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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5(2): 212-214, 2002
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Chemical Composition and Fatty Acid Analysis of Saudi Hassawi Rice *Oryza sativa* L.

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Abstract: A comparison of the chemical and fatty acid composition between cvs. Hassawi rice (*Oryza sativa* L.) and Hassa No. 2 was made. Although other parameters of proximate analysis were not significantly modified, content of crude protein in cv. Hassa No. 2 decreased from 10.68% to 9.24%, whereas carbohydrates increased from 75.69% to 77.38%. In response to breeding, mineral composition was unchanged. Saturated fatty acids content significantly decreased with cv. Hassa No. 2, whereas, unsaturated fatty acids increased in comparison with cv. Hassawi. Palmitic, oleic, and linoleic acids were the most abundant fatty acids in both genotypes. Linolenic acid was significantly higher and palmitic acid was significantly lower in cv. Hassa No. 2 than in cv. Hassawi but myristic, stearic, oleic, and linoleic acid content were comparable. Apparently modifications were detected in the concentration of chemical constituents in seeds of Hassawi rice hybrid, the extent of which was dependent on the parameter being considered.

Key words: Breeding, *Oryza sativa* L., rice improvement, mineral content, proximate analysis

Introduction

Rice (*Oryza sativa* L.) grains are the principal source of protein and calories for over half of the world's human population (Poehlman, 1979). In the Eastern Province of Saudi Arabia an indigenous brown rice of Indica type, known as cv. Hassawi, has been cultivated for centuries in Al-Hassa oasis (25° N, 49° E). Hassawi rice is characterized by wide adaptability to soil salinity and drought, is grown in 200 hectares of farms yielding 400 tons. However, it bears some undesired characteristics such as susceptibility for lodging, delayed maturity, and photoperiod sensitivity. To overcome these cultivation problems associated with cv. Hassawi rice, a breeding program was initiated to produce improved varieties. Hybridization between cv. Hassawi and an introduced superior Taiwanese rice variety, Ai Chiao Chien, has resulted in the production of improved hybrids, one of which named cv. Hassa No. 2 (CATM, 1985).

Ultimately, the chemical composition of seed crops is determined by genetic factors and hence varies widely among species and their varieties (Ahmed *et al.*, 1998; Taira, 1989; Taira and Chang, 1986). Breeding strategies can affect the physical properties and chemical composition of seed crops (Bewley and Black, 1985). Therefore, it is necessary to evaluate the nutritional composition of newly developed Hassawi rice hybrids to assure the maintenance of high nutritional value associated with this particular rice variety. To obtain information on the chemical composition of cv. Hassawi rice, this study was conducted to determine proximate analysis, mineral content, and fatty acid composition. Furthermore, this investigation involved a comparison between cv. Hassawi rice landrace and its improved hybrid, cv. Hassa No. 2, to detect any potential changes in nutritional composition as a consequence of breeding.

Materials and Methods

Plant material: Mature rice (*Oryza sativa* L.) grains of cv. Hassawi and cv. Hassa No. 2 were collected at the end of 1999 season from Hofuf Regional Agricultural Research Center, Ministry of Agriculture and Water, Kingdom of Saudi Arabia. The seeds were sown at the end of April, transplanted in late June, and harvested in November. Nitrogen fertilizer was applied at 150 kg/ha, with 10% applied as basal dressing and the remainder as four top dressing splits (20, 30, 20 and 30%) applied 15, 25, and 35 days after transplanting and at the panicle-initiation stage. Phosphorous fertilizer was applied at 100 kg/ha, with 50% as basal dressing and the remainder as top dressing, 15 days after transplanting. The seeds were dehulled using conventional seed-cleaning equipment and immediately analyzed.

Proximate analysis: Proximate analysis for moisture, crude protein, crude fat, crude fiber and ash was performed in accordance with the Official Methods of Analysis of the Association of Official Analytical Chemist (AOAC, 1984). Moisture content determination was based on the decrease in weight after 2-3 g of seeds have been dried in an air oven set at 85 °C for 3 days. Percentage of total carbohydrates was determined by subtracting the sum percentage of moisture, crude protein, crude fat, and ash from one hundred.

