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Morphological Observation and Correlation of Growth and Yield Characteristics with Grain Quality and Antioxidant Activities in Exotic Rice Varieties of Afghanistan

Kifayatullah Kakar [1,2], Tran Dang Xuan [1,*], Saidajan Abdiani [2], Imran Khan Wafa [3], Zubair Noori [4], Shakeib Attai [5], Tran Dang Khanh [6] and Hoang-Dung Tran [7]

1. Graduate School for International Development and Cooperation, Hiroshima University, Hiroshima 739-8529, Japan
2. Faculty of Agriculture, Nangarhar University, Nangarhar 2601, Afghanistan
3. Badam Bagh Research Station, Ministry of Agriculture, Irrigation and Livestock, Kabul 1001, Afghanistan
4. Kunduz Directorate of Agriculture, Irrigation and Livestock, Kunduz 3501, Afghanistan
5. Nangarhar Directorate of Agriculture, Irrigation and Livestock, Nangarhar 2601, Afghanistan
6. Agricultural Genetics Institute, Pham Van Dong Street, Hanoi 122000, Vietnam
7. Faculty of Biotechnology, Nguyen Tat Thanh University, 298A-300A Nguyen Tat Thanh Street, Ward 13, District 4, Ho Chi Minh 72820, Vietnam

* Correspondence: tdxuan@hiroshima-u.ac.jp; Tel./Fax: +81-82-424-6927

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Abstract: Rice is an important staple food for Afghans. Its production has been increased, and attention is needed to improve grain quality. Experiments were conducted to evaluate the growth, yield, physicochemical properties, antioxidant activity, and morphological structures of four exotic rice varieties widely grown in Afghanistan (Attai-1, Jalalabad-14, Shishambagh-14, and Zodrass). Antioxidant activities, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), of rice grain were determined. A scanning electron microscopic observation was conducted on the cross-cut section of dehulled rice grains. The results showed a wide variation among four rice varieties for growth, grain yield, physicochemical properties, antioxidant activities, and morphology. Tiller and panicle number per hill, 1000-grain weight, grain yield, and antioxidant activities were found to be highest in Jalalabad-14. Attai-1 showed lower amylose, protein, and lipid contents with a high number of perfect grains, consequently enhanced taste point (score of quality). Grain yield, protein, and amylose contents showed a negative correlation with antioxidant activities. Accumulated structures in Attai-1, Shishambagh-14, and Zodrass were normal; however, Jalalabad-14 increased protein bodies and its traces in the amyloplasts. Information on yield potential, grain quality, and nutritional value of these exotic rice varieties may useful for sustainable food provision and nutritional improvement of rice in Afghanistan.

Keywords: rice; growth; yield; grain quality; antioxidant activity; morphological observation; scanning electron microscope

1. Introduction

Rice (*Oryza sativa* L.), an edible starchy grain, is one of the leading food crops in the world, and a primary source of calorie intake for more than half of the world population [1]. Asian countries are the main producers and consumers of rice [2], and it is the second staple food, after wheat, for Afghans [3,4]. Recently, annual rice grain production has increased to 33,600 metric tons in Afghanistan [4,5]; however, great effort is needed to improve grain quality based on the preference and acceptance of consumers. Genetic and environmental factors are principally responsible for alteration in growth and quality composition of rice [6].

Factors that determine rice grain quality include physical and chemical characteristics; hence a specific customer class might need for an enthusiasm [7]. Amylose, protein, and lipid contents are the main components of grains that affect the cooking and eating qualities of rice [6]. The acceptance of rice grain quality in terms of appearance, cooking, and eating are mostly dependent upon cultural differences. Zhou et al. [8] has reported that rice grains with low amylose and protein contents are highly desirable in worldwide markets.

The rice endosperm primarily determines the nutritional value of its elements, including starches, proteins, and lipids [9]. The morphology of starch granules and their physicochemical properties have counteractive effects on each other and contribute to the functional properties of starches [10]. Therefore, morphological observation is conducting to explore information regarding morphostructural and accumulation synthesis of amyloplasts, starch granules, and protein bodies [11].

On the other hand, as rice is a staple food for more than half of the world population, an evaluation on the biochemical properties that are beneficial for human health is required. Food value declines with oxidative degradation and may have risky effects on human health [12]. Antioxidants, which play inhibitory roles against free radicle reactions [13], are the most important biochemical components of rice grain. Antioxidants are used as food additives to prevent oxidative rancidity and discoloration of foods [14]. Determination of the presence of antioxidants is crucial to describe an organism's defense against free radicals based on nutritional and biological characteristics [12].

