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Research Article

Organoleptic and grain quality traits of aromatic rice varieties as influenced by supplementation of Zn and 2-acetyl-1-pyrroline

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Abstract

Aromatic rice fetches premium prices in world markets due to its pleasant smell and enchanting flavor. In Bangladesh, the quality of aromatic rice is much inferior than those of other rice growing countries because of lack of improved variety and judicious agronomic management. Selection of appropriate variety and supplementation zinc (Zn) and 2-acetyl-1-pyrroline (2-AP) can improve the aroma. The present study exhibited the effects of 2-AP and Zn supplementation on yield and quality of aromatic rice. Two well-known aromatic cultivars, BRRI dhan80 and BRRI dhan34, were cultured separately in pot supplemented with 2-AP and Zn. The results showed that supplementation of 2-AP and /or Zn along with conventional practices had significant effects on organoleptic and some quality parameters studied in this study. The concentration of 2AP and Zn in rice grain increased with increasing their application rate. Additionally, grain 2-AP concentrations were significantly and positively correlated with organoleptic characters. Interactions of both these elements with the complex process of 2-AP formation remain to be explored.

Keywords: Zn; 2-acetyl-1-pyrroline; grain 2-AP; protein; amylase; aromatic rice.

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Introduction

Fine rice is mainly used by the people in the preparation of palatable dishes and sold at a higher price in the market due to its special appeal for aroma and acceptability. Rice imports hit the all-time high during the current financial year. So, the country has imported 3.7 million tons and the import might surpass 4.0 million tons by the end of next June month. So far, the public sector has imported over 1.0 million tons, while the private sector import also increased to a record 2.65 million tons, of which 90 per cent were from India and of which demand for the fine-quality rice now is around 30% (1). Most of the rice exported in the international market is par-boiled (Atap) and sticky rice. But 95 per cent of rice produced in Bangladesh is hard grained with hardly any demand in the international market. However, Bangladesh exports about 50,000 tons of fragrant rice to the Middle East, the US and Europe every year. The main consumers of this rice are expatriate Bangladeshis (2). Still Bangladesh has a bright prospect for export of this fine rice thereby earning foreign exchange. Islam (3) observed that the yield of aromatic rice is much lower than those of other rice growing countries because of lack of improved variety and judicious fertilizer management. Selection of appropriate variety and application of integrated nutrient management, the yield can be increased. Another drawback is that the aroma of rice is gradually decreased with advancing storage period. Researchers have identified approximately 200 different volatile compounds that contribute to rice aroma. Mathure (4, 5) reported that 2-acetyl-1-pyrroline (2-AP), (E)-2-nonenal, octanal, decanal (E, E)-2-decenal, hexanal, 4-vinylphenol, guaicol, indole and vanillin etc are the key contributors to rice aroma. Among these complicated multiple volatile substances that have been detected in fragrant rice, 2-AP was identified as the most prominent compound contributing to aromatic characters of scented rice (6, 7). The 2-AP production is genetically controlled but the concentration is also actually affected by other factors such as environment, climate, location and nutrient elements (8). These findings imply that good management on cultivation conditions could enhance 2-AP production and increase yield in fragrant rice (9). So yield and grain quality of aromatic rice may be improved by applying optimum amount of specific fertilizers and can also increase the aroma by applying with 2-AP in soil. Shivay (10) observed that the application of 7.5 kg Zn ha^{-1} increased Zn concentrations in the grain and straw of aromatic rice and Yadi *et al.* (11) also reported that the application of Zn is better for grain yield and some quality traits of rice. So, zinc may have the positive relation with aroma of rice. Moreover Yoshihashi (9) pointed out that N, P, Ca, Zn, Mn and Mg affects aroma formation in aromatic rice and showed a significant relationship with 2-AP biosynthesis. Among these, Zn is the key input for improving the grain quality of aromatic rice. Therefore, a study plan will be undertaken to find out the effect of Zn and 2-AP supplementation on organoleptic and grain quality traits of aromatic rice varieties.

2. Materials and Methods

2.1 Experimental period and site

The pot experiment was conducted in the farm shade house of Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, at *Aman* season during June, 2018 to November, 2018. The experimental area was belonged to 23°7'N latitude and 93°E' longitude at an altitude of 8.6 meter above the sea level. The experimental site belongs to the agro-ecological zone of "Madhupur Tract", AEZ-28. The soil was also characterized by pH-5.62 and organic carbon-0.456% (Analyzed from Soil Resources Development Institute, Dhaka). The research area is under sub-tropical humid climatic conditions with a total annual rainfall was 24.23 mm with average monthly maximum and minimum temperature of 29.64°C and 14.91°C, respectively.

2.2 Planting material

Popular two aromatic rice varieties *viz.,* BRRI dhan34 and BRRRI dhan80 were used in this study. The seeds of the test crop *i.e.*, BRRI dhan34 and BRRRI dhan80 were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur (Plant authentication number: Khaskani used to select BRRI dhan34 by local selection process and BR7697-15-4-4-2-2 for BRRI dhan80).

2.3 Treatments and design

The present experiment comprised of three factors

Factor A: Aromatic rice varieties (2)	**Factor B: Zinc dose (3)**	**Factor B: 2-AP concentration (3)**
1. BRRI dhan34	1. Zn_0= 10 kg $ZnSO_4$/ha(Recommended dose) + 0kg $ZnSO_4$/ha (supplementation)	1. AP_0=0 ml 2-AP mother solution/L water in 5 kg pot soil
2. BRRI dhan80	2. Zn_1=10 kg $ZnSO_4$/ha + 2 kg $ZnSO_4$/ha (supplementation)	2. AP_1= 0.503 ml 2-AP mother solution/L water in 5 kg pot soil
	3. Zn_2= 10 kg $ZnSO_4$/ha+ 4 kg$ZnSO_4$/ha (supplementation)	3. AP_2= 1.006 ml 2-AP mother solution/L water in 5 kg pot soil

The experiment was laid out in a 2×3×3 factorial design with three replications. There were 18 treatment combinations. The total numbers of unit pots were 54.