Mineral composition: Mineral composition was determined by digesting the ash with concentrated HNO_3 and $HClO_4$ in 4:1 ratio. Sodium and potassium contents were determined using flame photometer (Jenway, Model PEP7). Calcium and magnesium were quantified using atomic absorption spectrophotometer (Perkin-Elmer, Model 2380). Phosphorous was determined according to Chapman and Pratt (1978).

Fatty acid analysis: The methyl esters of the fatty acids were prepared directly from the seeds using gas chromatography according to the method described by Flood (1981) with some modifications reported by El-Shintinawy and Selim (1995). Samples of ground clean and dry seeds, 200 mg, were mixed with 4 ml of 0.66N KOH in methanol placed in a 20-ml screw capped tube. Tubes were flushed with N_2, sealed, and heated at 100 °C for 5 min. The mixture was cooled and 4 ml of boron trifluoride in methanol (14% w/v) (BDH Co.) was added. Tubes were flushed with N_2 again, capped, and heated at 100 °C for 5 min. The mixture was cooled and 4 ml of each of saturated NaCl, water, and petroleum ether (b.p. 40-60 °C) were added. The supernatant was removed, washed with water, dried over anhydrous Na_2S0_4, and concentrated by a stream of N_2 at room temperature to about 1 ml. Methyl ester of dodecanoic acid (2 µl) was added as internal standard. A sample of 0.5 µl of the supernatant was injected into a GLC-variant 6000 with Flame Ionization Detector (FID), 2 m length, 1/8 inch internal diameter stainless steel column, packed with 15% OV-275, Chrom P/acid wash/80-100 mesh stationary phase, operated at 175 °C. Injection and detector temperatures were 230 and 250 °C, respectively. The helium carrier gas was set at a flow rate of 25 ml/min, hydrogen flow rate at 30 ml/min, and airflow at 300 ml/min. Fatty acid methyl esters were identified by comparing their retention times with that of the standards under the same operating conditions. Fatty acid quantities were determined from the area obtained by KLB-2220 Recorder Integrator. The unsaturated/saturated fatty acid ratio was calculated by dividing the summed percentage of unsaturated fatty acids by the summed percentage of saturated fatty acids.

Statistical analysis: Data were subjected to analysis of variance based on triplicate samples and the means were separated using LSD at 5% significance.

Results and Discussion

Proximate analysis: Based on the proximate analysis (Table 1), breeding of cv. Hassawi rice appeared to modify the nutrient composition of the resultant cv. Hassa No. 2 hybrid. Percentages of nitrogen and crude protein significantly decreased while the percentage of carbohydrate significantly increased in cv. Hassa No. 2. Other parameters including moisture, crude oil, and ash content were also modified but the difference was statistically non-significant. The crude fiber content was the least affected parameter by breeding as both cv. Hassawi and cv. Hassa No. 2 showed similar percentages (Table 1). In comparison with other rice genotypes, both Hassawi rice genotypes are higher in crude protein, crude oil, and ash contents but lower in carbohydrate content (Adeyeye and Ajewole, 1992; Muzafarov and Mazhidov, 1997).

Mineral content: Different rice genotypes contain differing concentrations of minerals (Sangha and Sachdeva, 1999). However, the mineral analysis data of Na, K, Ca, Mg, and P in the seeds of the two rice genotypes tested in this study were not significantly different. Apparently the breeding for dwarfism, early maturity, and day-length insensitivity caused minor changes in the mineral composition (Table 1). Cultivar Hassa No. 2 had a higher K and P contents than cv. Hassawi. However, cv. Hassawi had a higher Ca and Mg than cv. Hassa No. 2. Content of Na was approximately the same for both genotypes. Similar results were obtained in other genotypes (Adeyeye and Ajewole, 1992; Oyenuga, 1968) for K, Ca, Mg, and P which ranged from 325 to 342, 51 to 120, 60 to 119, and 290 to 390 mg/100 g, respectively. However, Na content in both cv. Hassawi and cv. Hassa No. 2 were higher than those obtained from other rice genotypes in which Na level ranged from 15 to 78 mg/100 g.