There is little information on the physicochemical properties, morphology, and antioxidant activities of principal rice varieties in Afghanistan. To address these issues, this study was carried out to evaluate growth, grain yield, physicochemical properties, and antioxidant activities as well as morphological structures on selected exotic rice varieties using a scanning electron microscope.

2. Materials and Methods

2.1. Site Selection and Design

The experiments were conducted in the Shisham Bagh Research Station, Nangarhar, Afghanistan during rice growing seasons in 2017 and 2018. The experimental site is located at 34°25′29.7′′ N latitude and 70°28′14.3′′ E longitude, had an average annual temperature and rainfall of 28.7 °C (min = 4, max = 47) and 180 mm for 2017 and 27.8 °C (min = 3, max = 45) and 197 mm for 2018, respectively. The experiments were carried out in a randomized complete block design with 4 varieties and 3 replications based on open field conditions. Four well known and widely cultivated exotic rice varieties in Afghanistan with high yield potential (Table 1) were selected to evaluate their growth, yield, and quality performance along with their antioxidant activities and morphological structures of grain. Standard fertilizers (N: 68.4 g and P_2O_5: 41.4 g) were applied for each plot (9 m^{-2}) and hand weeding was conducted twice. The soil properties of the experimental field at 30 cm depth are summarized in Table 2.

Table 1. List and description of the selected rice varieties.

Varieties	Subtype	Group	Origin
Attai-1	Indica	Long grain	India
Jalalabad-14	Indica	Long grain	India
Shishambagh-14	Japonica	Short grain	Japan
Zodrass	Indica	Short grain	India

Varieties were provided by Shisham Bagh Research Station, Nangarhar, Afghanistan.

Table 2. Description of soil properties at 30 cm depth in the experimental field.

Soil Properties	Description
Texture class	Sandy clay loam
pH	7.5
Electroconductivity	0.05 dS m^{-1}
Organic matter	3.5%
Total nitrogen	3.2%
Phosphorus	7.5 mg kg^{-1}
Potassium	170 mg kg^{-1}
Calcium carbonate	5%

2.2. Seedling Management and Data Collection

Seeds of each variety were sown in a prepared muddy nursery bed and the 28-day-old rice seedlings of 3.5 plant age in leaf number were transplanted to the paddy field by hand at three seedlings per hill. The seedlings were planted with a density of 15 cm and 20 cm spaces between plants and rows, respectively. Plants were harvested at the maturity stage. Ten hills were chosen from each replication (90 plants per treatment) to evaluate growth parameters along with grain yield and its components, followed the technique described by Hoshikawa [15].

Grains were dehusked using an automatic rice husker machine (model TR-250, Kett Electric Laboratory, Tokyo, Japan). 200 g of brown rice grain samples with three replications were used to measure grain quality, measuring the amylose, protein, and lipid contents, as well as the taste points through a taste analyzer machine (RCTA11A; Satake Co., Ltd., Hiroshima, Japan). A cereal grain discriminator device (RGQI 10B; Satake Co., Ltd., Hiroshima, Japan) was used to record the percentages of perfect, imperfect and broken grains.

2.3. Antioxidant Activities

2.3.1. Extraction

The extraction was conducted based on the method described by Shao et al. [16] with slight modification. Briefly, two grams of brown rice flour of the samples were saturated in 30 mL methanol for 7 days at room temperature. Samples were filtered with filter paper (Advantec No.1, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and filtrates were evaporated to achieve methanol extracts. Subsequently, an adequate volume of hexane was mixed proportionately with the methanol extract in a separatory funnel. After 3 h at room temperature, the methanol layer was collected and filtered. The filtrate was then evaporated to obtain crude extracts which was eventually dissolved with methanol and kept at 4.0 °C for further analysis.

2.3.2. DPPH Radical Scavenging Activity

DPPH assay was determined according to the method described by Tuyen et al. [17] with some modifications. In brief, 500 µL of the extract was mixed with 250 µL of DPPH solution and 100 µL of 0.1 M acetate buffer (pH 5.5). The mixture was then incubated for 20 min at room temperature in a dark place. The absorbance was measured at 517 nm using a microplate reader (MultiskanTM Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan). The IC_{50} value was expressed as the concentration of the sample required to inhibit 50% of DPPH. Thus, the lower IC_{50} value denoted the higher antioxidant activity. Butylated hydroxytoluene (BHT) at 5–20 ppm was used as a standard sample. The activity was calculated with the following formula.

$$\text{DPPH radical scavenging activity (\%)} = (1 - A_1/A_0) \times 100$$

where A_1 is the absorbance of reaction with momilactones or positive control (BHT), and A_0 is the absorbance of reaction without momilactone or positive control.