2.4 Crop management

Healthy seeds were selected by specific gravity method and then immersed in water bucket for 24 hours and then those were kept tightly in gunny bags. The seeds started sprouting after 48 hours and were sown after 72 hours. The seedbed was prepared with 1 m wide adding nutrients as per the requirements of soil. Seed were sown in the seed bed @ 70 g m^{-2} on 10 June, 2018. Cowdung was applied in unit pot @ 8 ton per hectare and chemical fertilizer as urea, TSP, MOP, Gypsum, and Zinc sulphate, was applied @ 270-130-120-70-10 kg per hectare, respectively. All the fertilizer was applied as basal dose except urea which was applied as top dressing in 3 equal instalments and at 15 Days after transplanting (DAT), tillering and panicle initiation stages. The seedlings were transplanted in the pot on 15 July, 2018. The irrigation was provided to maintain a constant level of standing water up to 6 cm was equipped with watering cane for continuous flood irrigation throughout the rice-growing season. Different intercultural operations (Gap filling, removal of weed and plant protection measures) were done as per when needed.

2.5 Recording of data

2.5.1 Effective tillers per hill

The total numbers of effective tillers per hill were counted as the number of panicle bearing tillers during harvesting. Data on effective tillers hill^{-1} were counted from 5 selected hills and average value was recorded.

2.5.2 Filled grains per panicle

The total numbers of filled grains were collected randomly from selected 5 panicle of a plot on the basis of grain in the spikelet and then average numbers of filled grains per panicle was recorded.

2.5.3 Weight of 1000-grains

One thousand grains were counted randomly from the total cleaned harvested grains and then weighed in grams and recorded.

2.5.4 Grain yield per plant

The crops were harvested according to maturity from pot. After threshing, cleaning and sun drying, the grain weight was recorded in gram per plant.

2.5.5 Zn content in rice

The dried samples of rice grains were ground for Zn analyses with a milling machine and sieved. Thereafter, the samples were digested using a di-acid [perchloric acid ($HClO_4$) + nitric acid (HNO_3) in 3:10 ratio] method. After digestion in the aliquot of samples, total Zn was estimated with the help of atomic absorption spectrophotometer (Perkin Elmer; Model-A. Analyst 100) as described by (12).

2.5.6 Grain-2AP content in rice

The 2-AP content in grain was estimated by using method described by (13), prior to analysis, grains were ground by mortar and pestle. Approximately 10 g grains were mixed homogeneously with 150 ml purified water into a 500 ml round-bottom flask attached to a continuous steam distillation extraction head. The mixture was boiled at 150°C in an oil pot. A 30 ml aliquot of dichloromethane was used as the extraction solvent and was added to a 500 ml round-bottom flask attached the other head of the continuous steam distillation apparatus, and this flask was boiled in a water pot at 53°C. The continuous steam distillation extraction was linked with a cold water circulation machine in order to keep temperature at 10°C. After approximately 35 min, the extraction was complete. Anhydrous sodium sulfite was added to the extract to absorb the water. The dried extract was filtered by organic needle filter and analyzed for 2-AP content by GCMS-QP 2010 Plus. High purity helium gas was used as the carrier gas at flow rate of 2 ml/min. The temperature gradient of the GC oven was as follows: 40^0C (1 min), increased at 2°C min^{-1} to 65°C and held at 65°C for 1 min, and then increased to 220°C at 10°C min^{-1}, and held at 220°C for 10 min. The retention time of 2-AP was confirmed at 7.5 min. Each sample had three replicates, and 2-AP conc. was expressed as μg g^{-1}.

2.5.7 Protein content in rice

The protein content of rice grains was determined by the Micro-Kjeldahl method using automated nitrogen determination system (14).

2.5.8 Amylose content in rice

The amylose content of the rice samples was carried out using method by (15) with some modification. Hundred mg of the powdered rice sample was taken in a volumetric flask. To which 1 ml of 95% ethanol and 9 ml of 1 NaOH was added. It was then heated in boiling water bath to gelatinize starch. Five ml of the starch extract was taken in 100 ml volumetric flask. One ml of 1N acetic acid and 2 ml iodide solution was added to the starch extract and the volume was made up to 100 ml. The solution was shaken and allowed to stand for 20 min. Then the absorbance was measured at 620 nm using Agilent Technologies Cary 60 UV-VIS spectrophotometer. Then the amylose content of the sample was determined with reference to the standard curve of potato amylose and expressed in percent basis.

2.6 Statistical Analysis

Analysis of variance (ANOVA) technique was used to analyze the collected data on different parameters with the help of Statistix 10 (2013) computer program and LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5 % level of provability.

3. Results and Discussion

3.1 Effective tillers hill^{-1}

Application of Zn and/or 2-AP had significant effect on number of effective tillers plant $^{-1}$ irrespective of aromatic rice varieties. Between the two varieties, BRRI dhan34 (V_1) produced the maximum number of effective tillers plant^{-1} compared to that of BRRI dhan80. (Table 1). Generally different cultivars produced different number of effective tillers hill^{-1} although different biotic and abiotic factors also influenced it. Effective tillers plant^{-1} increased with increasing Zn level (Table 2), Mustafa (16) also reported maximum productive tillers m^{-2} increased with increasing its level. On the other hand, 2-AP application had no effect on this parameter (Table 3).

