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RESEARCH ARTICLE

Fertility restoration and agronomic performance of hybrid rice derived from CMS × NPT under optimum and low nitrogen environments

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ABSTRACT

The use of cytoplasmic male sterility (CMS) is a promising strategy for combining the potential of a new plant type (NPT) rice (*Oryza sativa* L.) to develop superior hybrids. However, another important aspect to consider is how well the plants can adapt to different environments. The objective of this experiment was to evaluate several CMS × NPT hybrid rice and identify hybrids with high productivity in both optimum and low-N environments. This study was conducted at the IPB University Experimental Station at Babakan, Indonesia, using a randomized complete block design (RCBD) with three replicates in each environment. The genetic material used consisted of several rice hybrids resulting from crosses of four CMS lines with eight NPT lines, as well as six commercial varieties as controls. The result showed that 52.94% of genotypes were partially fertile. Genotype × Environment interactions had a significant effect on most of the observed traits ($p < 0.05$). Several hybrids showed higher grain yields than some of the control varieties and comparable to others. One genotype had a yield stability index of 0.99, which was higher than that of all control varieties. The PCA analysis showed that PC1 (57.6%) was associated with yield components, while PC2 (42.2%) was associated with tolerance variation. The multi-trait genotype-ideotype distance index (MGIDI) selected five genotypes in an optimal environment. These findings emphasize the importance of NPT as a source of restoration in the development of hybrids that perform optimally with optimum N input and tolerance to low N conditions.

Key words: Cytoplasmic male sterility, fertility restoration, G × E analysis, low nitrogen.

INTRODUCTION

Rice (*Oryza sativa* L.) remains one of the most important primary sources of nutrition for over half of the global population. The Food and Agriculture Organization (FAO) projects that global rice consumption will increase by 2.2% in 2024-2025, reaching a record 539 million tons (FAO, 2025). In Indonesia, the average per capita weekly rice consumption in 2024 was 1.521 kg (BPS, 2024), making Indonesia one of the countries with the highest rice consumption in the world. Furthermore, amidst limited land and declining soil fertility, the need for higher rice production is becoming increasingly urgent.

Hybrid rice is one of the most promising approaches to increasing productivity through heterosis. However, the development of hybrid rice in Indonesia still faces challenges, one of which is limited seed availability, as seeds must be produced each season using complex hybrid seed production technology that is not yet readily accessible to farmers (Sari et al., 2025). One such complexity concerns the reproductive characteristics of rice, a self-pollinating plant with a very low rate of natural cross-pollination. Therefore, hybrid rice seed production generally utilizes the cytoplasmic male sterility (CMS) system to prevent self-pollination and produce pure F_1 hybrid seeds (Ashraf et al., 2024).

A previous study on CMS A05 conducted by Safitri et al. (2023) showed differences in restoration ability between lines. These differences are associated with allelic variation in fertility-restorer genes (Zhao et al., 2023), emphasizing the importance of selecting appropriate restorer lines. Furthermore, CMS systems require restorer lines that not only function to restore fertility but also support the agronomic performance of hybrids. The new plant type (NPT) concept offers a promising approach by optimizing plant architecture for high yield potential, with reported traits including moderate plant height, longer panicles, and a higher number of filled grains (Aswidinnoor et al., 2023). However, the potential of the CMS × NPT combination is highly dependent on the suitability of the parent combination and its adaptability to environmental variations.

The main limiting factor for productivity that is often encountered is N availability. Djaman et al. (2018) reported that increasing N doses can increase the yield of some hybrids, but at high application rates, can reduce efficiency. According to Zhou et al. (2023), optimum N application can increase yields by 30%-50% compared to N-deficient conditions. This N deficiency can induce chlorosis, suppress vegetative growth, and reduce tiller formation in rice plants (Lestari et al., 2016). Therefore, an important consideration is the plant's ability to adapt to environments with low nutrient availability, especially N. The objective of this study was to evaluate several CMS × NPT hybrid rice and identify hybrids with high productivity in optimum environments and low N availability.

MATERIALS AND METHODS

Experimental site and genetic material

The study was conducted at the IPB University Experimental Station at Babakan, Bogor, West Java, Indonesia. The F_1 rice (*Oryza sativa* L.) seed production was carried out from July to November 2024. Field evaluation of the hybrids was conducted from February to July 2025. The experimental site is located at 6.56352° S and 106.73551° E, at 192 m a.s.l. The average annual rainfall is approximately 3500-4000 mm. During the crop growth period, the mean monthly rainfall was 292.05 mm, with an average temperature of 26.37 °C and relative humidity of 85.20%.

