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Characterization of Biochemical and Proximate Composition in Rice Grains as Influenced by Germination

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Abstract: Present study was conducted to characterize ten rice (*Oryza sativa* L.) cultivars viz. IET-23466, Dhan-201, IET-23448, MAS-946, IET-23445, IET-23463, IET-23455, PR-123, PR-115 and IET-23449 based on biochemical (starch, amylose, amylopectin, total sugars, reducing sugars, total protein and amino acids) and proximate composition (moisture, crude protein, crude fiber, ash and fat) before and after germination with an aim to identify cultivars containing higher health promoting components after germination. Findings of our study demonstrated that germination significantly influenced the biochemical and proximate components in all the cultivars. Cultivars IET-23463, IET-23466, PR-123 performed better as revealed by higher content of crude protein, ash, crude fiber, reducing sugars and low content of amylose, amylopectin and starch after germination. These cultivars hold great potential after germination and would open up a useful opportunity for the food industries to use them as an ingredient in functional foods. Consumption of these cultivars as ingredients in functional foods after germination would afford greater health benefits to consumers because of increase of fiber, minerals, amino acids and simple sugars after germination.

INTRODUCTION

Whole grains are gaining popularity day by day because of presence of number of bioactive compounds that fights against chronic diseases[1]. The interest of germination of Brown Rice (BR) is enormously increasing, since, it is being demonstrated that the health benefits of BR are enhanced with germination. Health promoting bioactives present in whole grains makes them as a valuable ingredient in number of functional foods. This is the reason why the demands of consumers are also increasing for these functional foods and this led the scientists to develop more such cultivars that have health promoting components.

Rice (*Oryza sativa* L.) is one of the key cereal crop cultivated in the world and is the major staple food consumed by population globally. Brown rice contains three components, i.e, embryo, endosperm and bran after dehusking. Inspite of having nutritional components such as vitamins, dietary fibers and phytochemical compounds in the bran layer[2], brown rice is not consumed widely due to its poor cooking properties and hard texture.

Hence, germination of cereals is an economical and effective approach to improve nutritional and antioxidant properties of brown rice. During germination, hydrolytic enzymes are activated to decompose the large molecules such as starch, non-starch polysaccharides and proteins for the respiration which results in the synthesis of new cell components of germinated grains, i.e, simple sugars[3], peptides, oligosaccharides and amino acids. Simple nutrients formed after germination become easy to absorb and digest[4]. Germination has positive effects on amino acid composition and protein availability and also increases the contents of total sugars and bioactive components and decreases the contents of antinutrients[5]. Germinated Brown Rice (GBR) or their derived extracts and fractions have shown several properties such as antioxidant, antidiabetic, anticancer, neuroprotective and cholesterol lowering effects[6]. Due to these properties germinated brown rice combat against headache, colon cancer, heart disease, Alzheimer's diseases and Parkinson's like disease, blood pressure and blood sugar[7].

IET-23466, IET-23448, IET-23445, IET-23463, IET-23455 and IET-23449 are the advanced breeding lines developed for aerobic rice and collected from the Institute of Rice Research, Hyderabad, India. PR-123 and PR-115 are the commercial rice varieties released by Punjab Agricultural University and they are popular among farmers of that region. PR-123 differs from PR-115 because of having stay-green character and long growth duration. MAS-946 and Dhan-201 are the national checks for aerobic rice recommended by the Institute of Rice Research, Hyderabad. The growth duration of all the genotypes varies from 100-105 d under aerobic condition except PR-115 (115-120 d) and PR-123 (130-135 d). Water scarcity for rice cultivation in North-West India is really a problem. Due to looming water crisis and labour shortage for rice transplanting, rice farmers of North-West India are showing more interest in aerobic rice. Since, the grain development conditions and crop dynamics are different for aerobic rice genotypes, it would be interesting to record the possible genotypic difference of aerobic rice on biochemical and proximate composition of these cultivars.

Knowledge of the cultivars, that are more useful in promoting health benefits after germination could be useful for application in food products. Therefore, present study was conducted to compare changes in proximate and biochemical basis, in ungerminated and germinated ten brown rice cultivars.

MATERIALS AND METHODS

Procurement and germination of rice grain: Rice materials and germination procedure Rough rice of *Oryza sativa* L. cultivars viz. IET-23466, Dhan-201, IET-23448, MAS-946, IET-23445, IET-23463, IET-23455, PR-123, PR-115 and IET-23449 were selected for the study which were procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India. The preparation of germinated rice samples followed the methods studied and reported by Saetung. Rough rice grains were dehusked to obtain brown rice and then 100 g brown rice were sterilized with 25 mL of 0.07% sodium hypochlorite for 15 min. Then, the grains were steeped in distilled water for 12 h. The steeping water was changed after every 4 h and drained at the last of soaking. The steeped grains were distributed on germination paper and placed in petri plates. The germination took place in an incubating chamber for 48 h at 28-30°C with 90-95% relative humidity. The germinated grains were dried at 50°C to approximately 10% of moisture content. All the samples were finely grounded and stored at -20°C until used.

