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# Agronomic and molecular performance of rice lines carrying spikelet number and days to heading loci

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**Joko Prasetiyono[1*], Tasliah[1], Nafisah[2], Ma'sumah[2], Chaerani[3], Supriyanta[4], Andari Risliawati[3], Kurniawan Rudi Trijatmiko[5] and Mahrup[1]**

**Abstract:** *Increasing rice production can be achieved by increasing spikelet number and shortening the flowering period. This study evaluated the agronomic performance, genotype stability, and molecular profiles of 14 rice lines derived from backcrosses of Conde—an Indonesian nationally released variety targeted for improvement—with IR64, which carries qTSN4, a locus that enhances spikelet number, or qDTH8, which shortens days to heading. Five Conde-qTSN4 lines (A8-5, A16-5, B6-2, B12-2, and B22-1) exhibited statistically increased total grains (both filled and empty) per panicle, while two Conde-qDTH8 lines (G64 and G142) matured earlier. AMMI and GGE biplot analyses identified Conde and three qTSN4 lines (A8-5, B12-2, B15-2) as stable across nine environments. Molecular analysis confirmed the presence of qTSN4 or qDTH8 alleles, with all lines carrying the Xa7 resistance gene. The qTSN4 and qDTH8 loci contributed to yield improvements of 3% and 12.5%, respectively, demonstrating their potential to enhance rice productivity in Indonesia.*

**Keywords**: *Rice, Conde-*qTSN4*, Conde-*qDTH8*, multilocation test, stability test*

***Corresponding author:**
E-mail: joko038@brin.go.id

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[1] National Research and Innovation Agency Republic of Indonesia, Research Center for Applied Botany, KST Soekarno, Jl. Raya Jakarta-Bogor km 46, Cibinong, 16911, Bogor, West Java, Indonesial
[2] Ministry of Agriculture, Agricultural Assembly and Modernization Agency Republic of Indonesia, Jl. Raya Ragunan No 29, 12540, DKI Jakarta, Indonesia
[3] National Research and Innovation Agency Republic of Indonesia, Research Center for Food Crops, KST Soekarno, Jl. Raya Jakarta-Bogor km 46, Cibinong, 16911, Bogor, West Java, Indonesia
[4] Gadjah Mada University, Faculty of Agriculture, Jalan Flora, 55281, Yogyakarta, DI Yogyakarta, Indonesia
[5] International Rice Research Institute, Pili Drive, 4031, Manila, NCR, Philippines

## INTRODUCTION

Rice serves as a staple food for 90% of Indonesia's population, and ensuring its consistent availability is one of the national priorities. According to a survey conducted in 2022 by the Indonesian Central Statistics Agency (BPS 2023), the country's population reached 275.77 million, reflecting a 1.13% increase from the previous year. With the increase in population, the demand for rice also increases. To address this, Indonesia's rice breeding programs are dedicated to enhancing yield potential to safeguard national food security.

Developing new plant types that reduce tiller numbers and enhance panicle architecture is a promising approach to increasing rice yields (Peng et al. 2008). Additionally, improving the harvest index, that is the ratio of dry grain weight to total dry weight, is essential for maximizing yield potential. Rice productivity is determined by four key components: the number of panicles per plant, the total spikelet number, the weight of filled grain per panicle, and grain fertility (the proportion of filled grains relative to the total grain number per panicle). Considerable variation in grain number exists among cultivated rice varieties, making this trait a focal point for breeding programs aimed at increasing rice yield potential (Liu et al. 2021, Mai et al. 2021).

Flowering time is another crucial factor influencing rice yield. While yield components such as grain weight and grain number are important, agronomic traits also play a significant role. Recent studies have advanced the understanding of yield regulation, plant height, and flowering time in rice (Wei et al. 2010). Several genes affecting flowering time, collectively known as the heading date (*Hd*) gene family, have been identified, including *Hd1*, *Hd2*, and *Hd3a* (Xiang et al. 2013).

At the International Rice Research Institute (IRRI), rice parents carrying the *qTSN4* locus (YP9/IR68522-10-2-2) and the *qDTH8* locus (YP1/IR6500-87-2-2-3) have been developed (Fujita et al. 2012). The *qTSN4* locus contributes to a higher spikelet count, while *qDTH8* shortens the harvesting time. Together, these traits can be exploited to enhance the productivity of Indonesian rice varieties, such as Conde. Conde is a superior national rice variety developed from a cross between IR64 and IRBB7, carrying the *Xa7* gene that confers broad resistance to bacterial leaf blight pathogen (*Xanthomonas oryzae* pv. *oryzae*). Because Conde retains much of the IR64 genome, it is expected to be familiar to farmers; however, its yield performance remains modest, averaging 6 t ha$^{-1}$ (Suprihatno et al. 2009). To address this limitation, Conde was crossed with isogenic lines YP9/IR68522-10-2-2 and YP1/IR6500-87-2-2-3, both of which share the IR64 genetic background.

Following this breeding strategy, the selection of Conde lines containing each locus was conducted under greenhouse conditions using molecular markers, followed by preliminary and advanced yield evaluations in the field. The final phase involved multi-location testing of advanced lines across diverse environments. This study evaluates the agronomic performance, genotype stability, and molecular characteristics of these advanced rice lines derived from Conde across nine locations. The study will support the selection of promising lines for potential commercial release.

