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Original Research Article

Effect of plant establishment method on the growth and yield performance of hilly black rice cultivars in Charland ecosystem of Bangladesh

Shah Golam Azam, Md. Rashedur Rahman[*], Md. Parvez Anwar, Md. Romij Uddin and Umme Salma

Department of Agronomy, Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh

[*] Corresponding author's Email: rashedagron@bau.edu.bd

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ABSTRACT

Black rice, rich in anthocyanins, phenolic compounds, and essential micronutrients, offers a promising pathway for enhancing both productivity and nutritional security in the char land ecosystems of Bangladesh. This study aimed to identify suitable plant establishment methods and high-performing hilly black rice cultivars for these vulnerable production environments. Field experiments were conducted to evaluate the effects of establishment methods and genotypes on growth, physiological attributes, yield components, and productivity. Establishment method significantly ($p < 0.01$) affected tillering, panicle characteristics, grain weight, and grain yield. Puddled transplanting consistently enhanced crop performance, producing the tallest plants (115.09 cm at 60 DAT), highest tiller number (19.43 hill^{-1}), greater leaf production (75.96 hill^{-1}) and more grains panicle^{-1} (120.60), while reducing spikelet sterility to 11.24%. Genotypic responses were highly significant, with the Indonesian cultivar Ketan hitam achieving the highest grain yield (4.47 t ha^{-1}), straw yield (7.29 t ha^{-1}), biological yield (11.76 t ha^{-1}), and harvest index (40.47%), indicating superior biomass production and assimilate partitioning. Gelong Ni also demonstrated strong performance for tillering and panicle development, whereas Kongnam ene exhibited the lowest productivity. Overall, the combination of puddled transplanting and Ketan hitam emerged as the most productive establishment–genotype strategy. Beyond improving yield, this approach provides a practical entry point for integrating nutritionally riched black rice into charland farming systems, potentially converting marginal production environments into viable sources of both food and nutritional security.

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INTRODUCTION

Rice is a staple meal for population and primary source of energy that contain decent amounts of fiber, protein, vitamin B, iron and manganese; makes up 95 percent of food production in Bangladesh. Rice nourishes about half of the global population and is recognized as a major crop (Bin Rahman & Zhang, 2023). Since polished white rice loses many nutrients, enhancing seed-specific accumulation of iron, zinc, provitamin A, and other minerals is crucial (Bhandari *et al*., 2024). People of Bangladesh consume white rice, provides mainly carbohydrate to the people's diet and this rice is lack of other important essential nutrients for human health which ultimately enhance the hidden hunger of this mass people of Bangladesh (Harding *et al*., 2018). Black rice, a traditional rice cultivar, is being cultivating in the hilly area of Bangladesh especially in the Bandarban and Rangamati region, could be a potential way of eliminating the hidden hunger as it contains high amount of nutrients required for human body (Rahman *et al*., 2019). Black rice has been cultivated in Asia for about 9,000 years ago (Rahim *et al*., 2022). Black rice has shown increasing relevance in the **food industry**, with innovations in gluten-free product development, packaging technologies, and processing methods that influence the stability of bioactive substances. The global black rice industry to reach USD 15.14 billion by 2030, reflecting rising consumer demand for nutrient-dense super food (Javed *et al*., 2025). It has gained global attention as a nutrient-dense cereal, often termed a superfood due to its high anthocyanin content, antioxidant properties. Black rice is a rich source of phenolic compounds, including tannins, phenolic acids, and flavonoids that contribute to human health, and is inherently biofortified with iron, vitamin E, antioxidants, and anthocyanins such as cyanidin-3-glucoside, malvidin-3-glucoside, and peonidin-3-glucoside (Kaur *et al.*, 2025). Black rice is nutritionally rich and contains diverse bioactive compounds including essential amino acids, functional lipids, dietary fiber, vitamins, minerals, anthocyanins, phenolic compounds, γ-oryzanols, tocopherols, tocotrienols, phytosterols, and phytic acid (Ito & Lacerda, 2019). Black rice has potential to be incorporated into human diets as a means of combating hidden hunger and enhancing food and nutritional security in Bangladesh.