The proximate composition was determined by standard methods[8]

Estimation of moisture content: Sample weighing 2 g, was kept in a hot air oven at 130±1°C for 1 h and moisture content in percent was calculated according to the formula given below:

$$\text{Moisture content (\%)} = \frac{\text{WOS-WDS}}{\text{WDS}} \times 100$$

Where:
WOS = Weight of Original Sample
WDS = Weight of Dried Sample

Estimation of crude fiber: Finely ground fat free rice sample weighing 2 g was first boiled with 250 mL of 0.12 N sulphuric acid solution for half an hour, the residue was separated and washed thoroughly. The residue then boiled with 250 mL of 0.313 N sodium hydroxide followed by the separation, washing and drying of the residues. The dried residues obtained were weighed and ashed in a muffle furnace at 600°C and the crude fiber was calculated as per formula given:

$$\text{Crude fibre (\%)} = \frac{\text{weight loss on ignition (g)}}{\text{weight of sample (g)}} \times 100$$

Estimation of crude protein: Rice grain powdered sample (100 mg) was digested in Kjeldahl digestion tubes with 15 mL concentrated sulphuric acid and 5 g of digestion mixture until clear solution was obtained. The contents were cooled to room temperature and volume was made to 100 mL with distilled water. The aliquot (100 mL) was distilled with 50 mL of 40% NaOH solution using Kjeldahl distillation unit (FOSS Kjeltec 2100). About 100-150 mL of the distillate was collected in 25 mL of 4% boric acid solution and titrated against 0.1 N HCI acid till a grey colour was obtained as end point. Nitrogen content of the sample was calculated from the following formula:

$$N(\%) = [\{(X\text{-Blank})\times 0.00014\}/Y]\times 100$$

Where:
X = Titre value (mL)
Y = Weight of sample (g)

The crude protein content was calculated by multiplying N% with a factor of 5.95.

Estimation of crude fat: Fat analysis of samples was carried out using Soxtec 2045 (Foss instrument, Sweden) apparatus. Extraction cups were dried in oven at 130°C for 15 min and weight of empty cups was noted. Weighed sample (3 g) was taken in thimble. Dried empty cups were cooled and 70 mL petroleum ether was added. Firstly, preheat the instrument. When the temperature was attained, the extraction cups were attached to instrument and left them to boil for 30 min followed by rinsing for 20 min and all recovery of solvent was done for 10 min. The recovered ether was collected and fat contained in extraction cups was estimated by the following formula given below:

$$\text{Crude fat (\%)} = \frac{\text{Weight of fat (g)}\times 100}{\text{Weight of sample (g)}}$$

Estimation of ash content: Weighed 5 g powdered rice grain sample was first incinerated on hot plate until there were no more fumes. Afterwards, it was kept in muffle furnace at 550°C for 5 h; cooled to room temperature, weighed and the ash content was calculated as per the expression given below:

$$\text{Ash(\%)} = \frac{\text{Weight of ash}\times 100}{\text{Weight of sample}}$$

Estimation of biochemical composition

Extraction and estimation of total sugars: The estimation of total sugars was performed according to the method described by DuBois *et al*.[9]. Free sugars were extracted sequentially with 80 and 70% ethanol. The extracts containing sugars were concentrated by evaporating off the ethanol under vacuum. To 1 mL of appropriately diluted sugar extract, 1 mL of 5% phenol was added followed by addition of 5 mL concentrated sulphuric acid. The tubes were cooled at room temperature for 10 min. Intensity of the orange color developed was measured against reagent blank at 490 nm.

Estimation of reducing sugars: The estimation of reducing sugars was performed according to the method described by Nelson[10]. To 1 mL of appropriately diluted sugar extract, 1 mL of Nelson C was added. The tubes were covered with water condensors and then boiled in water bath for 20 min. After cooling the tubes to room temperature, 1 mL of Nelson D was added to each tube followed by addition of 7 mL of distilled water. The contents of the tubes were thoroughly mixed by shaking on a stirrer. The intensity of resulting blue colour was recorded at 510 nm against reagent blank.

Estimation of starch: The estimation of starch was performed according to the method described by Yoshida, etc. The sugar free residues from sugars extract were used for starch estimation. Sediment of the extract that was filtered was dried (60°C), weighed and boiled with 2 mL of deionised water for 30 min with occasional stirring. After cooling, 2 mL of 9.2 N perchloric acid ($HClO_4$) was added and the contents were stirred for 15 min. To this, 6 mL of water was added and centrifuged at 2000 g. Supernatant was collected and in the pellet 2 mL of 4.6 N $HClO_4$ was added. The volume was made to 10 mL with and centrifuged at 2000 g. Both the supernatants were mixed and the final volume was made to 25 mL. From this starch extract, total sugars were estimated by the procedure laid by DuBios *et al*.[9]. The concentration of starch was calculated by multiplying the value of glucose concentration so obtained in the test extract by a factor of 0.9.