## MATERIAL AND METHODS

### Plant materials

We utilized progenies from two backcrosses: Conde × YP9/IR68522-10-2-2 and Conde × YP1/IR6500-87-2-2-3. The YP9/IR68522-10-2-2 and YP1/IR6500-87-2-2-3 lines are isogenic derivatives of IR64, carrying the *qTSN4* and *qDTH8* loci, respectively, which were acquired from IRRI in 2012. The progeny of these crosses, Conde-*qTSN4* and Conde-*qDTH8*, underwent evaluation in multi-location trials alongside their female parent (Conde) and a nationally released control variety, Inpari 32 (Table 1). The remaining two donor parents were not included in the trials, as mandated by the Indonesian Ministry of Agriculture's field trial regulations.

These lines were developed through a marker-assisted backcross breeding program, utilizing five simple sequence repeat (SSR) markers associated with *qTSN4*, *qDTH8*, and *Xa7* loci (Table S1 and Figure S1). Final selections were based on agronomic performance and yield potential. Advanced yield testing of $BC_3F_3$ progenies were conducted at Sukamandi and Kuningan Field Stations in West Java in 2017, which identified superior lines.

### Field experiments

The experiments were conducted from 2018 to 2021 in irrigated rice fields across nine locations in five provinces of Sumatra and Java islands, each with distinct agro-climatic conditions (Figure S2). In each trial site, seeds of each genotype were sown in nursery blocks for three weeks before being transplanted into plots measuring 4 m × 5 m with a planting distance of 25 cm × 25 cm. The experiments followed a randomized complete block design with three replications per genotype. In each plot, 10 hills were selected for observation of key agronomic traits, including productive tiller number (PTN), total grains (filled and empty) per panicle (TGP), total filled grains per panicle (TFGP), weight of total grain per hill (WTGH), and the weight of 1,000 grains (1,000G). Additionally, harvesting time (HT) and yield were recorded at a seed moisture content of 14% to assess performance and yield.

### Molecular analysis

The presence of *qTSN4*, *qDTH8*, and *Xa7* loci in the lines was confirmed by molecular analysis (Table S1). Leaves from 10 plant hills per plot at the trial site in Bogor (West Java province, location number 8 in Supplementary Figure S2) in 2021 were sampled at three weeks after transplanting. DNA extraction was performed using the miniprep method (Dellaporta et al. 1983). The PCR reaction was carried out in a 20 µl volume containing 1× PCR buffer, 100 µM dNTPs, 0.5 µM primers,

***Table 1.*** Mean values of agronomic characters of Conde-*qTSN4* and Conde-*qDTH8* advanced generation rice lines across nine multi-location trials

