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Studies on biochemical parameters of hybrid rice varieties in the Faizabad region of eastern Uttar Pradesh, India

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Abstract
The experiment was carried out at the Department of Biochemistry, Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Faizabad as a part of PhD work. The seven varieties of hybrid rice namely- Dhani, US-312, 6201, 6302, 6444, JK-401, PHB-71 and 3 check varieties namely- Swarna, Sambhamah and Pusa Basmati were purchased from local market at Kumarganj, Faizabad from year 2012 to 14. The results highlighted that, the variety Pusa Basmati found highly superior overall the varieties and noticed, highest reducing sugar (0.34%), protein (8.43%), tryptophan (0.21 /16g N) and methionine (2.16g/16g N) content.

The highest (1.80 %) total mineral content found in Sambhamah followed by (1.66 %) Pusa Basmati and found highly significant and superior over 6201 and lysine (2.13g/16g N) followed by (1.92g/16g N) 6444 over US-312. The highest (74.71%) carbohydrate content was recorded in Swarna followed by (72.92%) Dhaniand highly superior over 6302. Like vise the variety PHB-71 found highest (1.52%) total sugar content followed by (1.25 %) Pusa Basmati and highly superior over 6302.

Keywords: carbohydrate, protein and total mineral, tryptophan, methionine and lysine

1. Introduction
Rice (*Oryza sativa* L.) is a plant belonging to the family of grasses, Poaceae. It is one of the three major food crops of the world with respect to acreage and production and forms the staple diet of about two third of the world's population. More than 90 per cent of the rice produced and consumed in Asian countries. Asia is the leader in rice production accounting for about 90 per cent of the world's production. Over 75 per cent of the world supply is consumed by people in Asian countries and thus rice is of immense importance to food security of Asia. In India, the rice is cultivated on 43.81 million hectare with production of 96.43 million tones (Anonymous, 2010) [1]. The demand for rice is expected to increase further keeping in view the expected increase in the population.

The projected demand for rice can only be met by maintaining steady increase in production over the years. Several breeding strategies are being employed in increasing the yield potential of rice and those among the available strategies; hybrid rice offers an immediate opportunity to break the yield plateau set by the semi-dwarf rice varieties after the first green revolution. In the recent years, much emphasis is given for the cultivation of hybrid varieties. Hybrid rice technology has proved to be one of the most feasible and readily adoptable approaches to break the yield barrier, as they produced about 15-20 per cent more than the best of the improved or high yielding varieties. Being convinced of the potential of hybrid rice technology in enhancing the production, India adopted this technique and has released nearly more than forty-three hybrids for commercial cultivation. Hybrid technology has been widely acclaimed and accepted. Hybrid rice is the commercial rice crop from F1 seeds of cross between two genetically dissimilar parents. Good rice hybrids have potential of yielding 15-20 per cent more than the best inbred variety grown under similar conditions (Nguyen van Suan, 1998) [14]. Hybrid rice was introduced commercially in China in 1976.

Keeping in view of above facts, potentially inclination towards hybrid rice by developed country and having vital chemical constituents hidden in it, the present study have been conducted.

2. Methodology

The present investigation was carried out at the Department of Biochemistry of Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Faizabad as a part of PhD work from 2012 to 2014. The details of material used and standard methods employed in present investigation has been described as under-Seven hybrids (Dhani, US-312, 6201, 6302, 6444, JK-401, PHB-71) and 3 checks (Swarna, Sambhamah and Pusa Basmati) were collected from local market at Kumarganj, Faizabad and Department of GPB, Narendra Deva University of Agriculture and Technology. The experiment was carried out at the Department of Biochemistry of Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Faizabad (U.P.) India, located in the Indogangetic plains of Eastern Uttar Pradesh at 26-46 ^{o}N latitude and 82.12 ^{o}E longitudes at an altitude of 113 meters above the mean sea levels (MSL). The study was started with equal level of moisture content in all the germplasm of hybrid rice (Rice 13%). The Completely Randomized Design (CRD) was employed with three replications. As per requirement the detailed method for different biochemical parameters are described below.