Extraction of free amino acids and total soluble proteins: Extraction of free amino acids and total soluble proteins was performed according to the method described by Singh *et al*.[11]. Rice grains (0.1 g) were extracted twice with 5 mL of 0.1 N NaOH followed by centrifugation at 14000×g. The supernatant was pooled. To 2 mL of supernatant, 2 mL chilled 20% TCA was added and mixed thoroughly. After aging for 1 h at 4°C, the contents were again centrifuged at 14,000×g and the precipitates were dissolved in 0.5 N NaOH for protein estimation. Supernatant was used for total free amino acid determination.

Estimation of free amino acids: Estimation of free amino acids was performed according to the method described by Lee and Takahashi[12]. To 0.2 mL of the extract, 5 mL of ninhydrin reagent prepared by mixing 1% ninhydrin in 0.5 M citrate buffer (pH 5.5), Pure glycerol and 0.5 M citrate buffer (5:12:2) was added. The reaction mixture was heated in water bath for 12 min and after cooling the test tubes under running tap water the absorbance was read at 570 nm against reagent blank.

Estimation of total soluble proteins: Estimation of total soluble proteins was performed according to the method given by Lowry *et al*.[13]. To 0.1 mL of the protein extract, 0.9 mL of distilled water and then added 5 mL of reagent 3. Mix well and after an interval of 10 min, 0.5 mL of reagent 4 was added, mixed and kept aside for 30 min at room temperature. The intensity of blue colour developed was read against reagent blank at 520 nm.

Estimation of amylose and amylopectin: Extraction of amylose and amylopectin was done according to the method described by Takeda *et al*.[14]. Dried rice grains were immersed in 0.2% NaOH at 4°C for 2 days. The soften rice grains were grounded and the homogenate obtained was squeezed through cheesecloth and filtered. The starch was washed with 0.2% NaOH followed by centrifugation at 3500 g for 10 min and washed with water and acetone and was air dried.

Separation of amylose and amylopectin: Separation of amylose and amylopectin was performed according to the method described by Pipe *et al*.[15]. Starch suspension (0.5 mL) in distilled water was laid on the top of 10 mL of 80% sucrose solution and centrifuged at 4000 g. The supernatant was separated containing small granules (amylose) and washed twice in distilled water and centrifuged. This process was repeated four times in fresh 80% sucrose solution. Starch pellet obtained in four extractions was combined containing large granules of amylopectin. Supernatants containing small granules were pooled and centrifuged at 3500 g and the resulting starch pellet containing large and small granules were washed three times in distilled water and acetone dried at 40°C. Dried powder was passed through 100 mesh sieve for further analysis.

Estimation of amylose and amylopectin was done according to the method given by Chrastil[16]. Dried pellet (100 mg) was washed in 1 mL of anhydrous absolute ethanol and 9.0 mL of 1 M NaOH and incubated in water bath at 95°C for 30 min. 0.1 mL of the extract solution was mixed with 5 mL TCA (0.5%) and KI (0.01 M) was added. After mixing thoroughly for 30 min, the intensity of the colour developed was measured at 620 nm.

Statistical analysis: The results were reported as mean±Standard Deviation (SD) of triplicate determinations. Data were analysed using SPSS program version 16.0 (SPSS Inc., Chicago, IL. USA). One way ANOVA was used to define significant differences at a level of $p<0.01$.

RESULTS

In the present investigation, levels of proximate and biochemical components were determined in ungerminated and germinated rice cultivars with an aim to identify cultivars having more health promoting nutrients after germination. Content of moisture, crude fiber, crude protein, ash, fat, starch, amylose, amylopectin, total sugars, reducing sugars, total protein and amino acids was quantified. Based on ANOVA results germination significantly affected all the biochemical and proximate components in all the cultivars. Difference in the range of biochemical and proximate components before and after germination is clearly depicted by Box plot, i.e., Fig. 1(a-l).

Biochemical composition

Starch, amylose and amylopectin: Starch, amylose and amylopectin contents in ungerminated brown rice extracts were determined (Table 1). Starch content before germination varied from 73.58-82.45% with an average value of 78.22%. IET-23448 showed highest content of starch. Amylose content was in the range of 27.80 (MAS-946)-31.98 (PR-115)%. Amylopectin content varied from 45.15-50.95%. PR-123 showed the highest content and IET-23463 showed the lowest content