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Table 1. Effect of Variety on number of effective tiller/plant and number of filled grains/panicle

Variety	Number of effective tillers plant $^{-1}$	Number of filled grains panicle $^{-1}$
V_1	26.185 a	180.12 a
V_2	20.889 b	105.21 b
CV (%)	13.63	15.70
LSD $_{(0.05)}$	1.7745	12.392
Level of Significance	**	*

V_1 =BRRI dhan34, V_2=BRRI dhan80

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

Table 2. Effect of Zinc on number of effective tiller/plant and number of filled grains/panicle

Zinc dose	Number of effective tillers plant^{-1}	Number of filled grains panicle^{-1}
Zn_0	21.889 b	140.31
Zn_1	23.667 ab	145.04
Zn_2	25.056 a	142.65
CV (%)	13.63	15.70
LSD $_{(0.05)}$	2.1733	15.177
Level of Significance	*	NS

Zn_0= 10 kg $ZnSO_4$/ha(Recommended dose) + 0kg $ZnSO_4$/ha (supplementation), Zn_1=10 kg $ZnSO_4$/ha + 2 kg $ZnSO_4$/ha (supplementation), Zn_2= 10 kg $ZnSO_4$/ha+ 4 kg$ZnSO_4$/ha (supplementation)

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

Table 3. Effect of 2-AP on number of number of effective tillers plant^{-1}and number of filled grains panicle^{-1}

2-AP conc.	Number of effective tillers plant^{-1}	Number of filled grains panicle^{-1}
AP_0	23.833	142.93
AP_1	23.278	144.17
AP_2	23.500	140.91
CV (%)	13.63	15.70
LSD $_{(0.05)}$	2.1733	15.177
Level of Significance	NS	NS

AP_0=0 ml 2-AP /L water in 5 kg pot soil, AP_1= 0.503 ml 2-AP /L water in 5 kg pot soil, AP_2= 1.006 ml 2-AP /L water in 5 kg pot soil

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

Combined effect of aromatic ice variety, Zn and 2-AP showed significant differences on effective tillers plant^{-1} (Fig. 1 & 2). Among the eighteen treatment combinations, the highest number of effective tillers plant^{-1} (29.667) was recorded from $V_1Zn_1AP_0$ and $V_1Zn_0AP_0$, $V_1Zn_1AP_2$, $V_1Zn_2AP_0$, $V_1Zn_2AP_1$, $V_1Zn_2AP_2$ and $V_2Zn_2AP_1$ treatment combinations also showed similar results, while the lowest number (17.33) was observed from $V_2Zn_0AP_0$ treatment combination.3.2 Filled grains panicle^{-1}.

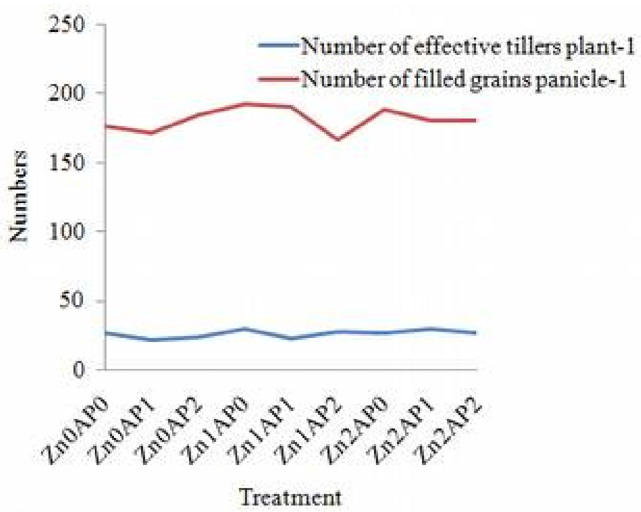

Fig. 1. Interaction effect of zinc and 2-AP on number of effective tillers plant^{-1}and number of filled grains panicle^{-1} of BRRI dhan34

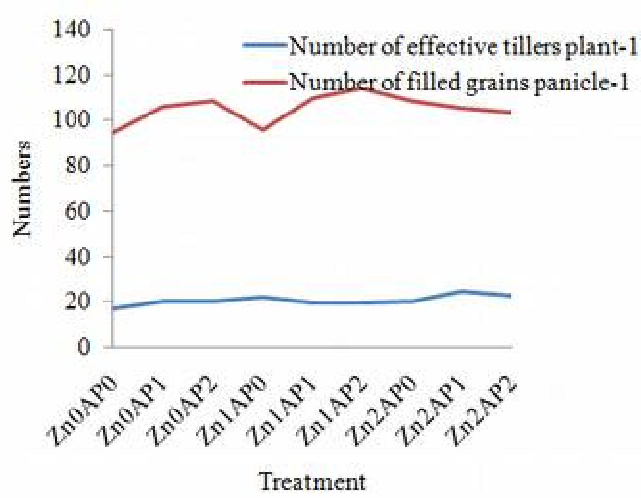

Fig. 2. Interaction effect of zinc and 2-AP on number of effective tillers plant^{-1}and number of filled grains panicle^{-1} of BRRI dhan80