Tadesse (2021) reported that that transplanting seedlings rather than directly spreading dry seed results in much higher grain yields and more economic benefits in rice cultivation. (Rahman *et al.*, 2019) reported black rice cultivar Gelong Se produced superior grain yield when transplanted on July 20 or August 5 during the aman season, while Kongnam ene showed remarkably high zinc content (65 mg/kg), surpassing other zinc-enriched varieties. In Arunachal Pradesh revealed significant variation among black rice cultivars, with BR1 (Imphal, Chalchao) produce highest tillers and grains per panicle, BR4 (Meghalaya, Nalapara) showed maximum plant height and longest crop duration, indicating their potential to enhance farmer income and nutritional security (Rian *et al.*, 2022). In Rampur, Chitwan, Nepal during 2015–2016 demonstrated that closer crop spacing (15 cm $\times$ 15 cm) combined with LCC-based nitrogen management maximized nitrogen uptake, economic return and benefit-cost ratio for sustainable black rice cultivation (Karkee *et al.*, 2019).

Black rice, cultivar Kongnam ene showed rapid growth, Gelong Ni achieved the highest yield (4.0 t/ha) in the boro season, highlighting its potential for year-round cultivation beyond the Chittagong Hill Tracts. (Palupi *et al.*, 2020; Rahman *et al.*, 2020) reported that four black rice varieties from West Kalimantan, Indonesia showed Senakin performing best in plant height, productive tillers, and grain weight, highlighting their potential as genetic resources for breeding programs. Kawatra *et al.* (2021) found that optimum seedling age ensures higher rice yields, while older seedlings can still achieve improved productivity through foliar application of kinetin or TRIA, with kinetin enhancing seed filling and TRIA increasing tiller number. (Cao *et al.*, 2022) found that in Wuxue, Hubei Province (2016–2018), seedling age up to 40 days not necessarily reducing yield when transplanting dates and crop durations were managed to avoid heat stress during panicle development and flowering. Transplanting 35-day-old seedlings at 2 seedlings hill^{-1} optimized growth, yield, and straw production of black rice, producing the highest grain yield (5.56 t ha^{-1}) and overall performance under Bangladesh conditions (Midya *et al.*, 2021). Unpuddled transplanting has emerged as a climate-smart alternative, conserving soil health and reducing transplanting shock while maintaining yield genotype $\times$ establishment interactions, with adaptable cultivars performing better under resource-efficient methods. Bastola (2020) studied rice plant establishment methods and found that transplanting produced 3.98 t ha^{-1} drum seeding 3.37 t ha^{-1} broadcasting 3.27 t ha^{-1} row seeding 2.95 t ha^{-1}. Transplanting black rice with pre-emergence application of pretilachlor (0.75 kg ha^{-1} at 30 DAT) followed by hand weeding at 40 DAT significantly reduced weed biomass and nutrient depletion along with enhanced weed control efficiency, nutrient uptake, and grain yield compared to other management practices (Dolie *et al.*, 2025). Black rice cultivar Gelong Se, produced the highest grain yield at transplanting on July 20 or August 5 and applying higher fertilizer doses, while late transplanting on October 5 resulted in the lowest yield (Rahman *et al.*, 2019). The main objective of the research is to develop a cultivation technique of nutrient enriched hilly black rice cultivars for the Charland of Bangladesh that would help to eliminate nutritional insecurity and boosting the economy of the char dwellers. The specific objectives were to find out the suitable method of cultivation of black rice cultivars

MATERIALS AND METHODS

Site description of the experimental field

The experimental field located in Sirajganj district of Bangladesh falls under Agro-Ecological Zone (AEZ) 8 the Active Brahmaputra Jamuna Floodplain. Geographically, the site is positioned at approximately 24°27′27″ N latitude and 89°42′28″ E longitude, with an average land elevation of about 19 meters above mean sea level. The hydrological regime is strongly influenced by the Jamuna River, which creates char lands (newly accreted lands) and alters the micro-topography annually. The soils are alluvial in origin and exhibit a sandy loam to silty loam texture with relatively low organic matter content. The pH value was 5.8–6.5. The soil contained <0.1% total nitrogen, deficient organic carbon (0.6–1.2%), phosphorus availability (8–12 mg kg^{-1}), while exchangeable potassium remains adequate. Secondary nutrients such as sulphur (<10 mg kg^{-1}), zinc (<0.8 mg kg^{-1}) and boron (<0.2 mg kg^{-1}), are widespread, constraining crop productivity. The soils possess a moderate cation exchange capacity (12–18 cmol kg^{-1}) and bulk density of 1.4–1.6 g cm^{-3} supporting reasonable root penetration. Climatically, the region experiences a subtropical monsoon with annual rainfall ranging between 1,800–2,000 mm, mean maximum temperatures of 30–34°C, and minimum temperatures of 18–22°C. Relative humidity remains high (70–85%) throughout most of the year.

