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Antioxidant Activity, Quality Parameters and Grain Characteristics of Rice Varieties of Afghanistan

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Abstract. Rice is one of the main source of food calories in the world, especially in Asia where the people eat it more than any other food. The goal of this study was to evaluate the antioxidant activity, grain traits and quality contents in Afghanistan's selected rice varieties. The antioxidant capacity was measured using DPPH (1,1-diphenyl-2-picrylhydrazyl) and Reducing Power methods. Grain length, grain width and grain thickness were in the range of 5.4-7.6, 1.7-3.2, and 1.5-2.2 mm respectively. Selah Zoodras, Taram Irani and Attai-1 had the longest grain length whereas Loke was associated with high width and thickness among the varieties. Protein, amylose and lipid content varied from 6.5-9.9, 20.3-23.5, and 10-18% respectively. Barah Kunduz showed the highest protein and lipid content while Selah zoodras was associated the highest amylose content. The selected rice varieties possessed moderate antioxidant activity and their physiochemical properties were not correlated with antioxidant capacity. Results of this study provided useful information on the selection and production of rice varieties with high nutritional and pharmaceutical values in Afghanistan.

Introduction

Rice (*Oryza sativa* L.) is the most important food crop in the world which is providing the primary source of calories for the 50% of the human population [1]. It is a significant stable food in Asia where about 90 % of the world's rice is produced and consumed [2]. Rice is the major stable food after wheat in Afghanistan and is cultivated widely in the north eastern, and south parts respectively [3]. Baghlan, Kunduz, Takhar provinces in the northeast and Nangarhar province in the south are considered to be the grain stock of rice in Afghanistan and playing a vital role for food security in the national level [4]. The rice yield production has a nonlinear relation with cultivation area in Afghanistan. Based on the report by ICARDA [5], the rice production has been increased from 3.22 t/ha in 2008 to 3.50 t/h in 2010. In contrary, the cultivation area has been decreased due to climate change impact [6].

Grain as the main product of rice and the source of nutritional elements, plays a crucial role in defending the body from the oxidative damages. Free radicals can cause oxidative damage to biomolecules such DNA, proteins and lipids and leads to chronic disease. Therefore, the consumption of whole grain can be a mechanism of prevention these diseases [7, 8]. Rice grain contains a large portion of antioxidants which are ingested daily by the people and many compounds such as phenolics and anthocyanin are found in this cereal [9] and its antioxidation is mostly related to phenolic compounds [10]. The daily consumption of the secondary metabolites as source of antioxidants in the diets will inhibit the development of chronic diseases and reduce the impact of oxidative stress [11]. It is reported that black rice bran has higher antioxidant activity than white rice bran. It was observed that rice potential health benefits seem to be related to its pericarp [12]. Some compounds mainly phenolics and anthocyanin have been studied and detected under germinated and normal conditions [9]. Alongside the rice yield and production, its quality has a crucial role in the adoption of new varieties. Grain quality properties is a dominant factor in economic returns for farmers and it really dictate market value in Afghanistan where the demand for the high-quality rice and its consumption is raising due to population growth. Rice varieties which selected for this research have only been evaluated by their yield production, and still there is lack of investigation for characterization of their genotypic variation. Therefore, the objective of this research was to evaluate the antioxidant activity, grain quality content and grain traits of Afghanistan selected rice varieties. These findings could help the breeders and commercial rice growers to choose high-quality varieties for the future rice production in Afghanistan.

Materials and Methods

Rice materials

The varieties selected for this research are cultivated widely in Afghanistan. These varieties could be representative of other native rice varieties in Afghanistan. Selah zoodras, Barah Kunduz and Sha Lawangi are famous aromatic rice varieties in Afghanistan. The seeds of 10 rice varieties (Table 1) were collected from the Departments of Agriculture, Irrigation and Livestock (DAILs), Afghanistan. The seeds moisture content was adjusted to 14 % using oven method. The dried seeds stored in air-tight plastic bags in the room temperature. The seeds were dehulled using Automatic Rice Husker Machine (model TR-250, Kett Electric Laboratory, Tokyo, Japan) to measure the quality content, then grounded to pass through a 100-mesh sieve for further analyses.

Table 1. Rice materials description.