2.1 Carbohydrate content (%)

Total carbohydrate content was determined as described by Yemme and Willis (1954) [21]. Accordingly 1 g of dried sample was transferred in 100 ml glass stoppard measuring cylinder. Added 10 ml distilled water to it and stirred with a long glass rod to disperse the sample thoroughly. Thereafter 30 ml of 52 percent perchloric acid was added and frequently stirred for 20 minutes. The volume was made up to 100 ml with water. It was then mixed properly and filtered the content using Whatman No. 42 filter paper in to a 250 ml volumetric flask. It was diluted to the mark with water and mixed properly. 10 ml of the sample extract was diluted to 100 ml with water. Three test tubes were taken and transferred 1 ml diluted filtrate; 1 ml distilled water and 1 ml standard glucose solution separately. 5 ml anthrone reagent (0.1% in concentrated sulfuric acid) was added in all the test tubes. The test tubes were kept in a boiling water bath for 12 minutes for development of colour and colour intensity was read at 630 nm on spectronic 20. Total carbohydrate content was calculated on per cent basis.

3. Estimation of sugar content

3.1 Preparation of sugar extract

10 ml 50 percent ethyl alcohol was added to 1.0 g well-grounded kernel of rice grains and then centrifuged at 5000 rpm for 15-20 minutes. Supernatant was collected and evaporated on hot plate until 2-3 ml of it remained. This was followed by addition of 10 ml CCl_4 and mixed well and left for 10 minutes. The content was transferred into separating funnel. After the addition of CCl_4, two layers were formed. The lower layer was discarded, while upper layer was separated and 10 ml of distilled water was added to it. This was used as sugar extract for the estimation of both total and reducing sugars.

3.1 (a) Total sugar (g/100g)

Total sugar was determined by the method of Dubois *et al.* (1956) [4] using phenol reagent 0.1 ml sugar extract was taken in a test tube and volume was made up to 1 ml with addition of distilled water. Then 0.1 ml of 80 per cent phenol and 4 ml concentrated H_2SO_4 was added by the side of the test tube and then cooled down. The intensity of colour was recorded at 480 nm by spectronic-20. The calculation was done with the help of standard glucose solution and results were expressed as grams per 100g of sample.

3.1 (b). Reducing sugar (g/100g)

Reducing sugar content in grain was determined by the method of Miller (1959) [12]. 1 ml sugar extract was mixed with 3 ml dinitro-Salicylic acid and kept over water bath for 10 minutes. The test tube was collected at room temperature and the intensity of colour was recorded on spectronic-20 at 575 nm.

4. Protein content (%)

Protein content in grain was determined by the Lowry's method (1951) [11]. This method depends on quantification of the colour obtained from the reaction of protein with Folin Ciocalteau's reagent. The colour thus obtained was due to reaction of alkaline copper reagent with protein and reduction of phosphomolybdate and phosphotungstatein Folin's reagent by tyrosine and tryptophan amino acids present in protein.

One gram sample was taken and homogenized in the presence of 10 ml of distilled water and centrifuged at 5000 rpm for 15 minutes. The residue was discarded. Thereafter, 1 ml supernatant was taken and mixed with 1 ml 10 per cent trichloric acid. It was kept for 30 minutes at room temperature. The whole content was filtered with the help of filter paper and residue obtained was dissolved in 5 ml of 0.1 N NaOH. 0.5 ml of sample extract was taken in another test tube and volume was made up to 1 ml with distilled water. Then 5 ml alkaline copper reagent was added and it was mixed properly. After 10 minutes, 0.5 ml folin's reagent was added and it was kept at room temperature for 30 minutes. Finally, colour intensity was recorded at 660 nm on spectronic-20 against blank solution. Calculation was done by standard curve prepared from Bovine Serum Albumin (BSA) solution and results were expressed as amount of protein in per cent.

5. Tryptophan content (g/16gN)

Tryptophan content was estimated by the method of Spies and Chamber (1949) [17]. 0.2 g of homogenized dried sample was transferred to 100 ml conical flask and 10 ml of 19 N H_2SO_4 was added. Conical flask was kept for 12 hours in dark place. After expiry of period, the whole content of the conical flask was filtered by using cotton and filtrate was collected into another flask. 1 ml p-dimethyl amino benzaldehyde (30 mg dissolved in 100 ml 2N H_2SO_4) and 0.1 ml of sodium nitrite (0.045 per cent in distilled water) was added and mixed the content. This was kept for 30 minutes for colour development. The intensity of colour was measured on spectronic-20 at 620 nm against blank solution. The calculation was done by using standard curve prepared from standard tryptophan solution and results were expressed as g per 16 g N.