The soil type at the experimental field was Latosol. Pre-experimental soil analysis indicated that the nutrient status of the topsoil layer (0-30 cm) had a pH (H_2O) 6.11, total N 0.25%, available P 8.7 mg P_2O_5 100 g^{-1} soil, and available K 22.45 mg K_2O 100 g^{-1} soil. The genetic materials (Table 1) used in this study consisted of several hybrid combinations derived from crosses between cytoplasmic male sterile (CMS) lines A02, A05, A322, and A410 and eight new plant type (NPT) rice, namely IPB 3S, IPB 8G, IPB 9G, IPB 12S, IPB 14S, IPB 15S, IPB 1R, and IPB 187-14-1-1. In addition, six commercial varieties were included as controls, comprising two inbred varieties (Inpari 32, Ciherang) and four hybrid varieties (Mapan P 02, Mapan P 05, Suppadi 61, Suppadi 284).

Table 1. List of rice genotypes used in the experiment.

Code	Cross combination	Code	Cross combination
G1	A02 × IPB 3S	G10	A322 × IPB 1R
G2	A02 × IPB 8G	G11	A322 × IPB 9G
G3	A02 × IPB 9G	G12	A322 × IPB 14S
G4	A02 × IPB 12S	G13	A410 × IPB 1R
G5	A02 × IPB 15S	G14	A410 × IPB 3S
G6	A02 × IPB 187-14-1-1	G15	A410 × IPB 8G
G7	A05 × IPB 3S	G16	A410 × IPB 9G
G8	A05 × IPB 8G	G17	A410 × IPB 14S
G9	A05 × IPB 9G		
Inpari 32	Inbred control	Mapan P 05	Hybrid control
Ciherang	Inbred control	Suppadi 61	Hybrid control
Mapan P 02	Hybrid control	Suppadi 284	Hybrid control

Experimental design and management practices

This study was conducted in two environments, namely optimum and low N, using a randomized complete block design (RCBD) with three replicates in each environment. A total of 69 experimental units were evaluated, each consisting of three rows with 12 plants per row. Seedlings aged 18-21 d were transplanted at a spacing of 30 cm × 25 cm, with one seedling per hill. In the optimum environment, fertilization consisted of total rates of 150 kg ha^{-1} urea (46% N) and 250 kg ha^{-1} compound NPK fertilizer (16% N, 16% P_2O_5, and 16% K_2O), applied in two stages: The first at 1 wk after planting (WAP) using 50 kg ha^{-1} urea and 150 kg ha^{-1} compound NPK fertilizer, and the second at 3 WAP using 100 kg ha^{-1} urea and 50 kg ha^{-1} compound NPK fertilizer. In the low-N environment, the fertilizers applied were 75 kg ha^{-1} urea (46% N), 200 kg ha^{-1} phosphate fertilizer (20% P_2O_5), and 66 kg ha^{-1} KCl (60% K_2O). Fertilizers were applied in two stages: 30 kg ha^{-1} urea, 150 kg ha^{-1} phosphate fertilizer, and 40 kg ha^{-1} KCl at 1 WAP, followed by 45 kg ha^{-1} urea, 50 kg ha^{-1} phosphate fertilizer, and 26 kg ha^{-1} KCl at 3 WAP. Water requirements were met through optimum irrigation. Pest and disease control using pesticides was carried out based on symptoms and infestation levels observed in the field. Weed control was performed manually.

Trait observation and statistical analysis

Observations comprised two groups of traits: (i) Seed-set percentage as the primary indicator of fertility, and (ii) agronomic traits, including plant height (PH), number of productive tillers (NPT), panicle length (PL), grain weight per hill (GWH), flowering age (FA), number of filled grains per panicle (FGN), filled grain percentage (FGP), 1000-grain weight (TGW), and total yield per plot (YLD). Each trait was recorded from five randomly selected sample plants within each plot. Seed-set percentage was calculated from one panicle per sample plant in each plot using the following formula:

$$\text{Spikelet fertility}(\%) = \frac{\text{Number of filled grains}}{\text{Number of filled grains + Number of unfilled grains}} \times 100$$

Statistical analysis was performed on each test environment. In the optimum environment, data were analyzed using an *F*-test at the 0.05 level. If a significant effect of genotype was found, a least significance difference (LSD) test was performed at the 0.05 level to evaluate differences between genotypes for each observed trait.

In the low-N environment, biotic disturbance from snails and rodents during the early vegetative stage resulted in incomplete observations for some genotypes until the generative phase, resulting in imbalanced data. To ensure the ANOVA assumptions were met and statistical validity maintained, a combined analysis was performed for only the 11 genotypes with complete data in both environments. A combined ANOVA was performed according to the following linear model to evaluate the effects of genotype, environment, and Genotype × Environment interactions on agronomic traits and spikelet fertility:

$$Y_{ijk} = \mu + E_k + B_{i(k)} + G_j + (GE)_{kj} + \varepsilon_{ij}$$

where i is the genotype, j is the environment, k is the block, Y_{ijk} is the observed response value, μ is the grand mean, E_k is the environment effect, $B_{i(k)}$ is the effect of block in environment, G_j is the genotype effect, $(GE)_{kj}$ is the Genotype × Environment interaction, and ε_{ij} is the experimental error.

