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Bio-Chemical and Functional Characteristics of Black Gram (*Vigna mungo)* Cultivars Grown in Himachal Pradesh,India

Rajni Modgil*, Shilpa Kaundal and Anupama Sandal

Department of Food Science Nutrition and Technology, College of Home Science, CSKHPKV Palampur HP176062, India

**Corresponding author*

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A B S T R A C T

The present study was undertaken to evaluate the nutritional quality and value addition of Black gram grown in H.P. Three cultivars/varieties of Black gram viz. UG-218, HIM MASH and Local cultivar were taken for the study. The various biochemical parameters studied were physico-chemical characteristics, proximate compositional, nutritional quality, functional properties and anti-nutritional constituents. The results of the investigation showed that UG-218 had maximum length, breadth and 1000 seed weight as compared to HIM MASH and Local cultivar. Whereas Local cultivar had highest amount of nutritional constituents followed by UG-218 and minimum was in HIM MASH. HIM MASH contained highest amount of proteins i.e. 34.41 per cent. Functional properties i.e. water absorption capacity was non-significantly higher in local cultivar whereas oil absorption capacity was for UG-218. Anti-nutrients were maximum in the local cultivar and minimum in the UG-218. For cultivar grading Local cultivar was the best.

Introduction

Black gram (*Vigna mungo* (L.) Hepper) or "urd bean" is the third important pulse crop in India. This legume originated in India, where it has been cultivated from ancient times and is one of the most highly priced legume. This leguminous crop has inevitably marked itself as the most popular legume and can be most appropriately referred to as the "King of legumes" FAO.

India is the world's largest producer as well as consumer of black gram. It produces about 1.5 to 1.9 million tons of urad annually from about 3.5 million hectares of area, with an average productivity of 500kg per hectare. Black gram output accounts for about 10% of India's total pulse production.(Anon.2018).It is mostly cultivated in Maharashtra, UP, AP, Orissa, Tamil Nadu, Rajasthan, Chhattisgarh and Madhya Pradesh.

India annually produces around 1.3-1.5 million tonnes of black gram, which is approximately 10% of India's total pulse production of 12-15 million tonnes (Anon 2018).

Black gram, also known as urd bean, mash, mah and black maple etc. is an important short-duration pulse crop grown in many parts of India. This crop is grown in cropping systems as a mixed crop, cash crop, sequential crop besides growing as sole crop under residual moisture conditions after the harvest of rice and also before and after the harvest of other summer crops under semi-irrigated and dry land conditions. This legume is consumed in many forms like whole, dehusked and split form. The legume is used in the preparation of many popular dishes like *idli, dosa, vada, papad, wadian,* etc.

In Himachal Pradesh Black gram is grown in Shivalik hill zone and Mid hill zones in an area of about 1.7 million hectares with a total production of 1.20 million tonnes with an average productivity of 480kg/hectares (Dwivedi *et al.,* 2015). It is main legume consumed in Himachal Pradesh as whole legume, dehusked and splitted pulse. Much work has not been done on the biochemical quality evaluation of black gram grown in different zones of Himachal Pradesh, so the present study was planned to evaluate these parameters.

Materials and Methods

Procurement of raw samples

Three cultivars of black gram viz. UG-218, HIM MASH and one local cultivar selected for study were procured from KVK CSKHPKV Berthin Distt. Bilaspur, H.P., HIM MASH was procured from KVK Una CSKHPKV one local cultivar was procured from the local farmers of Distt. Shimla Himachal Pradesh.

Preparation of samples

The procured dry, mature seed samples of black gram cultivars were cleaned manually for wholesomeness after discarding broken hull seeds, shriveled seeds, seeds having off colour and foreign material. The raw samples as well as the prepared product samples of all three cultivars were ground to form fine powder with the help of stainless steel mixer and were stored in properly labeled airtight glass containers at room temperature so as to prevent changes till further analysis. All the analyses were performed in triplicates.

Physical properties seed colour and shape

The seed colour and shape were observed from their physical appearance through visual perception.

Length and breadth

Ten Seeds were placed in a straight line and length was measured using vernier caliper and average length and breadth was calculated.