6. Methionine content (g/16gN)

Methionine content was analyzed as described by the Horn *et al.* (1946) [7]. 0.5 g sample was weighed and transferred in receiving flask. The 20 ml 6 N HCl was added to same flask. The material was refluxed for 20 hours. Thereafter, the content of flask was transferred into China dish. It was evaporated on water bath with addition of one gram of activated charcoal. Evaporation was continued until the content of china dish becomes viscous. Warm distilled water was added and filtered through Whatmann filter paper. The filtrate was collected in 25 ml volumetric flask. The china

dish was washed with little amount of hot water for about 5-6 times and filtered. The filtrate was collected in same volumetric flask and volume made upto 25 ml. This hydrolysate was used for calorimetric estimation of methionine. 10 ml of hydrolysate extract was transferred to 100 ml beaker with the addition of 4 ml of distilled water and 2 ml of 5N NaOH. Further, 0.1 ml sodium nitroprusside (1 g dissolved in 100 ml distilled water) and 2 ml of glycine solution (3% glycine solution) was also added. Finally, 4 ml of Meta phosphoric acid was added to develop colour. Intensity of colour was recorded with blank solution by sectronic-20 at 450 nm. The calculation was done by using standard curve prepared from standard methionine solution and results were expressed as g per 16 g N.

7. Lysine content (g/16gN)

Lysine content was estimated by method of Felker *et al.* (1978) [5]. 0.5 g of finely ground sample was taken in 250 ml volumetric flask. 50 ml of buffer solution (0.05 M tetra sodium pyrophosphate HCl buffer, pH 9.4) was added with gently shaking and kept on platform shaker for 2 hours at room temperature. Then it was centrifuged at 10000 rpm for 10 minutes. The supernatant was collected and absorbance was taken at 420 nm. Then 1 ml colouring reagent trinitro-benzene sulphonic acid (50 mg/ml) aqueous solution was added and again the solution was kept for gentle shaking one to one half hours. The absorption was recorded on 420 nm on spectronic-20. The difference in two reading was actual reading. The calculation was done by using standard curve prepared from standard lysine solution and results were expressed as g per 16 g N.

8. Total mineral content (g/ 100g)

The total mineral content was estimated by the method as described by Hart and Fisher, 1971 [6]. Material required for this estimation was silica crucible muffle furnace (600^0C) and dessicator with magnesium perchlorate desiccant. In this method the constant weight of silica crucible were achieved by placing clean crucible in Muffle furnance at 600^0C for one hours, transferring from furnance to dessicator weighing and repeating the above mentioned process till a constant weight of silica crucible were recorded, 2 g of the sample was transferred to ash less filter paper. Kept into the silica crucible and were placed into a hot plate to destroy the organic matter. Later on the crucible was transferred to a desiccators for cooling without absorption of moisture by the ash. The cold ash along with silica crucible was weighed and the result was calculated and reported on the moisture free basis into per cent.

$$\text{Total mineral or Ash content} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

9. Results and Discussion

The findings presented in the form of tables and salient features are described as under:-

10. Biochemical parameters

10.1 Total carbohydrate content

The highest carbohydrate content was found in Swarna (74.71%) followed by Dhani (72.92%).

The carbohydrate content of Swarna was recorded highly superior over 6302 and found statistically significant. These results have been supported by Muangkaeo *et al.* (2005) [13] and Oko *et al.* (2012) [15].

Table 1: Total Carbohydrate content of hybrid rice.

S. No.	Varieties	Total Carbohydrate (%)
	Dhani	72.92
	US-312	70.39
	6201	72.06
	6302	67.42
	6444	70.90
	JK-401	68.69
	PHB-71	71.56
	Swarna (C)	74.71
	Sambhamah (C)	70.03
	Pusa Basmati (C)	71.08
	SEm±	0.04
	CD at 5%	0.14

11. Total sugar content

The highest total sugar content was found in PHB-71 (1.52%) followed by Pusa Basmati (1.25 %). The total sugar content of PHB-71 was recorded highly superior over 6302. The variations in total sugar content may be due to differences in genetic variation and environmental factors of hybrid rice and traditional rice varieties. The results have a close agreement with the findings of Kapoor *et al.* (2011) [8] and Bhuyan *et al.* (2014).

12. Reducing sugar content

The highest reducing sugar content was found in Pusa Basmati (0.34%) and JK-401 (0.33%) and recorded highly superior over Swarna (0.19%) and Dhani (0.20%). The variations in reducing sugar content may be due to genetic diversity and environment factors. Similar results were also recorded by Kapoor *et al.* (2011) [8].