No	Local Name	Type	Genotype
1	Bara kunduz	Indica	Variety
2	Selah zoodras	Indica	Variety
3	Zerati Garma	Indica	Variety
4	Surkhah zerati	Indica	Variety
5	Taram irrani	Indica	Variety
6	Shah Lawangi	Indica	Variety
7	Loke	Japonica	Variety
8	Jalal Abad-14	Indica	Variety
9	Shesham bagh-14	Indica	Variety
10	Attai-1	Indica	Variety

Quality Contents

Protein, amylose and lipid contents were measured using quality tester machine PGC Shizuoka Seiki PS-500 machine (version 2-12, Shizuoka Seiki Co., Ltd., Shizuoka, Japan).

Physical Properties

Grain length, grain width, grain length and width ratio, and grain thickness were measured using a Vernier Caliper. The 1000-grain weight measured in triplicate from one hundred brown grain.

Extraction

The extraction was followed the method reported by Shao et al. [13]. Briefly, one gram of rice flour of the samples was extracted with 20 ml acidified methanol (95% methanol: 1M HCl 85:15, v/v) twice. The mixture was shaken under room temperature for 4 hours. The mixture was then centrifuged at 15,300 rpm for 10 min at 4 °C and the supernatant was collected and combined. The supernatant was evaporated using rotary evaporator (SB-350-EYELA, Tokyo, Japan) at 37 °C to remove the methanol after adjusting the pH 1.5-2.0. The precipitates were defatted by hexane three times then extracted with 60 ml of ethyl acetate three times. The ethyl acetate extracts evaporated to dryness by rotary evaporator. The dried extract was dissolved in 50% methanol for further analysis.

Antioxidant Activity

Measurement of DPPH Radical Scavenging Activity

Antioxidant activity was determined following the method described by Elzaawely et al. [14]. A 0.5 ml of the extract was mixed with 0.25 ml of DPPH solution and 0.1 ml of 0.1 M acetate buffer pH (5.5). The mixture was shaken and kept at room temperature for 30 min in the dark place. The absorbance was measured at 517 nm using microplate reader (Thermo Scientific Multiskan GO, Japan). The BHT (dibutyl hydroxytoluene) standard (5-20 ppm) was used as the positive control. The IC_{50} value was calculated, thus the lower IC_{50} indicates the higher antioxidant activity. The following formula is used to measure the percentage of DPPH radical scavenging activity.

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = [(Acontrol - Asample)/Acontrol] \times 100.$$

In the formula, *Acontrol* is absorbance of reaction without a sample, *Asample* is the absorbance of the sample.

Estimation of Reducing Power

Reducing power was measured following the method reported by Singh [15]. Briefly, 0.1 ml of the extract was added in 2.5 ml potassium ferricyanide (1%) and 2.5 ml of phosphate buffer (0.2 M, pH 6.5). After incubation at 50 °C for 30 min, 2.5 ml of trichloroacetic acid (10%) was added to the mixture. The mixture was centrifuged at 4000rpm for 10 min. Then, 2.5 ml of the supernatant was taken out and mixed with 2.5 ml of distilled water and 0.5 ml FeCl3 (0.1%) was added. The absorbance was measured at 700 nm using a microplate reader (Thermo Scientific Multiskan GO, Japan). The BHT standard (5-20 ppm) was used as the positive control. The IC_{50} value was calculated, thus the lower IC_{50} indicated the higher antioxidant activity. Reducing power capacity expressed as bellow.

$$Reducing\ Power\ Capacity\ (\%) = 100\text{-}\ (Ao\text{-}As/Ao \times 100),$$

where *Ao* is the absorbance without sample, *As* is the absorbance of the sample.

Statistical Analysis

The data were analyzed using Minitab 16.0 (Minitab Inc. State college, PA, USA). Results were presented means ± standard deviation (SD). Differences among verities were analyzed by one-way ANOVA (Analysis of Variance), followed by Tukey multiple comparison tests. Correlations among the traits were performed using Pearson's Method. Statistical significance was defined at 5% level.

Results and Discussion

Physical traits of the samples

The grain seizes, and other traits differed dramatically among the samples (Table 2, Fig. 1). The grain length ranged from 5.3 mm to 7.6 mm while the grain width varied from 1.7 mm to 3.2 mm. Grain thickness ranged from 1.5 mm to 2.2 mm, and the 1000-grain weight was between 16.3 g to 23.8 g. Selah Zoodras, Taram Irani and Attai-1 had the longest grain length (7.6 mm) and followed by Zerati Garma, Shalawangi, Bara Kunduz, Jalal Abad-14 and Loke, while Sheshambagh-14 showed the shortest grain length (5.4 mm) (Table 2). Loke was the plump one with largest grain width (3.2 mm), thickness (2.2 mm) and highest 1000-grain weight (23.8 g). The shortest grain width was recorded at Selah Zoodras and Attai-1 (1.7 mm). Previous studies reported that proper grain traits are considered by breeders when developing new varieties for release and marketable production [16]. Sheshambagh-14 had the lowest 1000-grain weight among the samples. Grains with long length, low amylose and high protein are preferable for the people in Afghanistan.

