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Original Paper

X-ray Induced Morpho-physicochemical Divergence in Peanut (*Arachis hypogaea L.*) Mutants

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***Abstract*—Peanut is a crucial source of edible oil and nutrients. Five peanut genotypes' yield and quality parameters were assessed to select superior genotypes. The experiment was conducted in Bangladesh Institute of Nuclear Agriculture HQ's experimental field, Mymensingh-2202. Correlation study identified significant positive correlation of pods plant^{-1}, kernel weight and shelling % with pod yield. Considering yield and oil content, the genotype B6/282/80 performed the best, with a yield of 2.25t/ha and 54.6% edible oil content. Oleic acid/Linoleic acid (O/L) ratio and iodine content were also higher in B6/282/80, followed by B6/282/63. RM-Kha-19 contains the maximum amount of protein (32.42%), while crude fibre and carbohydrate content were the highest in Binachinabadam-4. B6/282/64 possessed the maximum moisture (6.2%), while B6/282/80 had the highest amount of ash (2.35%). The principal component analysis identified that the first two principal components explained about 74.93% of the total variation. Biplot revealed that B, crude fibre, and ash content were higher in Binachinabadam-4. The genotype B6/282/63 was superior in K, S, Fe, moisture, and carbohydrate content. Genotype B6/282/80 was the best genotype for P content. Ca, Cu, N, Mg and Zn content was higher in the RM-KHA-19 genotype. Combining all the energy sources, the genotype B6/282/80 provides the maximum energy, i.e., 628.4 Kcal/100g. Considering the studied traits, the B6/282/80 has the potential to be set on a multilocational trial for the detection of stability as a new variety.**

Keywords*—Fatty acid, Correlation, Multivariate analysis, Food value.

I. INTRODUCTION

Peanut (Arachis hypogaea L.) is the second most important oil seed crop in Bangladesh, behind mustard (Brassica spp.) in terms of annual production and third in terms of acreage, behind sesame (Sesamum indicum L.) [1]. It has higher oil content than soybean (Glycine max) and mustard. As a major oil seed crop and food source, the peanut is grown on 80828 hectares in Bangladesh, with 60914 metric tons produced during the Rabi and Kharif seasons [2]. It is currently cultivated on approximately 35,000 ha, with 40,000 metric tons of peanuts produced annually in the Gangetic delta districts. Noakhali, Faridpur, Kishoreganj, Patuakhali and Rangpur districts. It is used as an edible oil in the food industry to produce cake, cookies, and baked goods. Oil cake is used as cattle feed and is traditionally eaten as fried 'badam.'

The nutritional quality of peanut products depends on the protein content, oil content, and composition. Peanut kernel contains 48- 50% oil, 25-28% protein, 8-14% soluble sugar, vitamin B, and vitamin E, as well as more than 30 essential nutrients. The oil contains about 30% linoleic acid, an essential fatty acid for humans. Peanut oil does not possess erucic acid like mustard oil. Except for leucine and methionine, peanut oil contains all essential amino acids. Peanuts' monounsaturated fats play an important role in a heart-healthy diet.

Moreover, an adult human requires 55 grams of protein daily, and peanuts alone can provide 5-6 grams (10%) of that requirement. Peanuts are high in niacin, folate, fibre, magnesium, manganese, phosphate, flavonoids, and isoflavones [3][33]. Each 100 gm of peanut contains 600 kcal, 50gm fat, 800mg sodium and 10mg fibre and no cholesterol. For this reason, World Health Organization recommends two servings of 100 gm of processed nuts as a survival base for African children per day. Even though legumes are low in S-containing amino acids [4], they increase the protein content of cereal-based diets. They can boost the nutritional status of cereal-based diets low in lysine [5]. Nuts are a good source of oil among legumes since they contain more unsaturated fatty acids than saturated fatty acids [6]. Peanut contains more protein than any other nut, with levels equal to or better than a serving of beans. The components in peanuts are highly digestible. The actual protein digestibility of peanuts is comparable with that of animal protein [7].