Stress tolerance indices under low-N conditions were calculated following Khan and Mohammad (2016), including tolerance (TOL) = Yp – Ys; stress index (SI) = $1 - \frac{\bar{Y}s}{\bar{Y}p}$; stress susceptibility index (SSI) = $\frac{(1 - Ys/Yp)}{SI}$; stress tolerance index (STI) = $\frac{Yp \times Ys}{\bar{Y}p^2}$; mean productivity (MP) = $\frac{Yp \times Ys}{2}$; geometric mean productivity (GMP) = $\sqrt{Yp \times Ys}$; and yield stability index (YSI) = $\frac{Ys}{Yp}$. In these equations, Ys represents the grain yield of each genotype under a low-N environment, Yp represents the grain yield of each genotype under an optimum environment, while $\bar{Y}s$ and $\bar{Y}p$ denote the corresponding mean yields across genotypes.

Principal component analysis (PCA) was performed using productivity data on Yp and Ys, and the stress tolerance index to evaluate relationships among variables and visualize genotype separation patterns based on responses to different N conditions. Furthermore, the multi-trait genotype-ideotype distance index (MGIDI) was used to identify genotypes with the best agronomic performance and closest to the ideotype (Olivoto et al., 2022) in the optimum environment. The traits used in the MGIDI analysis included PH, NPT, TGW, FGN, FGP, and YLD, with double weighting applied to TGW, FGN, and YLD. The MGIDI estimation, strengths and weaknesses analysis, and calculation of selection differentials were performed using R version 4.1.5 (R Foundation for Statistical Computing, Vienna, Austria) with the 'metan' package, while PCA was conducted using the ‘factoextra’ package. ANOVA for hybrid rice traits and correlation analysis among stress tolerance indices were performed using SAS On-Demand for Academics (welcome.oda.sas.com).

RESULTS

Classification of fertility restoration

The results of the spikelet fertility evaluation (Table 2) showed that 9 genotypes (52.94%) were classified as partially fertile and 8 genotypes (47.06%) as partially sterile. Meanwhile, the fertile and completely sterile categories were not found in this study. The distribution of spikelet fertility of 17 hybrid genotype combinations tested is presented in Figure 1. Spikelet fertility ranged from 13.09% to 72.16%, with the highest value recorded in G10 (72.16%) and the lowest in G5 (13.09%). Among the evaluated hybrids, G13 (71.74%), G9 (67.66%), and G16 (65.40%) also exhibited relatively high spikelet fertility.

Table 2. Fertility restoration categories in the hybrid rice genotype combinations tested. Source: Adapted from Raj and Virmani (1988), with modifications.

Genotype classification	Fertility criteria (%)	Number of genotypes	Frequency (%)
Fertile	≥ 80	0	0
Partially fertile	31-79	9	52.94
Partially sterile	11-30	8	47.06
Sterile	≤ 10	0	0
Total		17	100

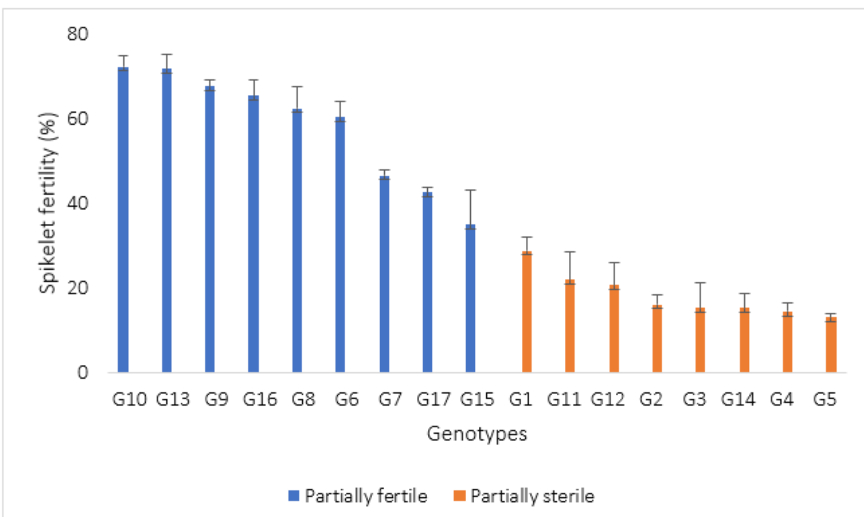

Figure 1. Distribution of fertility restoration rates for each rice genotype.

Combined analysis of variance

The combined ANOVA (Table 3) showed that the environmental effect (E) was highly significant ($p < 0.01$) for plant height (PH), number of productive tillers (NPT), panicle length (PL), 1000-grain weight (TGW), number of filled grains (FGN), filled grain percentage (FGP), and flowering age (FA), significant ($p < 0.05$) for grain yield (YLD), and nonsignificant for grain weight per hill (GWH). The genotypic effect (G) was highly significant ($p < 0.01$) for PH, PL, GWH, TGW, FGN, FGP, and FA, but nonsignificant for YLD. The Genotype × Environment interaction showed a highly significant effect ($p < 0.01$) on PL and FGP, a significant effect ($p < 0.05$) on PH and FGN, and a nonsignificant effect on NPT, GWH, TGW, FA, and YLD. The coefficient of variation (CV) ranged from 4.92% to 28.53%, with the highest value observed for YLD.