Table 2: Average total sugar and reducing sugar content of hybrid rice.

S. No.	Varieties	Total sugar (%)	Reducing Sugar (%)
1	Dhani	1.16	0.20
2	US-312	1.08	0.28
3	6201	1.18	0.32
4	6302	1.02	0.27
5	6444	1.17	0.31
6	JK-401	1.13	0.33
7	PHB-71	1.52	0.25
8	Swarna (C)	1.04	0.19
9	Sambhamah (C)	1.24	0.29
10	Pusa Basmati (C)	1.25	0.34
	SEm±	0.01	0.04
	CD at 5%	0.04	0.04

13. Protein content

The highest protein content was found in Pusa Basmati (8.43%) and 6302 (8.41%). The protein content of Pusa Basmati was recorded superior over Dhani and Swarna. Variations in protein content of rice varieties may be due to genetic diversity and environmental variations. Protein content of hybrid rice and traditional rice varieties varied significantly. Similar results of protein content were recorded by Thongbam *et al.* (2012) [19], Kim *et al.* (2013) [9] and Thomas *et al.* (2013) [18].

14. Total mineral content

The highest total mineral content was found in Sambhamah (1.80 %) followed by Pusa Basmati (1.66 %). The total mineral content of Sambhamah was recorded highly significant and superior over 6201.Variations in total mineral content of hybrid rice may be due to genetic variations and differences in salt accumulation during the metabolic

processes. Similar observations were also recorded by Yadav *et al.* (2007) [22] and Oko *et al.* (2012) [15].

Table 3: Protein and total mineral content of hybrid rice.

S. No.	Varieties	Protein (%)	Total Mineral (%)
1	Dhani	7.26	1.31
2	US-312	7.90	1.40
3	6201	7.63	1.13
4	6302	8.41	1.42
5	6444	7.47	1.27
6	JK-401	8.40	1.14
7	PHB-71	8.12	1.22
8	Swarna (C)	7. 31	1.33
9	Sambhamah (C)	8.32	1.80
10	Pusa Basmati (C)	8.43	1.66
	SEm±	0.01	0.01
	CD at 5%	0.05	0.05

15. Tryptophan content

The highest tryptophan content was found in Pusa Basmati (0.21 /16g N) followed by JK-401 (0.20 g/16g N). The tryptophan content of Pusa Basmati was recorded highly superior over 6302.Variation in tryptophan content of hybrid rice and traditional rice varieties may be due to varied in protein content and genetic variations in varieties. Similar results were also obtained by Reddy and Pushpamma (1986) [16] and Singh *et al.* (2008) [20].

16. Methionine content

The highest methionine content was found in Pusa Basmati (2.16 g/16g N) followed by Sambhamah (1.90g/16g N). The methionine content of Pusa Basmati was recorded highly superior over PHB-71.Variations in methionine content may be due to differences in genetic potential of hybrid rice and traditional rice varieties. Data on methionine content was recorded significant. The results have a close agreement with findings of Reddy and Pushpamma (1986) [16] and Singh *et al.* (2008) [20].

17. Lysine content

The highest lysine content was found in Sambhamah (2.13 g/16g N) followed by 6444 (1.92g/16g N) and Dhani (1.90g/16g N). All the treatments regarding Lysine content were found statistically significant. The lysine content of Sambhamah was recorded highly superior over US-312.Lysine content in hybrid rice and traditional rice varieties varied significantly. Variations in lysine content of hybrid rice and traditional rice varieties may be due to differences genetic potential. Similar findings were also reported by Singh *et al.* (2008) [20].

Table 4: Tryptophan, Methionine and Lysine content of hybrid rice.

S. No.	Varieties	Tryptophan (g/16g N)	Methionine (g/16g N)	Lysine (g/16g N)
1	Dhani	0.17	1.20	1.90
2	US-312	0.19	1.08	1.49
3	6201	0.17	1.22	1.90
4	6302	0.14	0.96	1.59
5	6444	0.16	1.71	1.92
6	JK-401	0.20	1.04	1.58
7	PHB-71	0.16	0.87	1.82
8	Swarna (C)	0.17	1.76	1.75
9	Sambhamah (C)	0.19	1.90	2.13
10	Pusa Basmati (C)	0.21	2.16	1.88
	SEm±	0.06	0.02	0.04
	CD at 5%	0.05	0.06	0.09

18. Conclusion

The study concluded that, the variety Pusa Basmati was found best among all the varieties in terms of its reducing sugar, protein, tryptophan and methionine. The highest Carbohydrate content noticed in Swarna followed by Dhani.