Characteristics of test varieties

Four black rice cultivars—three hilly Bangladeshi cultivars (Kongnam ene, Gelong ni, and Gelong se) and one Indonesian cultivar (Ketan hitam)—were evaluated. Seeds were collected from Bandarban, Bangladesh, and Indonesia. Kongnam ene is a traditional black rice cultivar widely associated with Southeast Asia, including China, India, and Thailand. Its black pericarp is attributed to anthocyanin pigmentation. It is a non-lodging transplant Aman cultivar, generally maturing within 95–105 days and producing approximately 3.0–3.5 t ha^{-1}. Plants are about 90–95 cm tall and show moderate tolerance to pests and diseases, particularly blast. Gelong ni and Gelong se are traditional cultivars adapted to hilly upland environments. They mature within approximately 110–120 days after transplanting during summer and attain about 120 cm plant height, with an average yield of around 3.5 t ha^{-1}. Both possess brown hull and bran and are nutritionally rich in minerals and B-group vitamins. Their morphological characteristics are largely similar, although Gelong ni has a slightly higher 1000-grain weight than Gelong se. Ketan hitam, meaning "black sticky rice" in Indonesia, is a glutinous *Oryza sativa* cultivar traditionally cultivated in several Indonesian regions. It matures in about 110 days after transplanting, with a reported yield potential of 5.5–6.0 t ha^{-1} and a total growth duration of approximately 150 days, depending on environmental conditions.

Experimental treatments and design

The experiment was comprised of two factors viz. Factor A: Cultivar (4), Kongnam ene (V_1), Gelong ni (V_2), Gelong se (V_3), Ketan hitam (V_4), Factor B: Establishment method (3): Wet seeding (Line sowing of sprouted seeds in puddled soil), Puddled transplanting, Unpuddled transplanting. The experiment was laid out in a split plot design where plant establishment methods were allocated in main plots and varieties were distributed in subplots. The treatments were replicated three times. Thus, the total number of plots were 36 ($4\times3\times3$). The size of each plot was 10 m^2 (4 m $\times$ 2.5 m).

Seed processing and seedling growth

Rice seed treatment was carried out following three sequential steps to ensure uniform germination and protection against seed-borne pathogens. **Fungicide seed dressing** was applied by coating seeds with a slurry of Benlate and Mancozeb at a rate of 3 g kg^{-1} seed, effectively reducing the incidence of seed-borne diseases such as blast and seedling rot. These treatments minimize early biotic stress, and ensure reliable performance of the experimental rice varieties under floodplain agro-ecosystems. Healthy seeds were immerged in water in bucket for 24 hours. Then the seeds were taken out of the water and keep thickly in a gunny bags. The seed started sprouting after 48 hours and were sown after 72 hours in the nursery beds.

Land preparation and layout

The land for rice cultivation was prepared thoroughly to ensure a fine tilth and uniform crop establishment. Initial ploughing was carried out with a power tiller, followed by cross-ploughing to break down soil clods and incorporate crop residues into the soil profile. Each ploughing operation was immediately followed by laddering, which levelled the surface and refined the seedbed structure. The experimental field was laid out according to the design. The field layout was made on 10 February 2021 according to experimental specification immediately after final land preparation.

Description of crop establishment methods

Wet seeding

In broadcasting method seed rate was 110-120 kg and in line sowing method seed rate was 60-80 kg ha^{-1}. In wet seeding method seed was normally pre-germinated prior to broadcasting onto recently drained, well puddled seedbed.

Puddle transplanting

In puddle transplanting irrigation was given as the farmers usually apply their own plot. In this method seedlings were transplanted in well prepared puddled field at the rate of two seedlings $hill^{-1}$. Maintained row and hill distance of 25 cm and 15 cm respectively. Water levels were maintained 5-7 cm from transplanting maximum tillering stage and water levels were maintained 10-15 cm from transplanting to hard dough stage.

Un- puddled transplanting

No tillage operation was done for an un-puddled transplanting. In case of Un- Puddled transplanting the seedling was transplanted in un-puddled field at the rate of two seedlings $hill^{-1}$. Maintained row and hill distance of 25 cm and 15 cm respectively. Water levels were maintained 5-7 cm from transplanting to hard dough stage.