Table 3. Mean squares from the combined ANOVA for agronomic traits and yield components under optimal and low N conditions. Env: Environment; Rep: replication; CV: coefficient of variation; PH: plant height; NPT: number of productive tillers; PL: panicle length; GWH: grain weight per hill; TGW: 1000-grain weight; FGN: number of filled grains per panicle; FGP: filled grain percentage; FA: flowering age; YLD: yield. *, **Significant at $p < 0.05$ and $p < 0.01$; ^{ns}nonsignificant.

Traits	Env (E)	Rep/E	Genotype (G)	G × E	CV (%)
PH	6393.36**	88.55^{ns}	392.75**	94.69*	6.47
NPT	225.41**	0.68^{ns}	8.71^{ns}	3.11^{ns}	15.09
PL	53.37**	2.90^{ns}	18.07**	5.22**	4.92
GWH	154.28^{ns}	13.69^{ns}	407.77**	99.60^{ns}	16.13
TGW	110.48**	6.46*	7.19**	3.56^{ns}	5.55
FGN	8137.32**	218.57^{ns}	3626.93**	2048.29*	23.39
FGP	1257.49**	100.96^{ns}	994.03**	484.06**	17.53
FA	307.03**	36.08^{ns}	94.19**	22.62^{ns}	7.03
YLD	5.56*	1.73^{ns}	1.35^{ns}	0.48^{ns}	28.53

Agronomic performance

The ANOVA showed significant differences between varieties for all observed traits (Table 4). Plant height of the tested genotypes ranged from 90.9 cm (G1), comparable to 'Inpari 32' and 'Ciherang', to 130.1 cm (G10), which was higher than all controls. The number of productive tillers varied from 11 to 17 tillers per hill. Genotypes G12, G14, and G16 had the lowest tiller counts among the controls, while G2, G5, and G7 had comparable tiller counts. Days to flowering ranged from 46 d for G5 and G3 to 70 d for G17. Genotypes G2, G3, and G5 had fewer days to flowering than all controls, while G8, G9, and G17 had comparable days to flowering.

Table 4. Agronomic performance and yield components of hybrid rice genotypes under optimal N conditions. PH: Plant height (cm); NPT: number of productive tillers; PL: panicle length (cm); GWH: grain weight per hill (g); TGW: 1000-grain weight (g); FGN: number of filled grains per panicle; FGP: filled grain percentage; FA: flowering age (d); YLD: yield (t ha^{-1}); CV: coefficient of variation. ^{a}Significantly different from 'Inpari 32'. ^{b}Significantly different from 'Ciherang'. ^{c}Significantly different from 'Mapan P 05'. ^{d}Significantly different from 'Suppadi 284'. ^{e}Significantly different from 'Mapan P 02'. ^{f}Significantly different from 'Suppadi 61'. Letters indicate higher values for all traits, except flowering age.

Genotype	PH	NPT	PL	GWH	TGW	FGN	FGP	FA	YLD
G1	90.9	16	29.6^{abdf}	28.5	28.7^{ad}	47	29	55^{acdf}	1.13
G2	89.3	17	29.3^{abcdf}	22.9	27.1	26	16	52$^{a\text{-}f}$	1.01
G3	93.5	16	26.6^{abdf}	23.1	26.5	20	15	46$^{a\text{-}f}$	1.52
G4	96.3	16	28.2^{abdf}	19.5	26.0	23	14	50^{acdf}	0.65
G5	93.9	17	27.3^{abd}	19.9	25.7	25	13	46$^{a\text{-}f}$	1.06
G6	92.3	15	23.9	31.8	25.3	86	60	51^{acdf}	2.32
G7	110.0	17	29.0^{abdf}	51.7^{ab}	30.6^{abd}	102	47	63	3.85
G8	102.2	15	26.6^{a}	34.2	26.8	96	62	66	1.14
G9	92.4	17	25.9^{a}	38.7	22.9	105	68	65	3.22
G10	130.1$^{a\text{-}f}$	16	28.9^{abdf}	41.5	23.2	155	72	55^{acdf}	3.76
G11	101.5	12	27.2^{abd}	22.8	24.6	54	22	62	1.45
G12	121.0^{abcef}	11	26.5^{a}	16.7	25.8	44	21	65	0.75
G13	120.7^{abcef}	13	27.2^{abd}	39.2	26.1	141	72	52^{acdf}	5.02^{bdf}
G14	112.7^{ab}	11	27.9^{abdf}	20.6	27.1	34	15	62	1.09
G15	113.9^{abef}	12	27.7^{abdf}	26.4	28.6^{a}	77	35	62	2.29
G16	112.9^{abef}	11	28.5^{abdf}	49.4^{b}	28.1^{a}	153	65	62	5.79^{abdf}
G17	100.2	13	26.2^{a}	25.6	27.4	72	43	70	0.39
Inpari 32	97.7	15	21.5	37.4	24.4	121	84	67	3.69
Ciherang	98.3	15	24.1	35.0	25.6	110	76	63	3.50
Mapan P 05	107.4	15	25.9	49.6	26.7	159	78	64	4.74
Suppadi 284	110.4	15	24.7	51.5	25.3	163	81	67	2.91
Mapan P 02	102.1	16	26.6	49.8	27.2	129	79	63	4.24
Suppadi 61	102.9	16	24.9	56.6	26.5	138	82	66	3.53
CV, %	5.14	15.66	5.54	17.88	7.53	29.09	24.52	7.28	32.40