Figure 1. Rice genotypes used in this study arranged according to their lengths.

Table 2. Grain characteristics of the varieties.

Variety	GL	GW	RLW	GT	1000-Grain Weight (g)
Bara Kunduz	7.1±0.5^{abc}	2.1±0.2^{bc}	3.3±0.4^{b}	1.7±0.1^{bcd}	23.7±0.4^{a}
Selah Zoodras	7.6±0.4^{a}	1.7±0^{d}	4.5±0.3^{a}	1.5±0^{ef}	16.7±0.1^{ef}
Zerati Garma	7.4±0.3^{ab}	2.2±0.2^{bc}	3.3±0.4^{b}	1.7±0^{bcd}	21.8±0.4^{b}
Surkhah Zerati	6.9±0.6^{bc}	2.3±0.2^{b}	3.1±0.2^{b}	1.8±0.1^{b}	21±0.2^{bc}
Taram Irani	7.6±0.5^{a}	2.3±0.4^{b}	3.4±0.6^{b}	1.7±0.1^{bcd}	20.3±0.2^{c}
Shah Lawangi	7.2±0.5^{abc}	2.3±0.2^{b}	3.2±0.4^{b}	1.8±0.1^{bc}	20.7±0.1^{c}
Loke	5.3±0.3^{d}	3.2±0.4^{a}	1.7±0.2^{d}	2.2±0.10^{8a}	23.8±0.3^{a}
Jalal Abad-14	6.7±0.4^{c}	2±0.3^{c}	3.3±0.4^{b}	1.7±0.1^{cd}	17.7±0.3^{d}
Sheshambagh-14	5.4±0.3^{d}	2.2±0.1^{bc}	2.4±0.1^{c}	1.6±0.1^{de}	16.3±0.2^{f}
Attai-1	7.6±0.5^{a}	1.7±0.1^{d}	4.4±0.3^{a}	1.5±0.1^{f}	17.4±0.5^{de}
ANOVA					
Variety	*	*	*	*	*

GL: Grain Length, GW: Grain Width, RLW: Ratio of Grain, Lenght, GT: Grain Thickness. Means with same letter in a column are not significantly different at 5% level. * indicates significant differences at (p <0.05).

Grain Quality Content

Grain quality contents are shown in Table 3. The result showed that there is a wide difference of quality contents except for amylose content among the varieties. In this study protein content ranged from 6.5% to 9.9%, amylose content varied from 20.3% to 23.5% and lipid content 10% to 18%. Jing et al. [17] reported that amylose content varied from 8% to 16% between places and from 5% to 22% among varieties. Protein content is different among varieties, but it is raised and accumulated in the grain due to fertilizer application [18]. Bara Kunduz associated with the highest significant protein content (9.9 %) followed by, Shah Lawangi, Sheshambagh-14, Tarram irrani, Surkha Zerati, Loke and Zerati Garma (9.2, 8.9, 8.8, 8.3, 7.8, and 6.5%) respectively. Protein content accumulation is remarkably affected by many factors, such environment, genetic interaction and mailing process [19]. All the values of the protein content in this study were lower than 10%. Based on the previous studies and increase to the protein content higher than 10% will decrease lysin which affects negatively the rice digestibility [20]. Attai-1 contained the highest amylose content (23.5%) while Selah Zoodras had the lowest (20.3%). Among the varieties, Surkhah Zerati associated with the highest lipid content (18 %), whereas the lipid contends declined to 10 % at Bara Kunduz.

Table 3. Grain quality contents of the varieties.

