

Optimization of fertilizer application and transplanting density across agro-ecological zones and cropping seasons for black glutinous rice cultivation

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Abstract: This study aimed to optimize fertilizer application and transplanting density for the black glutinous rice to enhance its growth and yield potential. This study evaluated the effects of different fertilizer rates (50kgN-50kgP₂O₅-50kgK₂O, 70kgN-70kgP₂O₅-70kgK₂O, 90kgN-90kgP₂O₅-90kgK₂O, and 110kgN-110kgP₂O₅-110kgK₂O /ha) and transplanting densities (30, 35, 40, and 45 hills/m²) on the growth, yield, and resistance to pests and diseases of black glutinous rice (ĐH8). The experiment was conducted over two cropping seasons including Summer (CPS1) and Spring (CPS2) across three regions: the Red River Delta, the Northern Mountainous Region, and the North Central Region. Experimental plots were randomized, with main plots (fertilizer levels) divided into sub-plots (transplanting densities). Results indicated that moderate fertilizer application (70kgN-70kgP₂O₅-70kgK₂O /ha) combined with medium transplanting densities (35-40 hills/m²) generally led to the best agronomic performance, optimizing yield without significant pest and disease pressure. However, higher fertilizer application levels and denser transplanting conditions resulted in increased susceptibility to pests and diseases, such as brown planthopper and sheath blight, particularly in the CPS1. The study's findings offer practical guidance to improve crop management techniques, support sustainable rice farming, and contribute to better economic benefit for black glutinous rice producers.

Keywords: ĐH8, black glutinous rice, transplanting density, fertilization rate, agronomical trait, yield.

Introduction

Glutinous rice (*Oryza sativa* var. *glutinosa*), also known as sticky rice, is a staple crop widely cultivated and consumed across Asia, particularly in China, Thailand, Laos, Vietnam, and Japan (Olsen and Purugganan, 2002). It is distinguished by its high amylopectin content, which gives it a characteristic sticky texture after cooking (Ali and Hashim, 2024; Qiangqiang, Xuhua et al., 2024). The cultivation of glutinous rice dates back thousands of years, originating in Southeast Asia, where it holds significant cultural, economic, and nutritional value (Olsen and Purugganan, 2002, Gross and Zhao, 2014). Beyond its role in daily consumption, glutinous rice is integral to traditional festivals, religious ceremonies, and specialty food products such as rice cakes, fermented rice wine, and medicinal foods (Buresova et al., 2023). In addition to its culinary importance, glutinous rice varieties, particularly pigmented types like black glutinous rice, have attracted attention due to their high antioxidant content, primarily anthocyanins, which provide health benefits such as reducing oxidative stress and improving cardiovascular health (Das et al., 2023).

The successful cultivation of glutinous rice depends on several agronomic factors, including soil fertility, water management, and planting density (Wang et al., 2018; Liu et al., 2022). Proper fertilization is crucial to achieving high yields while maintaining soil health and reducing environmental impacts (Lei et al., 2021). Similarly, transplanting density affects plant competition, light interception, and nutrient uptake, ultimately influencing yield and grain quality (Dou et al., 2021). As climate conditions and soil characteristics vary across regions, determination of optimal cultivation practices, particularly fertilization rates and planting densities, is essential to maximizing productivity and sustainability. Given the increasing demand for high-quality glutinous rice, research on improving cultivation techniques for specific varieties is essential. Among the many glutinous rice varieties in Vietnam, ĐH8 has gained recognition as a promising cultivar in the

northern provinces due to its adaptability, high yield potential, and resistance to common pests and diseases. Developed through selective breeding, ĐH8 exhibits a short growth duration, making it suitable for multiple cropping seasons, including late spring, early summer, and autumn. However, in order to maximize its production potential, it is essential to optimize agronomic practices, particularly fertilization rates and transplanting densities. Proper nutrient management and planting techniques can enhance the growth, yield, and grain quality of ĐH8. Therefore, determining the optimal cultivation practices for ĐH8 is vital to improving productivity and meeting the growing demand for high-quality glutinous rice in Vietnam.

Results

Effects of Cropping Season (CPS) on growth and yield of black glutinous rice

The ĐH8 variety in CPS2 exhibited a longer GD (Supplementary Fig. S1), higher number of panicles/m² (Supplementary Fig. S3), greater NoP excepted in North Central Region (Supplementary Fig. S8), and higher GFR (Supplementary Fig. S13) as compared to the CPS1. It also showed a higher higher AY in CPS2 (Fig. 1), accompanied by a lower incidence of major pests and diseases (Supplementary Fig. S23-34).