2.3.3. ABTS Radical Scavenging Activity

ABTS decolorization was measured according to the method described by Pellegrini et al. [18] with slight changes. A solution of ABTS was obtained by mixing aqueous ABTS (7 mM) solution and 2.45 mM potassium persulfate (1:1 *v*/*v*). The achieved solution was then incubated for 16 h in dark conditions at room temperature. The mixture was diluted in methanol (1:1 *v*/*v*) to obtain the specific absorbance. 25 µL of sample and 200 µL of the solution were put in a microplate, smoothly shaken, covered with aluminum foil, and kept for 30 min at room temperature. The absorbance of the sample was measured at 517 nm using the microplate reader. The IC_{50} value was expressed as the concentration of the sample required to inhibit 50% of ABTS. Thus, the lower IC_{50} value of ABTS indicated higher antioxidant activity. BHT was used as a standard sample with pure methanol. The activity was calculated with the following formula.

$$\text{ABTS radical scavenging activity (\%)} = (1 - A_1/A_0) \times 100$$

where A_1 is the absorbance of reaction with momilactones or positive control (BHT), and A_0 is the absorbance of reaction without momilactone or positive control.

2.4. Preparation for Scanning Electron Microscopic Observation

Observations of the endosperm by a scanning electron microscope were conducted through the method determined by Zakaria et al. [19]. Perfect brown rice grains were collected from each variety to observe internal structures of grains using a scanning electron microscopy (JSM6360A; JEOL, Japan). Thirty perfect brown rice grains per variety were freeze-dried with a freeze vacuum dryer (−60.0 °C, 10–3 Pa, LFD-100NDPS1; Nihon Techno Service Co., Ltd., Japan). After drying, grains that had been cross-cut using a razor blade were placed on specimens. They were coated with platinum (JUC-5000; JEOL, Japan) and were observed using a scanning electron microscopy.

2.5. Statistical Analysis

Data were analyzed using Minitab 16.0 statistical software (Minitab Inc., State College, PA, USA). They were subjected to a two-way analysis of variance (ANOVA), based on a factorial combination of two years × four varieties. Means were separated by Tukey's multicomparison test, when the F-test was significant. Statistical significances were defined at the $p < 0.05$ probability level. Person correlation was conducted to express the relationship between growth and yield parameters with quality traits and antioxidant activities.

3. Results

3.1. Growth and Yield Performances

Significant differences ($p < 0.05$) were found among four varieties in growth parameters including plant length, tiller number, leaf number, days to heading, and maturity (Table 3). The highest plant length, leaf number, and days to heading and maturity were observed for Attai-1 compared to other varieties regardless of year. Jalalabad-14 obtained a high tiller number per hill (23.6), followed by Zodrass (23.1), Attai-1 (19.7), and Shishambagh-14 (18.6). Zodrass was an early maturity variety, followed by Jalalabad-14, Shishambagh-14, and Attai-1. Identical results were found in 2017 and 2018 for growth parameters; however, for each variety, variations were observed for days to heading and maturity between years.

Table 3. Growth parameters of selected rice varieties.

Year	Varieties	Plant Length (cm)	Tiller No. Hill^{-1}	Leaf Number Plant^{-1}	Days to Heading	Days to Maturity
2018	Attai-1	115 ± 0.9	20.0 ± 0.7	17.5 ± 0.4	115 ± 0.3 b	145 ± 0.2 b
	Jalalabad-14	110 ± 0.8	23.6 ± 0.5	16.3 ± 0.2	103 ± 0.4 e	132 ± 0.4 e
	Shishambagh-14	101 ± 0.8	18.1 ± 0.8	14.2 ± 0.3	106 ± 0.1 d	134 ± 0.3 d
	Zodrass	115 ± 0.9	22.8 ± 0.6	15.2 ± 0.4	97 ± 0.3 f	125 ± 0.4 f
2017	Attai-1	115 ± 1.0	19.4 ± 0.6	16.8 ± 0.4	117 ± 0.4 a	148 ± 0.6 a
	Jalalabad-14	111 ± 0.8	23.7 ± 0.4	15.9 ± 0.3	106 ± 0.9 d	135 ± 0.4 d
	Shishambagh-14	102 ± 1.1	19.2 ± 0.9	14.0 ± 0.4	110 ± 0.6 c	139 ± 0.1 c
	Zodrass	114 ± 0.5	23.5 ± 0.7	15.6 ± 0.7	98 ± 0.5 f	126 ± 0.3 f
Variety (V)		***	***	***	***	***
Year (Y)		ns	ns	ns	***	***
V × Y		ns	ns	ns	***	***

Values are presented as means ± standard errors. Same letters in a column with significant V × Y interaction showed not significant differences within the column at $p < 0.05$ based on Tukey's multicomparison test. *** shows significant difference at $p < 0.001$ and ns means not significant.