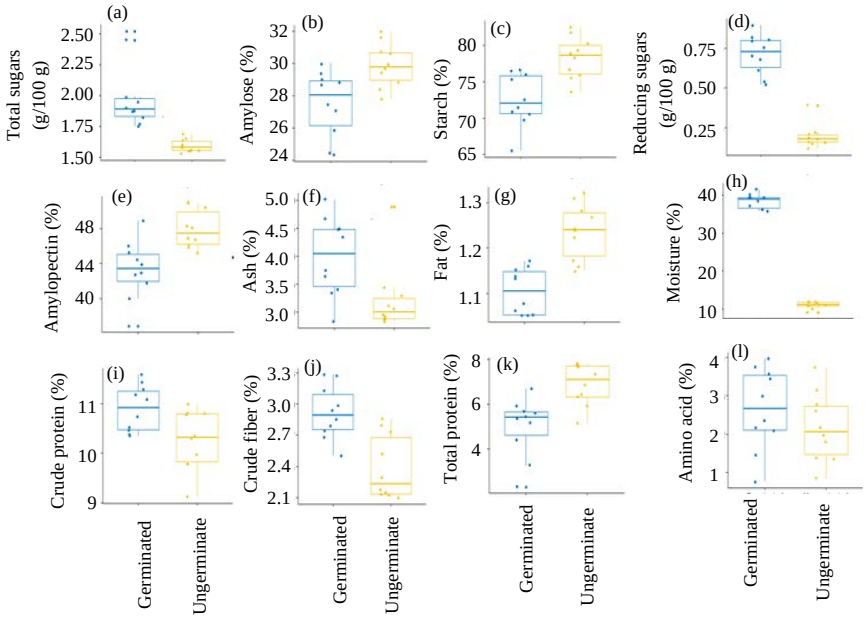

Fig. 1: Box plot showing the effect of germination on the biochemical and proximate components in rice grains (a-l)

Table 1: Effect of germination on contents of starch, amylose and amylopectin in rice grains

	Starch content (%)		Amylose content (%)		Amylopectin (%)	
Cultivar	Germinated	Ungerminated	Germinated	Ungerminated	Germinated	Ungerminated
Dhan-201	76.64±0.97 ^{a}	79.25±1.01^{bcd}	24.44±0.98^{e}	29.93±1.15^{abcd}	44.42±1.25^{bcd}	46.69±2.29^{bc}
IET-23445	70.52±0.97^{bc}	78.25±0.9^{cde}	28.8±1.08^{abc}	29.66±1.48^{abcd}	41.72±0.48^{de}	48.07±0.62^{abc}
IET-23448	75.27±1.42 ^{a}	82.45±1.26^{a}	29.99±0.85^{a}	31.58±0.46^{ab}	45.98±0.82^{bc}	50.87±1.58^{a}
IET-23449	71.47±0.60^{bc}	75.49±1.34^{ef}	27.43±0.61^{bcd}	29.43±0.45^{abcd}	42.7±1.09^{cde}	46.07±1.83^{c}
IET-23455	75.93±1.23^{a}	80.24±1.45^{abc}	27.06±0.55^{cd}	28.83±0.44^{bcd}	48.87±1.74^{a}	50.41±1.42^{ab}
IET-23463	65.51±0.91^{d}	75.84±1.05^{ef}	28.68±1.17^{abc}	30.59±1.06^{abcd}	36.83±1.92^{f}	45.15±1.6^{c}
IET-23466	69.68±0.63^{c}	76.61±1.25^{de}	24.32±0.81^{e}	28.38±1.29^{cd}	42.94±0.74^{bcde}	48.24±0.18^{abc}
MAS-946	72.53±0.97^{b}	73.58±1.08^{f}	25.84±0.79^{de}	27.8±0.89^{d}	43.85±1.28^{bcd}	45.78±1.8^{c}
PR-115	76.41±1.27^{a}	78.84±1.17^{bcd}	29.36±0.68^{ab}	31.98±0.71^{a}	45.97±1.52^{b}	46.86±1.36^{bc}
PR-123	70.83±0.64^{bc}	81.66±0.85^{ab}	28.99±0.69^{abc}	30.71±1.93^{abc}	39.94±0.85^{e}	50.95±1.58^{a}
Mean	72.48	78.22	27.49	29.89	43.32	47.91
A	1.63	1.48	2.2			
B	0.73	0.66	0.98			
AxB	2.3	2.09	3.11			

Values represent mean±S.D of triplicates; LSD values were analyzed by factorial CRD (Software CPCS1). A; Cultivar, B; Treatment, AxB; cultivar and treatment interaction; Values with different superscripts differ significantly

Table 2: ANOVA results of the effect of germination on the contents of starch, amylose, amylopectin, total sugars, reducing sugars, total soluble protein, amino acids, moisture, crude protein, crude fiber, fat and ash in rice grains

Mean squares													
Source of variation	df	Starch	Amylose	Amylopectin	Total sugars	Reducing sugars	Total protein	Amino acids	Moisture	Crude protein	Crude fibre	Fat	Ash
Cultivars	9	46**	15.13**	31.3**	0.104**	0.03**	4.8**	5.079**	10**	1.63**	0.414**	0.01368**	0.609**
Treatment	1	494.7**	86.23**	325.5**	2.3777**	3.973**	54.51**	3.422**	11226**	6.067**	4.256**	0.26401**	8.994**
Cultivars: Treatment	9	17**	2.81**	16.1**	0.1266**	0.031**	2.92**	0.659**	4**	0.107**	0.064	0.00501**	1.988**
Residuals	38	1.1	0.92	2	0.0022	0.004	0	0.001	0	0.485	0.056	0.00133	0.001

