Agriculture and Food

http://www.aimspress.com/journal/agriculture

AIMS Agriculture and Food, 9(4): 1027–1048.
DOI: 10.3934/agrfood.2024055
Received: 24 June 2024
Revised: 21 August 2024
Accepted: 10 September 2024
Published: 12 November 2024

Research article

Grain yield, cooking quality, and aroma of fragrant rice as affected by nitrogen source and method of application

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Abstract: One constraint is the incapacity of existing agronomic studies on rice yield, aroma, and cooking quality to fully assess the effects of various applications and sources of nitrogen (N) fertilizer. It is challenging for us to understand how different N inputs impact rice's sensory and cooking qualities in addition to crop productivity because of this research gap. So, a two-year pot experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka, under an open field plastic net house from July 15 to December 4 in 2020 and 2021. The study used Bangladesh Rice Research Institute (BRRI) dhan70 as the test crop. The experiment examined two factors: the methods and source of N application. Three methods of N application were tested: 100% soil application (NM_1), 2% foliar spray at tillering and booting stages (NM_2), and a combination of 50% soil application and 2% foliar spray at the booting stage (NM_3). Three sources of N were also evaluated: Urea (NS_1), ammonium nitrate (NS_2), and ammonium sulfate (NS_3). Total 41.0 kg N ha^{-1} was applied considering the nitrogen content in Urea (46%), ammonium nitrate (35%), and ammonium sulfate (21%). The experiment followed a randomized complete block design (RCBD) with three replications. The results indicated that the application of N significantly influenced most of the studied parameters. The combined application of N as a foliar dose and soil application, along with ammonium nitrate and ammonium sulfate, showed improved results for various parameters such as gelatinization temperature, gel consistency, protein content, cooking time, imbibition ratio, 2-AP content, grain aroma, and taste of BRRI dhan70 compared to 100% soil application of N. The highest grain yield and 2-AP was observed in the NM_3NS_3 treatment (35.437 $g{\cdot}pot^{-1}$ and 0.137 $\mu g{\cdot}g^{-1}$, respectively) which was statistical similar with NM_3NS_2 treatment while the lowest yield and 2-AP was recorded in NM_1NS_1 (24.877 $g \cdot pot^{-1}$ and 0.076 $\mu g \cdot g^{-1}$, respectively).

Keywords: ammonium sulpahte; ammonium nitrate; 2-AP; aromatic rice

1. Introduction

Fragrant rice, a rare variety highly valued in Asian cultures, is known for its unique flavor, scent, and aroma. It is often used in the preparation of exceptional dishes. As shown by Islam et al. [1], rice quality can be assessed based on grain size, shape, appearance, milling quality, volatile component concentration, and cooking qualities. Aromatic rice, due to its unique attributes, can fetch a premium price in both domestic and international markets. Bangladesh, which imports fragrant rice annually, has recently received government approval to sell fragrant rice to other countries [2]. This could lead to the export of high-quality, aromatic rice grains that retain their cooking qualities [2]. However, due to a lack of efficient agronomic methods, the yield, cooking quality, and aroma content of fragrant rice in Bangladesh are significantly lower and more variable than in other rice-growing nations. Despite these challenges, Bangladesh has a promising future in exporting high-quality, fragrant rice with a unique flavor and aroma. This could help establish its presence in the global rice market. The delightful aroma and rich flavor of fragrant or aromatic rice are attributed to volatile chemical molecules, such as hexanol, octanol, naphthalene succinate, and 2-AP (2-acetyl-1-pyrroline), the latter being the most noticeable volatile component for scent. Standards for exporting aromatic rice include long, thin grains, an intermediate amylase concentration, a high elongation ratio, a strong scent, softness, whiteness, stickiness, and less chalkiness [3].

Fragrant rice's aroma is primarily determined by genetic factors [4], but it can also be significantly influenced by various environmental conditions and farming practices [5]. By carefully selecting a variety and implementing agronomic measures, such as fertilizers, organic compounds, irrigation, and the mode and source of nutrient application, one can enhance the cooking quality and aroma of fragrant rice. In Bangladesh, farmers typically use nitrogenous fertilizers during the final stages of rice production. However, since rice seedlings rely on endospermic energy to complete the recovery stage, they do not absorb this nitrogen fertilizer in the first 8–12 days after transplanting in the field. As a result, more nitrogen than is necessary for the entire cropping season is applied, leading to a significant loss of nitrogen due to denitrification, volatilization, leaching, runoff, and other processes. This not only increases farmers' fertilizer costs and reduces crop yields, but also contributes to groundwater pollution and greenhouse gas emissions. Under climate change conditions, the primary goal is to reduce the amount of nitrogen used in crop production globally. Rice production is significantly impacted by the efficient use of nitrogen. Since N is available continuously throughout the entire crop cycle, it is preferable to apply N as a split dose to yield more rice. To ensure optimal uptake and utilization, as well as maximum output, it is crucial to balance crop demand with N supply [6]. Bao et al. [7] suggest that increasing N application during the booting stage of fragrant rice can enhance its aroma without compromising yield or other qualities. Nutrient-foliar feeding can boost crop productivity and influence the physiological and morphological traits of crops like rice (*Oryza sativa* L.). Foliarly sprayed fertilizer not only enhances crop yields but also reduces the amount of soil-applied fertilizer. Bhuyan et al. [8] found that foliar treatment can expedite the interval between application and plant uptake. Different utilizable sources of nitrogen can affect the scent of fragrant rice [9]. The known effect of nitrogen on the 2-AP level in fragrant rice means that sources such as ammonium bicarbonate (NH_4HCO_3), nitrate (NO_3), and carbamate (urea-H_2NCONH_2) can significantly impact crop growth, yields, and quality. There is limited research on how the scent and cooking attributes of fragrant rice grown in Bangladesh are affected by applying foliar nitrogen fertilizer from various sources. This study aims to explore how different nitrogen methods and sources affect the yield, aroma, and cooking quality of fragrant rice.