As peanuts are highly nutritious, peanuts and products based on peanuts can be promoted as nutritional foods to fight energy, protein, and micronutrient malnutrition among the poor [3]. The soil and climate of Bangladesh are ideal for growing peanuts. It is grown mainly in sandy soils and riverbeds [8]. Peanut is a major crop in Bangladesh's char lands, but farmers only make a small profit from it due to low yields. The productivity of peanuts depends on the proper selection of variety, fertilizer management, environmental factors, metal contents in soil and other management practices [9]. To make the country self-sufficient in edible oil, the yield and acreage of oil seed crops, including peanuts, must be increased.The proper selection of variety highly influences peanut productivity.

Improving the genetic potential of peanuts for qualitative and quantitative traits is one of the major objectives in most peanut breeding programs [10]. Wide genetic diversity for these traits is necessary for crop improvement. Using only a few elite germplasm lines and cultivars in breeding programs reduces the genetic variation, leading to a narrow genetic base in the peanut gene pool [11]. Sustainable peanut improvement programs, therefore, need to discover and incorporate genes from germplasm with high genetic variability for desired traits. Many genetic diversity studies have been conducted on peanuts for different regions [10],[12]. New desirable traits and genotypes have been revealed in these studies to select specific cultivars for growing in the target regions of the crop. Besides, mutation can play a massive role in diverging the peanut germplasm. Thus, this study aimed to investigate some advanced genotypes' quantitative and qualitative properties and to find potential peanut genotypes that can be released as a variety.

II. Materials and Methods

A. Plant materials

Seeds of peanut were gone under X-ray radiation at 282 Gy in Vienna, Austria to develop the M2 lines. Continuous evaluation and selection were made to advance the lines until M6.

B. Experimental design and plant materials

M_6 mutant lines viz. B6/282/63, B6/282/64, B6/282/80, RM-Kha-19 and Binachinabadam-4 of peanut were evaluated for high yielding with bold pods and kernels and higher shelling percentage as well as better qualitative characteristics. Binachinabadam-4 was included in this experiment as a check variety. The experiment followed an RCB design with three replicates. A unit plot size was 2.0 m × 3.0 m. Seeds were sown on 09 January to 02 February 2020 at 15cm distances within rows of 30cm apart. Recommended fertilizer dose and intercultural operations were also followed. For morphological characterization, data were recorded on plant height, pod number, pod yield plant^{-1}, 100-pod and kernel weight from randomly selected ten competitive plants at maturity. Pod yield was recorded from an area of 1.0m^{2,} later converted to tha^{-1}. The shelling percentage was calculated using the following formula-

$$\text{Shelling percentage} = \frac{\text{Kernel weight of 100 g pod}}{\text{Unshelled weight of 100 g pod}} \times 100$$

C. Physicochemical analysis

Oil contents of five genotypes were determined by the Soxhlet method [13] with some modifications by using the following formula:

$$\text{Oil (\%)} = \frac{(T + SW1) - (T + WS2)}{\text{SW1}} \times 100$$

Here, (T+SW1) = weight of thimble and sample before extract, (T+SW2) = weight of thimble and sample after extract, and SW1 = Weight of sample before extract.

Oil's fatty acid content was measured using gas chromatography (GC). Peanut oil was first converted to fatty acid methyl ester (FAME), which was then injected into a GC system equipped with an FID, where various peaks were observed as retention time increased. To confirm the presence of a particular fatty acid in the oil, the observed retention time was compared to the standard FAME. The fatty acid analysis protocol was modified by Danish *et al.* (2019) [14].

The iodine values of the selected five genotypes were calculated from the fatty acid composition by using the following formula [15]:

Iodine value = (% Oleic acid × 0.8601) + (% Linoleic acid × 1.7321)

The mineral components were determined by using spectrophotometric methods. Protein content was calculated by multiplying %N with a conversion factor. To convert measured nitrogen concentration to protein concentration, a conversion factor (CF) 6.25 (equivalent to 0.16 g nitrogen per gram of protein) was used. Moisture contents of the total five genotypes were determined directly by Grain Moisture Tester (PM-450). The code for peanut moisture measurement in this moisture meter is 21.