| Codes | Genotypes (cross) | HT | PTN | TFGP | TGP | WTGH | 1,000G | Y |
|---|---|---|---|---|---|---|---|---|
| G1 | Conde (female parent)[1] | $115.2^{a}$ | $22.2^{a}$ | $127.5^{d}$ | $158.2^{ef}$ | $52.97^{a}$ | $26.87^{ab}$ | $7.06^{ab}$ |
| G2 | Inpari 32 (control variety)[2] | $113.7^{abc}$ | $21.0^{ab}$ | $132.0^{cd}$ | $152.8^{f}$ | $54.17^{a}$ | $26.82^{abc}$ | $7.42^{ab}$ |
| G3 | A6-2 (BC$_1$ of Conde/IR64-*qTSN4*/Conde)[3] | $115.0^{a}$ | $20.9^{ab}$ | $138.6^{bcd}$ | $158.8^{def}$ | $49.66^{a}$ | $25.96^{d}$ | $6.62^{b}$ |
| G4 | A8-5 (BC$_1$ of Conde/IR64-*qTSN4*/Conde) | $114.0^{abc}$ | $20.0^{ab}$ | $147.1^{abc}$ | $181.6^{abc}$ | $51.96^{a}$ | $26.36^{a\text{-}d}$ | $7.09^{ab}$ |
| G5 | A10-1 (BC$_1$ of Conde/IR64-*qTSN4*/Conde) | $115.0^{a}$ | $20.4^{ab}$ | $140.7^{a\text{-}d}$ | $167.1^{b\text{-}f}$ | $48.65^{a}$ | $26.21^{bcd}$ | $6.75^{b}$ |
| G6 | A16-5 (BC$_1$ of Conde/IR64-*qTSN4*/Conde) | $114.7^{a}$ | $19.3^{b}$ | $154.2^{ab}$ | $183.5^{abc}$ | $54.26^{a}$ | $26.19^{bcd}$ | $6.85^{b}$ |
| G7 | B6-2 (BC$_2$ of Conde/IR64-*qTSN4*/Conde) | $114.5^{ab}$ | $20.2^{ab}$ | $154.6^{ab}$ | $185.6^{a}$ | $55.12^{a}$ | $26.06^{cd}$ | $6.72^{b}$ |
| G8 | B11-4 (BC$_2$ of Conde/IR64-*qTSN4*/Conde) | $114.9^{a}$ | $20.3^{ab}$ | $145.4^{abc}$ | $173.5^{a\text{-}e}$ | $53.58^{a}$ | $26.03^{cd}$ | $6.93^{b}$ |
| G9 | B12-2 (BC$_2$ of Conde/IR64-*qTSN4*/Conde) | $115.6^{a}$ | $21.2^{ab}$ | $144.3^{abc}$ | $176.3^{a\text{-}d}$ | $53.22^{a}$ | $26.64^{a\text{-}d}$ | $7.27^{ab}$ |
| G10 | B15-2 (BC$_2$ of Conde/IR64-*qTSN4*/Conde) | $113.7^{abc}$ | $20.5^{ab}$ | $139.7^{a\text{-}d}$ | $162.0^{def}$ | $51.58^{a}$ | $26.16^{bcd}$ | $7.06^{ab}$ |
| G11 | B22-1 (BC$_2$ of Conde/IR64-*qTSN4*/Conde) | $114.5^{ab}$ | $21.1^{ab}$ | $154.8^{a}$ | $184.3^{ab}$ | $55.34^{a}$ | $25.90^{d}$ | $7.25^{ab}$ |
| G12 | B24-4 (BC$_2$ of Conde/IR64-*qTSN4*/Conde) | $115.2^{a}$ | $20.8^{ab}$ | $146.0^{abc}$ | $168.6^{a\text{-}f}$ | $52.67^{a}$ | $26.18^{bcd}$ | $7.06^{ab}$ |
| G13 | C34 (BC$_3$ of Conde/IR64-*qTSN4*///Conde) | $115.0^{a}$ | $20.2^{ab}$ | $143.4^{a\text{-}d}$ | $169.6^{a\text{-}f}$ | $52.41^{a}$ | $26.40^{a\text{-}d}$ | $7.16^{ab}$ |
| G14 | E13-1 (BC$_1$ of Conde/IR64-*qDTH8*/Conde)[4] | $114.3^{abc}$ | $21.1^{ab}$ | $144.1^{abc}$ | $170.0^{a\text{-}f}$ | $51.56^{a}$ | $26.08^{bcd}$ | $7.36^{ab}$ |
| G15 | G64 (BC$_3$ of Conde/IR64-*qDTH8*///Conde) | $112.8^{bc}$ | $22.1^{a}$ | $139.8^{a\text{-}d}$ | $166.1^{c\text{-}f}$ | $56.12^{a}$ | $27.01^{a}$ | $7.94^{a}$ |
| G16 | G142 (BC$_3$ of Conde/IR64-*qDTH8*///Conde) | $112.6^{c}$ | $21.6^{ab}$ | $133.6^{cd}$ | $160.6^{def}$ | $50.88^{a}$ | $26.80^{abc}$ | $7.37^{ab}$ |
| Location (Province, island, year) | | | | | | | | |
| Lampung (Lampung, Sumatra, 2018) | | $110.5^{d}$ | $16.1^{cd}$ | $82.0^{f}$ | $130.1^{e}$ | $32.91^{de}$ | $26.57^{bc}$ | $3.28^{f}$ |
| Banten (Banten, Java, 2018) | | $108.2^{d}$ | $13.4^{d}$ | $156.0^{bc}$ | $186.2^{ab}$ | $21.23^{e}$ | $24.64^{d}$ | $4.12^{ef}$ |
| Karawang-18 (West Java, 2018) | | $106.5^{de}$ | $24.0^{ab}$ | $172.5^{ab}$ | $192.2^{ab}$ | $75.31^{b}$ | $26.28^{c}$ | $10.40^{a}$ |
| Karawang-19 (W. Java, 2019) | | $115.3^{c}$ | $27.2^{a}$ | $169.3^{abc}$ | $188.1^{ab}$ | $93.10^{a}$ | $27.60^{a}$ | $10.82^{a}$ |
| Cianjur (W. Java, 2019) | | $118.2^{bc}$ | $23.5^{ab}$ | $185.8^{a}$ | $203.0^{a}$ | $73.80^{b}$ | $27.39^{a}$ | $8.36^{b}$ |
| Malang (East Java, 2020) | | $115.8^{c}$ | $25.6^{a}$ | $132.1^{de}$ | $161.7^{cd}$ | $49.91^{c}$ | $24.09^{d}$ | $7.75^{bc}$ |
| Bogor (W. Java, 2021) | | $121.2^{b}$ | $19.4^{bc}$ | $114.1^{e}$ | $151.7^{de}$ | $40.15^{cd}$ | $27.07^{ab}$ | $6.59^{cd}$ |
| Brebes (Central Java, 2021) | | $103.1^{e}$ | $14.8^{cd}$ | $150.0^{cd}$ | $178.4^{bc}$ | $38.28^{cd}$ | $26.25^{c}$ | $7.31^{bc}$ |
| Klaten (C. Java, 2021) | | $131.2^{a}$ | $23.3^{ab}$ | $123.9^{e}$ | $137.9^{e}$ | $50.16^{c}$ | $27.29^{a}$ | $5.44^{de}$ |
| | Average (genotypes/locations) | 114.4 | 20.8 | 142.9 | 169.9 | 52.76 | 26.35 | 7.12 |
| | CV (%) | 1.67 | 12.62 | 11.61 | 10.82 | 16.45 | 3.23 | 13.72 |

HT=harvesting time (days after sowing); PTN=productive tiller number; TFGP=total filled grain per panicle; TGP=total grain (filled and empty) per panicle; WTGH=weight of total grain per hill (g); 1,000G=weight of 1,000 grain (g); and Y=yield (t $ha^{-1}$). Numbers followed by the same letter in the same column (Genotype or Location) are not significantly different based on the Tukey test at $\alpha=0.05$. [1] National variety released in 2001; [2] National variety released in 2013; [3] IR64-*qTSN4*=YP9/IR68522-10-2-2; and [4] IR64-*qDTH8*=YP1/IR6500-87-2-2-3.

50 ng of DNA, and 1 unit of Taq DNA polymerase. PCR cycles were adjusted for each SSR marker. DNA amplification results were then separated using an 8% polyacrylamide gel. DNA staining was performed with ethidium bromide.

### Data analysis

Agronomic data were analyzed using ANOVA through the web-based software PKBT-STAT v.3.1 (https://pbstat.com/pkbt-stat/). When significant differences among tested genotypes were detected, the Tukey test was applied at a 5% probability level. To evaluate genotype adaptability and stability across environments in terms of yield performance, stability analysis was conducted using the AMMI and GGE models (Gauch et al. 2008) via PBSTAT-GE v.3.5 (https://apps.pbstat.com/reports/pbstat-ge/).