2. Materials and methods

2.1. Experimental location with edaphic status and soil sampling

The study was conducted at the research area of Sher-e-Bangla Agricultural University, located in Dhaka-1207, under a net house. The research area is situated between 23°7'N and 93°E in latitude, and the experimental region, at 8.6 meters above sea level, is located in the agroecological zone of the Madhupur tract, AEZ-28. Significant climatic variations occur throughout the summer months at the experimental location, which are from July 15, 2020, to December 4, 2020, and July 14, 2021, to December 5, 2021 (*Aman* season), within the sub-tropical agricultural zone. The monthly meteorological data and the findings of the soil component analysis are shown in Tables 1 and 2.

The normal field soil (soil depth was 0–15 cm, cropping pattern was rice-rice-rice) was used in this pot experiment, where we cleared the surface layer of selected field and then we poured into respected pots. We have taken the soil from different location of selected field and then mixed for pot preparation. After pouring in pots, we supplied our respective pot soil to the analytical division of Soil Resource Development Institute (SRDI), Farmgate, Dhaka, Bangladesh for getting the initial soil components data. After the cultivation of aromatic rice, we collected the post-harvest soil samples from the respective pots and then supplied to SRDI for getting soil components data. The analytical division of SRDI supplied us the results of soil analysis by using the following methods of determination.

Table 1. Meteorological data summary during the experiment period (2020–2021).

Year	Month	Air temperature (°C)		Relative humidity (%)	Total rainfall (mm)
		Maximum	Minimum		
2020 (*Aman* season)	July	28.1	11.1	79.2	373.1
	August	25.4	15.0	78.3	316.5
	September	25.2	11.2	77.1	300.4
	October	23.8	9.6	80.5	67.8
	November	22.7	8.8	60.6	33.8
2021 (*Aman* season)	July	29.7	10.9	76.1	401.1
	August	25.9	14.8	77.7	321.2
	September	25.7	12.6	78.9	311.9
	October	22.9	8.5	82.5	75.5
	November	23.1	8.1	63.1	40.6

Source: Metrological Centre (Climate Division), Agargaon, Dhaka, Bangladesh.

Table 2. Soil analysis results pre- and post- experiment (2020–2021).

Constituents of soil	Pre-planting	After harvesting	Method of determination
2020			
pH	5.70	5.72	Glass electrode method
Organic matter (%)	1.25	1.91	Wet oxidation method
Total nitrogen (%)	0.125	0.248	Micro Kjeldahl method
K (meq/100 g soil)	0.131	0.167	Ammonium acetate extraction method
P (mg/g soil)	6.79	7.88	Ascorbic acid extraction method
S (mg/g soil)	24.39	27.42	Turbidimetric Method
B (mg/g soil)	0.45	0.51	Hot water extraction method
Zn (mg/g soil)	3.43	4.19	Hydrochloric acid extraction method
2021			
pH	5.66	5.71	Glass electrode method
Organic matter (%)	1.31	1.88	Wet oxidation method
Total nitrogen (%)	0.121	0.237	Micro Kjeldahl method
K (meq/100g soil)	0.128	0.154	Ammonium acetate extraction method
P (mg/g soil)	5.71	6.91	Ascorbic acid extraction method
S (mg/g soil)	21.31	26.39	Turbidimetric Method
B (mg/g soil)	0.42	0.53	Hot water extraction method
Zn (mg/g soil)	3.36	4.21	Hydrochloric acid extraction method

Source: Soil Resources Development Institute (SRDI), Framgate, Dhaka, Bangladesh.

2.2. Crop characters

The study utilized Bangladesh Rice Research Institute (BRRI) dhan70, a variety of rice with seeds sourced from the BRRI, Joydebpur, Gazipur. This variety grows to a height of 125 cm, has an erect and long flag leaf, and contains 21.7% amylose in its grain. It is noted for its pleasant aroma, similar to Thailand's jasmine rice.

2.3. Experimental treatments and design

The experiment was designed around two factors. The first factor was the methods of nitrogen application, which had three levels: NM_1 = 100% soil application, NM_2 = 2% (2 g/100 mL of water) foliar spray at tillering and booting stage, and NM_3 = 50% soil application + 2% foliar spray at booting stages. The second factor was the source of nitrogen application, also with three levels: NS_1 = urea, NS_2 = ammonium nitrate, and NS_3 = ammonium sulfate. Total 41.0 kg nitrogen (N) ha^{-1} was applied as per BRRI's recommendation [10], considering the nitrogen content in Urea (46%), ammonium nitrate (35%), and ammonium sulfate (21%). The experiment followed a randomized complete block design (RCBD) and was replicated three times, using a total of 27 pots.

2.4. Agronomic crop husbandry

Healthy seeds were selected using the specific gravity method and soaked in water for a day before being stored in gunny bags. The seeds germinated in 48 hours and were sown 72 hours later. Nutrients were added to a one-meter-wide seedbed based on soil requirements. The sprouting seeds were sown on July 14, 2020, and July 15, 2021, at a rate of 70 $g \cdot m^{-2}$. Each 12-cm-diameter plastic pot used in this research could hold ten kilograms of field soil. Twenty-five-day-old plants were transplanted into a puddled planter, with each pot initially holding three seedlings. After recovery, one healthy seedling was retained in each pot. Nitrogen was supplied to the soil in two parts: half at the base and the other half before flowering. Foliar nitrogen was applied during the stages of tillering and booting. The crop was managed according to BRRI Dhan70 approved fertilizers (10.0 kg P, 40.0 kg K, 8.0 kg S and 1.0 kg Zn from TSP, MoP, Gypsum and Zinc Sulphate, respectively per hectare) [10], based on a hectare slice and a weight of 2.0×10^6 kg of soil per hectare. The pots were watered (when needed for proper growth and development), weeded, and treated with pesticides and fungi as needed. The crop was harvested at full maturity, around when 80% of the seeds turned a golden yellow color, on November 30, 2020, and November 30, 2021. The harvested produce was brought to the threshing floor in separate bundles with proper tags. The grains from each pot were washed, dried, and weighed, with the weight adjusted to include 12% moisture content.