Density

Density is as weight volume ratio and calculated by using the formula
Density (g/ml) = W (g)/ V (ml). Where,

W= Weight of 1000 seeds and V= Rise in water level after adding seeds

The bulk density was measured according to the method given by Narain *et al.,* 1978.Water absorption and oil absorption capacity was measured by method described by Sosulski *et al.,* (1976) and Lin *et al.,* (1974) respectively.

Proximate composition

The proximate constituents viz. moisture content, crude fat, crude fiber and ash contents in the samples of seeds were analyzed by using standard methods of AOAC (2010). Nitrogen was analyzed by

Micro-kjeldhal method and was multiplied by the factor of 6.25 for converting it in to crude protein AOAC (2010).Total carbohydrates were calculated by the following formula:

Total carbohydrate (%) = 100- (Moisture + crude fat + crude protein + crude fiber+ crude ash)

Non Protein Nitrogen (NPN) in samples was determined by the method of Pellet and Young (1980). True protein was calculated by formula:

True protein (%) = (Crude protein nitrogen- Non Protein Nitrogen) x 6.25

Energy in the samples was determined by chromic oxide method of O' shea and Maguire (1962). Starch in samples of black gram cultivars was determined by the method of Clegg (1956) Neutral detergent Fiber (NDF), acid detergent fiber (ADF) and lignin in samples was estimated by the method as suggested by Van Soest and Wine (1967) Difference between ADF and Lignin gave values for cellulose and difference between NDF and ADF gave the hemicelluloses content Methionine was estimated by the method of Horn *et al.,* (1946).

Lysine in the samples was estimated by the method of Felker. *et al.,* (1978). Phytic acid was determined by the method of Haugh and Lantzch (1983). Trypsin inhibitor activity in the samples was estimated by the modified method of Ray and Rao (1971). The saponin content in samples was analyzed according to the method given by Obadoni and Ochuko (2001). Digestion of mineral was done by using diacid mixture i.e. nitric acid (HNO_3) and perchloric acid ($HClO_4$) in the ratio 5:1.Calcium. phosphoros, zinc and iron was analysed using determination by atomic absorption spectrophotometer (Perkin Elmer Analyst 400).

Difference among chemical constituents of raw cultivars and developed products were statistically analyzed using statistical tools as completely randomized design at 5% level of significance ($p \leq 0.05$) using computer aided design.

Cultivar grading in search of multipurpose cultivars

A part of superiority of individual cultivar for specific parameters, the cultivar grading was done by selecting desirable traits so as to find out overall nutritional superior multipurpose cultivar. Cultivars ranking was done in respect of ash, protein, crude fat, carbohydrate, methionine, saponins and energy. Versatile cultivars for multipurpose use were picked up in descending order for desirable nutrients i.e. ash, protein, crude fat, carbohydrate, methionine, lysine, saponins and energy. Diacid mixture was used for digesting the sample minerals viz; calcium, phosphorus, Iron and zinc were determined by using absorption spectrophotometer (Perkin Elmer Analyst 400). Cumulative grading was done on the basis of total points obtained after addition.

Data was analysed using statistical tools as completely randomized design at 5% level of significance ($p \leq 0.05$).

Results and Discussion

Physical characteristics

Colour and shape

The colour of UG-218 and HIM MASH were dull black, whereas Local cultivar was dark black in colour (Table 1). The results of present investigation are in accordance with Vasudeva (2013) who reported that black gram seeds were dull black in colour. Agarwal and Singh (2004) reported the colour

of black gram to be dull black whereas Pavithra *et al.,* (2006) reported the colour of black gram genotypes to be black in colour. The variation in the colour of different varieties/cultivars of black gram might have been due to varietal differences and also due to agro climatic conditions under which the crop was grown. All black gram cultivars were oblong in shape.

Size (Length and Breadth L/B Ratio)

Data in Table 1 reveals the average length, breadth and length breadth ratio of seeds of three black gram cultivars/varieties. Maximum length was for variety UG-218 (0.59cm) and minimum was in HIM MASH (0.57cm). An average length of UG-218 variety was non-significantly higher when compared with HIM MASH and Local cultivar. The average breadth of three black gram varieties/cultivars ranging between 0.45 to 0.46 cm. A non-significant difference was observed in all the varieties studied when compared with each other. Maximum L/B ratio was for UG-218 i.e. 1.31 and minimum was for HIM MASH i.e. 1.23. A significant ($p \leq 0.05$) difference was observed in all the three varieties/cultivars when compared with each other. Agarwal and Singh (2004) analyzed black gram and reported the length to be 5.20 mm. Vasudeva (2013) reported the average length of black gram to be 0.60 cm breadth of seeds to be 0.45 mm and L/B ratio to be 1.26.