Effects of transplanting density and fertilizer application on growth and yield of black glutinous rice cultivar in Red River Delta

In the CSP1, the growth and yield performance of the black glutinous rice cultivar in the Red River Delta were significantly influenced by both transplanting density and fertilization levels, excepted for GD. Transplanting density and fertilizer application, and their interaction, had no significant effect on the GD (Supplementary Fig. S1). The NoP was also influenced by higher transplanting densities, with the D4 treatment consistently showing the highest NoP across all fertilizer levels (Supplementary Fig. S3). However, the NoG exhibited an inverse relationship with transplanting density (Supplementary Fig. S8, S11). The GFR was slightly with moderate to high fertilizer levels, particularly P2D3, compared to lower fertilizer treatments such as P1D1 (Supplementary Fig. S13, S16). Similarly, the TY followed the same trend as NoP, with higher transplanting densities (D4) and higher fertilization rates (P2 and P3). For instance, the P2D3 treatment had the highest TY. The P3D4 treatment achieved a lower AY compared to P2D2 and P2D3, (Fig. 4).

In the CPS2, the growth duration of ĐH8 increased slightly with higher fertilization levels, ranging from 132 days in P1 treatments to 136 days in P3 and P4 treatments. This increase was consistent across transplanting densities, whereas transplanting density did not have a significant impact on the GD (Supplementary Fig. S1). Next, the NoP increased with transplanting density across all fertilizer levels (Supplementary Fig. S3). Particularly, the highest NoP was recorded at P2D2 (Supplementary Fig. S6). The NoG tended to decrease slightly with increasing density (Supplementary Fig. S8). The highest grain count was observed at P2D1 and P2D3, while lower values were observed in denser plantings including P1D4 and P4D4 (Supplementary Fig. S11). GFR were relatively stable across treatments with the highest value at P2D1 (Supplementary Fig. S16). The TY ranged from P4D1 to a maximum at P2D2 (Supplementary Fig. S19). In contrast, lower yields were associated with both insufficient fertilization (P1) and overly high fertilization combined with dense planting (P4D1) (Fig. 4).

The incidence of pests and diseases in the ĐH8 glutinous rice cultivar was influenced by both fertilization levels and transplanting densities during the CPS1 and CPS2 (Supplementary Fig. S23-S34). During the CPS1, pest and disease pressure was generally higher, with a clear trend showing increased severity under higher transplanting densities (D4) and elevated fertilization levels (P3 and P4). Brown planthopper incidence increased from a score of 3 at D1 to 5 at D3-D4, especially under P2-P4 treatments (Supplementary Fig. S27). Similarly, leaf folder and stem borer scores rose with increasing density and fertilizer, reaching score 5 under P3D3 and P4D3/D4, compared to minimal levels (score 1) at lower densities (D1) (Supplementary Fig. S29). Rice blast and sheath blight were more severe in high-input conditions (Supplementary Fig. S23). For instance, blast disease severity rose to score 3 at P3D3 and P4D4, while bacterial leaf blight was higher under P4D4. Most treatments recorded pest and disease scores between 0 and 1, with only slight increases in pressure under higher fertilizer levels and dense planting. Notably, brown planthopper, stem borer, and leaf folder remained at low levels (score 0-1) across all treatments (Supplementary Fig. S26, Fig. 28, Fig. S29 và Fig. S34). Blast disease, however, remained the most prevalent across seasons, especially under high-input treatments (Supplementary Fig. S24). Scores increased from 1 at P1 to 3 under P4D1-D4. Nonetheless, other diseases such as sheath blight and bacterial leaf blight showed minimal incidence during the CPS2, with maximum scores reaching only 1, even under high-density conditions.

Effects of transplanting density and fertilizer application on growth and yield of black glutinous rice cultivar in Northern Mountainous Region

Transplanting density and fertilizer application, and their interaction, had no significant effect on the GD (Supplementary Fig. S1). In both seasons, higher fertilization rates combined with moderate to dense transplanting densities tended to promote greater NoP (Supplementary Fig. S5). The maximum NoP meter was recorded at P4D4 in CPS1 and P2D4 in CPS2, NoG showed variation across treatments, with CPS1 values peaking at P2D3 and CPS2 values highest at P3D2 (Supplementary Fig. S10). GFR remained consistently high across treatments and seasons, with maximum values at P2D1 in CPS1 and at P2D2 in CPS2. In terms of yield, both TY and AY were highest at P2D3 in CPS 1 and at P2D2 in CPS2 (Fig. 3; Supplementary Fig. S20). These results indicated that a moderate fertilization rate (P2) combined with a transplanting density of D2 or D3 optimizes growth parameters and enhances grain yield (Fig. 3).