2.5. Data collection

The following parameters have been taken under the present study:

2.5.1. Grain yield ($g \cdot plant^{-1}$)

The harvested grains of a plant in a pot were sun dried and the weight of the total gains in gram was measured by using an electrical balance.

2.5.2. Water uptake ratio (WUR)

The water uptake ratio was determined by cooking 2.0 g of whole rice kernels from each cultivar in 20 mL of distilled water for a minimum cooking time in a boiling water bath, following the methodology used by Oko et al. [11]. After cooking, the surface water of the rice was drained. The cooked samples were then accurately weighed, and the WUR was calculated as follows: Water uptake ratio = weight of cooked rice/weight of uncooked rice.

2.5.3. Imbibition ratio (IR)

Head rice samples (4 g) were cooked in 40 mL of distilled water for a minimum cooking time in a boiling water bath. The cooked rice was then drained, and any remaining surface water was removed by pressing the samples against filter paper. The cooked samples were weighed accurately to calculate the IR [1].

2.5.4. Optimum cooking time (OCT)

Milled rice samples (5g) were placed in a graduated cylinder with 5 mL of water and then placed in a water bath. The cooking time was calculated by removing a few kernels at various points during cooking, once 90% of the rice had gelatinized, and pressing them between two glass plates [1].

2.5.5. Kernel elongation ratio (KER)

The elongation ratio was determined by dividing the cumulative length of 10 cooked rice kernels by the length of 10 uncooked raw kernels [1,12].

2.5.6. Gel consistency (GC)

The gel consistency was determined using the approach by Cagampang et al. [13]. A cold gel in a culture tube, maintained horizontally for 0.5–1 h, was measured, which was less than 61 mm. Ten whole-milled rice grains were ground for forty seconds using a Wig-L-Bug amalgamator to produce fine flour (100 mesh). A powder weighing one hundred milligrams was added to each culture tube in duplicate. Then, 2.0 mL of 0.2 M KOH and 0.2 mL of 95% ethyl alcohol containing 0.025% thymol blue were added using a pipette. The ingredients were mixed using a Vortex Genie mixer set at speed six. The samples were cooked in a rapidly boiling water bath for eight minutes, ensuring that the contents of the tubes reached two-thirds of their height. After removal from the water bath, the test tubes were allowed to stand at room temperature for five minutes. After cooling in an ice-water bath for twenty minutes, the tubes were placed horizontally on a laboratory bench lined with millimeter-sized graphing paper. The entire length of the gel, from the bottom of the tube to the gel front, was measured in millimeters. The GC was classified according to the method reported by Tang et al. [14]: length > 61 mm, soft consistency; 60 > length > 41 mm, medium consistency; length < 40 mm, hard consistency.

2.5.7. Apparent amylose content (AAC)

The amylose content in rice samples was determined using the methodology proposed by Juliano [15]. A volumetric flask containing 100 mg of powdered rice, 1 mL of 1M NaOH, and 1 mL of 95% ethanol was filled with boiling water to gelatinize the starch. Then, 5 mL of starch extract was added to a 100 mL volumetric flask. To bring the volume up to 100 milliliters, two milliliters of iodide solution and one milliliter of 1N acetic acid were added. The mixture was shaken and allowed to sit for twenty minutes. The amylose content was calculated using a standard curve of potato amylose and expressed as a percentage. The absorbance was measured at 620 nm using an Agilent Technologies Cary 60 UV-VIS spectrophotometer.

2.5.8. Alkali spreading value (ASV) and gelatinization temperature (GT)

The ASV was calculated using the methodology of Chemutai et al. [16]. Six whole-milled kernels free of cracks were selected and placed in a 5 cm × 5 cm × 2.5 cm plastic box. A 1.7% potassium hydroxide (KOH) solution was added to a volume of 10 mL. The samples were arranged to allow room for the kernels to spread. The covered boxes were then placed in an oven set at 30℃ for 23 hours. The starchy endosperm was visually evaluated using a 7-point numerical spreading scale. Alkali spreading rating of 1–3 was classified as high (75–79 ℃), 4–5 as intermediate (70–

74 °C), and 6–7 as low (55–69 °C) GT [17].

2.5.9. Protein content (%)

The protein content was determined using a near-infrared grain tester (AN-820, Kett Co., Ltd., Tokyo), a method also utilized by Nakamura et al. [18].

2.5.10. Grain 2-AP content (μg·g^{-1})

Before analysis, the grains were ground using a mortar and pestle following Huang et al.'s [19] procedure to determine the grain's 2-AP concentration. Approximately 10 g of grains and 150 mL of purified water were thoroughly mixed and then added to a 500 mL round-bottom flask, which was affixed to an extraction head for continuous steam distillation. The mixture was heated to 150 °C in an oil pot. Meanwhile, a 500-mL round-bottom flask attached to the opposite head of the continuous steam distillation equipment received the extraction solvent, a 30 mL aliquot of dichloromethane. This flask was then boiled in a water pot to 53 °C. To maintain a temperature of 10 °C, the continuous steam distillation extraction system was coupled to a cold water circulation unit. After approximately 35 min, the extraction process was completed. Anhydrous sodium sulfite was introduced to the extract to absorb the water. The dried extract underwent filtration using an organic needle filter, and the 2-AP content was determined using the GCMS-QP 2010 Plus. High purity helium gas, flowing at a rate of two milliliters per minute, served as the carrier gas. The temperature gradient of the GC oven proceeded as follows: 40 °C (1 min), increased at 2 °C min^{-1} to 65 °C and maintained at 65 °C for 1 min, then raised to 220 °C at 10 °C min^{-1}, and finally held at 220 °C for 10 min. The retention time of 2-AP was confirmed at 7.5 min. Each sample underwent three replications, and 2-AP was expressed as μg·g^{-1}.