## RESULTS AND DISCUSSION

### Agronomic performance at multi-location trials

In this study, plant materials were derived from three generations of backcross (BC$_1$, BC$_2$, and BC$_3$; Supplementary Figure S1). It is expected that genome recovery toward the Conde variety will be more efficient, as Conde and the *qTSN4* or *qDTH8* donor parents share the IR64 genetic background. Silva et al. (2023) reported that markers associated with desirable traits facilitate the selection of crosses of *Coffea arabica*, especially when combined with phenotypic observation.

ANOVA revealed that environmental factors influenced all agronomic traits, while genotype factors affected nearly all traits except for WTGH (Supplementary Table S2). Among the seven agronomic traits observed, only four exhibited genotype-by-environment interactions (GEIs): HT, WTGH, 1,000G, and yield. Such interactions highlight the adaptability and stability of genotypes across environments, contributing to the identification of superior varieties in a plant breeding program (Valenzuela-Antelo et al. 2023).

Except for WTGH, yield and other yield components exhibited significant differences (Table 1). Conde-*qTSN4* lines shared a similar harvest time of 114–115 days, whereas Conde-*qDTH8* lines had a shorter HT of 113–114 days. Meanwhile, PTN showed no significant differences between the lines and Conde. The impact of the *qTSN4* locus was evident in the TGP. Across all lines, TGP and TFGP surpassed those of Conde. Notably, three lines, A16-5, B6-2, and B22-1, demonstrated significantly higher TFGP than Conde (Table 1). The spikelet increases in the Conde-*qTSN4* lines resulted in a higher total grain count (TGP). Five Conde-*qTSN4* lines (A8-5, A16-5, B6-2, B12-2, and B22-1) showed an increase in TGP ranging from 11% to 17%.

All three Conde-*qDTH8* lines (E13-1, G64, and G142) demonstrated higher yields than Conde, although the differences were not statistically significant (Table 1). The highest yield was recorded in the G64 line, reaching 7.94 t ha$^{-1}$. In this line, the *qDTH8* locus not only shortened the HT by three days but also contributed to a larger grain size, as indicated by a 1,000G weight of 27.01 g. However, it remained unclear why the yield for two Conde-*qDTH8* lines (E13-1 and G142) exceeded that of Conde, given that both had lower 1,000G and WTGH. A possible explanation could be that these lines may have a lower percentage of empty grains compared to Conde, which, when extrapolated from small plot conversions, could result in a higher total yield per hectare. These Conde-*qDTH*8 lines exhibited increases in TGP of 1.5% (G142), 5.0% (G64), and 7.5% (E13-1).

The agronomic performance of the tested genotypes varied across nine multi-location trials, with average yields ranging from 3.28 to 10.82 t ha$^{-1}$ (Table 2). Among the trial locations, Karawang (-18 and -19) and Cianjur provided favorable environments for rice line production, supported by high yield and yield component values (Table 2). In contrast, Lampung and Banten exhibited lower yield potential and were likely unsuitable for cultivating Conde-*qTSN4* and Conde-*qDTH8* rice lines. During planting in Lampung, a blast disease outbreak led to a significant yield decline, while in the Banten area, brown planthopper severely hindered plant growth. In both locations, the high percentage of empty grains was the primary factor contributing to reduced yields. Across nine locations, the highest yields obtained

***Table 2.*** Yield performance of Conde-*qTSN4* and Conde-*qDTH8* advanced-generation rice lines across nine multi-location trials