There was a wide difference among yield and its components. Grain yield and panicle number per hill, spikelet number per panicle, repined ratio, and 1000-grain weight significantly differed ($p < 0.05$) among varieties (Table 4). Panicle number per hill and 1000-grain weight were higher in Jalalabad-14, which consequently increased grain yield. Spikelet number per panicle and repined ratio were higher in Shishambagh-14 regardless of year. Grain yield was greater in Jalalabad-14 at 9.2 t/ha, followed by Shishambagh-14 (8.6 t/ha), Zodrass (8.1 t/ha), and Attai-1 (7.9 t/ha). Spikelet number and repined ratio were lower in 2017 than in 2018; however, panicle number and 1000 grain weight were higher in 2017.

Table 4. Description of grain yield and its components in selected rice varieties.

Year	Varieties	Panicle No. Hill^{-1}	Spikelet No. Panicle^{-1}	Repined Ratio (%)	1000 Grain Weight (g)	Grain Yield (t ha^{-1})
2018	Attai-1	13.7 ± 0.33 cd	98.2 ± 0.60 d	87.7 ± 0.58	20.5 ± 0.21	8.0 ± 0.17
	Jalalabad-14	15.8 ± 0.25 a	96.2 ± 0.17 e	86.2 ± 0.45	20. 7 ± 0.18	9.1 ± 0.18
	Shishambagh-14	11.6 ± 0.15 e	125.0 ± 0.25 a	95.9 ± 0.23	19.1 ± 0.28	8.8 ± 0.05
	Zodrass	14.6 ± 0.40 bc	93.1 ± 0.37 f	90.3 ± 0.19	19.8 ± 0.23	8.1 ± 0.21
2017	Attai-1	12.9 ± 0.25 d	101.7 ± 0.86 c	85.7 ± 0.64	20.9 ± 0.33	7.8 ± 0.16
	Jalalabad-14	16.2 ± 0.34 a	97.9 ± 0.75 de	84.9 ± 0.49	20. 9 ± 0.54	9.3 ± 0.24
	Shishambagh-14	11.9 ± 0.16 e	118.0 ± 0.82 b	93.0 ± 0.52	19.5 ± 0.25	8.5 ± 0.21
	Zodrass	15.3 ± 0.17 ab	90.7 ± 0.26 g	88.5 ± 0.98	20.0 ± 0.12	8.2 ± 0.12
Variety (V)		***	***	***	***	***
Year (Y)		ns	**	***	*	ns
V × Y		**	***	ns	ns	ns

Values are presented as means ± standard errors. Same letters in a column with significant V × Y interaction showed not significant differences within the column at $p < 0.05$ based on Tukey's multicomparison test. *, **, and *** show significant difference at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns: not significant.

3.2. Grain Quality Traits and Antioxidant Activities

All rice grain quality traits including protein, amylose, and lipid contents, taste point, perfect, imperfect, and broken grains were significantly different ($p < 0.05$) among all varieties (Table 5). Attai-1 showed lower protein and amylose contents than other varieties, therefore, the taste point was increased. Attai-1 also showed a higher perfect grain and a lower imperfect grain percentage. Jalalabad-14 showed higher protein and amylose contents, and a consequently declined grain taste point. It also produced lower perfect grain and higher imperfect and broken grain percentages. Lipid content was higher in Shishambagh-14 and was lower in Zodrass variety. Protein, amylose, and lipid contents and broken grain percentage were increased in 2017 experiment; thus, taste score was declined in contrast to 2018.

Table 5. Grain quality categories of selected rice varieties.

Year	Varieties	Protein Content (%)	Amylose Content (%)	Lipid Content (%)	Taste Point (Score)	Perfect Grain (%)	Imperfect Grain (%)	Broken Grain (%)
2018	Attai-1	8.3 ± 0.02 f	20.0 ± 0.08	8.2 ± 0.04	65.3 ± 0.24 a	52.3 ± 0.85	41.2 ± 1.08	0.7 ± 0.06
	Jalalabad-14	11.0 ± 0.08 a	22.1 ± 0.06	8.6 ± 0.04	52.0 ± 0.41 e	42.0 ± 0.82	55.3 ± 1.22	0.8 ± 0.02
	Shishambagh-14	9.4 ± 0.02 d	20.9 ± 0.13	9.6 ± 0.02	58.7 ± 0.24 b	45.3 ± 0.86	51.0 ± 2.68	0.5 ± 0.02
	Zodrass	10.1 ± 0.02 bc	21.5 ± 0.21	7.6 ± 0.04	55.0 ± 0.41 d	44.7 ± 0.74	51.7 ± 1.03	0.7 ± 0.05
2017	Attai-1	8.9 ± 0.36 e	20.3 ± 0.34	8.9 ± 0.40	65.0 ± 0.09 a	51.0 ± 1.41	42.5 ± 1.40	0.8 ± 0.08
	Jalalabad-14	10.3 ± 0.34 b	22.8 ± 0.08	9.3 ± 0.39	51.7 ± 0.47 e	40.0 ± 0.94	56.5 ± 1.32	0.8 ± 0.05
	Shishambagh-14	9.8 ± 0.05 cd	21.2 ± 0.22	10.3 ± 0.49	57.3 ± 0.47 c	44.1 ± 1.60	52.9 ± 1.63	0.5 ± 0.06
	Zodrass	10.1 ± 0.14 bc	21.8 ± 0.28	8.0 ± 0.37	56.1 ± 0.34 d	44.3 ± 1.16	52.4 ± 1.31	0.8 ± 0.02
	Variety (V)	***	***	***	***	***	***	***
	Year (Y)	ns	***	***	ns	*	ns	**
	V × Y	***	ns	ns	**	ns	ns	ns