Overall, higher fertilization rates were associated with increased pest and disease pressure, particularly under dense

transplanting conditions (Supplementary Fig. S23-Fig. S34). During the CPS1, pest infestation remained low at fertilization

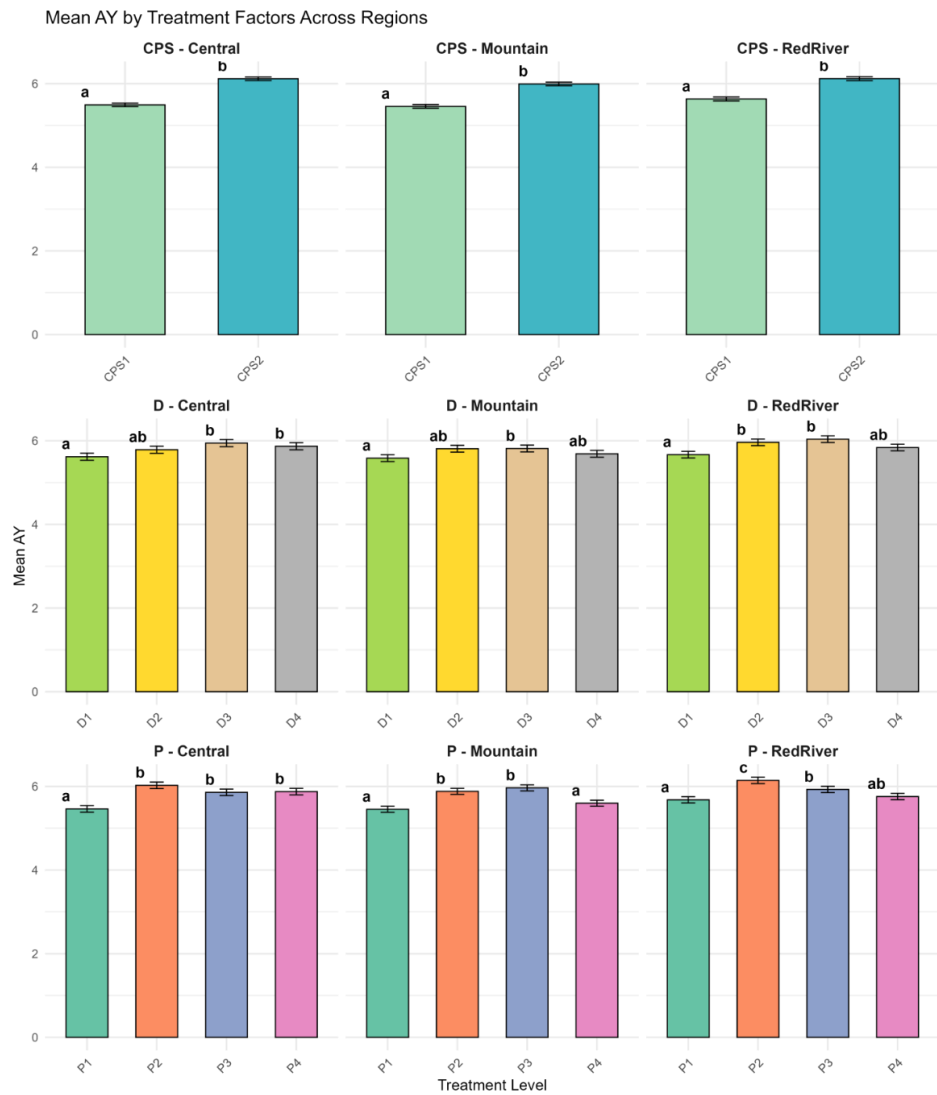


Fig. 1. Effects of cropping season (CS), plant density (D), and fertilizer dose (P) on the actual yield (AY) of black glutinous rice (DH8). Treatments with different letters are significantly different at $p < 0.05$.