| Code | Genotyp$^{e}$1 | Lampung | Banten | Karawang (2018) | Karawang (2019) | Cianjur | Malang | Bogor | Brebes | Klaten | Average (t ha$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G1 | Conde | 3.80$^{a}$ | 4.60$^{a}$ | 10.35$^{a}$ | 10.45$^{a}$ | 7.27$^{b}$ | 7.00$^{bc}$ | 7.44$^{ab}$ | 6.85$^{ab}$ | 5.76$^{a}$ | 7.06$^{ab}$ |
| G2 | Inpari 32 | 4.88$^{a}$ | 4.66$^{a}$ | 11.05$^{a}$ | 11.33$^{a}$ | 7.06$^{b}$ | 11.72$^{a}$ | 6.74$^{ab}$ | 4.96$^{b}$ | 4.38$^{a}$ | 7.42$^{ab}$ |
| G3 | A6-2 | 2.91$^{a}$ | 4.05$^{a}$ | 9.27$^{a}$ | 9.75$^{a}$ | 7.26$^{b}$ | 7.06$^{bc}$ | 5.68$^{b}$ | 8.45$^{a}$ | 5.15$^{a}$ | 6.62$^{b}$ |
| G4 | A8-5 | 3.06$^{a}$ | 4.15$^{a}$ | 10.49$^{a}$ | 10.24$^{a}$ | 7.73$^{b}$ | 8.55$^{bc}$ | 6.85$^{ab}$ | 7.74$^{ab}$ | 5.01$^{a}$ | 7.09$^{ab}$ |
| G5 | A10-1 | 2.64$^{a}$ | 3.86$^{a}$ | 10.02$^{a}$ | 9.88$^{a}$ | 7.83$^{b}$ | 7.30$^{bc}$ | 5.80$^{b}$ | 8.25$^{a}$ | 5.19$^{a}$ | 6.75$^{b}$ |
| G6 | A16-5 | 3.55$^{a}$ | 4.49$^{a}$ | 10.08$^{a}$ | 9.99$^{a}$ | 7.45$^{b}$ | 8.91$^{ab}$ | 5.15$^{b}$ | 6.40$^{ab}$ | 5.63$^{a}$ | 6.85$^{b}$ |
| G7 | B6-2 | 2.97$^{a}$ | 3.96$^{a}$ | 9.22$^{a}$ | 9.59$^{a}$ | 8.13$^{b}$ | 7.10$^{bc}$ | 5.92$^{b}$ | 7.32$^{ab}$ | 6.23$^{a}$ | 6.72$^{b}$ |
| G8 | B11-4 | 2.90$^{a}$ | 4.52$^{a}$ | 10.47$^{a}$ | 9.55$^{a}$ | 7.79$^{b}$ | 8.35$^{bc}$ | 5.89$^{b}$ | 6.34$^{ab}$ | 6.52$^{a}$ | 6.93$^{b}$ |
| G9 | B12-2 | 2.96$^{a}$ | 3.83$^{a}$ | 10.66$^{a}$ | 11.51$^{a}$ | 8.48$^{b}$ | 7.35$^{bc}$ | 7.16$^{ab}$ | 7.38$^{ab}$ | 6.07$^{a}$ | 7.27$^{ab}$ |
| G10 | B15-2 | 3.21$^{a}$ | 4.05$^{a}$ | 10.65$^{a}$ | 11.00$^{a}$ | 8.66$^{b}$ | 7.28$^{bc}$ | 6.44$^{ab}$ | 6.21$^{ab}$ | 6.08$^{a}$ | 7.06$^{ab}$ |
| G11 | B22-1 | 3.24$^{a}$ | 4.52$^{a}$ | 11.17$^{a}$ | 12.04$^{a}$ | 9.36$^{ab}$ | 6.67$^{bc}$ | 6.55$^{ab}$ | 6.67$^{ab}$ | 5.03$^{a}$ | 7.25$^{ab}$ |
| G12 | B24-4 | 2.76$^{a}$ | 3.97$^{a}$ | 10.15$^{a}$ | 10.23$^{a}$ | 7.26$^{b}$ | 7.68$^{bc}$ | 6.58$^{ab}$ | 8.60$^{a}$ | 6.30$^{a}$ | 7.06$^{ab}$ |
| G13 | C34 | 3.27$^{a}$ | 3.82$^{a}$ | 9.32$^{a}$ | 11.88$^{a}$ | 8.71$^{ab}$ | 7.45$^{bc}$ | 7.78$^{ab}$ | 8.04$^{a}$ | 4.12$^{a}$ | 7.15$^{ab}$ |
| G14 | E13-1 | 3.41$^{a}$ | 3.80$^{a}$ | 11.48$^{a}$ | 11.97$^{a}$ | 9.37$^{ab}$ | 6.65$^{bc}$ | 6.69$^{ab}$ | 7.81$^{ab}$ | 5.06$^{a}$ | 7.36$^{ab}$ |
| G15 | G64 | 3.16$^{a}$ | 3.54$^{a}$ | 10.48$^{a}$ | 11.78$^{a}$ | 11.52$^{a}$ | 9.06$^{ab}$ | 9.12$^{a}$ | 7.60$^{ab}$ | 5.17$^{a}$ | 7.94$^{a}$ |
| G16 | G142 | 3.69$^{a}$ | 4.07$^{a}$ | 11.51$^{a}$ | 11.97$^{a}$ | 9.86$^{ab}$ | 5.83$^{c}$ | 5.72$^{b}$ | 8.33$^{a}$ | 5.37$^{a}$ | 7.37$^{ab}$ |
| *Average (t ha$^{-1}$)* | | *3.28* | 4.12 | 10.40 | 10.82 | 8.36 | 7.75 | 6.59 | 7.31 | 5.44 | 7.12 |

Numbers followed by the same letter in the same column are not significantly different based on the Tukey test at α=0.05. $^{1}$See Table 1 for information about the genotype.

for Conde-*qTSN4* and Conde-*qDTH8* were 3% and 12.5% greater than Conde, respectively, indicating that both loci contributed significantly to increasing rice production in Indonesia (Table 1).

The diverse agronomic performance of tested genotypes across locations highlights the significant impact of environmental factors on phenotype expression. This effect is further supported by the observed GEIs. GEIs often pose challenges in breeding programs, as they can reduce trait heritability and complicate the selection of suitable lines for advancement to the next generation (Yan and Fregeau-Reid 2018). Therefore, understanding GEI effects is crucial for selecting superior varieties, and this can be analyzed through multi-location trials (Pour-Aboughadareh et al. 2022).

## Analysis of yield stability of Conde-*qTSN4* and Conde-*qDTH8* lines using AMMI and GGE biplot methods

### *AMMI biplot analysis*

AMMI biplot analysis is commonly used to identify stable genotypes across different environments. In this analysis, the Average Environment Coordinate (AEC) serves as a central axis that facilitates the evaluation of genotype performance and stability across environments. The horizontal axis represents the mean yield of genotypes, while the vertical axis reflects the interaction effects, indicating the stability of each genotype. Genotypes farthest from the AEC line show higher interaction with specific environments, while those closer to the line are considered more stable.

The AMMI analysis provided a visualization of rice yield stability, with principal components PC1 and PC2 accounting for 68.3% of the total yield variation (Figure 1a). This high percentage suggests that the data accurately represent yield variance across all examined genotypes, making it a reliable tool for genotype assessment across nine locations. Based on Figure 1a, Malang exhibited the longest environment vector, indicating its strong influence on GEIs and its suitability for testing adaptability extremes. Conversely, Karawang-18 had the shortest environmental vectors, signifying its minimal impact on GEIs and its limited representation of actual yield potential. The AMMI biplot identified four genotypes, Conde (G1), A8-5 (G4), B12-2 (G9), and B15-2 (G10), positioned close to the axis (0.0) and within the circle's radius, suggesting that these genotypes maintained stable yields across all locations.

