mol. Breed.* 26(4): 719-727. <https://doi.org/10.1007/s11032-010-9482-5>.
- Thippeswamy S, Chandramohan Y, Srinivas B, Padmaja D (2016). Selection of diverse parental lines for heterotic hybrid development in rice (*Oryza sativa* L.). *SABRAO J. Breed. Genet.* 48(3): 285-294.
- Virmani SS, Sun ZX, Mou TM, Ali AJ, Mao CX (2003). Two-line Hybrid Rice Breeding Manual. IIRI. Retrieved from http://books.irri.org/9712201856_content.pdf.
- Wang K, Qiu F, Larazo W, Dela Paz MA, Xie F (2015). Heterotic groups of tropical indica rice germplasm. *Theor. Appl. Genet.* 128(3): 421-430. <https://doi.org/10.1007/s00122-014-2441-5>.
- Wegary D, Vivek B, Labuschagne M (2013). Association of parental genetic distance with heterosis and specific combining ability in quality protein maize. *Euphytica* 191(2): 205-216. <https://doi.org/10.1007/s10681-012-0757-2>.
- Wilson LT, Morace B, Wang J, Samford J, Yang Y (2022). *Texas rice crop survey*. Accessed 10 December 2022 from <http://beaumont.tamu.edu/CropSurvey>.
- Xie F, He Z, Esguerra MQ, Qiu F, Ramanathan V (2014). Determination of heterotic groups for tropical indica hybrid rice germplasm. *Theor. Appl. Genet.* 127(2): 407-417. <https://doi.org/10.1007/s00122-013-2227-1>.
- Yingheng W, Qiuhua C, Hongguang X, Fangxi W, Ling L, Wei H, Liping C, Hua'An X, Jianfu Z (2018). Determination of heterotic groups and heterosis analysis of yield performance in indica rice. *Rice Sci.* 25(5): 261-269. <https://doi.org/10.1016/j.rsci.2018.08.002>.
- You-Xin Y, Yan-Hong L, Jing-Fei T, Qasim SM, Zhi-Xiong C, Lan W, Jin-Quan L, Xiang-Dong L, Yong-Gen L (2012). Wide-compatibility gene *s5n* exploited by functional molecular markers and its effect on fertility of intersubspecific rice hybrids. *Crop Sci.* 52(2): 669-675. <https://doi.org/10.2135/cropsci2011.04.0232>.
- Zhang T, Ni X-L, Jiang K-F, Deng H-F, He Q, Yang Q-H, Yang L, Wan X-Q, Cao Y-J, Zheng J-K (2010). Relationship between heterosis and parental genetic distance based on molecular markers for functional genes related to yield traits in rice. *Rice Sci.* 17(4): 288-295. [https://doi.org/10.1016/s1672-6308\(09\)60029-9](https://doi.org/10.1016/s1672-6308(09)60029-9).