2.5.11. Aroma (Retronasal) and taste (Orthonasal)

Five grams of rice were mixed with 15 milliliters of water, soaked for ten minutes, cooked for fifteen minutes, transferred to a Petri dish, and refrigerated for twenty minutes. Subsequently, the cooked rice was evaluated by a random panel of individuals who categorized it as other than basmati (OTB), slightly scented (MS), strongly fragrant (SS), or non-scented (NS) [20]. Ten graduate and undergraduate students were randomly selected to serve on the panel and provided with the samples. The responses from each of the two replications, each comprising five students, were aggregated into the SS, MS, NS, and OTB categories as previously described. The same procedure was employed to assess whether the cooked rice exhibited sour, salty, or sweet characteristics.

2.5.12. Percent grain yield and grain 2-AP increase

The percentage increase in grain yield and the percentage increase in grain 2-AP were calculated using an excel spreadsheet. To evaluate the better combination, here we used 100% soil application as compared treatment and other two methods as expected treatment.

$$\textbf{(\%) increase} = \frac{(\text{Value of expected treatment} - \text{value of compared traetment})}{\text{Value of compared treatment}} \times 100$$

2.5.13. Statistics used

The data obtained for different parameters were subjected to statistical analysis using the computer-based software Statistix-10 (Analytical Software, Tallahassee, FL, USA) employing the ANOVA technique, and means were adjusted by LSD at a 5% level of significance. Correlation analysis was performed using the mean values of the investigated parameters to examine the correlation between different grain quality traits, also utilizing Statistix-10.

3. Results

3.1. Grain yield (g·plant^{-1})

The application of nitrogen from various sources and in different methods (Table 4) significantly influenced the grain yield of fragrant rice. The highest grain yield was observed in the NM_3NS_3 treatment (35.437 g·pot^{-1}), statistically similar to NM_3NS_2 (34.227 g·pot^{-1}), NM_3NS_1 (33.647 g·pot^{-1}), and NM_2NS_3 (34.667 g·pot^{-1}), while the lowest yield was recorded in NM_1NS_1 (24.877 g·pot^{-1}).

3.2. WUR

The quantity of water absorbed by rice during boiling directly impacts cooking quality, affecting the expansion of cooked rice and cooking duration. Comparing WURs across different nitrogen sources and application methods revealed significant differences (Table 4). The highest water uptake ratio was observed in the NM_1NS_1 treatment (7.526), statistically similar to NM_1NS_2 (7.276), while the lowest ratios were found in NM_3NS_3 (4.236) and NM_3NS_2 (4.236).

3.3. IR

An important indicator of fragrant rice cooking quality is the ratio of cooked grain length to milled grain swelling, reflecting water absorption by rice kernels (imbibition ratio). Analysis of IRs across nitrogen application methods and sources showed significant impacts (Table 4). While the combined effect was not statistically significant, both nitrogen methods and sources exerted a notable influence. NM_1NS_1 treatment exhibited the highest imbibition ratio (4.796), while NM_3NS_3 showed the lowest ratio (3.396).

3.4. OTC

Culinary customs vary across countries, influencing food quality. Factors like amylose content, protein levels, GC, GT, and ASV affect rice cooking and taste. Cooking time, influenced significantly by nitrogen sources and methods (Table 4), varied. The shortest cooking time was observed for rice from the NM_3NS_3 treatment (13.030 min), while the longest was recorded for NM_1NS_1 (18.870 min.), favoring shorter cooking times for practical cooking.

3.5. KER

The KER, comparing cooked to uncooked rice length, is another valuable indicator of cooking quality. Significant correlations were observed between nitrogen application methods and sources and KER (Table 4). The highest ratio was observed in the NM_3NS_3 treatment (2.406), statistically similar to NM_3NS_2 (2.286), while the lowest was in NM_1NS_1 (1.336), indicating a preference for longer-cooked kernel.

3.6. GC

Gel consistency plays a crucial role in aromatic rice cooking quality. Significant impacts on GC were observed across nitrogen application methods and sources (Table 4). The highest consistency was seen in NM_3NS_3 treatment (97.827 mm), similar to NM_3NS_2 (96.907 mm), while the lowest was in NM_1NS_1 (75.557 mm).

3.7. Apparent amylose content

The hardness and softness of cooked rice kernels, which significantly affect the eating experience, are influenced by the apparent amylose concentration. The study found that the methods and sources of nitrogen application had a substantial impact on the perceived amylose content (Table 5). The highest AAC was observed in the NM_1NS_1 (26.137%) treatment, which was statistically similar to NM_1NS_2 (25.127%). The lowest amylose content was found in the NM_3NS_3 (20.027%) treatment, which was statistically similar to NM_3NS_2 (20.167%) and NM_3NS_1 (20.407%).

3.8. ASV

The ASV is used to estimate the GT, a key determinant of cooking quality. A low ASV corresponds to a high GT, while a high ASV indicates a low GT, as per Oko et al. [11]. The study found significant variations in ASV based on the methods and sources of nitrogen application (Table 5). The highest ASV was observed in the NM_3NS_3 (7.0) treatment, which was statistically similar to NM_3NS_2 (6.8), while the lowest ASV was found in the NM_1NS_1 (3.0) treatment.

3.9. GT

The GT, a critical factor in assessing the cooking quality of fragrant rice, was found to be significantly influenced by the methods and sources of nitrogen application (Table 5). A low GT was observed in the NM_3NS_3 and NM_3NS_2 treatments, while a high GT was found in the NM_1NS_1, NM_1NS_2, and NM_1NS_3 treatments. Other treatments exhibited an intermediate GT.

Table 3. Analysis of variance (ANOVA) for grain yield, cooking qualities and grain 2-AP content of aromatic rice (BRRI dhan70).