Crop husbandry

The experimental plots were fertilized with urea 150 kg/ha in three equal instalments (33.33% each at the first, second, and third stages), while triple super phosphate (TSP) and muriate of potash (MoP) are applied fully as basal doses at 100 kg/ha each. Gypsum at 60 kg/ha and borax at 10 kg/ha are also applied entirely as basal. Various intercultural operation viz. irrigation and drainage, weeding, disease and pest management etc were done when necessary to maintain a favourable condition for normal growth and development of crop. After transplanting, a water depth of approximately 5–7 cm was maintained uniformly across the plots throughout the entire crop cycle.

Observation and data collection

Harvesting was performed at full maturity when approximately 80–85% of the grains in the panicle had turned golden yellow. The harvested crop from each plot was bundled separately, properly tagged and brought to the threshing floor for processing. Threshing was done manually, followed by cleaning and drying of grains to a safe storage moisture level of about 14%. Plant height was measured at maturity, while tillering was assessed through total and effective tillers $hill^{-1}$, with the effectivity index showing tiller productivity. Yield components were derived from 10 panicles per plot, including panicle length, grains $panicle^{-1}$, sterile spikelets and 1000-grain weight. Finally, grain yield, straw yield, and biological yield were recorded, with the harvest index calculated to reflect efficiency of assimilate partitioning.

Statistical analysis

All collected data on growth, yield, yield-contributing traits, and greenhouse gas emissions were statistically analyzed using R-Studio to ensure precision and reproducibility. Treatment effects were evaluated through analysis of variance (ANOVA), and mean separation was conducted using Duncan's Multiple Range Test (DMRT) at both 1% and 5% levels of significance, following the principles outlined by Gomez & Gomez (1984).

RESULTS AND DISCUSSION

Effect of plant establishment method

The effects of establishment method were highly significant for most growth and yield traits ($p \leq 0.01$), with relatively low coefficients of variation (3.32–10.07%), indicating good experimental precision. The establishment method markedly influenced vegetative growth and reproductive development. Puddled transplanting (M_2) produced the tallest plants (123.00 cm), highest total tillers (14.01 $hill^{-1}$), effective tillers (11.15 $hill^{-1}$), longest panicles (24.17 cm), and maximum grains $panicle^{-1}$ (120.60). It also produced the highest grain yield (4.47 t ha^{-1}), straw yield (6.92 t ha^{-1}), biological yield (11.39 t ha^{-1}), and harvest index (39.31%). Unpuddled transplanting (M_3) generally ranked second, whereas wet seeding (M1) recorded the lowest overall values for most traits. Thus, compared with M_1, M_2 increased grain yield by approximately 26.6%, while M3 increased it by 20.1% (Table 1). The superiority of puddled transplanting can primarily be attributed to better crop establishment and early root–soil interaction. Puddling reduces percolation, improves soil water retention, and creates a softer soil environment for transplanted seedlings. These conditions can promote early recovery after transplanting, tiller initiation, nutrient acquisition, and canopy development. The present results support this interpretation because puddled transplanting simultaneously increased plant height, total and effective tillers, panicle length, grains panicle^{-1}, and ultimately grain yield. Similar evidence has been reported recently in rice: conventional transplanting produced greater plant height, panicle number, and panicle length than aerobic establishment (Zayed *et al.*, 2023). More specifically, a 2024 Bangladesh study in salt-affected coastal rice found that conventional transplanting increased grain yield by 13–63% compared with direct-seeding systems, largely because direct-seeded treatments produced fewer panicle-bearing tillers and grains panicle^{-1} (Paul *et al.*, 2024).

Table 1. Effect of plant establishment method on the growth and yield performance of hilly black rice cultivars.

Establishment Methods	Plant height (cm)	No. of total tillers hill^{-1}	No. of effective tillers hill^{-1}	Panicle length (cm)	No. of grains panicle^{-1}	Weight of 1000-grain (g)	Grain Yield (tha^{-1})	Straw yield (tha^{-1})	Biological yield (tha^{-1})	Harvest index (%)
M_1	116.64 b	9.20 c	8.10 c	19.48 b	96.89 c	23.15 b	3.53 b	5.78 b	9.72 c	25.34 c
M_2	123.00 a	14.01 a	11.15 a	24.17 a	120.60 a	25.67 a	4.47 a	6.92 a	11.39 a	39.31 a
M_3	119.68 ab	11.93 b	10.23 b	23.99 a	112.58 b	24.76 a	4.24 a	6.19 b	10.32 b	36.25 b
Level of significance	**	**	**	**	**	**	**	**	**	**
CV%	3.32	10.07	8.31	4.33	7.33	3.76	4.79	5.9	3.78	5.27

** =Significant at 1% level of probability, * =Significant at 5% level of probability, NS = Not significant; M_1 = Wet seeding (Line sowing of sprouted seeds in puddled soil), M_2 = Puddled transplanting, M_3 = Unpuddled transplanting.