Genotypes identified as adaptive to a specific environment were those positioned near the respective location's vector line. For example, B22-1 (G11) and C34 (G13) demonstrated adaptability in Karawang-19, while G14 was adaptive in Cianjur. Additionally, genotypes A6-2 (G3), B6-2 (G7), A10-1 (G5), and B24-4 (G12) were associated with the Klaten location. Utami et al. (2023) demonstrated that evaluating genotypes in highly contrasting environments facilitates the identification of those with stable performance across diverse conditions. Their study, conducted across six distant and highly contrasting agro-climates on four islands (Java, Sumatra, Kalimantan, and Papua), identified five stable genotypes. By contrast, although our study encompassed nine environments, only four genotypes exhibited stability, likely due to its restriction to two islands (Sumatra and Java) with relatively similar agro-climatic conditions.

The AMMI method is widely applied to assess the stability of lines grown across multiple regions with varying environmental conditions. Supriadi et al. (2024) demonstrated that maize hybrids G01 and G04 had minimal deviation from the AEC axis, signifying consistent performance across diverse Indonesian environments. Ahmed et al. (2024) evaluated 20 Green Super Rice lines across 12 locations in Pakistan and found that several lines exhibited yield stability. Similarly, Akter and Hassan (2014) successfully identified two adaptive lines in four locations in Bangladesh after testing six selected lines.

### *GGE biplot analysis*

According to Figure 1b, which displays the GGE biplot, PC1 and PC2 accounted for 38% and 32.5% of the total variation, respectively. Field tests are generally classified into three types: Type I environments are characterized by short vectors and provide limited information about the tested genotypes, making them unsuitable for environmental evaluations. Type II environments have long vectors with small angles relative to the average abscissa, making them optimal for selecting superior genotypes. In contrast, Type III environments are defined by long vectors with large angles relative to the mean abscissa, rendering them less ideal for selecting superior genotypes but valuable for identifying unstable ones (Yan et al. 2000). In this study, the Type I category includes Banten, Lampung, Karawang-18, and Klaten, which are not suitable for environmental testing. Bogor and Karawang-19 are categorized as Type II environments, as they have