Weight

Table 1 shows the 1000 kernel seed weight of different black gram varieties ranging between 40.56 to 43.14 g/1000 seeds. Maximum weight was for UG-218 (43.14g) followed by Local cultivar (41.86g) and minimum was for HIM MASH (40.56g). A significant ($p \leq 0.05$) difference was observed in the weight of all the three cultivars when compared with each other. Vasudeva (2013) reported the weight of black gram seeds to be 42.75 g whereas, Sharma *et al.,* (2004) reported the seed weight of black gram genotypes to be 40 to 42 g and Malhotra *et al.,* (1998) reported the weight of 1000 kernel seeds of black gram genotypes to be 43 g. Kumar (2006) found that 1000 seed weight varied from 40.57 to 43.15 g.

Density and bulk density

Weight volume ratio of the seed or mass per unit volume is called as density. Density is important because it gives an idea that how closely the atoms of substance are packed. The space required for the storage of grains will be less if the bulk density is higher. Lower the bulk density more will be the space required for storage of the grains.

As depicted in 1 the density was maximum in UG-218 (2.33 g/ml) and minimum in Local cultivar (1.26 g/ml). whereas the bulk density of black gram cultivars/varieties was 0.83, 0.81 and 0.85 g/ml respectively for UG-218, HIM MASH and Local cultivar respectively A significant ($p \leq 0.05$) difference was observed in the density and bulk density of HIM MASH and Local cultivar when compared with each other. Vasudeva (2013) analyzed black gram and reported the average density to be 2.24 g/ml whereas, Rehman *et al.,* (2004) reported the average density of black gram genotypes to be 1.48- 1.85 g/ml and Malhotra *et al.,* (1998) reported black gram density to be 1.15 g/ml. It is evident from data a significant ($p \leq 0.05$) difference was thee in the bulk density of three black gram cultivars/varieties when these were compared with each other Similar findings have been reported in the present investigation. The variation in the density and bulk density of different cultivars might have been due to seed size, length, weight since density is weight volume ratio so the cultivar

which had higher weight resulted in more rise in water.

Variation the physical characteristics might have been due to difference in genotypes or varieties used in present investigation which might affect the genetic make-up of seeds followed by variation in agro-technical processes and environmental conditions.

Proximate composition

Data depicted in Table 2 shows the values for moisture content of three varieties of black gram. The mean values for moisture ranged from 5.47 to 6.27 per cent. HIM MASH (6.27%) had significantly ($p \leq 0.05$) higher moisture content as compared to UG-218 (5.47%) and Local cultivar (5.53%). Aparana *et al.,* (2000) reported the moisture content of black gram genotypes to be 5.6 to 6.4 per cent

Ash content in different varieties/cultivars of black gram is depicted in Table 2. As is clear from the table the minimum ash content was in the Local cultivar 3.17 per cent and maximum was in the UG-218 3.47 per cent. Ash content of all the varieties/cultivars differed significantly from each other. Sharon and Kavitha (2015) reported the ash content in black gram genotypes to be 2.2 to 3.4 per cent whereas Vasudeva (2013) reported that there was 3.8 to 4.0 per cent ash in the different genotypes of black gram.

Crude protein in black gram varieties ranged from 27.13 to 34.41 per cent. A significant ($p \leq 0.05$) difference was observed in protein content all the three varieties of black gram when these were compared with each other. Kakati *et al.,* (2010) observed crude protein content in raw seeds of the cultivars of black gram ranged from 19.87 to 21.15 per cent. Tresina and Mohan (2011) found crude protein content of three varieties of *Vigna mungo* ranged from 21.37 to 23.22 per cent.

It is clear from the data that the values of crude fat varied significantly ($p \leq 0.05$) among the cultivars/varieties as presented in Table 2. The values for crude fat in three cultivars/varieties ranged from 1.20 to 1.77 per cent. The crude fat content of UG-218 (1.63%) and Local cultivar (1.77%) was significantly higher than other variety HIM MASH (1.20%). Similarly, UG-218 (1.63%) showed non-significant difference from Local cultivar (1.77%) but UG-218 (1.63%) was significantly higher than HIM MASH (1.20%). Saharan *et al.,* (2002) reported black gram genotype crude fat as 1.30 per cent.