Effect of cultivars

Cultivar effects were also pronounced. Ketan hitam (V_4) was the tallest (123.85 cm), produced the highest total and effective tillers (14.20 and 12.01 hill^{-1}), grains panicle^{-1} (122.44), 1000-seed weight (26.80 g), grain yield (4.47 t ha^{-1}), straw yield (7.29 t ha^{-1}), biological yield (11.76 t ha^{-1}), and harvest index (40.47%). Gelong Ni (V_2) was the second-best cultivar for grain yield (4.15 t ha^{-1}), whereas Kongnam ene (V_1) had the lowest yield (3.88 t ha^{-1}). Ketan hitam therefore, exceeded Kongnam ene by approximately 15.2% in grain yield (Table 2). The strong cultivar effect demonstrates substantial genotypic variability in yield formation and adaptation. Ketan hitam combined high tillering, long panicles, high grain number, greater 1000-seed weight, biomass accumulation, and harvest index. Its 4.47 t ha^{-1} yield was therefore not attributable to a single yield component but to the coordinated expression of several components. Recent black-rice research similarly demonstrates substantial genotype-dependent variation. A 2025 study evaluating 19 black rice genotypes across multiple environments found significant genotype $\times$ environment effects and substantial variation in yield-related traits (Brunet-Loredo *et al.*, 2025). Another recent study reported grain yields of approximately 4.0 t ha^{-1} for a high-performing fine black-rice genotype, compared with only 1.93 t ha^{-1} for coarse black rice, confirming that genetic background strongly determines productivity (Bhandari *et al.*, 2024).

Combined effect of establishment method and cultivars

The establishment and cultivar interaction was significant for all measured traits except that the level of significance varied among variables. The highest grain yield was recorded in M_1V_1 (4.69 t ha^{-1}), followed by M_2V_1 (4.50 t ha^{-1}), M_2V_2 (4.41 t ha^{-1}), M_1V_2 (4.45 t ha^{-1}), and M_2V_4 (4.64 t ha^{-1}). Interestingly, Ketan hitam responded poorly to unpuddled transplanting, where its yield declined to 3.63 t ha^{-1}, compared with 4.64 t ha^{-1} under puddled transplanting. The lowest grain yield was observed in M_3V_3 (2.97 t ha^{-1}), followed by M_3V_2 (3.28 t ha^{-1}). An interesting interaction was observed for yield components. Puddled transplanting of the cultivar Ketan hitam (M_2V_4) produced a relatively high grain yield (4.64 t ha^{-1}) together with 25.87-cm panicles and 122.83 grains panicle^{-1}, whereas M_3V_2 produced the maximum grains panicle^{-1} (125.77) but only 3.28 t ha^{-1} grain yield. This indicates that grain number alone did not determine final productivity; effective tillering, grain filling, and seed weight collectively contributed to yield. The interaction also revealed considerable phenotypic plasticity among cultivars, suggesting that establishment method should be considered together with genotype rather than independently (Table 3). The interaction results provide the most important biological insight. Ketan hitam was highly productive under puddled transplanting (4.64 t ha^{-1}), but its yield fell sharply under unpuddled transplanting (3.63 t ha^{-1}). This suggests that its high yield potential may depend on favourable soil-water conditions during establishment. Conversely, Kongnam ene maintained relatively high productivity under wet seeding (4.69 t ha^{-1}), indicating greater establishment plasticity. Such genotype-specific responses are consistent with recent evidence that black rice genotypes differ substantially in their responses to environmental and management conditions (Brunet-Loredo *et al.*, 2025). The finding that M_3V_2 had the highest grains panicle^{-1} (125.77) but only 3.28 t ha^{-1} yield highlights another important point: maximum grain number does not necessarily translate into maximum grain yield. Grain filling, productive tiller number, and individual grain weight must also be optimized. Recent work on black rice has likewise shown that high-yielding genotypes can differ substantially in grain number and grain weight, demonstrating the importance of coordinated yield-component expression (Veni *et al.*, 2025).