Values are presented as means ± standard errors. Same letters in a column with significant V × Y interaction showed not significant differences within the column at $p < 0.05$ based on Tukey's multicomparison test. *, **, and *** show significant difference at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns: not significant.

The antioxidant scavenging activities of rice grain in selected rice varieties are presented in Table 6 and are expressed as the IC_{50} value, thus the smaller value indicates greater activity. Significant differences ($p < 0.05$) were found among the values observed in the DPPH and ABTS assays. Jalalabad-14 showed high antioxidant activities, which were 2574 µg per mL for DPPH and 2970 µg per mL for ABTS in the 2018 experiment. Zodrass showed lower antioxidant activities which were 7411 µg per mL for DPPH and 8548 µg per mL for ABTS. Based on the showed levels of antioxidant activities, Shishambagh-14 and Attai-1 placed at the second and third positions, respectively. Antioxidant activities were higher in 2018 experiment compared to 2017 except for Zodrass variety.

Table 6. Antioxidant activities of rice grain in terms of IC_{50} inhibition in selected rice varieties.

Year	Varieties	IC_{50} of DPPH (µg/mL)	IC_{50} of ABTS (µg/mL)
2018	Attai-1	6097 ± 10 d	7036 ± 98 d
	Jalalabad-14	2574 ± 43 g	2970 ± 43 g
	Shishambagh-14	5275 ± 54 f	6085 ± 56 f
	Zodrass	7411 ± 43 a	8548 ± 43 a
2017	Attai-1	6157 ± 34 c	7226 ± 78 c
	Jalalabad-14	2691 ± 28 g	3012 ± 43 g
	Shishambagh-14	5319 ± 25 e	6249 ± 74 e
	Zodrass	7329 ± 21 b	8417 ± 26 b
	Variety (V)	***	***
	Year (Y)	***	***
	V × Y	**	**

Values are presented as means ± standard errors. Same alphabetical letters indicated not significant differences within a column at the $p < 0.05$ probability level based on Tukey's multicomparison test. ** and *** show significant difference at $p < 0.01$ and $p < 0.001$, respectively.

The correlation coefficient of growth parameters, grain yield and its components with the quality traits and antioxidant activities of rice grains in selected varieties are illustrated in Table 7. Tiller number showed a significantly positive correlation with panicle number, protein content, amylose content, and imperfect grain percentages. It displayed a negative correlation with spikelet number, lipid content, and taste point. Days to heading and maturity exhibited a positive correlation with taste point and perfect grain percentage. However, these parameters revealed a negative correlation with protein content, amylose content, and imperfect grain percentages.

Table 7. The correlation coefficient of growth parameters, grain yield and its components with quality traits and antioxidant activities among exotic rice varieties.

	TN	DH	DM	PN	SN	GY	PC	AC	LC	TP	PG	IG	DPPH
DH	−0.486 *												
DM	−0.446	0.994 ***											
PN	0.919 ***	−0.328	−0.294										
SN	−0.762 ***	0.173	0.159	−0.851 ***									
GY	0.140	−0.205	−0.172	0.153	0.351								
PC	0.631 **	−0.743 ***	−0.737 ***	0.580 **	−0.218	0.553 *							
AC	0.681 **	−0.772 ***	−0.755 ***	0.598 **	−0.254	0.531 *	0.947 ***						
LC	−0.596 **	0.232	0.229	−0.623 **	0.913 ***	0.596 **	−0.003	−0.073					
TP	−0.610 **	0.850 ***	0.841 ***	−0.535 *	0.196	−0.502 *	−0.974 ***	−0.952 ***	0.048				
PG	−0.460	0.779 ***	0.769 ***	−0.341	−0.022	−0.614 **	−0.888 ***	−0.824 ***	−0.174	0.878 ***			
IG	0.527 *	−0.732 ***	−0.716 **	0.324	0.048	0.661 **	0.828 ***	0.815 ***	0.172	−0.827 ***	−0.923 ***		
DPPH	−0.221	−0.063	−0.071	−0.342	−0.077	−0.741 ***	−0.595 **	−0.504 *	−0.464	0.449	0.453	−0.407	
ABTS	−0.231	−0.065	−0.075	−0.351	−0.070	−0.747 ***	−0.592 **	−0.505 *	−0.459	0.446	0.451	−0.409	0.999 ***