Source	**Grain yield**			**Water uptake ratio**			**Imbibition ratio**			**Optimum cooking time**			**Kernel elongation ratio**		
	Mean Square	F value	P value	Mean Square	F value	P value	Mean Square	F value	P value	Mean Square	F value	P value	Mean Square	F value	P value
Replication	0.181			0.0073			0.00348			0.0584			0.00088		
N Method	195.201	91.97	0.0000	13.3651	196.47	0.0000	2.92000	99.39	0.0000	34.4800	67.09	0.0000	1.52003	292.16	0.0000
N Source	7.190	3.39	0.0593	1.7263	25.38	0.0000	0.19000	6.47	0.0087	5.3374	10.38	0.0013	0.19723	37.91	0.0000
Interaction	11.853	5.58	0.0052	0.1994	2.93	0.0539	0.03500	1.19	0.3523	1.5363	2.99	0.0509	0.02933	5.64	0.0050
Error	2.122			0.0680			0.02938			0.5140			0.00520		
Source	**Gel consistency**			**Apparent amylose content**			**Alkali spreading value**			**Protein content**			**Grain 2-AP content**		
	Mean Square	F value	P value	Mean Square	F value	P value	Mean Square	F value	P value	Mean Square	F value	P value	Mean Square	F value	P value
Replication	1.965			0.0851			0.0022			0.0332			0.000000588		
N Method	496.878	24.19	0.0000	48.2757	53.23	0.0000	21.9900	691.99	0.0000	19.3297	112.44	0.0000	0.004735	608.9389	0.0000
N Source	93.249	4.54	0.0274	1.7296	1.91	0.1808	2.2900	72.06	0.0000	1.1941	6.95	0.0067	0.000797	102.4437	0.0000
Interaction	58.618	2.85	0.0583	2.7242	3.00	0.0502	0.2800	8.81	0.0006	0.4919	2.86	0.0578	0.000463	59.51768	0.0000
Error	20.538			0.9069			0.0318			0.1719			0.000007775		

Note: N indicates Nitrogen.

Table 4. Impact of nitrogen application methods and sources on BRRI dhan70 grain yield and cooking quality (*average data from a two-year experiment)*.

Treatments	**Grain yield (g·plant^{-1})**	**Water uptake ratio (WUR)**	**Imbibition ratio (IR)**	**Optimum cooking time (OCT) (Min.)**	**Kernel elongation ratio (KER)**	**Gel consistency (GC) (mm)**
NM_1NS_1	24.877 c	7.526 a	4.796	18.870 a	1.336 e	75.557 d
NM_1NS_2	25.117 c	7.276 a	4.696	16.527 b	1.386 de	76.337 cd
NM_1NS_3	25.887 c	6.746 b	4.696	16.027 bc	1.486 d	83.627 bc
NM_2NS_1	28.677 b	6.286 c	4.196	15.777 bc	1.506 d	85.547 b
NM_2NS_2	30.867 b	5.976 cd	3.996	15.107 cd	1.676 c	84.587 b
NM_2NS_3	34.667 a	5.766 d	3.996	14.407 df	1.736 c	84.007 bc
NM_3NS_1	33.647 a	5.536 d	3.896	13.347 ef	1.916 b	85.177 b
NM_3NS_2	34.227 a	4.466 e	3.496	13.307 ef	2.286 a	96.907 a
NM_3NS_3	35.437 a	4.236 e	3.396	13.030 e	2.406 a	97.827 a
CV (%)	4.80	4.36	4.15	4.73	4.12	5.30
LSD $_{(0.05)}$	2.521	0.451	0.296	1.240	0.124	7.844
F-test (AB)	**	*	NS	*	**	*
Nitrogen method (A)	**	**	**	**	**	**
Nitrogen source (B)	*	**	**	**	**	*

Notes: In a column, having similar letter(s) is statistically similar, and having dissimilar letter(s) differs significantly at the 0.05 level of probability. * indicates significant at the 5% level of probability; ** indicates significant at the 1% level of probability; NS indicates non-significant. NM_1 = soil application (100%), NM_2 = foliar application of 2% spray solution (SS) at tillering and booting stage and NM_3 = soil application (50%) + foliar application of 2% SS at booting stage; NS_1 = urea, NS_2 = ammonium nitrate and NS_3 = ammonium sulphate.

Table 5. Effect of nitrogen application methods and sources on BRRI dhan70 cooking quality and grain 2-AP content (average data from a two-year study).

Treatments	Apparent amylose content (AAC) (%)	ASV	Gelatinization Temperature (GT)	Protein content (%)	Grain 2-AP content ($\mu g \cdot g^{-1}$)
NM_1NS_1	26.137 a	3.000 g	High	7.784 d	0.076 f
NM_1NS_2	25.127 a	3.500 f	High	7.857 d	0.079 ef
NM_1NS_3	23.217 b	3.500 f	High	7.885 d	0.081 e
NM_2NS_1	22.107 b	4.100 e	Intermediate	8.036 d	0.086 d
NM_2NS_2	22.077 b	4.500 d	Intermediate	8.107 cd	0.088 cd
NM_2NS_3	22.777 b	5.000 c	Intermediate	8.786 c	0.091 bc
NM_3NS_1	20.407 c	5.500 b	Intermediate	9.724 b	0.095 b
NM_3NS_2	20.167 c	6.800 a	Low	10.999 a	0.135 a
NM_3NS_3	20.027 c	7.000 a	Low	11.022 a	0.137 a
CV (%)	4.24	3.74	---	4.65	2.89
LSD (0.05)	1.648	0.308	---	0.717	0.0251
F-test (AB)	*	**	---	*	**
Nitrogen method (A)	**	**	---	**	**
Nitrogen source (B)	NS	**	---	**	**

Note: In a column, having similar letter(s) is statistically similar, and having dissimilar letter(s) differs significantly at the 0.05 level of probability. * indicates significant at the 5% level of probability; ** indicates significant at the 1% level of probability; NS indicates non-significant, NM_1 = soil application (100%), NM_2 = foliar application of 2% spray solution (SS) at tillering and booting stage and NM_3 = soil application (50%) + foliar application of 2% SS at booting stage; NS_1 = urea, NS_2 = ammonium nitrate, and NS_3 = ammonium sulfate.