The following methodology was used to determine the ash content of the sample [16]:

Ash % = {(W2−W1)/ W3)} × 100

Here, W1= weight of empty crucible, W2= weight of crucible with ash, and W3= weight of the sample.

The following equation was used to calculate crude fibre in the sample:

CF % = {(W1 – W2)/ Ws} x 100

Here, CF = Crude fibre, W1 = Weight of crucible with sample before ashing, W2 = weight of crucible with sample after ashing, Ws = weight of the sample

The following equation determined carbohydrates:

Carbohydrates % = 100 – (moisture + ash + fat/oil + fiber + protein)

The total energy content generated from the five selected genotypes of peanut were determined by multiplying the percentages of crude protein, crude fat and carbohydrates by factors of 4, 9 and 4 respectively [17].

D. Statistical Analysis

The recorded data of the study for all characters were analyzed statistically using Statistix 10 package program. The mean for all treatments was calculated, and the F variance test performed variance analysis. The mean differences were evaluated by the least significant difference (LSD) test [18]. Correlation study was done by Past and R studio software.

Principal component analysis was done by origin pro 2021 software.

III. Results and Discussion

A. Yield and yield contributing traits

Results showed significant variations among the mutants and check for most of the characters (Table I). The plant height ranged from 90.05 to 59.814cm. The highest plant height was observed in RM-KHA-19 (90.05cm), followed by B6/282/63 (69.3 cm). The shortest plant was found in B6/282/64 (59.814cm). Nigam and Aruna (2008) [19] indicated that short plant stature with fewer days to the first flowering, and accumulation of the maximum numbers of early flowers are important traits to develop short duration peanut cultivars. Usually, the short statured plant requires fewer days to flower, meaning these are the early genotypes [20].

The number of pod/plants ranged from 9.235 to 16.009. The maximum number of pods was observed in Binachinabadam-4 (16), while RM-KHA-19 had the lowest (9.2).The genotypes having a higher number of pods per plant offer an opportunity for improving seed yield in peanut[8],[21],[22]. In the present investigation, number of pods per plant indicated positive correlation with seed yield, kernel weight and shelling % (Fig.1). Similar relationship was observed in Chinese peanut mini-core collection [23] and Asian peanut core collection [24] showing number of pods per plant is one of the selection criteria to obtain higher seed yield in peanut breeding.

Pod weight/plant was ranged from 80.81g to 87.562g. The highest pod weight/plant was observed in B6/282/64 while the lowest was observed in the genotype RM-KHA-19. Kernel weight/plant ranged from 59.409g to 68.238g. The highest kernel weight was found in the genotype B6/282/80 while the lowest kernel weight was observed in in the genotype RM-KHA-19. Although the pod weight per plant was higher in RM-KHA-19, the kernel weight was the lowest. It indicates that the shell of the genotype was much heavier, which increased the pod weight.

100-pod weight ranged from 81.1g to 125.44g. The genotype RM-KHA-19 had the highest 100 pod weight but possessed the lowest shelling percentage (62.247%). Shelling percentage is an index of the percentage of grains or seeds [25]. It is one of the essential selection criteria in peanut breeding [26]. Obtaining higher seed yield for different environmental conditions is one of the most important challenges in plant breeding. The seed yield ranged from 1.8745 t/ha to 2.4465 t/ha. The genotype B6/282/80 was the highest yielder (2.45t/ha). This trait was significantly and positively correlated with pod/plant, kernel weight and shelling% (Fig. 1). The results indicated that direct selection based on either of the traits might be rewarding for the improvement of the yield of peanuts.

B. Correlation among the yield and yield contributing traits

Correlation study indicated that pod plant^{-1} was significantly and positively correlated with yield and shelling %. At the same time, it has a significant negative correlation with pod weight plant^{-1}. Again, pods plant^{-1} and plant height were negatively correlated (Fig. 1). The results showed that yield positively correlated with pod plant^{-1}, kernel weight and shelling %. Many researchers reported that seed yield was positively correlated with the number of pods/plant that supported the present experimental results [25].