TN: tiller number; DH: days to heading; DM: days to maturity; PN: panicle number; SN: spikelet number; GY: grain yield; PC: protein content; AC: amylose content; LC: lipid content; TP: taste point; PG: perfect grain; IG: imperfect grain; DPPH: 1,1-diphenyl-2-picrylhydrazyl; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); *, **, and *** indicated significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Panicle number showed a significantly positive correlation with protein and amylose contents. It demonstrated a negative correlation with spikelet number, lipid content, and taste point. Spikelet number exhibited a positive correlation with lipid content. Grain yield had a positive correlation with protein, amylose, and lipid contents, as well as imperfect grain percentage. However, it revealed a negative correlation with taste point, perfect grain, and DPPH and ABTS activities. Growth parameters and yield components did not show significant correlation with DPPH and ABTS activities.

Protein and amylose contents had a positive correlation with imperfect grain percentage and displayed a negative correlation with taste point, perfect grain percentage, and DPPH and ABTS activities. Taste point and perfect grain percentage showed a negative correlation with imperfect grain percentage. Perfect grain is positively associated with taste point. Additionally, DPPH and ABTS had a strong positive correlation with each other.

3.3. Morphological Observation of Grain

Micrograph observations by scanning electron microscope revealed several differences in the internal structures of endosperm in selected rice varieties. Attai-1 rice grain had few protein bodies and their traces compared to other varieties (Figure 1a). The formation of amyloplasts and starch granules were also different. Amyloplasts were round with different sizes but starch granules were bigger than in other varieties. Starch granules were spherical and gathered without airgaps which lead the way for normal accumulation structure.