3.10. Protein content

The grain protein concentration significantly limits the overall acceptance of aromatic rice, particularly concerning health issues. The study observed a notable effect of the methods and sources of nitrogen application on the protein content (Table 5). The highest protein content was found in the NM_3NS_3 (11.022%) treatment, which was statistically similar to NM_3NS_2 (10.999%). The lowest protein content was found in the NM_1NS_1 (7.784%) treatment, which was statistically similar to NM_1NS_2 (7.857%), NM_1NS_3 (7.885%), and NM_2NS_1 (8.036%).

3.11. Grain 2-AP content

When fragrant rice is boiled, the grain's 2-AP content releases a delightful aroma, leading consumers to prefer rice varieties with stronger aromas. The study observed significant differences in the grain 2-AP content based on the methods and sources of nitrogen application (Table 5). The highest grain 2-AP content was found in the NM_3NS_3 (0.137 $\mu g \cdot g^{-1}$) treatment, which was statistically similar to NM_3NS_2 (0.135 $\mu g \cdot g^{-1}$). The lowest 2-AP content was found in the NM_1NS_1 (0.076 $\mu g \cdot g^{-1}$) treatment, which was statistically similar to NM_1NS_2 (0.079 $\mu g \cdot g^{-1}$).

3.12. Aroma (Retronasal-by nose)

Since 2-AP is the precursor of grain aroma, the results of a random panel test provide an estimate of the amount of 2-AP in aromatic rice grains. It is believed that a higher grain scent corresponds to a higher grain 2-AP content. The grain scent has been significantly impacted by the application of nitrogen in various forms and from various sources (Table 6). The stronger aroma was found in the NM_3NS_3, NM_3NS_2, NM_3NS_1, and NM_2NS_3 treatments, while NM_1NS_1, NM_1NS_2, NM_2NS_1, and NM_2NS_2 treatments resulted in a mild aroma upon cooking.

3.13. Taste (Orthonasal-by mouth)

A random panel test revealed that the methods and sources of nitrogen have an impact on the flavor of cooked rice kernels (Table 6). Consumers typically prefer sweet cooked rice over other varieties when offered as a market option, and they prefer good quality, fragrant rice. From the panel test, it is clear that NM_3NS_3, NM_3NS_2, NM_2NS_3, NM_2NS_2, NM_1NS_3, and NM_1NS_2 treatments performed better for the taste of cooked rice. This indicates that the foliar application of nitrogen in the form of nitrate and sulfate, along with soil, has a significant impact on this feature, resulting in a sweet kernel after cooking, whereas other treatments produced a salty taste.

Table 6. Evaluation of nitrogen application methods and sources on BRRI dhan70 kernel aroma and taste (panel evaluation from a two-year study).

Treatments	Aroma (Retronasal-by nose)	Taste (Orthonasal-by mouth)
NM_1NS_1	MS	Salty
NM_1NS_2	MS	Sweet
NM_1NS_3	OTB	Sweet
NM_2NS_1	MS	Salty
NM_2NS_2	MS	Sweet
NM_2NS_3	SS	Sweet
NM_3NS_1	SS	Salty
NM_3NS_2	SS	Sweet
NM_3NS_3	SS	Sweet

Note: NM_1 = soil application (100%), NM_2 = foliar application of 2% spray solution (SS) at tillering and booting stage and NM_3 = soil application (50%) + foliar application of 2% SS at booting stage; NS_1 = urea, NS_2 = ammonium nitrate, and NS_3 = ammonium sulphate. MS, mildly scented; SS, strong scented; OTB, other than basmati.

3.14. Grain yield increase (%) and grain 2-AP increase

The results indicated that the methods and sources of nitrogen have a significant influence on the percentage of yield and grain 2-AP increase of aromatic rice (Figures. 1 and 2). According to the calculation, the maximum percent of yield increase was found in the NM_2NS_3 (38.02%) combination, which was near NM_3NS_3 (36.89%), followed by NM_3NS_1 (33.96%) and NM_3NS_1 (32.21%). On the other side, the maximum percent of grain 2-AP increase was exhibited in the NM_3NS_3 (69.13%) combination, which was near NM_3NS_2 (66.66%). From the present study, it is clear that the application of nitrogen from three sources and foliar application along with soil performed better than 100% soil application of nitrogen.

3.15. Pearson correlation (r)

This study demonstrated that the application of nitrogen, from various sources and in different methods, influenced the cooking quality and aroma of fragrant rice. Various traits related to cooking quality and aroma was found to correlate either positively or negatively with each other (Table 7). Grain yield (GY) showed a negative correlation with WUR, IR, OCT, and AAC but a positive correlation with

KER, GC, ASV, protein content, and grain 2-AP. WUR was positively correlated with IR, OCT, and AAC, but negatively correlated with KER, GC, ASV, protein content, and grain 2-AP. IR showed a positive correlation with OCT and AAC but a negative correlation with KER, GC, ASV, protein content, and grain 2-AP. OCT was positively correlated with AAC but negatively correlated with KER, GC, ASV, protein content, and grain 2-AP. KER showed a negative correlation with AAC but a positive correlation with GC, ASV, protein content, and grain 2-AP. GC was negatively correlated with AAC but positively correlated with ASV, protein content, and grain 2-AP. AAC showed a negative correlation with ASV, protein content, and grain 2-AP. ASV and protein content both showed a positive correlation with grain 2-AP. Contrary to Shivani et al.'s [21] report of a positive relationship among water uptake, amylose content, and GC, this study hypothesized that kernels with higher GC require less water to cook and contain a lower percentage of amylose, resulting in more stickiness after cooking. In line with Renuka et al.'s [22] depiction that the amount of soluble protein increases the amount of the 2-AP precursor in aromatic rice, this study also found a positive correlation between protein content and grain 2-AP content.