Panicle length varied from 21.5 to 29.6 cm. Genotype G2 showed higher panicle length than the comparison ‘Inpari 32’, ‘Ciherang’, ‘Mapan P 05’, ‘Suppadi 284’, and ‘Suppadi 61’, and equivalent to ‘Mapan P 05’. Several other genotypes, including G1, G3, G4, G7, G10, G14, G15, and G16, also showed higher panicle length than all comparison varieties, except ‘Mapan P 02’ and ‘Mapan P 05’. Grain weight per hill (GWH) ranged from 16.74 g (G12) to 56.62 g (‘Suppadi 61’). Tested genotypes with high GWH were also observed in G7 (51.73 g) and G16 (49.36 g), which were higher than the comparison ‘Inpari 32’ and ‘Ciherang’, and comparable to the comparison ‘Mapan P05’, ‘Suppadi 284’, ‘Mapan P 02’, and ‘Suppadi 61’. The 1000-grain weight (TGW) ranged from 22.8 to 30.6 g. Genotype G7 had a higher 1000-grain weight than the comparison ‘Inpari 32’, ‘Ciherang’, and ‘Suppadi 284’, and comparable to ‘Mapan P 05’, ‘Mapan P 02’, and ‘Suppadi 61’.

The FGN varied widely, ranging from 20 to 163. Genotypes G3, G4, and G5 showed the lowest values, while G10, G13, and G16 showed several filled grains per panicle, comparable to those of all the comparison varieties. This variation pattern was also evident in the FGP trait, with G10 (72%), G13 (72%), and G16 (65%) showing the highest percentages. Grain yields ranged from 0.39 t ha^{-1} in G17 to 5.79 t ha^{-1} in G16. The G16 had a higher grain yield of 2.1 t ha^{-1} compared to ‘Inpari 32’, 2.29 t ha^{-1} higher than ‘Ciherang’, 2.88 t ha^{-1} than ‘Suppadi 284’, and 2.26 t ha^{-1} than ‘Suppadi 61’, which was on a par with ‘Mapan P 02’ and ‘Mapan P 05’. Another genotype with a high grain yield is G13, higher than ‘Ciherang’, ‘Suppadi 284’, ‘Suppadi 61’, and equivalent to ‘Inpari 32’, ‘Mapan P 05’, and ‘Mapan P 02’.

Genotype tolerance indices

Tolerance indices were calculated for each genotype, and the results are presented in Table 5. Substantial variation was observed among genotypes for all estimated indices. Grain yield under low-N conditions (Ys) ranged from 1.48 t ha^{-1} in G10 to 3.83 t ha^{-1} in G7, whereas yield under optimum N conditions (Yp) ranged from 2.32 t ha^{-1} in G6 to 5.02 t ha^{-1} in G13. Tolerance (TOL) and tress susceptibility index (SSI) values, which indicate the magnitude of yield reduction and sensitivity to N stress, respectively, varied considerably among genotypes, with the highest TOL (2.28) and SSI (3.06) observed in G10. In contrast, the ability of genotypes to maintain high yield under both optimum and low-N conditions, as reflected by the stress tolerance index (STI), ranged from 0.32 in G6 to 1.29 in ‘Mapan P 05’.

Table 5. Grain yield and stress tolerance indices of rice genotypes under optimum and low N environments. Yp: Grain yield under optimum environment (t ha^{-1}); Ys: grain yield under low-N environment (t ha^{-1}); TOL: tolerance; STI: stress tolerance index; SSI: stress susceptibility index; MP: mean productivity; GMP: geometric mean productivity; YSI: yield stability index.

Genotype	Yp	Ys	TOL	STI	SSI	MP	GMP	YSI
G6	2.32	1.87	0.45	0.32	0.97	2.09	2.08	0.81
G7	3.86	3.83	0.03	1.07	0.04	3.84	3.84	0.99
G9	3.22	3.13	0.09	0.73	0.15	3.18	3.18	0.97
G10	3.76	1.48	2.28	0.40	3.06	2.62	2.36	0.39
G13	5.02	3.12	1.91	1.14	1.91	4.07	3.96	0.62
Inpari 32	3.69	3.23	0.46	0.87	0.63	3.46	3.45	0.88
Ciherang	3.50	3.27	0.24	0.83	0.34	3.38	3.38	0.93
Mapan P 05	4.74	3.73	1.01	1.29	1.07	4.24	4.20	0.79
Suppadi 284	2.91	2.86	0.05	0.61	0.09	2.89	2.89	0.98
Mapan P 02	4.24	3.35	0.90	1.03	1.07	3.79	3.77	0.79
Suppadi 61	3.53	2.85	0.68	0.73	0.97	3.19	3.17	0.81