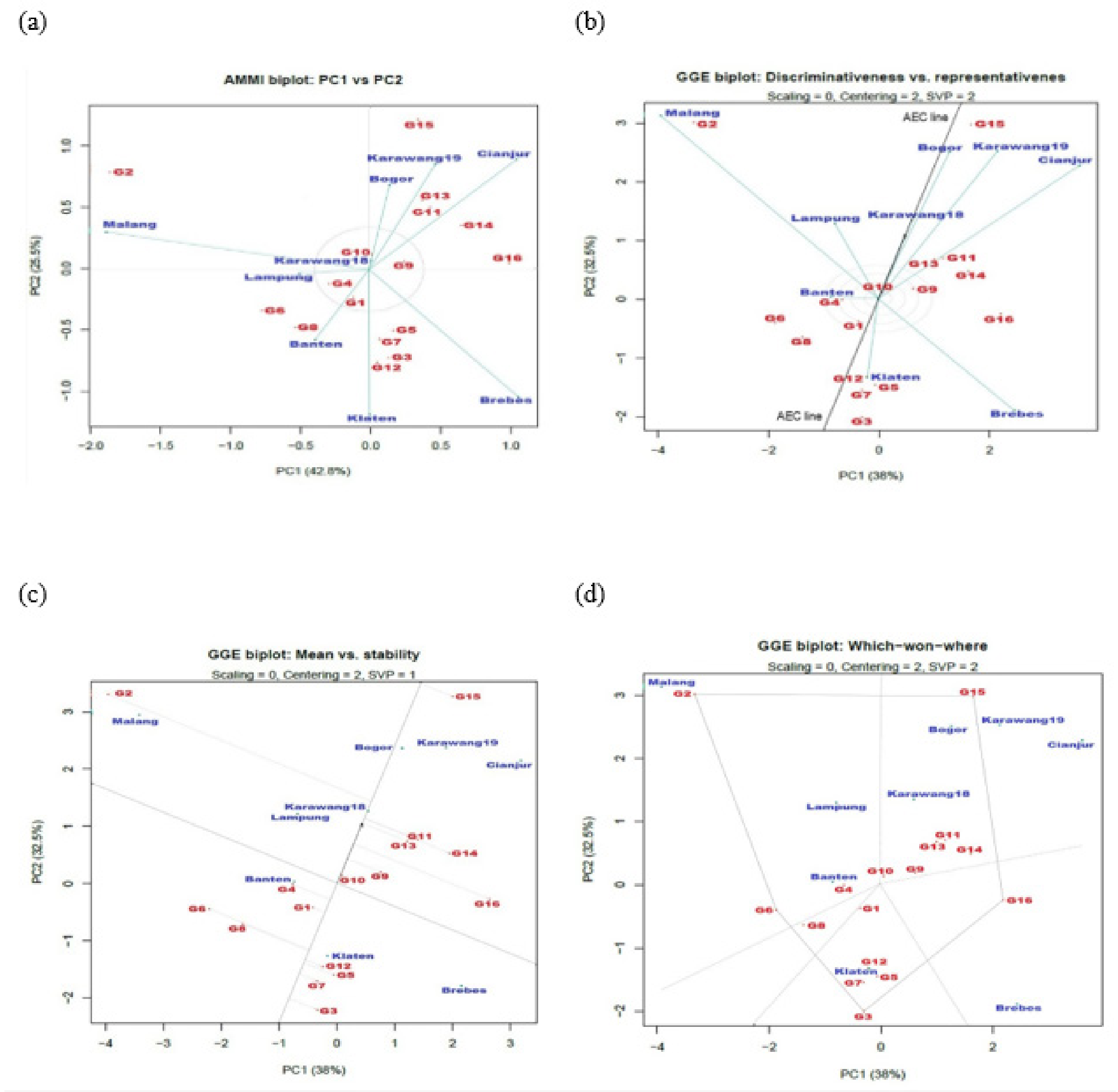


***Figure 1.*** AMMI biplot analysis (a) and GGE biplot analysis illustrating the relationship between discriminativeness and representativeness (b), the relationship between mean performance and stability (c), and the "which-won-where" pattern (d) for 16 rice genotypes across nine environments. G1 = Conde, G2 = Inpari 32, G3 = A6-2, G4 = A8-5, G5 = A10-1, G6 = A16-5, G7 = B6-2, G8 = B11-4, G9 = B12-2, G10 = B15-2, G11 = B22-1, G12 = B24-4, G13 = C34, G14 = E13-1, G15 = G64, and G16 = G142.

long vectors and small angles to AEC, making them ideal locations for selecting superior genotypes. Meanwhile, Malang, Cianjur, and Brebes belong to the Type III environment category. While these locations are not suitable for identifying superior genotypes, they can be used to assess adaptive or region-specific genotypes.

Figure 1c illustrates the relationship between genotype mean performance and stability. The Y-axis represents the average yield of each genotype, while the X-axis indicates the stability of yield across tested genotypes. Genotypes positioned farther from the center of the Y-axis are expected to exhibit higher-than-average yields, with G64 (G15) and Inpari 32 (G2) fitting this criterion. Genotypes Conde (G1), A8-5 (G4), B12-2 (G9), and B15-2 (G10) are considered stable, as they are positioned near the intersection of the X- and Y-axes. Meanwhile, genotypes located to the right of the Y-axis are expected to yield below the average, though this trend does not appear to hold in this study.

Figure 1d shows the classification of tested genotypes into five sectors. Two of these sectors (Sectors 2 and 5) comprise multiple environments, forming a mega-environment. Sector 1, led by genotype Inpari 32 (G2), corresponds to the Malang environment. Sector 2, with G64 (G15) as the leading genotype, includes Karawang-18, Karawang-19, Bogor, and Cianjur. Sector 3, represented by G142 (G16), corresponds to the Brebes environment. Sector 4, dominated by A6-2 (G3), is associated with Klaten. Sector 5, with A16-5 (G6) as the peak genotype, encompasses Banten and Lampung. Genotypes Conde (G1), A8-5 (G4), B12-2 (G9), and B15-2 (G10) are positioned near the zero axis, indicating a small GEI effect, meaning they exhibit stable performance across locations.

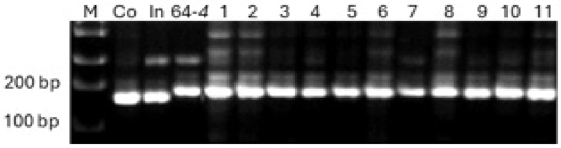
Several researchers have utilized the GGE biplot separately to assess the yield stability of tested lines, as it is considered to provide more detailed insight than the AMMI biplot. Lee et al. (2023) successfully selected six early-maturing genotypes, six medium-maturing genotypes, and four medium-late-maturing genotypes from 276 Korean rice cultivars grown in three different environments using the GGE biplot. Similarly, Shrestha et al. (2020) examined seven genotypes planted in three mid-hill environments in Nepal. Their analysis using the GGE biplot identified only one genotype (NR10676-B-5-3) that demonstrated stability across all three environments, despite not being among the highest yielders. Based on the AMMI and GGE biplots, we found that the genotypes identified as stable across all environments were the same for both analysis methods: Conde (G1) with a yield of 7.06 t ha$^{-1}$, A8-5 (G4) with 7.09 t ha$^{-1}$, B12-2 (G9) with 7.27 t ha$^{-1}$, and B15-2 (G10) with 7.06 t ha$^{-1}$. Meanwhile, the yields of five other genotypes, Inpari 32 (G2) at 7.42 t ha$^{-1}$, B22-1 (G11) at 7.25 t ha$^{-1}$, E13-1 (G14) at 7.36 t ha$^{-1}$, G64 (G15) at 7.94 t ha$^{-1}$, and G142 (G16) at 7.37 t ha$^{-1}$ were higher, but these genotypes exhibited instability across the nine study locations.

Although stability test results can be analyzed using a single method, researchers generally prefer to combine AMMI and GGE-biplot approaches simultaneously, as this is believed to yield more accurate data (Premi et al. 2023, Ashwini et al. 2024, Ghazy et al. 2024, Justo et al. 2024). Utami et al. (2023) took this further by employing three methods, AMMI, GGE biplot, and the Sustainability Index (SI), to identify Fe-tolerant lines with high and stable yields across all test locations.