TABLE I. Yield and yield components of peanut mutants/Variety

Mutant	Plant height (cm)	Pods/plant (no)	Pod weight/ plant (g)	Kernel weight/ plant (g)	100-pod weight (g)	Shelling%	Pod yield (t/ha)
B6/282/80	62.7bc	16.0a	86.64ns	68.24a	82.95b	72.7a	2.45a
B6/282/63	69.3b	14.7a	87.13	66.15ab	81.10b	68.7bc	2.17b
RM-KHA-19	90.1a	9.2b	87.56	59.41b	125.4a	62.2d	1.87c
B6/282/64	59.8c	13.8a	80.81	64.59ab	84.66b	66.3c	2.121b
Binachinabadam-4	61.2c	16.0a	87.17	63.75ab	91.96b	70.9ab	2.21ab
CV (%)	9.18	14.2	12.5	10.59	12.32	5.28	8.2

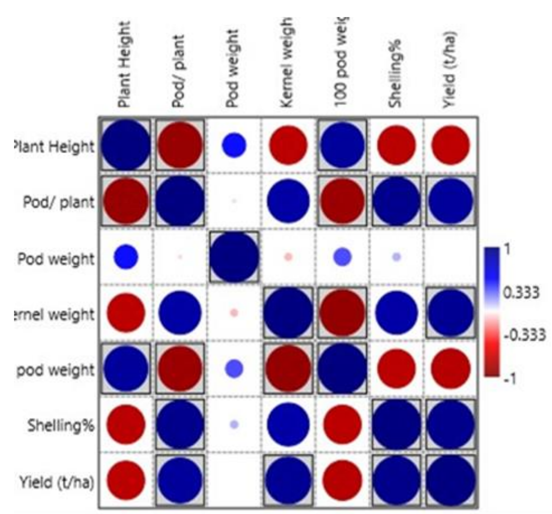


Fig. 1. Correlation among the yield contributing traits. Positively correlated traits were shown by the blue color and negatively correlated traits were shown by red color. The volume and intensity of color increased with the value of correlation. Significantly correlated traits were boxed. Example of a figure caption.

C. Oil content

The oil content of 5 peanut genotypes varied from 54.6% to 47.33 %. The highest oil content (54.6%) was found in B6/282/80 followed by RM-Kha-19 (50.8%), B6/282/64 (50.6%), B6/282/63 (50.1%) and Binachinabadam- 4 (47.33%) (Table II). Asibuo *et al.* (2008) [27] stated that significant differences were observed in 20 cultivars for oil.

TABLE II. OIL CONTENTS AND O/L RATIO OF 5 PEANUT MUTANTS/VARIETY

Sl. No.	Name of the genotypes	O/L ratio	Oil %
1	B6/282/63	2.87 b	50.1 bc
2	B6/282/64	1.75 e	50.6 b
3	B6/282/80	3.68 a	54.6 a
4	RM-Kha-19	2.52 c	50.8 b
5	Binachinabadam-4	0.95 d	47.33 c
	CV (%)	0.64	1.54

D. Oleic acid and Linoleic acid (O/L) ratio

The O/L ratio of the genotypes ranged from 3.68 to 1.75. The highest ratio of 3.68 was found in B6/282/80; followed by B6/282/63 (2.87), RM-Kha-19 (2.52), B6/282/64 (1.75) and Binachinabadam-4 (0.95) (Table II). Gulluoglu *et al.* (2016) [28] observed that the O/L ratio of peanut varieties varied between 1.09-3.78 and 1.05-2.47 in the primary and double-cropped growing seasons, respectively.

E. Genotype by trait biplot for the nutrient components

Biplot analysis is mainly used to determine varietal stability in multi-environmental trials [29]. Genotype by Trait (GT) biplot analysis describes the association among the traits across different genotypes [30]. Biplot can also be used to determine the gene expression of plants [31]. The Principal Component Analysis (PCA) identified 14 Principal Components (PCs) for the nutrient components. Among the PCs, four havean eigenvalue greater than 1. The first two PCs explained about 74.93% of the total variation.

The Genotype by Trait biplot analysis visualized the association between nutrient component traits among the genotypes. The acute angle between two traits represented positive correlation, while the obtuse angle between two traits represented negative correlation [30].