PCA bi-plot of method and genotype

The PCA biplot clearly separated treatments by method and genotype, revealing distinct trait associations. The first two principal components explained the majority of variation, with PC_1 capturing yield-related traits and PC_2 reflecting sterility and seed weight.

Table 2. Effect of plant establishment method on the growth and yield performance of hilly black rice cultivars.

Cultivars	Plant height (cm)	No. of total tillers hill^{-1}	No. of effective tillers hill^{-1}	Panicle length (cm)	No. of grains panicle^{-1}	Weight of 1000- grain (g)	Grain Yield (tha^{-1})	Straw yield (tha^{-1})	Biological yield (tha^{-1})	Harvest index (%)
Kongnam ene	106.70 c	12.12 b	9.61 b	23.99 a	111.00 c	21.40 b	3.88 c	5.44 c	9.51 c	36.79 b
Gelong Ni	121.06 a	14.11 a	11.46 a	23.51 a	117.78 ab	26.03 a	4.15 b	6.65 b	10.48 b	38.56 ab
Gelong Se	116.50 b	13.09 ab	10.22 b	23.87 a	116.88 b	24.75 b	4.06 b	6.31 b	10.19 b	37.04 b
Ketan hitam	123.85 a	14.20 a	12.01 a	24.15 a	122.44 a	26.80 a	4.47 a	7.29 a	11.76 a	40.47 a
Level of significance	**	**	*	NS	**	**	**	*	*	**
CV%	3.32	10.07	8.31	4.33	7.33	3.76	4.79	5.9	3.78	5.27

** =Significant at 1% level of probability, * =Significant at 5% level of probability, NS = Not significant.

Table 3. Interaction effect of plant establishment method on the growth and yield performance of hilly black rice cultivars.

Methods × Cultivars		Plant height (cm)	No. of total tillers hill^{-1}	No. of effective tillers hill^{-1}	Panicle length (cm)	No. of grains panicle^{-1}	Weight of 1000- grain (g)	Grain Yield (tha^{-1})	Straw yield (tha^{-1})	Biological yield (tha^{-1})	Harvest index (%)
M_1	V_1	116.93 de	13.40 bc	11.02 cde	23.11 cd	113.83 abcd	26.71 bcd	4.69 a	7.49 a	12.18 a	38.52 abc
	V_2	110.33 ef	13.65 abc	11.44 bcd	22.70 d	121.23 abcd	24.30 ef	4.45 abc	6.78 bcd	11.23 bc	39.61 ab
	V_3	140.13 a	13.22 bcd	9.80 efg	24.80 abc	121.30 abcd	24.09 ef	4.32 bc	7.42 a	11.75 ab	36.80 bcd
	V_4	111.33 ef	15.44 ab	12.67 ab	23.32 cd	111.20 cd	25.50 cde	3.90 d	6.23 de	10.13 d	38.44 abc
M_2	V_1	108.56 f	15.86 a	13.44 a	23.36 cd	124.57 ab	26.89 bc	4.50 abc	7.04 abc	11.54 abc	39.15 ab
	V_2	132.51 b	13.24 bcd	11.82 bc	24.35 abcd	120.33 abcd	27.24 ab	4.41 abc	6.50 cde	10.91 c	40.39 a
	V_3	122.33 cd	13.55 abc	10.44 cdef	23.07 cd	114.67 abcd	23.84 f	4.34 bc	6.88 abc	11.22 bc	38.66 ab
	V_4	128.60 bc	10.14 e	8.89 fg	25.87 a	122.83 abc	24.73 ef	4.64 ab	7.26 ab	11.90 ab	39.02 ab
M_3	V_1	127.60 bc	13.33 bc	9.93 defg	25.48 ab	112.23 bcd	24.50 ef	4.22 c	7.34 ab	11.56 abc	36.45 bcd
	V_2	120.33 d	15.44 ab	12.77 ab	23.48 bcd	125.77 a	28.87 a	3.28 e	6.03 ef	9.31 e	35.40 cd
	V_3	109.09 f	12.51 cde	8.58 g	23.73 bcd	109.03 d	25.27 def	2.97 e	5.54 f	8.51 f	34.92 d
	V_4	109.55 f	10.77 de	9.11 fg	23.26 cd	119.30 abcd	24.03 ef	3.63 d	5.87 ef	9.50 de	38.22 abc
Level of significance		**	**	*	**	**	**	*	**	**	**
CV%		3.32	10.07	8.31	4.33	7.33	3.76	4.79	5.9	3.78	5.27

** =Significant at 1% level of probability, * =Significant at 5% level of probability, NS = Not significant; M_1 = Wet seeding (Line sowing of sprouted seeds in puddled soil), M_2 = Puddled transplanting, M_3 = Unpuddled transplanting; V_1 = Kongnam ene, V_2 = Gelong Ni, V_3 = Gelong Se and V_4 = Ketan hitam.