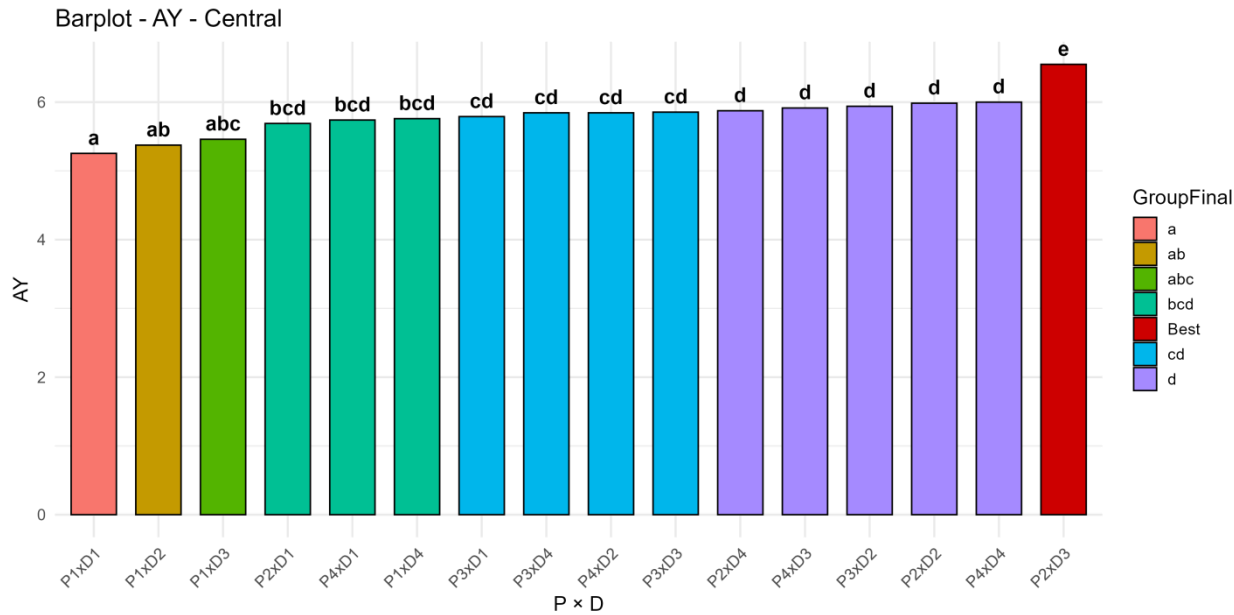


Fig. 2. Interaction effects of plant density (D) and fertilizer dose (P) ($P \times D$) on the actual yield (AY) of black glutinous rice (DH8) in the North Central Region. Treatments with different letters are significantly different at $p < 0.05$.

levels P1 and P2, with scores of 1 for brown planthopper, stem borer, and leaf folder across most transplanting densities (Supplementary Fig. S27; Fig. S29; Fig. S31). Notably, no sheath blight was observed under P1D1-D3, and bacterial leaf blight incidence was limited to scores of 0-1. However, at higher fertilization levels (P3 and P4), brown planthopper infestation increased to score 3 across all densities, while sheath blight and bacterial leaf blight also reached maximum scores of 3 (Supplementary Fig. S26). Leaf folder incidence remained low (score 1) in all treatments, whereas stem borer infestation increased slightly (score 3) under P4D2-D4 (Supplementary Fig. S30). Insect scores for brown planthopper and stem borer remained at 1 in most P1 and P2 treatments, while leaf folder was absent (score 0) in all but the P4 treatments, where it reached a score of 1 (Supplementary Fig. S27). Disease incidence also followed a similar trend, with blast, sheath blight, and bacterial leaf blight mostly at score 0-1 under P1 and P2 (Supplementary Fig. S23-S26). However, at higher fertilization levels (P3 and P4), blast and sheath blight consistently reached score 3 under D3 and D4 densities (Supplementary Fig. S23; Supplementary Fig. S25). Bacterial leaf blight showed a slight increase under P4, with a score of 1 across all transplanting densities.

Effects of transplanting density and fertilizer application on growth and yield of black glutinous rice cultivar in North Central Region

The study conducted in the North Central Region during the CPS1 and CPS2 highlights the significant influence of transplanting density and fertilization levels on the growth and yield performance of the black glutinous rice cultivar ĐH8 (Fig. 2; Supplementary Fig. S23-S34). Transplanting density, fertilizer application, and their interaction had no significant effect on GD (Supplementary Fig. S1) in the North Central Region, showing a similar trend to that observed in the Red River Delta and the Northern Mountainous Region. A clear trend was observed wherein both NoP and AY improved with increasing fertilization and appropriate transplanting densities (Supplementary Fig. S3; Fig. 2). In the CPS1, the combination of moderate fertilization (P2) and medium transplanting densities (D2 and D3) led to improved agronomic performance. The highest NoP were recorded under P2D3 (Supplementary Fig. S4). Although higher transplanting density (D4) under P2 produced comparable NoB, NoG declined. The treatment P2D3 again stood out, achieving the highest AY and TY, with balanced NoP, NoG, and GFR (Fig. 2; Supplementary Fig. S19). Other treatments under P3 and P4 showed similar patterns, with high yields maintained across D2-D4 densities (Fig. 1).