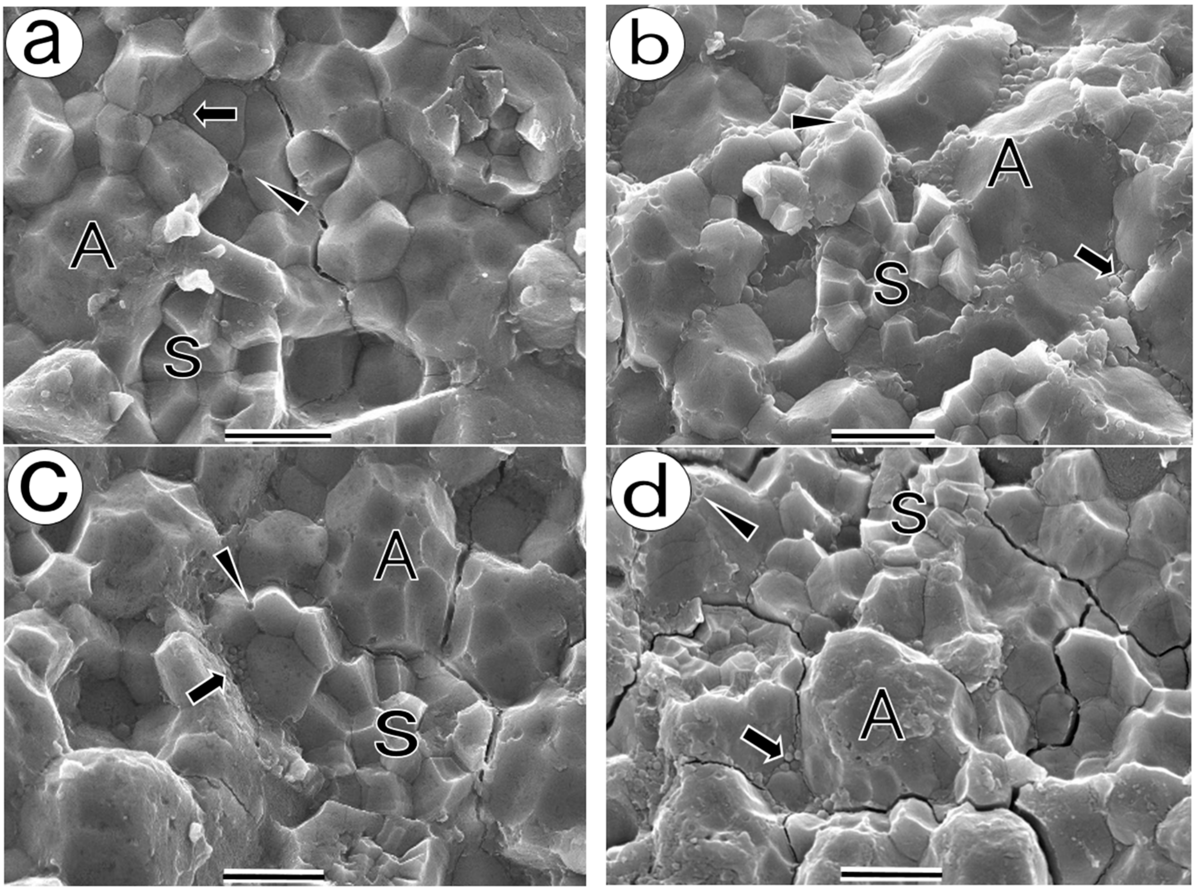

Figure 1. Accumulation structures of rice grain in selected varieties: (**a**) Attai-1, (**b**) Jalalabad-14, (**c**) Shishambagh-14, and (**d**) Zodrass. A: amyloplast; S: starch granules; arrow: protein bodies; arrowhead: traces of protein bodies. Bars: 10 μm.

Jalalabad-14 had polygonal amyloplasts with a high number of protein bodies and their traces in the endosperm (Figure 1b). Protein bodies were located around and on the surface of the amyloplasts. Shapes of the amyloplasts were round and polyhedral in Shishambagh-14 (Figure 1c) and Zodrass (Figure 1d) varieties. Starch granules of Shishambaagh-14 and Zodrass were polygonal with sharply defined edges. There were protein bodies and their traces in both varieties, but the number was fewer than in Jalalabad-14 and higher compared to Attai-1. The shapes of protein bodies were spherical or ovoid and were visible between starch granules.

Larger amyloplasts with smaller starch granules were observed in Jalalabad-14, Shishambagh-14, and Zodrass varieties in contrast to Attai-1. Attai-1 and Shishambagh-14 mostly accumulated protein bodies in the periphery portion of endosperm rather than the central part.

4. Discussion

In Afghanistan, rice is the second most cultivated crop after wheat with total paddy production amount of 532,000 metric tons [4]. Recently, rice production has been increased, but attention is needed to improve grain quality and nutritional value. Both Indica and Japonica rice varieties are cultivated in different zones and provinces. In our previous study, we listed 19 top rice cultivars throughout the country [4]. In this experiment, four widely grown and high yielding cultivars were selected to evaluate their grain quality and nutritive value. Thus, Afghan breeders will consider using these varieties in breeding strategies. Researchers and breeders have been working to develop new rice varieties with high yielding capacity and preferable quality. Genetic and environmental factors are mainly responsible for variation in yield and quality of rice [6].

A point to be noted is that rice grains produced in Afghanistan do not have standard value in regional markets, and are processed and distributed irregularly to local or city markets within the country [3,4]. Lack of detailed information regarding the quality and nutritional value of Afghan rice cultivars has resulted in a reduction of customers. Moreover, a huge amount of rice grains is being imported from neighboring countries, particularly from Pakistan and India, without standard certification [4]. Therefore, it is difficult to compare Afghan exotic rice cultivars with the cultivars from these countries, or to select a check variety.

In the current study, the four most popular exotic rice varieties were tested, and of them, Jalalabad-14 and Zodrass varieties produced high tiller number per hill. Badshah et al. [20] reported that tiller production is the most crucial factor for panicle development in rice plant. Tiller and panicle numbers were higher in 2017 than in 2018, this might be due to the temperature difference in these years. Krishnan et al. [21] reported that raise in temperature could increase tiller, panicle and biomass production in rice. On the other hand, Attai-1 produced more leaves per plant compared to other varieties which may lead to higher assimilation of product in this variety. Hosoya [22] found that assimilation products produced by photosynthesis in the leaf transfer to the grain via the transport system.

Attai-1 was found to be a late mature variety and Zodrass was found to be an early mature variety. This may help producers to manage the crop calendar, which is an important tool for production. Shortening of the vegetative phase in Zodrass variety led to decreased spikelet number per panicle, which matches the results reported by Vergara et al. [23]. Panicle number per hill, 1000-grain weight, and grain yield were higher in Jalalabad-14. Duan et al. [24] stated that panicle, spikelet, and 1000-grain weight are the most important traits and key enhancers of yield, therefore the grain yield was higher in Jalalabad-14. Reduction in spikelet number and ripened grain in 2017 might also be due to raise in temperature as stated that high temperature caused spikelet sterility and shortening grain filling duration [25].