Variety	PC (%)	AC (%)	LP (%)
Bara Kunduz	9.9±0.1^{a}	20.9±0.1^{ab}	10±0^{f}
Selah Zoodras	7.5±0.15^{ef}	20.3±5.7^{b}	11±0^{e}
Zerati Garma	6.5±0.1^{g}	22.8±0.1^{ab}	12.7±0.6^{d}
Surkhah Zerati	8.3±0.2^{cd}	22.8±0.1^{ab}	18±0^{a}
Taram Irrani	8.8±0.12^{bc}	23.1±0.1^{ab}	13.7±0.6^{c}
Shah Lawangi	9.2±0.12^{b}	22.7±0.1^{ab}	16±0^{b}
Loke	7.8±0.2d^{e}	22±0.6^{ab}	13±0^{cd}
Jalal Abad-14	7.1±0.06^{f}	22.1±0.2^{ab}	11±0^{e}
Sheshambagh-14	8.9±0.2^{b}	22.5±0.1^{ab}	10±0^{f}
Attai-1	7.8±0.15^{de}	23.5±0.3^{a}	11±0^{e}
ANOVA			
Variety	*	NS	*

PC: Protein Content; AC: Amylose Content; LP: Lipid Content. Means with same letter in a column are not significantly different at 5% level. * indicates significant differences at ($p <0.05$) and NS indicates non-significant.

The relationship between AC and other grain properties is shown in Fig. 2. The results indicate that there is a nonlinear relation between AC and PC, LP, GL and GW ($R^2 = 0.02$, $R^2 = 0.16$, $R^2 = 0.00$, and $R^2 = 0.01$) respectively.

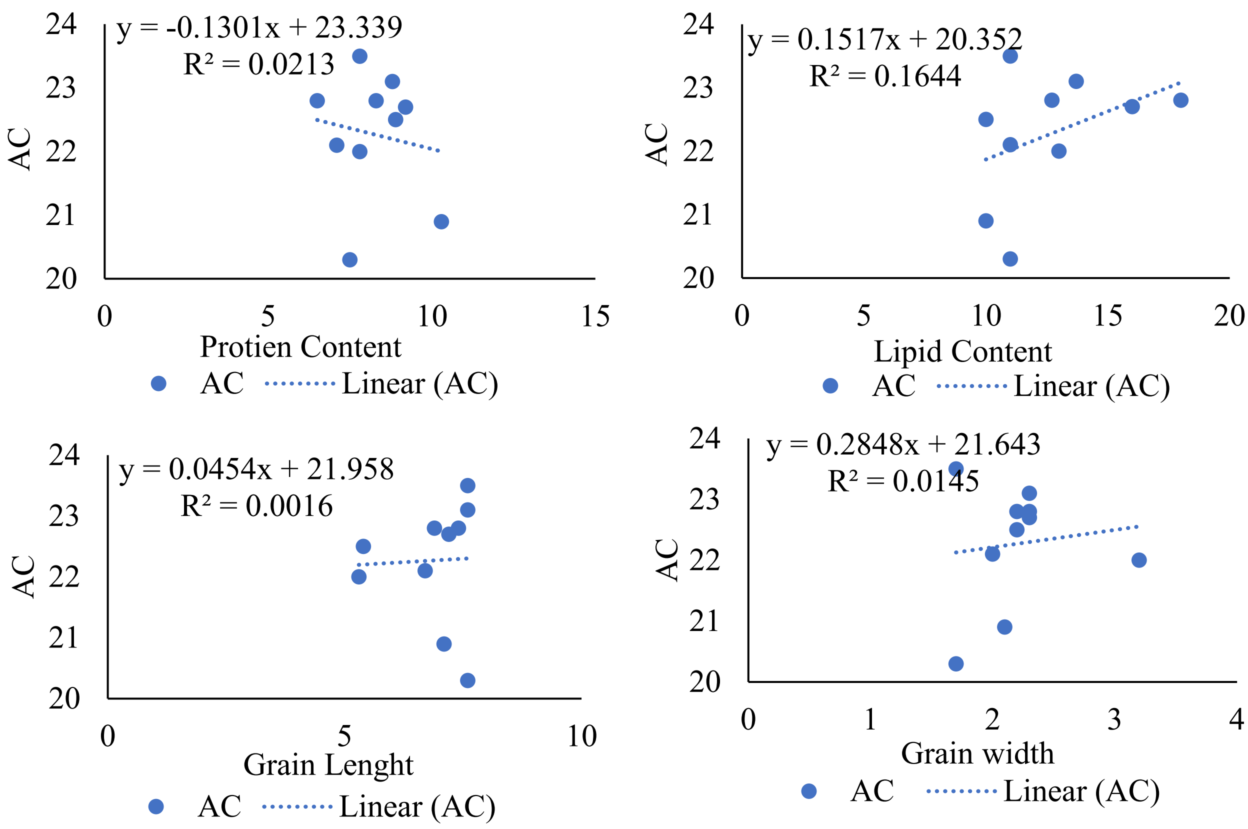


Figure 2. The relation of AC with, protein, content, lipid content, grain length and grain width.