As per the data (Table 2) values of crude fiber for three different cultivars/varieties that ranged from 3.27 to 3.67 per cent. Crude fiber of UG-218 (3.67%) was significantly same as that of Local cultivar (3.67%) and HIM MASH (3.27%) showed a non-significant difference when compared with each other. Alagsundaram and Kanchana (2015) investigated three different legumes mash, mung and lentil and found that fiber content was 3.2, 4.0 and 2.3 per cent respectively.

Carbohydrate content in the cultivars/varieties of black gram was observed between 51.48 to 58.73 per cent (Table 2). The maximum content of carbohydrate was found in Local cultivar (58.73%) followed by UG-218 (56.23%) and minimum in HIM MASH (51.48%). A significant ($p \leq 0.05$) difference was observed in the three varieties/cultivars when compared with each other. Tresina and Mohan (2011) reported the carbohydrate content in three varieties of *Vigna mungo* ranged from 51.24 to 63.60 per cent respectively

Data in Table 2 depicts the starch content of various black gram cultivars which was 52.33, 56.92 and 50.13 per cent in UG-218, HIM MASH and Local cultivar respectively. A significant ($p \leq 0.05$) difference was there in

starch content of Local cultivar when compared with HIM MASH and UG-218. Srivastava *et al.,* (2000) studied three varieties of legumes and found that starch content ranged from 50.2 to 55.5 per cent. Berhanu *et al.,* (2014) studied six varieties of black gram and found that starch content was 50 per cent. Barampama and Simard (1995) reported the starch content in legumes was 50.80 per cent.

A glance at Table 2 shows the energy value of various black gram cultivars. The maximum energy content was present in Local cultivar (359.37 Kcal/100g) and minimum was found in HIM MASH (354.36 Kcal/100g). A non-significant difference was observed in the energy content of all the three varieties/cultivars when compared with each other. Malhotra *et al.,* (1998) analyzed black gram and reported the energy content as 365.74 Kcal/100g. Oboh *et al.,* (1998) found that energy content ranged from 360.60 to 362.15 Kcal/100g.

The highest calorific energy content in Local cultivar might have been due to the reason that this cultivar had high fat content and carbohydrate content which is the main contributing factor for calorific value.

Non protein nitrogen

Non Protein Nitrogen is the nitrogen present in food stuffs which does not contribute towards the proteins in the body. This includes the nitrogen from all nitrogenous substances other than proteins which includes uric acid, urea, creatinine, creatine, some peptides and free amino acids. Significantly lowest NPN content was present in HIM MASH when it was compared with other cultivars (Table 2) A significant ($p \leq 0.05$) difference was observed in the NPN content of UG-218, HIM MASH and Local cultivar

True protein

The true protein content was the highest in HIM MASH (31.47%) and the lowest (23.63%) in Local cultivar. A significant ($p \leq 0.05$) difference was observed in all the three varieties of black gram when compared with each other. Yousaf *et al.,* (1991) reported that the true protein per cent in black gram to be 23.26 per cent. Parihar *et al.,* (1996) determined true protein content to be 21.28 to 33.48 per cent. Malhotra *et al.,* (1998) studied black gram and found the true protein content to be 23.62 per cent.

The variation in proximate constituents and nutritional parameters from other workers might be due to difference in genotypes or varieties used in present investigation which might affect the genetic make-up of seeds followed by variation in agro-technical processes /conditions and environmental conditions under which the crops was grown.

Dietary fiber constituents, mineral, limiting amino acid content and anti nutritional factors

Data pertaining to dietary fiber constituents mineral, limiting amino acid content and anti nutritional factors of black gram is depicted in Table 3.

Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF)

The NDF content of UG-218, HIM MASH and Local cultivar was 3.47, 3.07 and 3.53 per cent respectively. A non-significant difference was observed in all the three varieties when compared with each other (Table 2). A significant ($p \leq 0.05$) difference was observed in the ADF content of three varieties/cultivars i.e. UG-218, HIM MASH and Local cultivar when compared with each other. The ADF content in various cultivars/varieties ranged

from 2.64 to 3.05 per cent with the maximum value in Local cultivar (3.05%) and the minimum value was in UG-218 (2.64%). Perez-Hidalgo *et al.,* (1997) reported the insoluble dietary fiber NDF content to be 3.80 % and ADF content to be 1.69 per cent, whereas Sudha (1993) reported 3.71 per cent NDF and 2.69% ADF in black gram.