The hybrid variety PHB-71 was reported best in terms of total sugar content and Sambhamahin lysine and total mineral content respectively.

The variety Pusa basmati contains many biochemical qualities has the great potential to open up new vista for benefiting to farmers and people of high class of urban society and thus have enormous marketing scope in the domestic as well as global scenario. The other varieties like Sambhamah, Swarna and PHB-71 also have good potential.

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20. References

1. Anonymous. FAO statistical data base on agriculture, 2010. http//apps. FAO.org.
2. Bhuyan DJ, Barooah MS, Bora SS, Singaravadi K. Biochemical and nutritional analysis of rice beer of North East India. Indian Journal of Traditional Knowledge. 2014; 13(1):142-148.
3. Boraua I, Ahmed SA, Sarkar CR, Das D. Biochemical evaluation of scented rice of North India Bio-prospecting of commercially important plants. In proceeding of the national symposium on Biochemical approaches for utilization and exploitation of commercially important plant, Jorhat, India, 2003, 79-85.
4. Dubois M, Cilles KA, Homilton JK, Rebers PA, Smith F. Calorimetric method for estimation of sugar. Annal. Chem. 1956; 28:350-356.
5. Felker C, Libanuskas CK, Wainer G. Determination of lysine content in protein. Crop Science. 1978; 18(3):489-490.
6. Hart FL, Fisher HS. Modern food analysis. Springer Verlog: New York, 1971.
7. Horn JM, Jones DB, Blum AE. Calorimetric determination of methionine in protein and food. Journal of Biochemistry, 1946, 116- 313.
8. Kapoor N, Arya A, Siddiqui MA, Kumar H, Amir A. Physiological and biochemical changes during seed deterioration in aged seeds of rice (*Oryza sativa* L.). American Journal of Plant Physiology. 2011; 6(1):28-35.
9. Kim JW, Kim BC, Lee JH, Lee DR, Rehman S, Yun SJ. Protein content and composition of waxy rice grains. Pak. J Bot. 2013; 45(1):151-156.
10. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227:680-685.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (). Protein measurement with the folin·s reagent. J Biol. Chem. 1951; 193(1):265-275.
12. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Annal. Chem. 1959; 31:426-428.

13. Muangkaeo R, Srichuwong S, Vearsilip S. Influence of packaging material and storage time on seed viability and chemical component of rice seed. Conference on International Agriculture Research for Development, 2005, 11-13.
14. Nguyen VN, Tran DV, Quach NH. The International Workshop held from 28 to 30 May 1998, Hanoi, Vietnam Ed. in Procd, 1998, 40-44.
15. Oko AO, Ubi BE, Dambaba N. Rice cooking quality and physico-chemical characteristics: a comparative analysis of selected local and newly introduced rice varieties in ebonyi state, Nigeria. Food and Public Health. 2012; 2(1):43-49.
16. Reddy MV, Pushpamma P. Effect of storage on amino acid and biochemical quality of protein in different varieties of rice and sorghum. Nutrition Report Int. 1986; 33(5):703-706.
17. Spies JT, Chamber DC. Chemical determination of tryptophan in protein. Annal. Chem. 1949; 21:12-49.
18. Thomas R, Wan-Nadiah WA, Bhat R. (). Physiochemical properties, proximate composition and cooking qualities of locally grown and imported rice varieties marketed in Penang, Malaysia. International Food Research Journal. 2013; 20(3):1345-1351.
19. Thongbam PD, Tarentoshi, Raychaudhary M, Durai A, Das SP, Ramya KT *et al*. Studies on grain and food quality traits of some indigenous rice cultivars of north-eastern hill region of India. Journal of Agricultural Science. 2012; 4:(3).
20. Singh D, Metha R, Talati JG. Identification of rice (*Oryza sativa* L.) varieties through biochemical markers. Indian J Agric. Biochem. 2008; 22(1):18-25.
21. Yemme EW, Wills AJ. The estimation of carbohydrate in the plant extracted by anthrone. Biochem. J. 1954; 87:508-514.
22. Yadav RB, Khatkar BS, Yadav BS. Morphological, physicochemical and cooking properties of some Indian rice (*Oryza sativa* L.) cultivars. Journal of Agricultural Technology. 2007; 3(2):203-210.