Based on mean productivity (MP) and geometric mean productivity (GMP), genotypes G7 and G13 exhibited relatively high average and geometric mean grain yields, with MP values of 3.84 and 4.07 t ha^{-1} and GMP values of 3.84 and 3.96 t ha^{-1}, respectively. Meanwhile, yield stability index (YSI) values close to unity were observed for G7 (0.99), G9 (0.97), and G6 (0.81). The MP and GMP indices showed patterns that differed from those of SSI and YSI. However, the TOL index was consistent with MP and GMP for genotypes G7 and G9, and also showed agreement with SSI for genotypes G6, G7, and G9.

Principal component analysis

The principal component analysis (PCA) revealed variability among the tested rice genotypes (Figure 2). The first (PC1) and second (PC2) components justified 57.6% and 42.2% of the data variation, respectively, for a total of 99.8%. The PC1 was positively correlated with Yp, Ys, STI, MP, GMP, and YSI, while PC2 was positively correlated with Yp, TOL, STI, SSI, MP, and GMP. The genotypes showed a pattern across the four quadrants. The G13, together with the controls 'Mapan P05' and 'Mapan P02', was located in Quadrant I, close to the vectors Yp, MP, GMP, and STI. The G10 was positioned in the same quadrant as the SSI and TOL indices, while G7, G9, and the other control varieties were distributed in Quadrants III and IV, in close association with the Ys and YSI vectors.

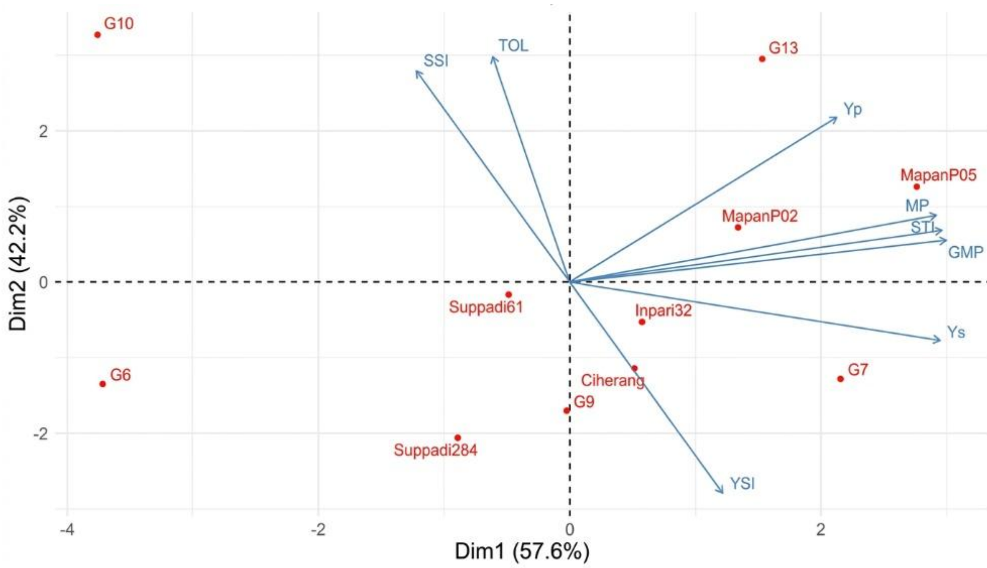

Figure 2. Principal component analysis of rice genotype distribution based on stress tolerance index. Yp: Grain yield under optimum environment (t ha^{-1}); Ys: grain yield under low-N environment (t ha^{-1}); TOL: tolerance; STI: stress tolerance index; SSI: stress susceptibility index; MP: mean productivity; GMP: geometric mean productivity; YSI: yield stability index.

MGIDI selection under optimum environments

The distribution of multi-trait genotype-ideotype distance index (MGIDI) values for 17 cytoplasmic male sterility (CMS)-based rice hybrids evaluated under optimum environmental conditions is presented in Figure 3a. The MGIDI values ranged from 0.96 to 3.89, indicating variation in performance among genotypes. Based on the MGIDI ranking, the five genotypes with the lowest MGIDI values were G13 (0.96), G10 (1.31), G16 (1.35), G7 (2.18), and G8 (2.47). The remaining genotypes exhibited higher MGIDI values, with the maximum value observed in G2 (3.89). The relative strengths and weaknesses of genotypic performance in relation to the ideotype are illustrated in Figure 3b. All analyzed traits were grouped into two main factors, namely FA1 and FA2. Factor FA1 comprised plant height (PH), filled grain number (FGN), filled grain percentage (FGP), and yield (YLD), whereas FA2 consisted of number of productive tillers (NPT) and 1000-grain weight (TGW).