Antioxidant Activity (AA)

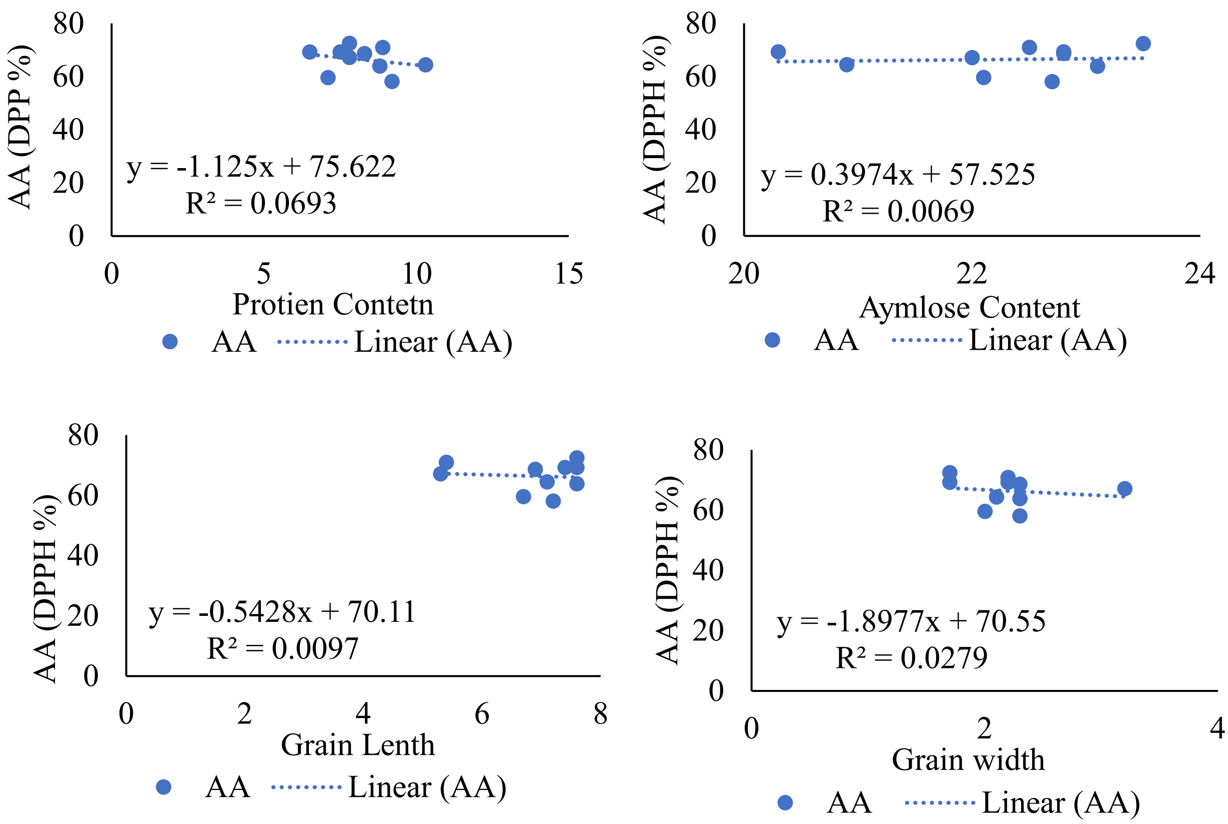
Table 4 showed the result of the samples antioxidant activity. The varieties revealed a highly significant variation (p<0.05) for antioxidant activity in both DDPH and Reducing assays. The IC_{50} values for DPPH assay ranged from 3.1 mg/l (Shah Lawangi) to 8.3 mg/ml (Selah Zoodras). These findings are consistent with IC_{50} values (6.01 - 14.47 mg/ml) of DPHH assay in Bangaladish rice varieties which reported by Dutta et al. [21]. The grain extract of Shah Lawange in DPPH assay expressed the highest scavenging activity with the value of 3.1 mg/ml followed by Loke, Surkha Zerati, Sheshambagh-14, Attai-1 (4.29, 5.66, 6.18 and 7 mg/ml) respectively. All the IC_{50} values of the sample extracts were higher than BHT standard of DDPH (2.9 mg/ml) and reducing power (11.14 mg/ml) not shown in Table 4. Antioxidant activity in the reducing power assay was lower than DPPH assay (Table 4). The highest IC_{50} value for reducing power observed in Jalal Abad-14 (29.28 mg/ml), whilst the lowest was revealed in Sha Lawangi (10.06 mg/ml). The current finding indicates that genetic variation, soil types and environmental conditions may have contributed to the antioxidant capacity of varieties and the data suggests that the varieties have moderate antioxidant activity [22, 23].