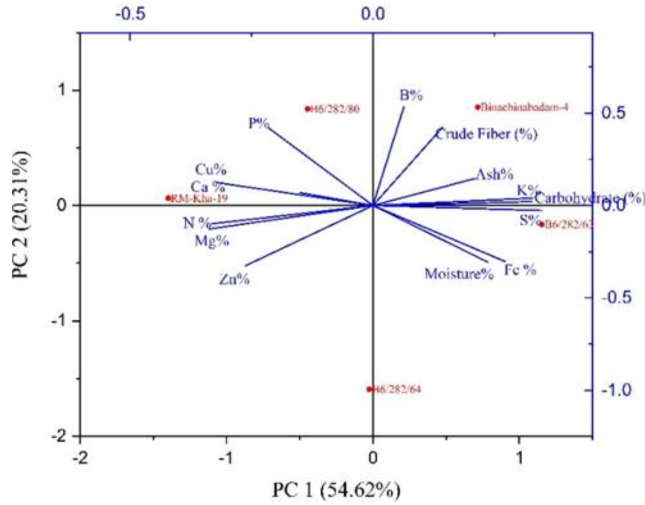


Fig. 2. Genotype by trait biplot showing the relationship among the nutrient components and the mutants.

Acute angle was found between B and Crude fibre, B and ash, B and P, B and K, B and carbohydrate, B and Cu, B and Cu, Crude fibre and ash, Crude fibre and K, Crude fibre and carbohydrate, Crude fibre and S, Crude fibre and P, Crude fibre and Fe, Crude fibre and moisture, ash and K, ash and S, ash and Fe, ash and moisture, K and carbohydrate, K and S, K and Fe, K and moisture, S and Fe, S and moisture, P and Cu, P and Ca, P and N, P and Mg, P and Zn, Cu and Ca, Cu and N, Cu and Mg, Cu and Zn, N and Mg, N and Zn, Mg and Zn indicating all of them have a positive correlation between them (Fig. 2). The obtuse angle between B and Zn, B and Mg, Crude fibre and Zn, K and N, and K and Mg indicatesa negative correlation. All these results indicate that direct selection for any trait will give positive rewards for the other positively correlated traits. At the same time, it will bring negative results for the negatively related traits.

Again, biplot analysis shows the trait profiles of the genotypes, especially those that are positioned far away from the origin [30]. For example, the biplot revealed that B, crude fibre, and ash content was higher in Binachinabadam-4. The mutant B6/282/63 was superior in K, S, Fe, moisture and carbohydrate content. The mutant B6/282/80 was the best genotype for P content. Ca, Cu, N, Mg and Zn content was higher in the RM-KHA-19 genotype. So, during the selection of any genotype, this relationship should be kept in mind to get good results.

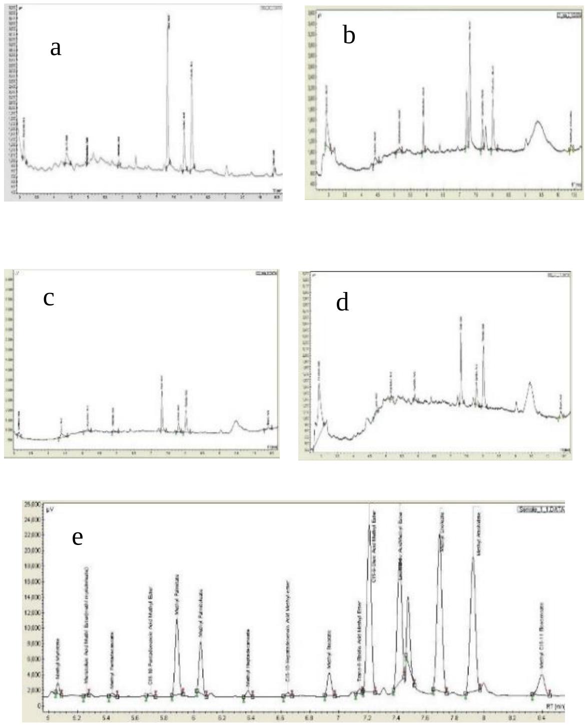
F. Protein

The protein contents of 5 peanut genotypes ranged from 32.42% to 27.34%. The highest protein content, 32.42%, was measured in RM-Kha-19; followed by B6/282/64 (29.41%), B6/282/80 (28.68%), Binachinabadam-4 (28.12%) and B6/282/63 (27.34%) (Table III). Asibuo *et al.*(2008) [27] found 21.15% to 30.53% protein content among 20 varieties of peanuts.