The results from the pest and disease incidence study in the North Central Region for both the CPS1 and the CPS2 showed that pest and disease pressure was generally low but increased under higher fertilization rates and dense transplanting densities, particularly in the CPS1 (Supplementary Fig. S23-S34). During the CPS1, brown planthopper infestation was consistently high, scoring 3 across all transplanting densities and fertilization levels (P1-P4). The incidence of other pests, including stem borer and leaf folder, remained low, with scores of 1 across all treatments. Disease pressure, however, was more variable. Blast disease showed increased severity in high-fertilization and high-density conditions, with scores rising to 3 in the P4D4 treatment (Supplementary Fig. S23). Sheath blight and bacterial leaf blight also became more pronounced at higher fertilization levels, especially in the P3 and P4 treatments, where scores of 3 were recorded, particularly in higher transplanting densities (D3 and D4) (Supplementary Fig. S25). In contrast, the CPS2 exhibited lower overall pest and disease pressure. Insect scores for brown planthopper and stem borer remained low (score 1) across most treatments, with leaf folder being absent (score 0) in most plots. Blast disease continued to be the most common, with a consistent score of 1 across treatments, except under higher fertilization levels (P4), where it reached a score of 3. Sheath blight and bacterial leaf blight showed minimal presence, with scores never exceeding 1, even in higher-density treatments (Supplementary Fig. S23-S34).

The effects of plant density (D) and fertilizer dose (P) ($P \times D$) on the growth and yield of black glutinous rice (ĐH8) across all regions

There was a significant interaction between fertilizer dose and plant density (Fig. 5; Supplementary Fig. S2, Fig. S7, Fig. S7, Fig. S12, Fig. S17, Fig. S22, Fig. S24, Fig. S26, Fig. S28, Fig. S30, Fig. S32 and Fig. 34). The combinations P2D2 and P2D3 consistently resulted in optimal GD, high NoP, large NoG, high GFR, and higher AY across both seasons and all three zones. In contrast, P3D4 and P4D4 showed longer GD, lower AY and higher levels of pest and disease incidence.

Discussion

The optimization of fertilizer application and transplanting density plays a pivotal role in improving the yield and sustainability of rice production. By integrating the results of the current study on black glutinous rice cultivar ĐH8 across three distinct ecological regions of Northern Vietnam including Red River Delta, Northern Mountainous Region, and North Central Region, this study identified region-specific strategies for enhancing the growth and yield of black glutinous rice. Our study observed that increasing fertilization rates promoted the growth of black glutinous rice cultivar ĐH8, extending the growth duration in all regions. In the Red River Delta, the growth duration ranged from ~110 days in the CPS1 to ~134 days in the CPS2, with the duration increasing as the fertilization level was raised. The increase in N fertilization by 90% led to an extended physiological maturity period, suggesting that higher rates of fertilization stimulate vegetative growth, causing delays in maturity. In the Northern Mountainous Region, growth duration was slightly longer, ranging from ~113 days in the CPS1 and ~133 days in the CPS2, also increasing with higher fertilization. This extended growth period can be

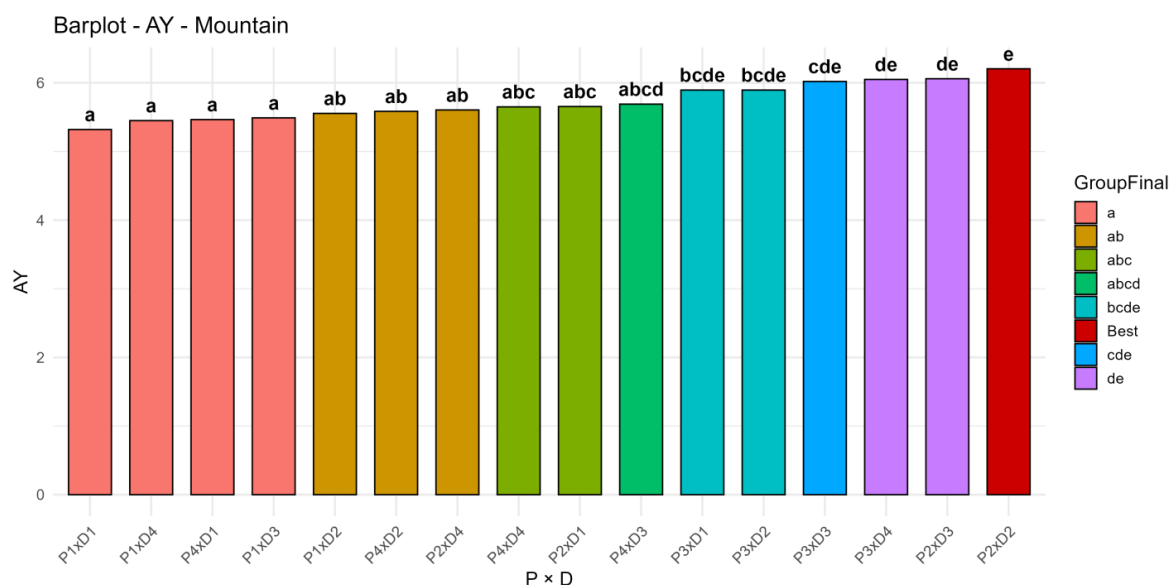


Fig. 3. Interaction effects of plant density (D) and fertilizer dose (P) ($P \times D$) on the actual yield (AY) of black glutinous rice (DH8) in the Northern Mountainous Region. Treatments with different letters are significantly different at $p < 0.05$.