** Significant at 1% probability level ($p<0.01$)

(Table 1). Based on the ANOVA results (Table 2), significant differences were found between cultivars, treatment and their interactions, after germination. The contents of starch, amylose and amylopectin decreased significantly after germination (Table 1). Starch content varied from 65.61-76.64% with an average value of 72.48%. Cultivar Dhan-201 showed highest while IET-23463 showed lowest content of starch after germination. Mean content of amylose was 27.49% and their level was found to vary in the range of 24.32 (IET-23466)-29.99 (IET-23448)%. Amylopectin content was found to vary in the range of 36.83 (IET-23463) to 48.87 (IET-23455)% with a mean content of 43.32%.

Total sugar and reducing sugar: According to the ANOVA results, contents of total sugars and reducing sugars were significantly affected by cultivars, treatment and their interactions (Table 2). The mean content of total sugars in ungerminated brown rice cultivars was 1.59 g/100 g and their amount was found to vary from 1.53 (IET-23466) to 1.69 (MAS-946) g/100 g (Table 3). As shown in Table 2, significant increase was found in the contents of total sugars after germination. The content of total sugars varied in the range of 1.75-2.52 g/100 g (Table 2). Cultivar MAS-946 was found to have lowest total sugar content, i.e., 1.75 g/100 g. Reducing sugar content varied from 0.12-0.39 g/100 g with an average value of 0.19 before germination. After germination, reducing sugar content also increased significantly as depicted by ANOVA results (Table 2). Its value varies in the range of 0.52-0.89 g/100 g with a mean value of 0.71 g/100 g. Cultivar PR-123 was found to have highest value of reducing sugars, i.e., 0.89 g/100 g after germination and cultivar IET-23455 was having lowest content of reducing sugars.

Total soluble protein and amino acids: Total soluble protein content was determined and varied from 5.12-7.81% with an average value of 6.89% in the ungerminated grains of brown rice cultivars (Table 2). Cultivar PR-115 was found to have the highest total soluble protein content while IET-23448 was found to have the lowest total soluble protein content before germination. Germination process reduced the content of total soluble protein significantly in all the cultivars in terms of ANOVA result (Table 2) which ranged from 2.30-6.69% (Table 3). PR-123 was found to have lowest protein content after germination. The level of amino acids in ungerminated brown rice extracts ranged from 0.85 (IET-23448)-3.73 (IET-23466)% with a mean value of 2.17%. After 48 h, germination improved the content of amino acids significantly as shown in ANOVA table and ranged from 0.77-3.96% (Table 2). IET-23466 was found to have the highest content of free amino acids.

Changes in proximate composition

Moisture, crude protein and crude fiber: Based on ANOVA results, germination influenced the contents of moisture, crude protein and fiber positively (Table 2). Moisture content before germination varied from

Table 3: Effect of germination on contents of total sugar, reducing sugar, total soluble protein and amino acid in rice grains

	Total sugars (g/100 g)		Reducing sugars (g/100 g)		Total soluble protein (%)		Amino acids (%)	
Cultivar	Germinated	Ungerminated	Germinated	Ungerminated	Germinated	Ungerminated	Germinated	Ungerminated
Dhan-201	1.88±0.09^{bcd}	1.55±0.02^{cd}	0.68±0.08^{bcd}	0.22±0.11^{b}	5.46±0.03^{d}	6.84±0.08^{d}	2.36±0.02^{f}	1.96±0.03^{f}
IET-23445	1.82±0.08^{cd}	1.65±0.02^{ab}	0.8±0.04^{abc}	0.15±0.1^{b}	5.58±0.03^{c}	6.42±0.05^{e}	2.09±0.05^{h}	2.17±0.02^{e}
IET-23448	1.99±0.08^{b}	1.59±0.01^{bcd}	0.82±0.04^{ab}	0.18±0.06^{b}	4.42±0.05^{f}	5.12±0.03^{h}	0.77±0.02^{j}	0.85±0.02^{i}
IET-23449	1.87±0.01^{bcd}	1.64±0.02^{ab}	0.61±0.03^{cd}	0.19±0.05^{b}	5.66±0.05^{c}	7.73±0.05^{ab}	2.98±0.05^{e}	2.77±0.03^{c}
IET-23455	1.77±0.03^{d}	1.56±0.02^{cd}	0.52±0.06^{d}	0.16±0.09^{b}	6.69±0.04^{a}	7.34±0.03^{c}	3.44±0.02^{d}	1.35±0.03^{h}
IET-23463	2.45±0.08^{a}	1.56±0.03^{cd}	0.79±0.07^{abc}	0.18±0.03^{b}	5.34±0.09^{d}	5.92±0.03^{g}	2.17±0.03^{g}	1.79±0.04^{g}
IET-23466	1.95±0.01^{bc}	1.53±0.02^{d}	0.75±0.04^{abc}	0.39±0.05^{a}	3.26±0.03^{g}	7.73±0.05^{ab}	3.96±0.03^{a}	3.73±0.04^{a}
MAS-946	1.75±0.05^{d}	1.69±0.03^{a}	0.54±0.16^{d}	0.21±0.07^{b}	5.18±0.07^{e}	7.66±0.03^{b}	3.56±0.04^{c}	3.15±0.02^{b}
PR-115	1.9±0.07^{bcd}	1.58±0.02^{cd}	0.7±0.08^{abcd}	0.12±0.07^{b}	5.92±0.04^{b}	7.81±0.04^{a}	3.74±0.05^{b}	2.6±0.03^{d}
PR-123	2.52±0.04^{a}	1.6±0.05^{bc}	0.89±0.07^{a}	0.16±0.05^{b}	2.3±0.06^{h}	6.3±0.05^{f}	1.46±0.04^{i}	1.37±0.05^{h}
Mean	1.99	1.59	0.71	0.19	4.98	6.89	2.65	2.17
A	0.07		0.1		0.08		0.05	
B	0.03		0.05		0.03		0.02	
AxB	0.1		0.14		0.11		0.07	