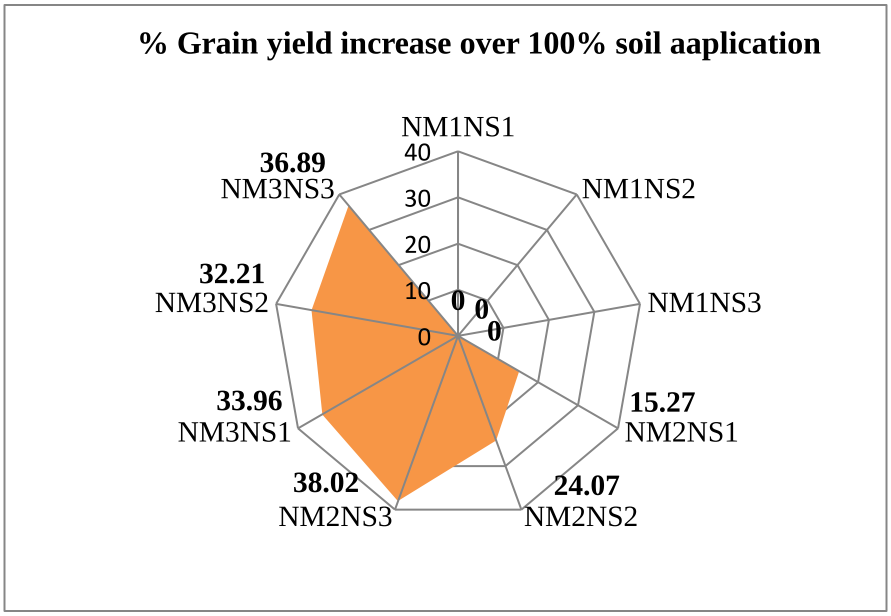

Figure 1. Impact of nitrogen application methods and sources on BRRI dhan70 grain yield increase percentage (average data from a two-year study).

Notes: NM_1 = soil application (100%), NM_2 = foliar application of 2% spray solution (SS) at tillering and booting stage and NM_3 = soil application (50%) + foliar application of 2% SS at booting stage; NS_1 = urea, NS_2 = ammonium nitrate and NS_3 = ammonium sulphate.

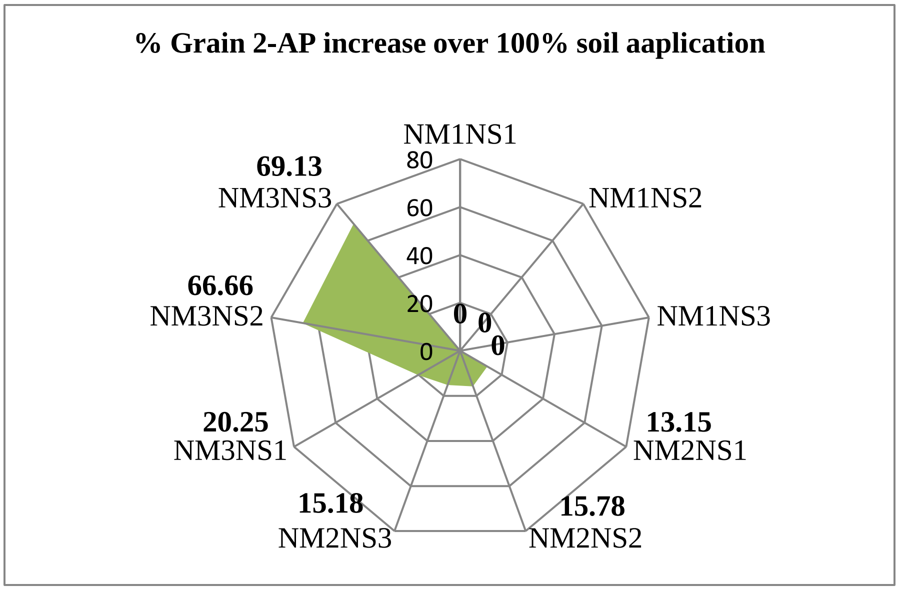

Figure 2. Effect of nitrogen application methods and sources on BRRI dhan70 2-AP increase percentage (average data from a two-year study).

Notes: NM_1 = soil application (100%), NM_2 = foliar application of 2% spray solution (SS) at tillering and booting stage and NM_3 = soil application (50%) + foliar application of 2% SS at booting stage; NS_1 = urea, NS_2 = ammonium nitrate and NS_3 = ammonium sulphate.

Table 7. Correlation matrix for various cooking quality traits and grain 2-AP content of aromatic rice.

	GY	**WUR**	**IR**	**OCT**	**KER**	**GC**	**AAC**	**ASV**	**Protein**	**Grain 2-AP**
GY	1.0000									
WUR	−0.7673*	1.0000								
IR	−0.7608*	0.9786**	1.0000							
OCT	−0.7435*	0.9201**	0.9118**	1.0000						
KER	0.8056*	−0.9219**	−0.8614**	−0.7820*	1.0000					
GC	0.7230*	−0.7703*	−0.6760*	−0.5875*	0.9004**	1.0000				
AAC	−0.6736*	0.9220**	0.9187**	0.9477**	−0.7568*	−0.6089*	1.0000			
ASV	0.8479**	−0.9471**	−0.9065**	−0.8369**	0.9885**	0.8728**	−0.7999*	1.0000		
Protein	0.7876*	−0.8436*	−0.7718*	−0.6943*	0.9754**	0.8840*	−0.6593*	0.9601**	1.0000	
Grain 2-AP	0.6652*	−0.8946**	−0.8289**	−0.7040*	0.9679**	0.8894*	−0.7001*	0.9505**	0.9513**	1.0000

Note: grain yield (GY), water uptake ratio (WUR), imbibition ratio (IR), optimum cooking time (OCT), kernel elongation ratio (KER), gel consistency (GC), apparent amylose content (AAC), alkali spreading value (ASV). * significant at $p < 0.05$ where n = 9. ** significant at $p < 0.01$ where n = 9.