Statistically significant difference was observed on filled grains panicle^{-1} of two aromatic rice varieties (Table 1). The highest number of filled grains panicle^{-1} (180.12) was found in V_1 while lowest was recorded in V_2 due to genetic performance. But there was no effect on this parameter for application of different levels of Zn and 2- AP application. Although Lei (17) reported that exogenous application of mixed micro-nutrients Zn notably increased filled grains. Statistically significant variation was recorded in terms of filled grains panicle^{-1} for different rice varieties (Table 1). The highest number of filled grains panicle^{-1} (185.30) was observed from V_1 which was statistically similar (179.47) to V_4 and closely followed (176.33) by V_2, whereas the lowest number (163.62) was found from V_3. Sarkar (18) revealed that the number of grains panicle^{-1} (152.3) in BRRI dhan34. Combined effect of these treatments showed significant differences on filled grains panicle^{-1} (Fig. 1 & 2). BRRI dhan34 produced higher number of filled grains panicle^{-1} than BRRI dhan80 irrespective of Zn and 2-AP levels. Numerically, $V_1Zn_1AP_0$ produced 192.67 filled grains panicle^{-1} and 94.67 was recorded in $V_2Zn_0AP_0$ (Fig. 1 & 2).

3.3 Weight of 1000-grains

Aromatic rice variety had significant effect of 1000- grain weight, but single effect of on Zn and 2-AP had no effect on this yield contributing parameters. (Tables 4, 5 and 6). Combined effect of rice varieties and different levels of Zn and 2-AP also showed significant effect on 1000-grains weight (Fig. 3 & 4). The maximum weight of 1000-grains was found in variety, BRRI dhan80 (22.3467-21.367 g) whereas the minimum was recorded in BRRI dhan34 (12.700-12.133 g) irrespective different levels of Zn and 2-AP (Fig. 3 & 4).

Table 4. Effect of variety grain yield plant^{-1} and 1000- grains weight

Variety	Grain yield plant^{-1} (g)	1000- grains weight (g)
V_1	52.122	12.259b
V_2	50.755	22.067a
CV (%)	15.89	5.43
LSD $_{(0.05)}$	--	0.515
Level of Significance	NS	**

V_1=BRRI dhan34, V_2=BRRI dhan80

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

Table 5. Effect of Zinc on grain yield plant^{-1} and 1000- grains weight

Zinc dose	Grain yield plant^{-1} (g)	1000- grains weight (g)
Zn_0	45.000 c	17.361
Zn_1	51.624 b	17.172
Zn_2	57.692 a	16.956
CV (%)	15.89	5.43
LSD $_{(0.05)}$	5.5356	-
Level of Significance	**	NS

Zn_0= 10 kg $ZnSO_4$/ha(Recommended dose) + 0kg $ZnSO_4$/ha (supplementation), Zn_1=10 kg $ZnSO_4$/ha + 2 kg $ZnSO_4$/ha (supplementation), Zn_2= 10 kg $ZnSO_4$/ha+ 4 kg$ZnSO_4$/ha (supplementation)

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

Table 6. Effect of 2-AP on grain yield plant^{-1} and 1000- grains weight

2-AP conc.	Grain yield plant^{-1} (g)	1000- grains weight (g)
AP_0	50.780	17.067
AP_1	53.341	17.383
AP_2	50.195	17.039
CV (%)	15.89	5.43
LSD $_{(0.05)}$	-	-
Level of Significance	NS	NS

AP_0=0 ml 2-AP /L water in 5 kg pot soil, AP_1= 0.503 ml 2-AP /L water in 5 kg pot soil, AP_2= 1.006 ml 2-AP /L water in 5 kg pot soil

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

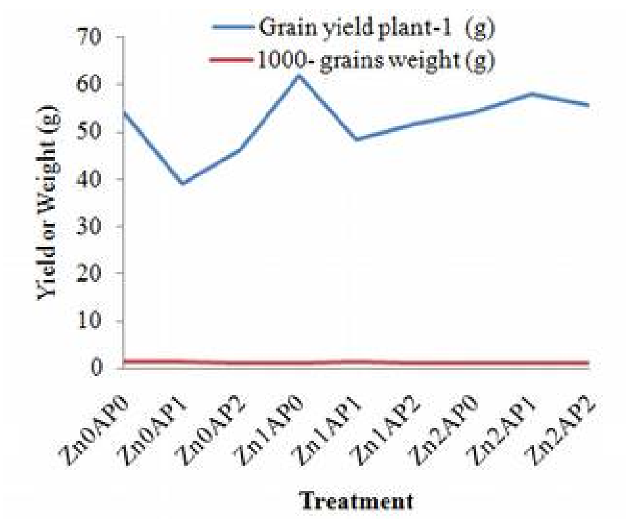

Fig. 3. Interaction effect of zinc and 2-AP on grain yield^{-1} (g) and 1000-grains weight (g) of BRRI dhan34

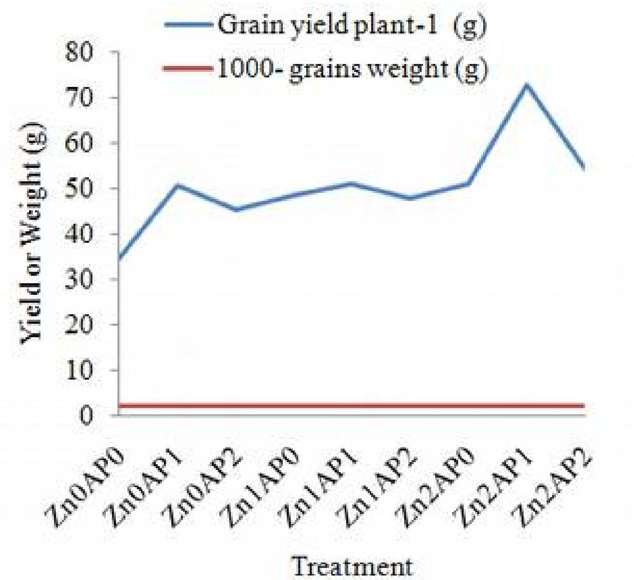

Fig. 4. Interaction effect of zinc and 2-AP on grain yield^{-1} (g) and 1000-grains weight (g) of BRRI dhan80