Genotypes under method M_3 clustered in the positive PC_1 axis, strongly associated with grain yield, biological yield, and harvest index, confirming their superior performance. In contrast, genotypes under method M_1 grouped toward the negative PC_1 axis, linked with higher sterile spikelets and reduced yield efficiency. Method M_2 treatments occupied intermediate positions, reflecting moderate associations with both yield and sterility traits. Overall, the PCA highlighted that yield improvement was primarily driven by method–genotype combinations favoring assimilate partitioning and reduced sterility, while low-performing clusters were constrained by spikelet abortion and poor harvest index (Figure 1).

Pearson correlogram among yield parameters

The correlation matrix revealed strong positive associations among yield-related parameters. Grain yield exhibited highly significant correlations with biological yield (r = 0.92), harvest index (r = 0.92), and straw yield (r = 0.84), underscoring their collective role in yield determination. Similarly, biological yield and harvest index were tightly linked (r = 0.94), reflecting efficient assimilate partitioning. Moderate positive correlations were observed between sterile spikelets and yield traits, though their contribution was less consistent. 1000-seed weight showed significant associations with straw yield (r = 0.68) and biological yield (r = 0.59), but its relationship with grain yield remained weak (r = 0.35). Overall, the analysis emphasized that yield improvement was primarily driven by biological yield and harvest index, while seed weight and sterility exerted secondary influences (Figure 2).

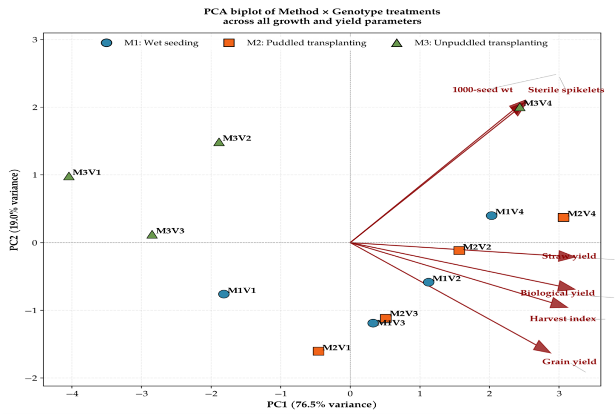

Figure 1. *PCA biplot of method and genotype.*

Hierarchical cluster heat map of treatments and parameters

The hierarchical clustering analysis distinguished clear treatment groupings and parameter associations. High-performing treatments (M_3V_1, M_3V_2, M_3V_3) clustered together, characterized by elevated grain yield, biological yield, and harvest index. In contrast, treatments such as M_1V_4 and M_2V_4 grouped with higher sterile spikelets and lower yield traits, reflecting poor efficiency. Parameter clustering revealed strong alignment among grain yield, biological yield, and harvest index, while straw yield formed a distinct cluster, indicating its contribution to biomass rather than economic yield. 1000-seed weight remained relatively independent, suggesting limited influence on overall productivity. Overall, clustering emphasized that superior yield performance was driven by assimilate partitioning efficiency and reduced sterility, whereas low-yielding treatments were constrained by spikelet abortion and poor harvest index (Figure 3). Also, Veni *et al.* (2025) also reported the similar findings regarding the yield performance in rice.