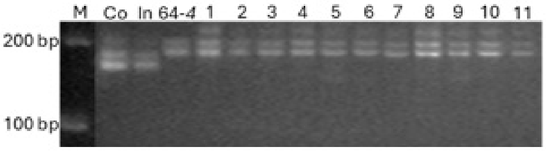
### *Molecular profile of Conde-qTSN4 and Conde-qDTH8 lines*

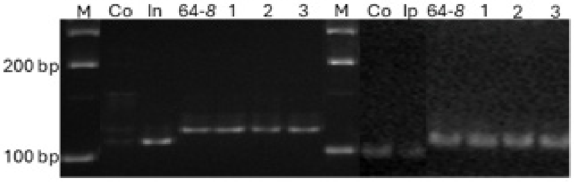
Molecular analysis of the Conde lines used in multi-location trials is essential to ensure genetic purity and prevent unintended mixing with other plants. Two SSR markers, RM178483 and RM6909, were employed to analyze 11 Conde-*qTSN4* lines, using IR64-*qTSN4* as the standard parent, which produces bands at sizes of 200 bp and 195 bp, respectively (Figure 2a1, a2). Similarly, three Conde-*qDTH8* lines were analyzed with two SSR markers, RM6838 and RM5556, with IR64-*qDTH8* serving as the standard parent,

(a1) RM17483 (*qTSN4*)



(a2) RM6909 (*qTSN4*)




(b1) RM6838 (*qDTH8*) (b2) RM5556 (*qDTH8*)

(c) RM20582 (*Xa7*)

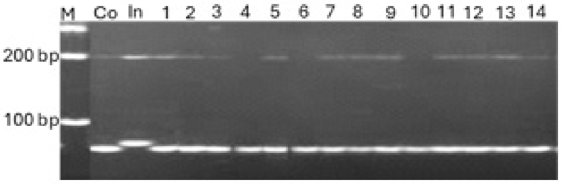


***Figure 2.*** Gel electrophoresis (8% polyacrylamide) results for Conde-*qTSN4* lines with *qTSN4* primers (RM17483 and RM6909) (a1, a2), for Conde-*qDTH8* lines with *qDTH8* primers (RM6838 and RM5556) (b1, b2), and all rice genotypes with *Xa7* primers (RM20582) (c). M = 100 bp ladder; Co = Conde, In = Inpari 32, 64-*4* = IR64-*qTSN4*, 64-*8* = IR64-*qDTH8*; 1 = A6-2, 2 = A8-5, 3 = A10-1, 4 = A16-5, 5 = B6-2, 6 = B11-4, 7 = B12-2, 8 = B15-2, 9 = B22-1, 10 = B24-4, 11 = C34, 12 = E13-1, 13 = G64, and 14 = G142.

which produces bands with sizes of 120 bp and 110 bp, respectively (Figure 2b1, b2). Additionally, allele detection was performed to identify the presence of the *Xa7* resistance gene, which produces a band with a size of 80 bp (Figure 2c).

Within the *qTSN4* locus, the spikelet number (*SPIKE*) gene is located near the RM17483 marker (Figure 2a1). This gene shares similarities with the *Narrow Leaf 1* (*NAL1*) gene, which has been shown to enhance spikelet production by increasing leaf size, improving root systems, and expanding the number of vessels involved in assimilate transport (Fujita et al. 2013). In addition to spikelet number, factors such as the number of productive tillers, the number of empty grains, and the percentage of filled grains also contribute to total yield per hill. Therefore, improving yield per unit area requires a holistic approach. Reliance on spikelet number alone is insufficient, and optimizing plant architecture is equally essential.

In the three Conde-*qDTH8* lines, SSR markers RM6838 and RM5556 successfully amplified DNA fragments identical to those of the parent line (IR64-*qDTH8*) (Figure 2b1, b2). Mishra et al. (2022) described that *qDTH8* from IR64 encodes a putative HAP3/NF-YB/CBF subunit of the CCAAT-box binding protein (HAP complex). *qDTH8* has been shown to positively influence yield, heading date, and stress tolerance in IR64. It functions as a positive regulator of gene networks associated with early flowering/heading, higher yield, and tolerance to salinity and drought stress, thereby enabling crops to adapt to diverse climatic conditions. In our study, *qDTH8* successfully increased TFGP, thereby enhancing yield (Table 1).

Despite the uniform appearance of the evaluated lines in the field, molecular analysis proved valuable in distinguishing genetic variations (Chen et al. 2008). This approach also helps detect seed mixing, particularly among parental lines with similar morphological characteristics (Chen et al. 2008). Molecular analysis confirmed that lines exhibiting banding patterns similar to Conde carry the *Xa7* resistance gene (Figure 2c). The *Xa7* gene, which originated from the Bangladesh variety DV85, together with the *xa5* gene, remains effective in combating *X. oryzae* pv. *oryzae* in Indonesia (Tasliah et al. 2013). Therefore, lines with banding patterns similar to Conde that possess the *Xa*7 resistance gene have the potential to be resistant to X. *oryzae* pv. *oryzae*.

## CONCLUSIONS

The identification of *qTSN4* and *qDTH8* loci as positive contributors to yield highlights their potential in developing high-productivity rice lines, as demonstrated by the superior performance of Conde-*qTSN4* and Conde-*qDTH8* lines compared to the parent variety (Conde). With Cianjur and Karawang recognized as optimal planting sites, and stable genotypes such as Conde (7.06 t ha$^{-1}$), A8-5 (7.09 t ha$^{-1}$), B12-2 (7.27 t ha$^{-1}$), and B15-2 (7.06 t ha$^{-1}$) showing consistent yields across environments, these lines can be advanced as promising commercial varieties. The Conde-*qTSN4* lines carry the *qTSN4* allele, the Conde-*qDTH8* lines carry the *qDTH8* allele, and all test lines possess the *Xa7* allele. The presence of the *Xa7* allele across all test lines further strengthens their value by conferring disease resistance, underscoring the importance of integrating molecular breeding with multi-environment trials to accelerate varietal release, enhance farmer adoption, and ensure resilient rice production under diverse tropical conditions.

## ACKNOWLEDGEMENTS

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## DATA AVAILABILITY

The supplementary file and datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

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