Lignin

Lignin is an organic substance binding the cells, fibers and vessels which constitute wood and the lignified elements of plants. Data in Table 3 show the lignin content of different black gram cultivars. As is evident from the Table the lignin content of various black gram varieties/cultivars viz. UG-218, HIM MASH and Local cultivar was 1.06, 1.34 and 1.67 per cent respectively. A significant ($p \leq 0.05$) difference was observed between three varieties when compared with each other.

Cellulose

Cellulose is a very important polysaccharide because it is the most abundant organic compound. In the human intestine, cellulose acts as an insoluble fiber, which can absorb water and thus increase the bulk of the stool.

Data in Table 3 show the cellulose content of different black gram cultivars. As is evident from the Table the cellulose content of various black gram varieties/cultivars viz. UG-218, HIM MASH and Local cultivar was 1.58, 1.34 and 1.38 per cent respectively. A significant ($p \leq 0.05$) difference was observed between three varieties when compared with each other.

Hemicellulose

Hemicelluloses are the polysaccharides containing pentoses, hexoses and uronic acid.

As is evident from the data the hemicellulose content of black gram cultivars viz. UG-218, HIM MASH and Local cultivar was 0.83, 0.39 and 0.49 per cent respectively. Non-significant difference was observed in all the three varieties of black gram when compared with each other.

The variation in dietary fiber constituents in different genotypes might have been due to differences in stage of maturity, seasonal variation, variety or genotypes, geographical location and the method of analysis.

Methionine and lysine

Methionine and lysine are the essential amino acid which is required for the proper growth of the body. Legumes are deficient in methione whereas cereals are deficient in lysine.

It is evident from Table 3 that the methionine and lysine content in UG-218, HIM MASH and Local cultivar was 0.54, 0.18 and 0.75 and 1.06, 1.57 and 1.32 g/100g protein respectively. A significant ($p \leq 0.05$) difference was observed in the methionine and lysine content of UG-218, HIM MASH and Local cultivar when compared with each other.

The results of present investigation are in accordance to Rani and Heera (1998) who reported that the methionine content in black gram flour was 0.3 to 0.6g/16g N in whole black gram. The variation in different genotypes might have been due to difference in varieties which might effect the genetic makeup of various black gram cultivars and also due to agro climatic conditions.

Minerals

As is evident from data, the highest calcium content was observed in HIM MASH (155.62mg/100g) and the lowest in Local

cultivar (147.79mg/100g). Non-significant differences in calcium content were observed between Local cultivar (147.79mg/100g) and UG-218 (147.90mg/100g) when compared with each other but differed significantly ($p \leq 0.05$) from HIM MASH. Whereas local cultivar of black gram had maximum amount of iron i.e. 7.55 mg/100g and Him Mash had higher amount of phosphorous and zinc. Similar findings have been reported by Vasudeva (2013) reported in black gram. Hira and Kaur (1993) reported the phosphorus content in black gram to be 375.13 mg/100g.

Table.1 Physical characteristics of black gram cultivars

Parameter/ Cultivar	UG-218	HIM MASH	Local cultivar	CD ($p \leq 0.05$)
Colour	Dull black	Dull black	Black	-
Shape	Oblong	Oblong	Oblong	-
Length(cm)	0.59	0.57	0.58	NS
Breadth(cm)	0.45	0.46	0.46	NS
L/B ratio	1.31	1.23	1.26	0.01
Seed weight (g/1000seeds)	43.14	40.56	41.86	0.05
Density(g/ml)	2.33	1.43	1.26	0.02
Bulk density(g/ml)	0.83	0.81	0.85	0.02
Water absorption capacity(ml/100g)	2.33	2.40	2.66	NS
Oil absorption capacity(ml/100g)	2.13	1.20	1.33	0.08

* Non-Significant

Table.2 Proximate composition and nutritional constituents of black gram cultivars