4. Discussion

Typically, various fertilizers are applied to the surface of fields, but a majority of these fertilizers are lost due to different chemical reactions in the soil. Therefore, alternative strategies need to be considered to retain more nutrients in the soil for healthy crop growth and quality. In light of this, we conducted a two-year pot trial using a range of aromatic rice to demonstrate the impact of different nitrogen application methods and sources. Our investigation revealed that foliar application in conjunction with soil application outperformed 100% soil application as a mode of nitrogen application. This could be because rice leaves absorb more nitrogen when it is applied foliarly, providing the right nutrients for the crop during various stages of the rice plant's growth. In terms of nitrogen sources, ammonium nitrate and sulfate performed better than traditional urea. We also discovered that ammonium sulfate and ammonium nitrate had better cooking properties than urea. This could be due to fewer nitrogen losses, leading to higher soil and crop uptake of nitrogen and increased nitrogen absorption. Our findings concur with those of other investigators. According to Hafez and Kobata's [23], urea lost more nitrogen than ammonium sulfate. When administered nitrogen levels increased, so did the amount of nitrogen absorbed by plants; this was more in plants given nitrogen from ammonium sulfate than from urea. Additionally, extra sulfur is supplied to the soil reaction via ammonium sulfate. According to Jamal et al. [24], the availability of sulfur in soil also enhances the availability of nitrogen. Hafez and Kobata [23] suggested that ammonium sulfate should be twice as effective as urea or ammonium nitrate in acidifying soil. They also proposed that, in spring wheat cultivars, ammonium sulfate is a more effective way to enhance the number of spikelets than urea. These findings align with our current investigation. The findings of this study align with previous research [25,26], which found that the foliar application of N enhances grain production more effectively than traditional soil treatment. Compared to the conventional method of applying 100% of N in the soil, the combined application of N in the soil and foliage significantly increased the GY. Alam et al. [27] found that the topical application of a 2% urea solution, along with soil application, greatly improved GY. Sandoval-Contreras et al. [28] identified ammonium sulfate as the nitrogen source that, under field conditions, led to higher increases in tillering, dry mass of shoots, N content in the dry mass of shoots, and GY. Furthermore, El Sharkawi and Zayed [29] reported that rice plants treated with $(NH_4)_2SO_4$ outperformed those treated with NH_4NO_3 or $Ca(NO_3)_2$ in terms of photosynthesis, growth, yield, and yield components.

According to Oko et al. [11], a low ASV is associated with a high gelatinization temperature, and a high ASV is associated with a low GT. We observed similar trends in both years of our study. AAC and IR were found to correlate positively, as reported by Shozib et al. [30]. This suggests that rice with a higher AAC content will absorb more water during cooking, thereby increasing the volume of cooked rice. Zhu et al. [31] reported a strong positive correlation between AAC and the quality of fragrant rice during cooking or eating. Tayefe et al. [32] found that the amount of AAC decreased as the availability of nitrogen increased. We observed a similar outcome: foliar treatment improved the supply of nitrogen, which in turn decreased the AAC. This could be because the foliar application of grain protein is increasing, thereby lowering the AAC value. The tendency of cooked rice to solidify upon cooling is also measured by its GC. Cooked rice tends to be softer and stickier with a high GC, while firmer cooked rice is associated with a firmer GC. This characteristic is especially noticeable in high-amylose rice, which requires longer boiling times [33,34]. Similar trends were observed in our research. The use of rice grains with intermediate amylose (19%–22%) in infant food and senior citizen diet formulations has become more common in recent years in Bangladesh [29]. Changlan et al. [35] reported that rice types with low amylose concentrations often have good eating and cooking qualities, while those with high amylose content have poor eating and cooking qualities. Matin et al. [36] found a negative correlation between ASV and cooking time, indicating that varieties with lower alkali spreading values required longer cooking times. Similar trends were observed in our study. The N fertilizer applied during the booting stage boosted the 2-AP content without reducing the fragrant rice grain production [37]. The N treatment also had an impact on the production of 2-AP in fragrant rice [38]. Mo et al. [39] found that varying N treatment levels altered the 2-AP content in fragrant rice. Ren et al. [40] revealed that administration of N at high doses during the tillering stage may significantly increase the 2-AP content in grain. This is consistent with our findings.

5. Conclusions

The two-year experiment demonstrated that the application of nitrogen using different methods and sources significantly influenced the grain yield, cooking quality, and aroma of fragrant rice. The study concluded that the combined application of N (41 kg·ha^{-1}) as a foliar dose (2%) and soil application (50%), along with ammonium nitrate and ammonium sulfate, showed improved results for various parameters, such as gelatinization temperature, gel consistency, protein content, cooking time, imbibition ratio, 2-AP content, grain aroma, and taste of BRRI dhan70 compared to 100% soil application of N. The highest grain yield and 2-AP was observed in the NM_3NS_3 treatment which was statistical similar with NM_3NS_2 treatment while the lowest yield and 2-AP was recorded in NM_1NS_1. The maximum percent of yield increase was found in the NM_2NS_3 combination and the maximum percent of grain 2-AP increase was exhibited in the NM_3NS_3 combination. Furthermore, it is suggested that additional research should be conducted on other sources of nitrogen and their physiological efficiency.

Author contributions

Rajesh Chakraborty: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing-original draft; Tuhin Suvra Roy: Review; Jun-Ichi Sakagami: Conceptualization, Supervision, Fund acquisition, Critical review and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The first author expresses his gratitude to the Japan Society for the Promotion of Science for offering full funding in the form of a Dissertation Ph.D. fellowship through the RONPAKU program (Grant Number: 12204) to conduct this study. The author expresses gratitude to the GQN (Grain Quality and Nutrition) section of the Bangladesh Rice Research Institute (BRRI) in Gazipur, Bangladesh, for providing technical assistance with the chemical analysis.

Conflict of interest

The authors declare no conflict of interest in the present research work.

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