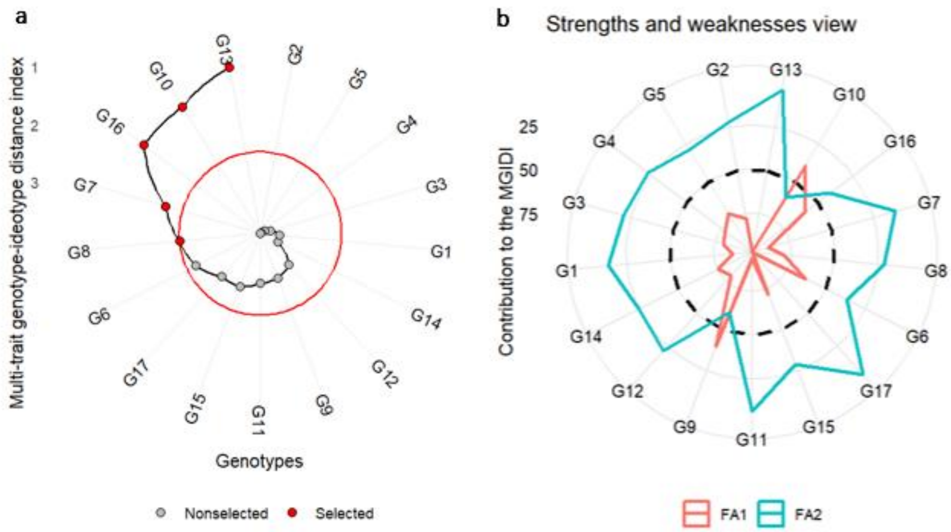

Figure 3. Visualization of multi-trait genotype-ideotype distance index (MGIDI) analysis results for 17 rice hybrids under an optimum environment: Ranking and application of a 30% selection intensity (3a) and strengths and weaknesses based on factor contributions (3b).

DISCUSSION

Classification of fertility restoration

The results of this study indicate differences in fertility restoration ability among cytoplasmic male sterility (CMS) × new plant type (NPT) combinations. Two restoration classifications were identified, namely partially fertile and partially sterile. These results are consistent with those of Vanitha et al. (2020) and Safitri et al. (2023), who also reported that CMS-based hybrid combinations generally exhibit partial fertility dominance relative to fertile or completely sterile.

According to Virmani et al. (1997), released varieties and elite breeding lines are generally not specifically developed for fertility restoration. Consequently, these lines exhibit differential restoration in testcross results. This phenomenon of differential restoration means that each cross-breeding combination results in varying levels of fertility restoration. Therefore, the medium fertility dominance observed in this study is likely related to the suboptimal purity and restorer capacity of the new rice type used as the male parent, as emphasized by Virmani et al. (1997) regarding the importance of purifying restorer lines before their use in hybrid rice breeding.

The absence of fertile genotypes indicates that the test genotypes still require further selection and evaluation before they can be used as effective restorers. Meanwhile, the absence of fully sterile genotypes indicates that all tested genotypes retain the ability to restore fertility, although the level of restoration varied among genotypes. This variation reflects the complexity of the fertility restoration mechanism. Previous studies have shown that fertility restoration is governed by interactions among the cytoplasmic background, nuclear restorer genes, and environmental conditions that may enhance or suppress restoration during plant development (Li et al., 2020; Xu et al., 2023). Therefore, the differences in spikelet fertility observed among the hybrids likely reflect variation in the restoration ability of each NPT line against the CMS cytoplasm used in this study.

Combined analysis of variance

The number of genotypes successfully evaluated in the combined ANOVA was relatively limited due to plant loss in the second environment during the early vegetative stage caused by rat and snail infestations. Nevertheless, significant Genotype (G) × Environment (E) interactions for several agronomic traits still indicated differential genotype responses to environmental conditions. In general, the environment exerted a greater influence than genotype and G × E interaction, as also reported by Huang et al. (2021). This pattern is likely attributable to the contrasting environmental conditions between the optimum and low N environments.

Under low-N conditions, limited N supply becomes the primary constraint (Wang et al., 2017), leading to plant phenotypic responses being more strongly governed by environmental factors than by genetic differences among genotypes. Nevertheless, recent studies have shown that modern hybrid rice cultivars can achieve higher grain yield than improved inbred cultivars under favorable growing conditions (Tao et al., 2022).

Agronomic performance

Evaluation of agronomic performance and yield components is essential to comprehensively assess the productivity potential and overall performance of hybrids. The genotypes tested in this study fall into the semi-dwarf and medium-dwarf categories, although some reach heights of 130 cm. Li et al. (2019) found that *japonica* and *indica* hybrid rice varieties have higher plant heights than inbred varieties, which could lead to higher yields. Previous research has reported that plant height positively affects yield (Miao et al., 2024), but the potential for lodging must still be considered.