G. Correlation among the Fatty acids

Fatty acid profiles of the studied mutant were shown in

Fig. 3. Correlation study revealed the relationship among the fatty acids. A significant positive correlation was found between palmitic and erucic acid (0.81) (Fig. 4).



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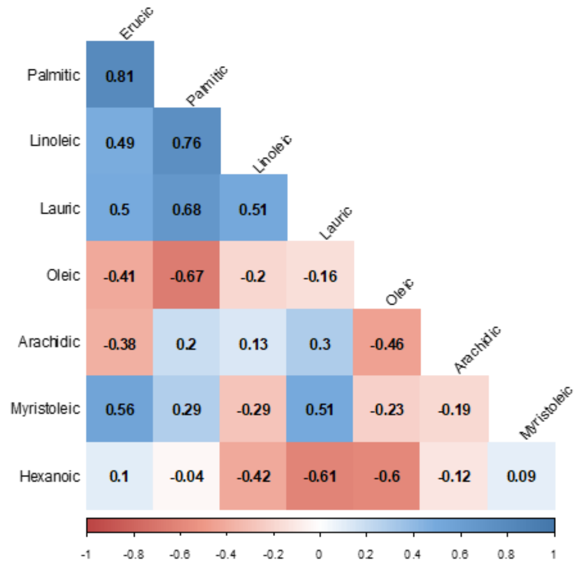

Fig. 4. Correlation among the fatty acids.

H. *Total Energy*

The total energy evolved from the selected genotypes ranged from 628.4to 594.58Kcal. The highest total energy, 628.4Kcal was found in B6/282/80; followed by RM-Kha-19 (609.64Kcal), B6/282/64 (604.68Kcal), B6/282/63 (603.46Kcal) and Binachinabadam-4 (594.58Kcal) (Table III). Aryal *et al.* (2015) [32] stated that the mean total energy found from 100 g of peanut was 567 kcal.

TABLE III. THE TOTAL ENERGY OF 5 PEANUT MUTANTS/VARIETY

Genotypes	**Carbohydrate (%)**	**Energy from Carbohydrate (kcal/100g)**	**Protein (%)**	**Energy from Protein (kcal/100g)**	**Oil (%)**	**Energy from Oil (kcal/100g)**	**Total Energy (kcal/100g)**
B6/282/63	10.80	43.2	27.34	109.36	50.1	450.9	603.46 c
B6/282/64	7.91	31.64	29.41	117.64	50.6	455.4	604.68 c
B6/282/80	5.57	22.28	28.68	114.72	54.6	491.4	628.4 a
RM-Kha-19	5.69	22.76	32.42	129.68	50.8	457.2	609.64 b
Binachinabadam-4	11.4	45.6	28.12	112.48	48.5	436.5	594.58 d
CV (%)							0.29

Palmitic acid was also highly correlated with linoleic acid (0.76) and lauric acid (0.68). Highly negative correlation was found between palmitic acid and oleic acid (-0.67), lauric acid and hexanoic acid (-0.61), and oleic acid and hexanoic acid (-0.6). The results of the correlation study indicated that selection for palmitic acid content would also lead to the increase of erucic acid, linoleic acid and lauric acid. Again, it will lead to a decrease in oleic acid as they were negatively correlated.

IV. CONCLUSION

It can be concluded that the selected advanced mutants of peanut genotypes are good in chemical analysis, which might be an excellent source of nutrition. Induced mutation through radiation could be the mentionable variations in the

nutritional and functional properties of genotypes. Considering O/L ratio (3.68), Iodine value (74.12), % protein, % crude fibre, % carbohydrate and total energy (628.4 Kcal/100g), mutant B6/282/80 is the best performer among all mutants used in this study. The findings presented in this comparative study of biochemical properties will be helpful for the breeders for the further breeding program.

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