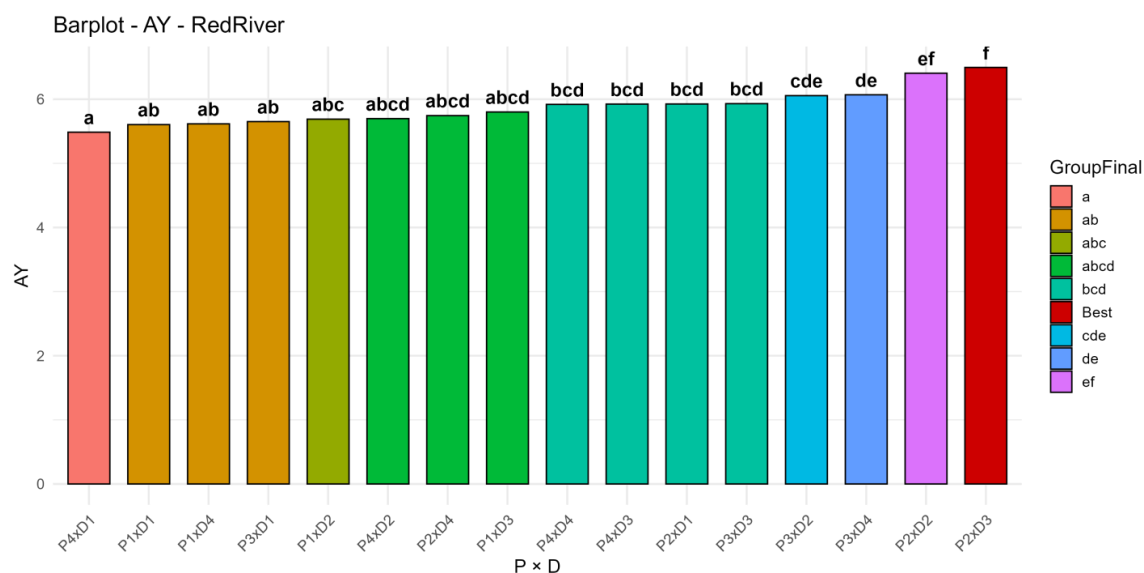


Fig. 4. Interaction effects of plant density (D) and fertilizer dose (P) ($P \times D$) on the actual yield (AY) of black glutinous rice (DH8) in the Red River Delta Region. Treatments with different letters are significantly different at $p < 0.05$.

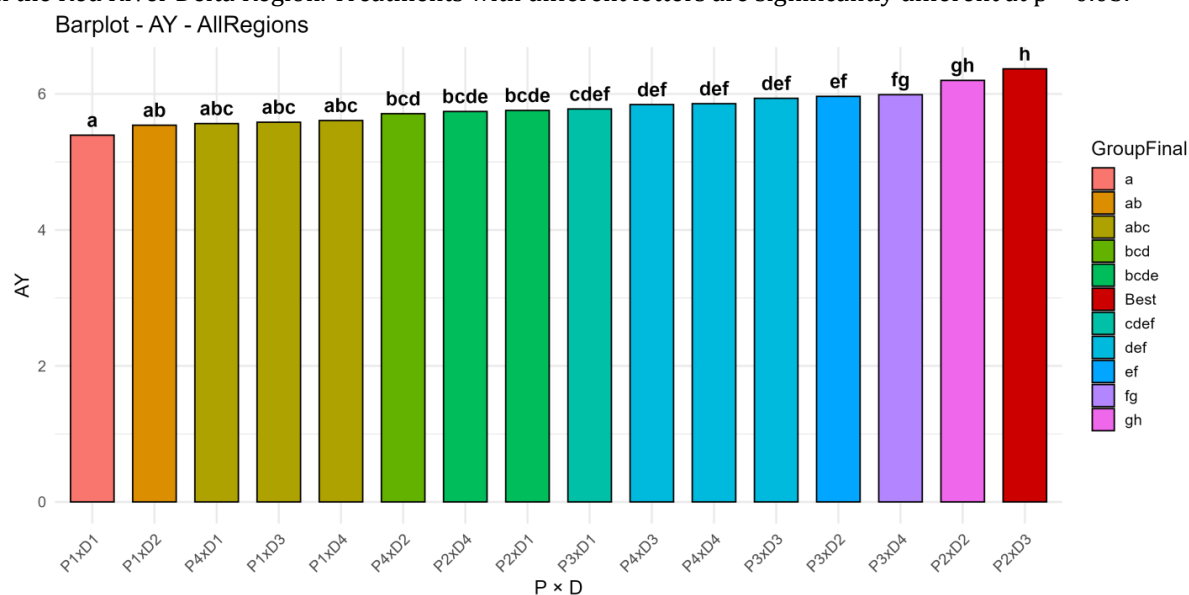


Fig. 5. Interaction effects of plant density (D) and fertilizer dose (P) ($P \times D$) on the actual yield (AY) of black glutinous rice (DH8) across all regions. Treatments with different letters are significantly different at $p < 0.05$.