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3.4 Grain yield plant^{-1}

Grain yield plant^{-1} (g) of aromatic rice varieties showed statistically non-significant differences due to different levels of Zn and 2-AP (Table 4). Supplementation of Zn had significant on grain yield plant^{-1} (Table 5). The result revealed that grain yield plant^{-1} increased with increasing Zn level, whereas, the application of 2-AP had no effect on this parameters. The combined effect of variety, Zn and 2-AP showed significant differences on weight of grain yield plant^{-1} (Fig. 3 & 4). The combination of $V_2Zn_2AP_2$ produced the highest weight of grain yield plant^{-1} (72.813 g) which was statistically similar to $V_1Zn_1AP_0$ (61.946) while the lowest was recorded from $V_2Zn_0AP_0$ (34.832) (Fig. 3 & 4).

3.5 Zn content in rice

Zn content in rice showed statistically significant differences due to different levels of zinc (Table 7). The highest Zn content (0.154 mg g^{-1}) was found from Zn_2 while the lowest (0.113 mg g^{-1}) was observed from Zn_1. Studies reported that high zinc groups showed better uptake ability in Zn content (19). Statistically significant variation was recorded in terms of Zn content in different rice varieties (Table 8). The highest Zn content (0.137 mg g^{-1}) was observed from V_2 while the lowest (0.123 mg g^{-1}) was found in V_1. The 2-AP concentration has significant influence on Zn content of rice grain (Table 9). The highest Zn content (0.141%) was recorded from AP_2 followed by (0.131%) to AP_1, while the lowest (0.119%) was found from AP_0. Combined effect of different levels of 2-AP, zinc and rice varieties showed non-significant differences on Zn content in rice (Fig. 5 & 6). But, numerically the highest Zn content (0.168 mg g^{-1}) was observed from $V_2Zn_2AP_2$ treatment combination and the lowest (0.101 mg g^{-1}) was found from $V_1Zn_0AP_1$ treatment combination.

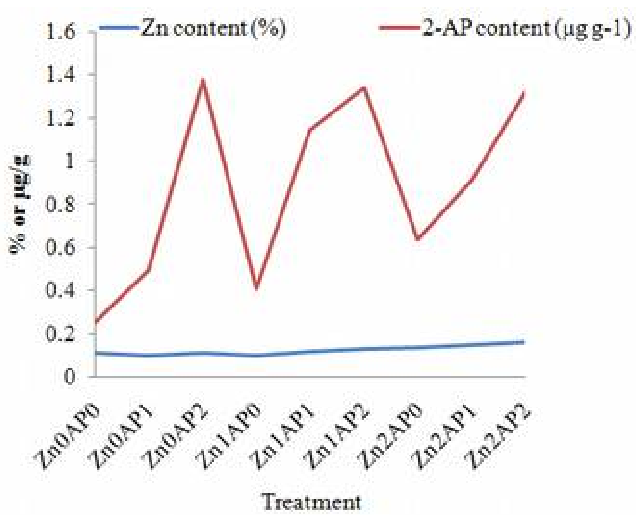

Fig. 5. Interaction effect of zinc and 2-AP on Zn content (%) and 2-AP content (µg g^{-1}) of BRRI dhan34

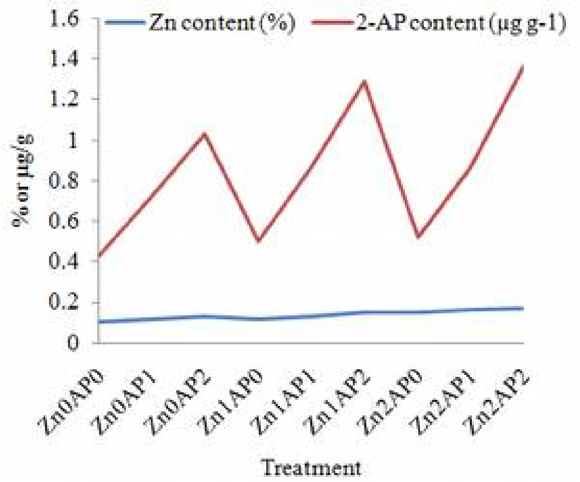

Fig. 6. Interaction effect of zinc and 2-AP on Zn content (%) and 2-AP content (µg g^{-1}) of BRRI dhan80

Table 7. Effect of variety on zinc and 2-AP content

Variety	Zn content (%)	2-AP content (µg g^{-1})
V_1	0.1239 b	0.8772 a
V_2	0.1376 a	0.8430 b
CV (%)	6.31	3.05
LSD $_{(0.05)}$	3.794	0.0121
Level of Significance	**	**

V_1 =BRRI dhan34, V_2=BRRI dhan80

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

Table 8. Effect of zinc on zinc and 2-AP content

Zinc dose	Zn content (%)	2-AP content (µg g^{-1})
Zn_0	0.1131 c	0.7175 b
Zn_1	0.1243 b	0.9244 a
Zn_2	0.1547 a	0.9383 a
CV (%)	6.31	3.05
LSD $_{(0.05)}$	5.442	0.0173
Level of Significance	**	**

Zn_0 = 10 kg $ZnSO_4$/ha(Recommended dose) + 0kg $ZnSO_4$/ha (supplementation), Zn_1 = 10 kg $ZnSO_4$/ha + 2 kg $ZnSO_4$/ha (supplementation), Zn_2 = 10 kg $ZnSO_4$/ha + 4 kg$ZnSO_4$/ha (supplementation)