Improvement of rice grain quality is one of the main targets of the breeding program to release high-quality varieties. Amylose, protein, and lipid contents are crucial factors to determine rice grain quality [26]. In this study, higher amounts of amylose and protein contents were recorded in Jalalabad-14 and lower amounts were exhibited in Attai-1 varieties. Amylose and protein contents are considered components of rice grain and varied based on genotypic and environmental factors [7]. Grain with low amylose content becomes soft and sticky while high amylose content makes the grain become hard and separate [27]. High amylose, protein, and lipid contents decrease the grain taste point, thus Jalalabad-14 exhibited lower taste point and Attai-1 showed a higher taste point. Protein and lipid contents are also major indicators of the nutritional value of rice grain [28]. High temperature in 2017 caused to increase protein and decrease taste score; this is true because high temperature leads the way for chalkiness and declines grain quality [29].

Appearance quality, consisting of grain size, chalkiness, and the ratio of perfect to imperfect grain, is an important trait in rice production and consumption. Hoshikawa [30] classified brown rice into perfect and imperfect grins; the perfect grain is the one that normally and perfectly ripens, while, the imperfect grain is the one with some abnormality or defect in shape. The yield of perfect grain widely depends on variety and cultural practices [31]. In this study, the highest and lowest perfect grains were produced in Attai-1and Jalalabad-14 varieties, respectively. We found that lengthening in the maturity period may increase the percentage of perfect grains. Qiao et al. [31] reported that high nitrogen application associated with a high ratio of imperfect grains.

Antioxidants also play an important role in food value. Shimamura et al. [14] mentioned that oxidative degradation reduces the value of food and may have harmful effects on human health. Antioxidants inhibit oxidation of oxidizable substrates and are widely distributed in many food sources [32], thus they can support human health [13]. In this study, Jalalabad-14 showed high antioxidant activity compare to other varieties, meaning that the variety has high potential to act against oxidation reactions. Antioxidant activities showed a negative correlation with the physicochemical properties of the grain. Rayee et al. [33] reported that differences in antioxidant capacity might be due to genetic variation and environmental factors that contributed to the antioxidant capacity of varieties. Mrad et al. [34] stated that high temperature degraded bioactive compounds and decreased antioxidant activity in plants.

Rice endosperm plays an important role in the observation of accumulated structures of amyloplasts, starch granules, and protein bodies [11]. Rice grain starch granules are the smallest starch granules among the grain of plants and average from 3 to 8 μm in size [35]. The starch granules are the accumulation of several starch molecules that can be fractionated as liner amylose or branched amylopectin. The main variation in the composition of rice starch is caused either by amylose or amylopectin, together with chain length frequency and distribution [8] which has a profound influence on the physicochemical properties of starch [36].

In our study, normal accumulation of amyloplasts and starch granules were found in Attai-1, Shishambagh-14, and Zodrass varieties; however, Jalalabad-14 displayed enhanced protein bodies and their traces in the endosperm. Protein accounts for about 8% of the endosperm in rice grain and fills the space between starch granules and amyloplasts [11]. Such protein bodies are the main form of endosperm protein and vary from 0.5 to 4 μm in size, and are spherical in shape [37–39].

Protein bodies occurred most frequently at the periphery when compared to the central endosperm. A high concentration of protein bodies at the periphery may mean that these grains are less susceptible to breakage during milling [40]. This might be a factor in the observation that Attai-1 and Shishambagh-14 produced a high percentage of perfect grain and reduced the percentage of broken grain.

Zodrass showed some holes and internal cavities in the endosperm. Fannon et al. [41] mentioned that holes and internal cavities lead to enzyme susceptibility and chemical reactivity; this situation has a positive impact on modified starch production. Pores, channels, and cavities are therefore potential sources of starch which may be amenable to enzyme manipulation for a range of industrial uses. According to the above results, each variety has its strong and weak points. The outcome of this study will contribute information for rice breeders in Afghanistan in order to release new varieties based on regions, production aim, and consumer preferences.

5. Conclusions

Wide variations were observed in growth, yield, physicochemical properties, antioxidant activities, and scanning electron microscopic analysis among selected rice varieties. Generally, Jalalabad-14 showed the best performances at growth and yield attributions, as well as antioxidant activities, however Attai-1 exhibited better physicochemical properties and morphological analysis. Scanning electron microscopic observation revealed that Attai-1, Shishambagh-14, and Zodrass accumulated amyloplasts and starch granules without abnormality. Normal accumulation of grain leads to higher grain quality. These findings might help Afghan breeders to release new preferable varieties and advance information on the multi-faceted nature of grain from the perspective of starch and protein accumulation and antioxidant activities.

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