attributed to cooler temperatures and lower soil fertility in this region, which necessitate higher fertilization to ensure optimal rice growth. Similarly, the North Central Region showed a similar trend, with growth duration increasing from 103 days at the lowest fertilizer rate (P1) to 105-126 days at higher rates (P3-P4). These results are consistent with findings of previous studies where higher fertilization rates extended vegetative growth and delayed maturity, supporting the conclusion that the increase in light-use efficiency, leaf area duration, and vegetative growth at a higher N rate prolongs the maturity period required by the plant (Xiong et al., 2018; Ye et al., 2019; Wu et al., 2021; Song et al., 2025). Higher fertilization levels enhanced tillering and panicle production across all regions. However, excessive fertilization, particularly at the highest levels (P3-P4), led to increased pest and disease incidences, including brown planthopper, sheath blight, and bacterial leaf blight. This was particularly evident in the Red River Delta, where pest and disease pressure increased at higher fertilization rates and dense planting densities (D3-D4).

Transplanting density had a significant impact on rice growth across all regions, with higher densities generally leading to more panicles per square meter but at the cost of decreased NoG. In the Red River Delta, higher transplanting densities (D4) resulted in the highest NoP at P2D4 in the CPS1 and at P2D2 in the CPS2. However, this increase in NoP was accompanied by a decrease in NoG, which remained stable around 83-86% for GFR. The highest AY in this region were at P2D3 and P2D2 in the CPS1 and CPS2, respectively. The Northern Mountainous Region exhibited a similar pattern, with higher transplanting densities and moderate fertilization (P2D3-D4) promoting tillering and achieving peak NoP at P4D4 in CPS1 and P2D4 in CPS2. However, the GFR was highest at P2D1 in CPS1 and at P2D2 in CPS2. Despite higher NoP, pest and disease pressure, especially in the form of brown planthopper and sheath blight, increased significantly at high fertilization and transplanting densities. These results align with the study of Tang et al. (2020), which indicates that higher planting densities lead to more panicles but reduced GFR, as observed in our study. Additionally, the interaction between higher density and N fertilizer application has been shown to delay physiological maturity, leaf senescence, and sustained leaf photosynthesis, thus extending days to maturity (Getachew, 2004). In the North Central Region, a moderate transplanting density (D2-D3) combined with moderate fertilization (P2) led to optimal performance, with the highest panicle density achieved at P2D3 in the CPS1. In the CPS2, the highest AY was at P2D3. Interestingly, excessive transplanting density (D4) led to a reduction in the number of grains per panicle, further demonstrating the importance of balancing these factors.

Yang et al. (2019) suggested that appropriate N application combined with balanced planting density can achieve high and stable rice yields, emphasizing the necessity of managing both factors to avoid negative environmental consequences. The combination of higher fertilization and transplanting density resulted in increased growth and yield across all regions but also led to a decline in the NoG. In the Red River Delta, for instance, at P2D4 (high fertilization and density), but NoG decreased. This was also evident in the Northern Mountainous Region, where peak NoP at P4D4 in CPS1 and at P2D4 in CPS2 were observed, but the GFR remained stable, and pest pressure increased significantly at these levels. This phenomenon is consistent with the findings of Liu et al. (2019) and Zhao et al. (2022), who suggested that excessive fertilizer application and high transplanting density tend to suppress yield due to their negative effects on the number of grains per panicle. In the North Central Region, moderate transplanting density and fertilization resulted in the highest yield, suggesting that this region is more suited to balanced fertilization and transplanting practices. Excessive transplanting density (D4) resulted in reduced grain production, emphasizing that high-density planting is only beneficial under moderate fertilization. This supports the conclusion by Dong et al. (2012), who found that yield increases with N application up to a certain point, after which further increases lead to yield reduction and some yield components, particularly due to competition for light, nutrients, and space among plants. Moreover, the interaction between N fertilizer and transplanting density is crucial in sustaining photosynthesis during the grain-filling stage, thus increasing economic yield (Hou et al., 2019).