Values represent mean±S.D of triplicates; LSD values were analyzed by factorial CRD (Software CPCS1). A; Cultivar, B; Treatment, AxB; cultivar and treatment interaction; Values with different superscripts differ significantly

9.1-11.92% with an average vale of 11.02%. IET-23466 showed lowest moisture content while MAS-946 had highest content of moisture in rice grains. Crude protein content was in the range of 9.11 (IET-23448)-10.99 (MAS-946)%. Crude fiber content ranged from 2.10-2.86%. IET-23455 showed lowest content of fiber whereas MAS-946 had highest content of fiber in grains. Moisture content after germination varied from 35.74-41.49% with a mean value of 38.37% (Table 4). Mean value of crude protein was 10.90% and their content was found to vary from 10.35-11.58% after germination. IET-23466 cultivar had highest crude protein content. Crude fiber content was in the range of 2.50-3.28%. Mean value was found to be 2.92%. Cultivar IET-23463 had highest content of crude fiber after germination out of all the cultivars.

Fat and ash contents: Content of fat in ungerminated brown rice extracts ranged from 1.15 (IET-23449)-1.32 (IET-23463)%. Mean value of fat content was 1.24% before germination. Significant differences were found based on ANOVA results in the content of fat after germination for two days, the content of fat reduced in all the cultivars and ranged from 1.05-1.16% with an average value of 1.10% (Table 4). Ash content before germination varied from 2.82-4.88%. Dhan-201 had highest content of ash before germination. Ash content increased significantly after germination as shown by ANOVA results in our study (Table 2). Its content was found to vary in the range of 3.34-5.01% (Table 4). Cultivar IET-23463 was found to have highest ash content after germination.

Multivariate analysis: Multivariate cluster analysis was used to cluster the cultivars before and after germination on the basis of biochemical and proximate components (Fig. 2 and 3). Figure 2 represents the dendrogram of ungerminated cultivars on the basis of contents of starch, amylose, amylopectin, total sugars, reducing sugars,

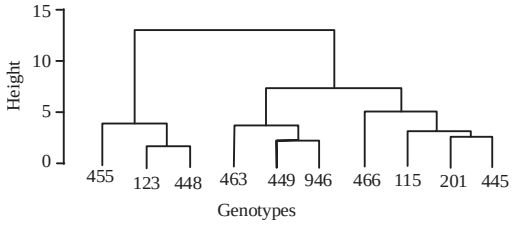

Fig. 2: Dendrogram of ungerminated rice grains on the basis of of starch, amylose, amylopectin, total sugars, reducing sugars, total protein, amino acids, crude protein, moisture, crude fiber, ash and fat content

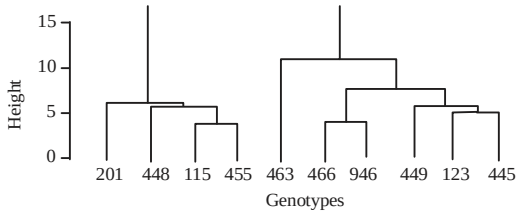

Fig. 3: Dendrogram of germinated rice grains on the basis of starch, amylose, amylopectin, total sugars, reducing sugars, total protein, amino acids, crude protein, moisture, crude fiber, ash and fat content

protein, amino acids, crude protein, fiber, fat and ash. This indicated the presence of diversity among the studied cultivars. Cluster I had two sub-clusters. Sub-cluster I had only one cultivar, i.e., IET-23455. Sub-cluster II had two cultivars namely PR-123 and IET-23448. Cluster II also contained two sub-clusters. Sub-cluster I had IET-23463 whereas sub-cluster II contained IET-23449 and MAS-946. Cluster III had further three sub-clusters. Sub-cluster I had IET-23466. Sub-cluster II had PR-115 and sub-cluster III had two cultivars namely Dhan-201 and IET-23445. Cultivars Dhan-201 and IET-23445 were