Table 4. Grain antioxidant activity in term of IC_{50}.

Variety	DPPH (IC_{50} mg/ml)	Reducing Power (IC_{50} mg/ml)
Bara Kunduz	4.41±0.83[bcd]	13.02±0.74[bc]
Selah Zoodras	8.3±0.72[a]	12.35±2.48[bc]
Zerati Garma	4.95±0.77[bcd]	15.36±3.78[abc]
Surkhah Zerati	5.66±0.6[abcd]	11.72±1.45[bc]
Taram Irrani	3.74±0.46[cd]	11.56±0.43[bc]
Shah Lawangi	3.1±0.3[d]	10.06±0.87[c]
Loke	4.29±0.01[bcd]	14.66±3.09a[bc]
Jalal Abad-14	3.49±0.12[cd]	29.28±11.12[a]
Sheshambagh-14	6.18±1.75[abc]	22.46±9.46[abc]
Attai-1	7±2.31[ab]	25.98±6.44[ab]
ANOVA		
Variety	*	*

Means with same letter in a column are not significantly different at 5% level. * indicates significant differences at (p <0.05).

The relation of AA and other grain properties is shown in Fig. 3. The results showed that there is a nonlinear relation between AA and PC, AC, GL, LC and GW (R^2 = 0.069, R^2 = 0.007, R^2 = 0.0097, R= 0.061 and R^2 = 0.027) respectively. Based on these findings AA correlation could not be considered in the breeding or production of rice varieties.



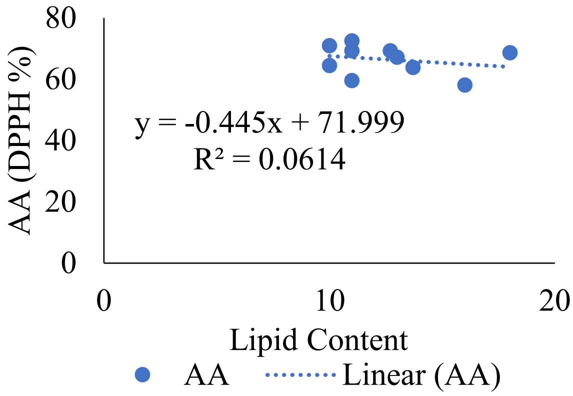


Figure 3. The relationship of AA with, protein content, amylose content, grain length grain width, and lipid content.

Inter correlation of grain physiochemical characteristics

The correlation coefficient of antioxidant activity, quality content and grain traits are presented in Table 5. The indicates that grain length showed a significant positive correlation with grain length-width ratio (r= 0.87, p<0.05) Table 5, while it had a significant negative association with grain width (r= -0.67, p<0.05). Grain width correlated negatively significant with grain length-width ratio (r= -0.89, p<0.05). The data showed that there is no significant correlation between quality content and other grain traits.

Table 5. Correlation coefficients of grain physical and biochemical traits.

	AA	**GL**	**GW**	**LWR**	**AC**	**PC**	**LC**
AA	1						
GL	-0.10 ns	1					
GW	-0.17 ns	-0.67 *	1				
LWR	0.14 ns	0.87 *	-0.89*	1			
AC	0.08 ns	0.04 ns	0.12 ns	-0.12 ns	1		
PC	-0.26 ns	-0.08 ns	0.08 ns	-0.16 ns	-0.15 ns	1	
LC	-0.25 ns	0.13 ns	0.36 ns	-0.17 ns	0.41 ns	0.02 ns	1

* indicates significant, ns non-significant correlation at 0.05 level; AA: antioxidant activity; GL: grain length; GW: grain width; LWR: ratio of length to width; AC: amylose content; PC: protein content; LC: lipid content

Conclusion

The results of this study imply that there was a high variation for the grain traits, quality contents and antioxidant activity. The varieties showed moderate protein content, but the amylose values were high in all varieties. These selected varieties possessed a moderate antioxidant activity. The demand for rice with high quality and yield is raising in Afghanistan as population is increased. These findings will bring better possibilities for breeders to easily choose the elite varieties for the rice production purposes and increase the production.

Conflict of interest

The authors declare no conflict of interests.

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