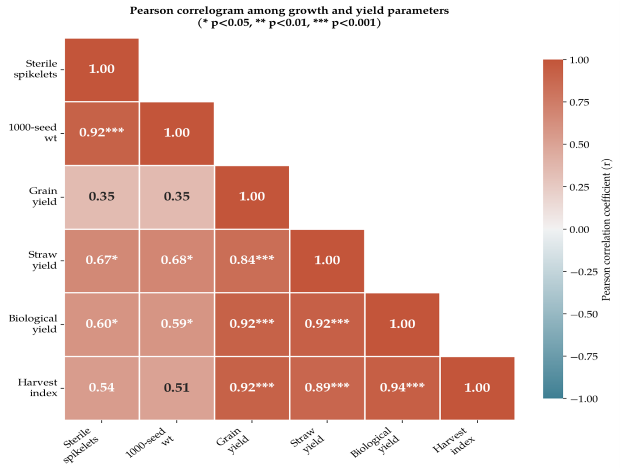

Figure 2. *Pearson correlogram among growth and yield parameters.*

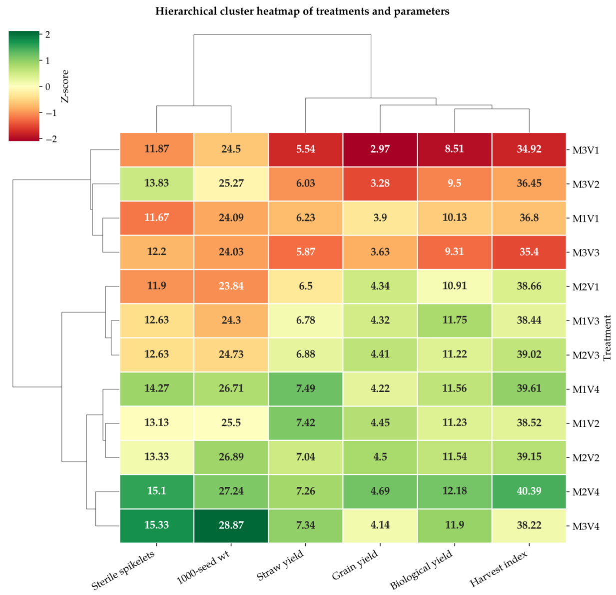

Figure 3. *Hierarchical Cluster heat map of treatments and parameters.*

Pearson correlogram among yield and yield contributing traits

Correlation analysis highlighted clear trait–yield relationships. Grain yield was strongly and positively associated with effective tillers, grains per panicle, and biological yield ($p < 0.001$), underscoring their primary role in yield determination. Straw yield correlated positively with plant height and total tillers, reflecting biomass accumulation. In contrast, non-effective tillers and sterile spikelets were negatively correlated with grain yield ($p < 0.01$), indicating yield penalties. The harvest index showed positive association with grain yield but negative with straw yield, confirming its role in assimilate partitioning. Panicle length moderately correlated with grains per panicle, while 1000-seed weight exhibited weak associations (Figure 4). The results are in agreement with Brunet-Loredo *et al.* (2025) who also reported the yield attributes of rice.

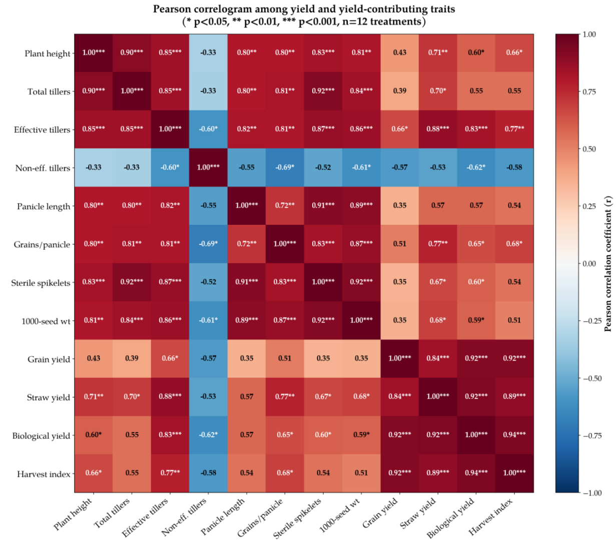

Figure 4. *Pearson correlogram among yield and yield contributing traits.*

Conclusion

Puddled transplanting was the most effective overall establishment method for enhancing growth, yield components, and productivity of hilly black rice, producing 4.47 t ha^{-1} grain yield, compared with 3.53 t ha^{-1} under wet seeding. Among cultivars, Ketan hitam exhibited the greatest yield potential (4.47 t ha^{-1}), biomass production (11.76 t ha^{-1}), and harvest index (40.47%). However, significant establishment × genotype interactions indicate that cultivar performance was management-dependent. Thus, puddled transplanting, particularly with Ketan hitam, provides a promising genotype–management combination for intensifying black rice production in Bangladesh's hilly and marginal ecosystems.

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DECLARATIONS

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