Fatty acid analysis: Previous research has shown that the fatty acid content of rice differ depending on genotypes (Deka *et al.*, 2000; Taira, 1989). The three most abundant fatty acids in both Hassawi rice genotypes were palmitic, oleic, and linoleic acids (Table 2). This was in agreement with previous results obtained with other rice genotypes (Adeyeye and Ajewole, 1992; Ali *et al.*, 1998). Apparently, breeding had little or no influence on the level of myristic, stearic, oleic and linoleic acids since these were not significantly different between the parental line cv. Hassawi and its hybrid cv. Hassa No. 2. Changes in palmitic and linolenic acids were most responsive to breeding since these showed significant differences between the two genotypes. The ratio of fatty acids of cv. Hassawi to cv. Hassa No. 2 were 1.18, 1.22, 1, 0.9, 1.02, and 0.65 for myristic, palmitic, stearic, oleic, linoleic, and linolenic acids, respectively.

In comparison with our results (Table 2), mean fatty acid composition of Indica and Japanica cultivars of brown rice obtained by Taira and Chang (1986) showed a lower myristic, palmitic, stearic, linoleic and linolenic acid contents but higher oleic acid content compared with the Hassawi cultivar. This was also true for cv. Hassa No. 2, except that palmitic acid content was higher in other Indica types.

Table 1: Proximate analysis and mineral composition of rice seeds of cv. Hassawi and cv. Hassa No. 2.

	Genotype	
Analysis	cv. Hassawi	cv. Hassa No. 2
Proximate analysis (%)		
Moisture	9.20a*	8.83a
Nitrogen	1.86a	1.60b
Crude protein	10.68a	9.24b
Crude oil	2.04a	2.29a
Crude fiber	0.84a	0.85a
Carbohydrate	75.69b	77.38a
Ash	1.55a	1.40a
Mineral content (mg/100 g seeds)		
Sodium	140a	139a
Potassium	315a	320a
Calcium	122a	118a
Magnesium	118a	112a
Phosphorous	354a	361a

*Values followed by the same letter within a row are not significantly different at 5% significance level.

Table 2: Percentage fatty acid composition of rice seeds of cv. Hassawi and cv. Hassa No. 2

	% of total fatty acids	
Fatty acid composition	cv. Hassawi	cv. Hassa No. 2
Myristic (14:0)	0.80a*	0.68a
Palmitic (16:0)	20.15a	16.55b
Stearic (18:0)	2.60a	2.59a
Oleic (18:1)	31.94a	35.39a
Linoleic (18:2)	42.79a	42.16a
Linolenic (18:3)	1.72b	2.63a
Total saturated fatty acids	23.55a	19.82b
Total unsaturated fatty acids	76.45b	80.18a
Unsaturated/saturated fatty acids ratio	3.25b	4.05a

*Values followed by the same letter within a row are not significantly different at 5% significance level.

Apparently the breeding program significantly altered the content of total saturated and unsaturated fatty acids in cv. Hassa No. 2 hybrid. Cultivar Hassa No. 2 exhibited significantly higher ratio of unsaturated to saturated fatty acids (4.05) than cv. Hassawi (3.25). This reflects the significantly observed higher level of total unsaturated and the lower level of total saturated fatty acids compared with the Hassawi cultivar (Table 2).

In conclusion, this study has provided detailed analysis of the chemical composition of Hassawi rice seeds. Hassawi rice is richer in some nutrients than other international rice genotypes. In addition, the chemical constituents of Hassawi rice were compared with its hybrid, cv. Hassa No. 2. The levels of some chemical constituents were modified; such variations between the two cultivars may be attributed to the genetic differences introduced by the breeding program.

Acknowledgement

The authors wish to extend their thanks to Mr. Hijji Hussein Al-Ashoor of Hofuf Regional Agricultural Research Center, Ministry of Agriculture and Water, Kingdom of Saudi Arabia for providing expertise in rice fields and seed collection.

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