Table 4: Effect of germination on contents of moisture, crude protein, crude fiber, fat and ash in rice grains

	Moisture (%)		Crude protein (%)		Crude fiber (%)		Fat (%)		Ash (%)	
Cultivar	Germinated	Ungerminated	Germinated	Ungerminated	Germinated	Ungerminated	Germinated	Ungerminated	Germinated	Ungerminated
Dhan-201	38.48±0.68^{bc}	11.82±0.21^{a}	10.74±0.94^{a}	10.33±0.44^{a}	2.94±0.22^{ab}	2.12±0.29^{c}	1.08±0.04^{abc}	1.28±0.06^{ab}	3.43±0.02^{b}	4.88±0.03^{a}
IET-23445	35.74±1.04^{d}	10.01±0.24^{d}	11.08±0.74^{a}	10.29±0.39^{a}	2.5±0.28^{b}	2.15±0.18^{c}	1.05±0.04^{c}	1.16±0.03^{d}	4.34±0.04^{d}	3.11±0.03^{d}
IET-23448	37.16±0.21^{cd}	10.74±0.12^{c}	10.37±0.71^{a}	9.11±0.67^{ab}	3.13±0.23^{ab}	2.79±0.18^{ab}	1.14±0.02^{abc}	1.22±0.03^{bcd}	3.64±0.03^{f}	3.31±0.02^{b}
IET-23449	41.49±1.52^{a}	11.77±0.22^{a}	11.17±0.83^{a}	10.8±0.47^{ab}	2.79±0.37^{ab}	2.52±0.18^{abc}	1.05±0.03^{bc}	1.15±0.02^{d}	3.34±0.04^{g}	2.87±0.05^{efg}
IET-23455	39.34±0.43^{b}	10.96±0.26^{bc}	11.28±0.43^{a}	10.8±0.51^{ab}	2.98±0.31^{ab}	2.1±0.28^{c}	1.17±0.04^{a}	1.27±0.02^{ab}	4.48±0.02^{c}	3.29±0.02^{c}
IET-23463	40.2±0.62^{ab}	11.24±0.24^{b}	10.52±0.9^{a}	9.96±0.28^{ab}	3.28±0.13^{a}	2.73±0.3^{ab}	1.13±0.08^{abc}	1.32±0.03^{a}	5.01±0.02^{a}	3.05±0.04^{d}
IET-23466	36.21±0.45^{d}	9.1±0.21^{e}	11.58±1.35^{a}	10.77±0.45^{ab}	2.85±0.26^{ab}	2.17±0.10^{c}	1.05±0.02^{c}	1.17±0.02^{cd}	3.4±0.02^{g}	2.94±0.03^{e}
MAS-946	36.3±1.04^{d}	11.92±0.06^{a}	11.43±0.62^{a}	10.99±0.73^{b}	3.27±0.17^{a}	2.86±0.17^{a}	1.16±0.04^{ab}	1.24±0.03^{abc}	4.47±0.05^{c}	2.92±0.02^{ef}
PR-115	39.43±0.62^{b}	11.77±0.13^{a}	10.45±1.38^{a}	9.77±0.45^{a}	2.68±0.27^{ab}	2.13±0.14^{c}	1.06±0.03^{bc}	1.31±0.02^{ab}	4.66±0.04^{b}	2.86±0.03^{fg}
PR-123	39.37±0.55^{b}	10.85±0.04^{bc}	10.35±0.17^{a}	9.78±0.51^{a}	2.74±0.14^{ab}	2.29±0.18^{bc}	1.15±0.04^{abc}	1.24±0.04^{abc}	3.74±0.04^{e}	2.82±0.02^{g}
Mean	38.37	11.02	10.9	10.26	2.92	2.39	1.1	1.24	4.05	3.2
A	0.92		1.07		0.36		0.06		0.04	
B		0.41		0.48		0.16		0.25	0.02	
AxB	1.3		1.52		0.52		0.08		0.06	

Values represent mean±S.D of triplicates; LSD values were analyzed by factorial CRD (Software CPCS1). A-Cultivar, B-Treatment, AxB-cultivar and treatment interaction: Values with different superscripts differ significantly

in close proximity before germination. In cluster I, PR-123 and IET-23448 were in close proximity before germination. Figure 3 represents the dendrogram of contents of germinated cultivars on the basis of contents of starch, amylose, amylopectin, total sugars, reducing sugars, protein, amino acids, crude protein, fiber, fat and ash. Cluster I had two sub-clusters. Sub-cluster I contained Dhan-201. Sub-cluster II had three cultivars namely IET-23448, PR-115 and IET-23455. Cultivars PR-115 and IET-23455 came in close proximity after germination. Cluster II had two sub-clusters. Sub-cluster I had only one cultivar, i.e, IET-23463. Sub-cluster II had further sub-clusters. In this sub-cluster I contained IET-23466 and MAS-946 and sub-cluster II had IEt-23449, PR-123 and IET-23445. After germination cultivars PR-123 and IET-23445 came in close proximity. Similarily PR-115 and IET-23455 came in close proximity after germination.