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

Table 9. Effect of 2-AP concentration on zinc and 2-AP content

2-AP conc.	Zn content (%)	2-AP content (µg g^{-1})
AP_0	0.1194 c	0.4581 c
AP_1	0.1313 b	0.8356 b
AP_2	0.1414 a	1.2867 a
CV (%)	6.31	3.05
LSD $_{(0.05)}$	5.442	0.0173
Level of Significance	**	**

AP0=0 ml 2-AP /L water in 5 kg pot soil, AP1= 0.503 ml 2-AP /L water in 5 kg pot soil, AP2= 1.006 ml 2-AP /L water in 5 kg pot soil

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

3.6 Grain-2AP content in rice

Grain 2-AP content in rice showed statistically significant differences due to different levels of zinc (Table 7). The highest grain-2AP content (0.938 µg g^{-1}) was observed from Zn_2 which was statistically similar (0.924 µg g^{-1}) to Zn_1, whereas the lowest (0.717 µg g^{-1}) was recorded from Zn_0. Statistically significant variation was recorded in terms of grain-2AP content in different rice varieties (Table 8). The highest grain-2AP content (0.877 µg g^{-1}) was found from V_1 while the lowest (0.842 µg g^{-1}) was recorded from V_2. The 2-AP concentration has significant influence on grain 2-AP content of rice grain (Table 9). The highest grain 2-AP content (1.286%) was recorded from AP_2 followed by (0.835%) to AP_1, while the lowest (0.458%) was found from AP_0. Combined effect of different levels of zinc, 2-AP and rice varieties showed significant differences on grain-2AP content in rice (Fig. 5 & 6). The highest grain-2AP content (1.376 µg g^{-1}) was recorded from $V_1Zn_0AP_2$ treatment combination which was statistically similar to $V_2Zn_2AP_2$ and $V_1Zn_1AP_2$, whereas the lowest (0.255 µg g^{-1}) was observed from $V_1Zn_0AP_0$ treatment combination.

3.7 Protein content in rice

Protein content in rice showed statistically significant differences due to different levels of zinc (Table 10). The highest protein content (9.00 %) was recorded from Zn_2 which was statistically similar (8.91) to Zn_1, while the lowest (8.85%) was found from Zn_0. Statistically significant variation was recorded in terms of protein content in different rice varieties (Table 11). The highest protein content (9.05%) was found from V_1, whereas the lowest (8.79%) was observed from V_2. The 2-AP concentration has significant influence on protein content of rice grain (Table 12). The highest protein content (9.10%) was recorded from AP_2 followed by (8.89%) to AP_1, while the lowest (8.77%) was found from AP_0. Combined effect of different levels of 2-AP, zinc and rice varieties showed non-significant differences on protein content in rice (Fig. 7 & 8). But numerically the highest protein content (9.32%) was observed from $V_1Zn_1AP_1$ treatment combination which was statistically similar (90.26) to $V_1Zn_2AP_2$, while the lowest (8.62%) was found from $V_2Zn_0AP_0$ treatment combination.

Table 10. Effect of varity on protein and amylose content

Variety	Protein content (%)	Amylose content (%)
V_1	9.0537 a	23.668
V_2	8.7956 b	23.821
CV (%)	1.99	2.30
LSD $_{(0.05)}$	0.0982	0.3023
Level of Significance	**	NS

V_1 =BRRI dhan34, V_2=BRRI dhan80

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

Table 11. Effect of zinc on protein content and amylose content

Zinc dose	Protein content (%)	Amylose content (%)
Zn_0	8.8539 b	23.717
Zn_1	8.9117 ab	23.724
Zn_2	9.0083 a	23.792
CV (%)	1.99	2.30
LSD $_{(0.05)}$	0.1203	0.3702
Level of Significance	*	NS

Zn_0= 10 kg $ZnSO_4$/ha(Recommended dose) + 0kg $ZnSO_4$/ha (supplementation), Zn_1=10 kg $ZnSO_4$/ha + 2 kg $ZnSO_4$/ha (supplementation), Zn_2= 10 kg $ZnSO_4$/ha+ 4 kg$ZnSO_4$/ha (supplementation)

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

Table 12. Effect of 2-AP concentration on protein content and amylose content

2-AP conc.	Protein content (%)	Amylose content (%)
AP_0	8.7772 b	23.268 c
AP_1	8.8933 b	23.666 b
AP_2	9.1033 a	24.299 a
CV (%)	1.99	2.30
LSD $_{(0.05)}$	0.1203	0.3702
Level of Significance	**	**

AP_0=0 ml 2-AP /L water in 5 kg pot soil, AP_1= 0.503 ml 2-AP /L water in 5 kg pot soil, AP_2= 1.006 ml 2-AP /L water in 5 kg pot soil

LSD (Least Significant Difference) test was done to determine the significance in statistical analysis at 5% (0.05) level of provability.

3.8 Amylose content in rice

Amylose content in rice showed statistically non-significant differences due to different levels of zinc (Table 10). The highest amylose content (23.35%) was recorded from Zn_4 and the lowest (22.18%) was observed from Zn_1. Statistically significant variation was recorded in terms of amylose content in different rice varieties (Table 11). The highest amylose content (24.99%) was observed from V_3, while the lowest (21.93%) was found from V_1 which was statistically similar (21.97% and 22.28%) to V_4 and V_2. The 2-AP concentration has significant influence on amylose content of rice grain (Table 12). The highest amylose content (24.29%) was recorded from AP_2 followed by (23.66%) to AP_1, while the lowest (23.26%) was found from AP_0. Combined effect of different levels of zinc and rice varieties showed non-significant differences on amylose content in rice (Fig. 7 & 8). But numerically the highest amylose content (26.05%) was recorded from Zn_4V_3 treatment combination, whereas the lowest (20.86%) was observed from Zn_1V_2 treatment combination.