Parameter/ Cultivar	UG-218	HIM MASH	Local cultivar	CD ($p \leq 0.05$)
Moisture (%)	5.47	6.27	5.53	0.31
Crude fat (%)	1.63	1.20	1.77	0.13
Crude protein (%)	29.53	34.41	27.13	0.18
Crude fiber(%)	3.67	3.27	3.67	NS
Ash (%)	3.47	3.37	3.17	NS
Total Carbohydrates (%)	56.23	51.48	58.73	2.04
Reducing sugars (% maltose)	0.29	0.85	0.48	0.36
Non-reducing sugars (% sucrose)	2.16	2.27	2.90	0.31
Total sugars (% glucose)	2.46	3.12	3.38	0.63
Starch (%)	52.33	56.92	50.13	0.04
Energy (Kcal/100g)	357.71	354.36	359.37	NS
Non protein nitrogen (%)	0.61	0.47	0.56	0.07
True protein (%)	25.72	31.47	23.63	0.24

Table.3 Dietary fiber constituents, mineral and limiting amino acid content of black gram cultivars anti-nutrients

Parameter/ Cultivar	UG-218	HIM MASH	Local cultivar	CD ($p \leq 0.05$)
NDF	3.47	3.07	3.53	NS
ADF	2.64	2.68	3.05	0.02
Lignin	1.06	1.34	1.67	0.04
Cellulose	1.58	1.34	1.38	0.06
Hemicellulose	0.83	0.39	0.49	NS
Calcium	147.90	155.62	147.79	2.06
Phosphorus	377.71	384.67	377.74	5.20
Zinc	3.04	3.30	3.17	NS
Iron	4.00	5.91	7.55	0.07
Methionine (g/100g protein)	0.54	0.18	0.75	0.24
Lysine (g/100g protein)	1.06	1.57	1.32	0.13
Phytic acid(mg/100g)	1093.77	1177.14	1262.51	18.87
Phytate phosphorus(mg/100g)	308.23	331.72	355.76	3.29
Non-phytate phosphorus (mg/100g)	69.48	52.95	21.98	1.54
Saponin (mg/100g)	4.64	5.74	5.71	0.05
TIA (TIU/mg)	2171.33	2454.48	2505.57	24.53

Table.4 Cultivar grading in search of multipurpose cultivar

Parameter/ Cultivars	UG-218	HIM MASH	Local cultivar
Ash	1	2	3
Protein	2	1	3
Crude fat	2	3	1
Carbohydrate	2	3	1
Methionine	3	1	2
Saponins	3	1	2
Starch	2	1	3
Energy	2	3	1
Total	17	15	16
Cumulative	3	2	1
Grading			

Anti-nutritional factors

The analysis of the anti-nutrients in seeds revealed that the phytic acid and trypsin inhibitor activity were maximum in Local cultivar i.e. 1262.51 mg/100g and 2505.57 TIU/mg respectively whereas saponins content was highest in HIM MASH i.e. 5.74

mg/100g and was lowest in UG-218 i.e. 4.64 mg/100g. The phytic acid and phytate phosphorus content was maximum in Local cultivar (Shimla) i.e. 1262.51 mg/100g and 355.76 mg/100g and minimum in UG-218 i.e. 1093.77 mg/100g and 308.23 mg/100g respectively whereas, non-phytate was maximum in UG-218 i.e. 69.48 mg/100g and was minimum in Local cultivar i.e. 21.98 mg/100g.

Cultivar grading

The cultivar grading made it convenient to identify multipurpose cultivars suited for direct inclusion in the human diet. Moreover the study opened further options for nutritional improvement of crops.

During the course of study it was noticed that none of the cultivars were excellent in every parameter. So, to identify overall nutritionally superior multipurpose cultivar the entire cultivars under study were graded for their overall excellence to strike a balance of maximum superiority among different characters, although some of the characters had to be sacrificed.

Therefore, grading of cultivars for prominent characters viz. ash, protein, crude fat, carbohydrate, methionine, saponins, starch and energy was done and the pertinent information in this respect is presented in Table 4. As is evident from the data in table that Local cultivar was graded as first, followed by HIM MASH and the last place was acquired by UG-218 cultivar on the basis of various nutritional parameters. On the basis of genotypic grading it can be expounded that the Local cultivar was nutritionally superior to other cultivars which might have been due to excellent amount fat, carbohydrates, ash (minerals) and energy content present in the seeds of this black gram cultivar.

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