DISCUSSION

Starch, a main reserve compound in cereal grains, consists of amylose and amylopectin before germination. α-amylase, β-amylase, debranching enzyme becomes active during germination and degrades these polysaccharides into simple sugars and improves digestibility[17]. These simple sugars resulted from starch degradation are the major sources of energy for all the metabolic reactions taking place in growing seedling[18]. Our results revealed that contents of starch, amylose and amylopectin of brown rice of all studied cultivars reduced after germination for two days whereas contents of sugars increased. This increase in the level of sugars was attributed to the activation of starch degrading enzymes. Our results are consistent with the results reported by Nissar *et al*.[5] in three paddy cultivars viz. Shalimar rice-1 (S), Jhelum (J), Kohsar (K). They found that after germination, starch content decreased and content of reducing increased with the increase of germination time in all the three cultivars. The effect of germination was also studied by Wunthunyarat *et al*.[19] in long grain brown rice cultivar. The results of their study also showed similar trend in the content of amylose and sugars. The increase of total free amino acids, after germination was a result of the degradation of protein by protease and a synthesis of new enzymes which helped to liberate the free amino acid. Veluppillai *et al*.[20] found similar results in rice grains, they soaked whole rice grains in distilled water for 12 h at 30°C and germinated for 5 days. The observations of their results showed that after germination process, the total protein content decreased significantly and the soluble protein content decreased up to the second day and then began to increase up to the fifth day. The levels of most amino acids also increased significantly after germination. Veluppillai *et al*.[20] observed that the free amino acid content increased significantly from 1.96-3.69 mg g^{-1} on a dry matter basis during brown rice germination for up to 5 days.

The increase in water uptake after germination could be due to the increasing: Number of hydrophilic cells from enzymatic degradation. These molecules bind with water and increase the bound water[21]. The results of the present study are well in agreement with the results reported by Rusydi *et al*.[22] in white, black, red, brown and barrio rice varieties. They also found significant increase in the moisture content in all rice varieties after germination. Increase in moisture content after germination was also observed by Moongngarm and Khomphiphatkul[23] in RD-8 rice cultivar. They reported that moisture content in RD-8 increased from 11-44.24% after one day of germination.

Different changes in the protein contents in brown rice grains for each cultivar could probably be due to metabolic changes that occur as a result of activation of various enzymes during germination process which led to the change in chemical composition of germinated rice[24]. During proteolysis, protein is degraded and hydrolysed into free amino acids, non-protein nitrogen substances: such as nucleic acids, these can cause protein level to be increased which can be transformed into new protein compounds rapidly. Our results for crude protein are

similar to the results reported by Nissar *et al*.[5] in three paddy cultivars viz. Shalimar rice-1 (S), Jhelum (J) and Kohsar (K).

The crude fiber comprising of components such as cellulose, lignin and hemicelluloses, increase significantly after germination[25] as the plant cells synthesize different cellular components. Chinma *et al*.[26] reported similar increase in soluble dietary fiber content after germination, in three Nigerian rice varieties (Jamila, Jeep and Kwandala). Our results for crude fiber are in well accordance with the results observed by Sibian *et al*.[3] They also found increase in the content of crude fiber in wheat, brown rice and triticale after germination.

The ash content of the rice cultivars gives an insight into their mineral composition. Higher ash content in germinated grains may be because of the formation of minerals such as magnesium, potassium and zinc from enzymatic activities during germination process[27]. Results of our study coincided well with the previous results reported by Xia *et al*.[28] in brown rice grains of Daohuaxiang JZX. Sharma *et al*.[29] reported similar results in yellow maize. They reported that ash content increased from 2.02-2.10% in yellow maize after germination for three days.

The fat content decreased with increase in germination time as the lipase activity increased during germination which breakdown the fat content into fatty acids and glycerol. These compounds inturns promotes embryonic growth. Similar results for fat content were observed by Kaur and Gill[30] in four cereals, wheat (*Triticum aestivum*) (HD-3086), rice (*Oryza sativa*) (PR-123), oats (*Avena sativa*) (OL-9) and maize (*Zea mays*) (Super-777). They found that fat content decreased upto 3 days after germination.

CONCLUSION

Significant variations were observed in the contents of starch, amylose, amylopectin, total sugars, reducing sugars and proximate composition after germination for all the rice cultivars. Cultivars IET-23463, IET-23466, PR-123 performed better and had higher content of fiber, crude protein, ash and lower starch, amylose and amylopectin contents after germination and these cultivars can be further incorporated in health improving products.

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