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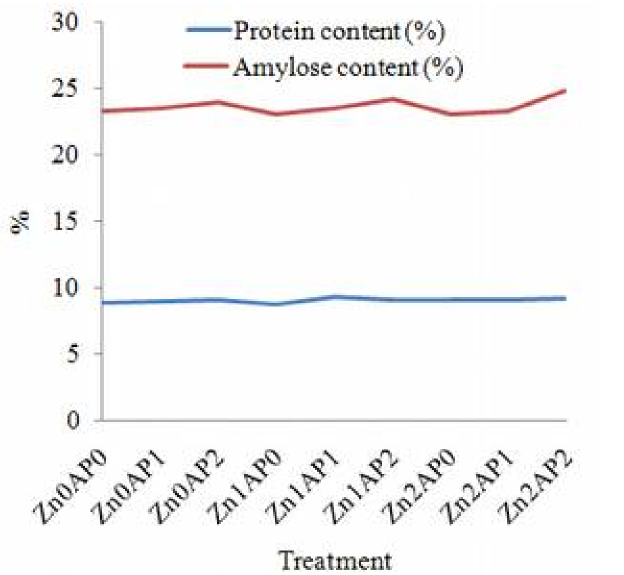

Fig. 7. Interaction effect of zinc and 2-AP on protein content (%) and Amylose content (%) of BRRI dhan34

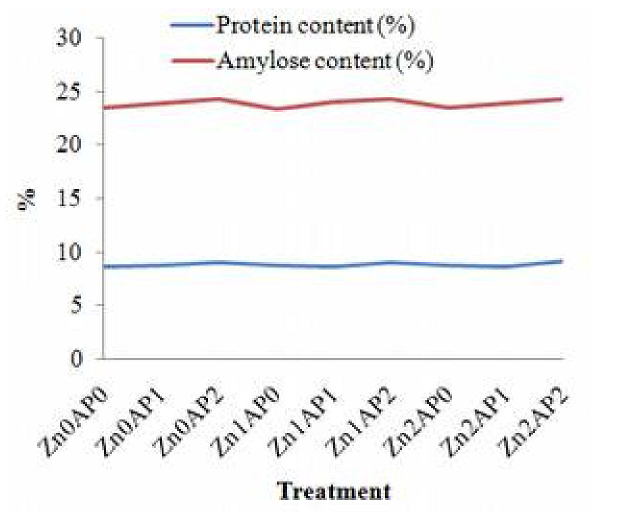

Fig. 8: Interaction effect of zinc and 2-AP on protein content (%) and Amylose content (%) of BRRI dhan80

3.9 Discussion

The study mostly emphasize on effect of Zn and 2-AP on yield and quality of popular aromatic rice variety of Bangladesh. Some nutrients *viz*., N, P, Zn, Ca, Mg and Mn could enhance the yield and quality of rice (20). 2-AP biosynthesis and accumulation in rice is an important phenomenon that is affected by several factors and the location for rice cultivation is affected on 2-AP contents (8). As expected, we have successfully investigated the effects of Zn and 2-AP on Zn, 2-AP, protein and amylose content. In present study, addition of Zn and 2-AP show the higher 2AP, Zn, protein content compared to those of no supplementation of Zn and 2-AP. Zn and 2-AP affected the ProDH activity resulted in proline conversion to 1-pyrroline-5-carboxylic acid then converted to 2-AP (21). Our observations regarding Zn and 2-AP induced changes in grain 2-AP concentrations of grains tell us that both elements may be involved in complicated biosynthetic mechanisms of aroma formation directly, or the elements might have indirect effects on 2-AP biosynthesis through regulation of enzymatic activities involved in 2-AP formation. Furthermore, the bio-chemical pathways leading to 2-AP biosynthesis are quite complicated and have not been fully resolved yet. Different mechanisms have been reported. For instance, the pathway might work as follows: conversion of proline, glutamic acid and ornithine to 1-pyrroline-5-carboxylic acid by proline dehydrogenase, pyrroline-5-carboxylic acid synthetase and ornithine aminotrans-ferase, respectively, followed by conversion to 2-AP via enzymatic (acetyl-CoA groups) or non-enzymatic (methylglyoxal) pathways (22). The results of this study indicate that higher grain 2-AP concentrations in grain seem to be due to increased transportation of 2-AP from the soil into grains. The increased Zn concentration is due to Zn supplementation. Application of both Zn and 2-AP at high levels might be helpful for enhancing rice aroma, and this supplementation can be easily practiced. To explore the possible roles of Zn and 2-AP in rice aroma formation and its interactions with physico-chemical processes of 2-AP biosynthesis, intensive research is needed at the molecular level.