Materials and Methods

Plant materials

This study was conducted using the black glutinous rice cultivar ĐH8 developed by the Crops Research and Development Institute of Vietnam National University of Agriculture. In addition to the rice seeds, various fertilizers were applied to evaluate their effects on growth and yield. The inorganic fertilizers, including urea (46% N), superphosphate (16.7% P₂O₅), and potassium chloride (50% K₂O) were used in different formulations to assess optimal nutrient requirements.

Rice cultivation

The black glutinous rice cultivar ĐH8 was used in this experiment. Seedlings were raised using the wet-bed method and transplanted at 35 days after sowing in the CPS2 and 20 days after sowing in the CPS1. From transplanting to the tillering stage, a standing water depth of approximately 5 cm was maintained. Thereafter, water depth was increased to around 10 cm and maintained until two weeks before harvest. Weeds were controlled manually by hand hoeing, ensuring that all plots remained weed-free throughout the growing period.

Experimental design

The study used a split-plot design to evaluate the effects of different fertilizer levels and transplanting densities on the growth and yield of the black glutinous rice cultivar across agro-ecological zones and cropping seasons. The main plot factor was fertilizer application (P treatments) with four levels: P1 (50kgN-50kgP₂O₅-50kgK₂O), P2 (70kgN-70kgP₂O₅-70kgK₂O), P3(90kgN-90kgP₂O₅-90kgK₂O), and P4 (110kgN-110kgP₂O₅-110kgK₂O)/ha. The sub-plot factor was transplanting density including D1 (30 hills/m²), D2 (35 hills/m²), D3 (40 hills/m²), and D4 (45 hills/m²). Each treatment combination was

replicated three times across three representative ecological regions: the Red River Delta (Yen Khanh District, Ninh Binh Province), the Northern Mountainous Region (Dien Bien District, Dien Bien Province), and the North Central Region (Yen Thanh District, Nghe An Province), where the experimental sites were located respectively. The three selected locations represent distinct ecological regions in Northern Vietnam, characterized by clear differences in topography and climatic conditions (Acharya and Bennett, 2021). The experiment was conducted in two seasons including Summer Cropping Season(CPS1) – Spring Cropping Season(CPS2) to evaluate seasonal variations in agronomic performance in two years.

Measurement of agronomical characteristics

Agronomic and yield-related indicators were measured at key growth stages to evaluate the effects of transplanting density and fertilizer application on the ĐH8 glutinous rice cultivar. Yield components were assessed by measuring the number of panicles per square meter (NoP), number of grains per panicle (NoG), and grain filling rate (GFR, %). Actual grain yield (tons/ha) was determined by harvesting and weighing grains from a standardized plot area. Pest and disease resistance were evaluated based on visual assessments of symptoms and damage levels using standard scales. The incidence of brown planthopper (BP), leaf folder (LB), stem borer (StB), bacterial leaf blight (BLB), blast disease (B), and sheath blight disease (ShB) was recorded. All measurements are based on the Standard Evaluation System (SES) for rice developed by the International Rice Research Institute (IRRI, 1996).

Statistical analysis

ANOVA was performed to assess treatment effects on growth, yield, and pest resistance, with LSD (p -value < 0.05) used for mean comparisons. A two-way ANOVA evaluated interactions between fertilizer and density. Descriptive statistics, including means and standard deviations, were calculated. Analyses were conducted using R software.

Conclusion

In conclusion, the results from the three regions and seasons studied provide valuable insights into the complex interactions between transplanting density, fertilization, and the growth, yield, and pest and disease incidence of the black glutinous rice cultivar ĐH8. In the Red River Delta, both transplanting density and fertilization levels significantly influenced growth and yield, with moderate fertilization (P2) combined with medium transplanting densities (D2 and D3) optimizing panicle density and actual yield, particularly in the CPS2. Pest and disease pressure was higher in the CPS1, particularly under higher fertilization and dense planting, with blast, sheath blight, and bacterial leaf blight becoming more prominent. In the Northern Mountainous Region, similar trends were observed, where moderate fertilization and medium transplanting densities (P2D3) resulted in the highest actual yield. Pest and disease pressure was generally low, but higher fertilization levels (P3 and P4) combined with dense planting (D3 and D4) increased the incidence of brown planthopper, blast, and bacterial leaf blight, particularly in the CPS1. In the North Central Region, moderate fertilization combined with medium transplanting density (P2D3) also yielded the best results in both seasons, with high panicle density and optimal grain number per panicle. While pest and disease pressure remained generally low in the CPS2, higher fertilization levels and dense planting resulted in increased disease incidence, particularly for blast and sheath blight. Overall, the findings highlight the importance of balanced agronomic practices that optimize growth while minimizing pest and disease risks, with region-specific strategies needed to address the varying environmental conditions and challenges faced by ĐH8 rice cultivation.

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