Recent studies have shown that the genetic mechanisms of tiller number and flowering time interact and are influenced by day length. However, the relationship between earlier flowering time and lower productive tiller numbers also depends on growing conditions and the genetic background of the genotype (Lee et al., 2025). This is consistent with the persistently high number of productive tillers in several genotypes with relatively early flowering times in this study. However, Kalaitzidis et al. (2025) stated that excessive tillering can lead to ineffective grain filling and small panicles. This is consistent with the finding that several genotypes with high total grain numbers are also associated with high numbers of unfilled grains. In addition, Li et al. (2020) identified, through a genome wide association studies (GWAS) analysis of 337 rice accessions, significant loci associated with fertility restoration that colocalize with the *Rf4* and *Rf5* genes, underscoring the importance of the restorer genetic background in ensuring F_1 hybrid fertility across different CMS types.

Adaptability and response of grain yields in two environments

Variations in tolerance indices between genotypes indicate differences in the level of yield response to N stress. High tolerance (TOL) and stress susceptibility index (SSI) values reflect genotype sensitivity to low N, while lower values indicate better yield stability (Fischer and Maurer, 1978). This suggests that the ability to maintain grain yield under N limitation is related to genotype characteristics, in line with the report by Padhan et al. (2023), which showed differences in N metabolism and remobilization between genotypes.

Genotypes with relatively high mean productivity (MP) and geometric mean productivity (GMP) values demonstrated more consistent grain yield performance across both test environments. This aligns with Hallajian et al. (2024), who stated that MP and GMP reflect a combination of productivity and yield stability. The difference in response between sensitivity-based indices and average performance-based indices indicates that each index assesses a different dimension of response (Jalili et al., 2026). The values of the TOL, MP, GMP, and SSI indices for specific genotypes indicate that the decrease in grain yield under low N conditions is influenced by a combination of yield potential and the level of genotype sensitivity to N stress (Fischer and Maurer, 1978).

Principal component analysis

Principal component analysis (PCA) was used to reduce agronomic traits into fewer components, facilitating the identification of variation patterns and superior genotypes. This approach is consistent with the findings of Haranal et al. (2024), who reported that PCA successfully reduced 11 traits in a rice population into four principal components. The PCA results in this study revealed the presence of genetic variation among the tested rice genotypes, with PC1 and PC2 explaining nearly all of the phenotypic variation. Genotype G13, located close to the grain yield under optimum environment (Yp) and MP vectors, can be considered highly responsive to optimal environments. Meanwhile, the G7, located near the grain yield under low-N environment (Ys) and yield stability index (YSI) vectors, suggests potential adaptation to low-N environments. This pattern can be interpreted as a relatively good ability to maintain grain yields in various environments (Al-Ghumaiz et al., 2025), although G7 performance remained below the average of the other genotypes.

MGIDI selection under optimum environments

The multi-trait genotype-ideotype distance index (MGIDI) under optimum environmental conditions was used as an integrated approach to simultaneously evaluate agronomic performance and fertility restoration within a single index. Olivoto and Nardino (2021) demonstrated that MGIDI can overcome the limitations of traditional indices, particularly in cases of multicollinearity. Several previous studies have also applied MGIDI to evaluate early vigor and various yield-related traits in rice (Pallavi et al., 2024).

Genotypes with the lowest MGIDI values in this study included G13, G10, G16, G7, and G8, indicating performance closest to the defined ideotype. Olivoto and Nardino (2021) explained that genotypes with the lowest MGIDI values are nearest to the ideotype, reflecting the most desirable combination of traits. To understand the contribution of each trait to this proximity, MGIDI values were analyzed using PCA. Analysis of strengths and weaknesses shows that G17, G8, and G13 exhibited strengths in the FA1 trait group but weaknesses in the FA2 trait group. In contrast, G10 and G16 showed a more balanced distribution of contributions across both factors (FA1 and FA2). These findings indicate that MGIDI enables the identification of superior CMS hybrids that balance agronomic performance and yield components, including spikelet fertility, while simultaneously highlighting the strengths and weaknesses of genotypes for further improvement.

CONCLUSIONS

A total of 52.94% of cytoplasmic male sterility (CMS) × new plant type (NPT) hybrids were classified as partially fertile. Genotype × Environment interactions had a significant effect on plant height, panicle length, number of filled grains, and filled grain percentage. Genotype G16 showed the highest yield under optimal conditions, while G7 showed a value of 0.99 on the yield stability index. The first (PC1) and second (PC2) components justified 57.6% and 42.2% of the data variation, respectively, for a total of 99.8%, while the multi-trait genotype-ideotype distance index (MGIDI) selected G13, G10, G16, G7, and G8 as genotypes closest to the ideotype.

Author contribution

Conceptualization: H.A. Methodology: W.B.S., H.A., A.Y. Formal analysis: N.M. Investigation: N.M. Resources: A.Y., H.A. Data curation: N.M. Writing-original draft: N.M. Writing-review & editing: W.B.S., H.A. Visualization: N.M Supervision: W.B.S., H.A. Funding acquisition: H.A. All co-authors reviewed the final version and approved the manuscript before submission.

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