3.10 Coefficient of Determinant (R^2)

Correlation analyses showed significant positive relation among grain 2-AP, protein and amylase content of grain in response to zinc application for both rice cultivars. There was a positive relation ($R^2 = 0.2688$) of between zinc content (g) and grain yield per plant (g) (Fig. 9). Sarwar (23) reported that with the increase in dose level from 20 kg to 30 kg Zn ha^{-1}, there was corresponding increase in grain yield regardless of the two varieties. A positive linear relation ($R^2 = 0.2689$) was found between zinc content (g) and grain 2-AP content ($\mu g\ g^{-1}$) (Fig. 10). Mo et al. (24) observed that a positive and significant relation between supplementation of zinc and increment of grain 2-AP content in aromatic rice. A positive linear relation ($R^2 = 0.0774$) was found between zinc content (g) and grain protein content ($\mu g\ g^{-1}$) (Fig. 11). It was observed that the application of nitrogen along with zinc significantly increased protein content of rice grain (25). The results supported the findings of present study. In Fig. 12,

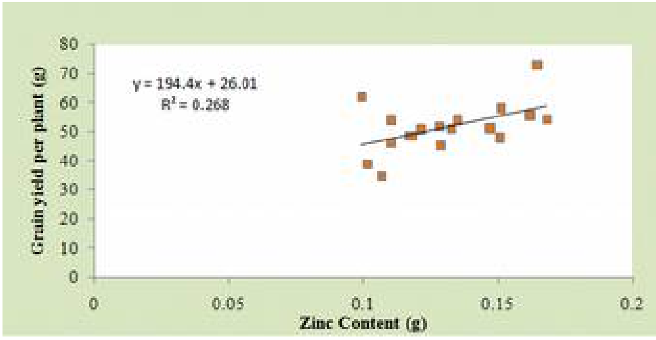

Fig. 9. Relationship between zinc content (g) and grain yield per plant (g)

a positive linear relation (R^2 = 0.2556) between zinc content (g) and grain alylose (%). It was found that amylose content was significantly and linearly increased with increasing zinc application rates (26).

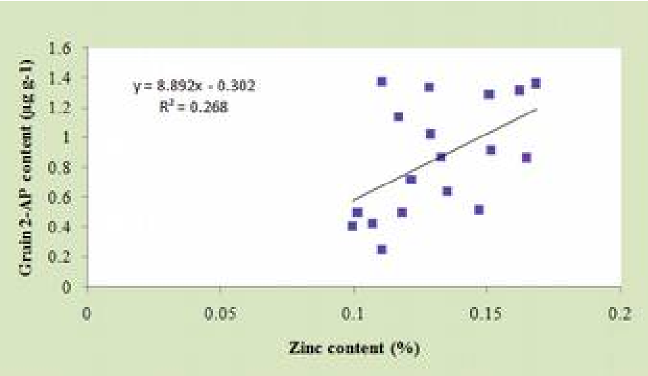

Fig. 10. Relationship between zinc content (g) and grain 2-AP content (µg g^{-1})

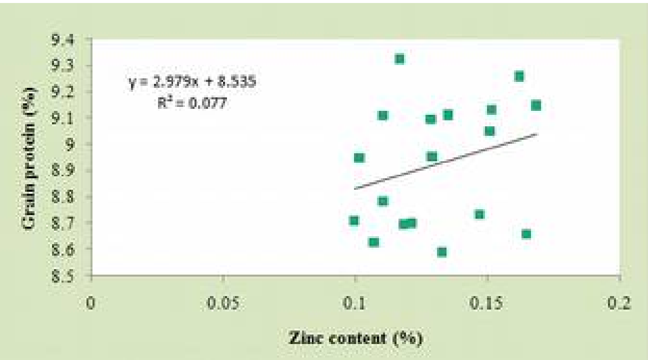

Fig. 11. Relationship between zinc content (g) and grain protein (%)

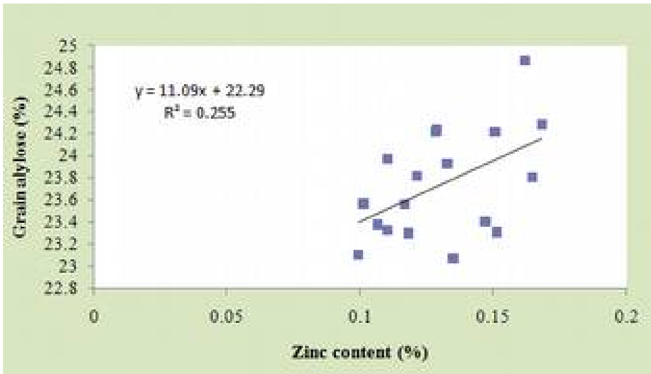

Fig. 12. Relationship between zinc content (g) and grain alylose (%)

4. Conclusion

The experiment illustrated the effect of Zn and 2-AP on yield and quality of Bangladeshi fragrant rice. It was found that supplementation of Zn and 2-AP had no significant effect on most of the yield contributing parameters. In this study, we demonstrated that supplementation of Zn and 2-AP significantly increased Zn, 2-AP, protein and amylose content. Transformation of 2-AP, directly from the soil due to Zn supplementation might also be responsible for the higher grain 2-AP concentration. Thus, Zn might have some relationship with 2-AP formation; however, more critical researches are still needed to understand exactly how Zn is involved in 2-AP formation. It may be concluded that a good management can also increase Zn, protein, amylose content in fragrant rice.

Conflict of Interest

No part of this research has been published elsewhere in any form. So, the authors declared that they have no conflict of interest.

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Author's contribution

Dr. Roy and Mr. Chakraborty thought the research ideas. Dr. Roy, Mr. Chakraborty and Mr. Anup conducted the research. Mr. Mostofa, Mr. Mahato, Mr. Sumon and Mrs. Sultana helped during data collection. Mr. Chakraborty analyzed the data. Dr. Roy and Mr. Chakraborty interpreted the data and drafted, read. Dr. Roy and Dr. Ali approved the final manuscript. After all, Dr. Roy supervised the team work and reviewed the manuscript to make a good frame work. After all, the authors were afforded their efficiency to the research work at the level best.

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