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Evaluation of phenotypic and genotypic variation of local pumpkin germplasm for improved breeding purposes

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Abstract

Pumpkin (*Cucurbita moschata*), a popular vegetable in Bangladesh, has a wonderful source of minerals, fibers, vitamins, antioxidants, and nutrients. Attempts were taken in the present work to select the best parents of pumpkin (*Cucurbita moschata*) among the 20 local germplasms for improvement breeding. The collected germplasms were evaluated for phenotypic and genotypic variations based on flowering days, vine length, fruits length and weight, yield and pericarp length that were found to differ significantly ($p< 0.01$). Time required to flowering varied from 57 to 83 days, and the vine length ranged from 11 to 23 ft. Fruit per plant varied from 1 to 4, weight varied from 0.90 to 4.35 kg. The extent of genotypic variance for flowering, vine length, fruits length, fruits weight and pericarp length were higher than the environmental variance. The detected significant differences among the germplasms for the traits suggest the presence of an intrinsic genetic variability that could potentially be used for the effective and successful breeding to improve the desirable traits in the future.

Keywords: Pumpkin; germplasm; phenotype; genotype; variation; improved breeding

1. Introduction

Pumpkin (*Cucurbita moschata*), a climbing plant belongs to Cucurbitaceae family is one of the popular vegetables in Bangladesh and other Asian countries. It is rich in minerals, fibers, vitamins, antioxidants and phytonutrients [1-4]. Besides the nutritional value, pumpkin are also well-documented in several reports for high medicinal values [5-8] such as action against diabetic [9], fungal [10], bacterial infection along with anti-inflammation properties [11] and antioxidant effects [12].

The people in Bangladesh extensively consume all parts of it such as fruits, vines, flowers, and seeds. Due to its high popularity, it is commonly grown in every part of the country in the agricultural field for commercial purposes and in the homestead areas for home consumption. Plenty of Pumpkins growers are from Jessore, Kustia, Chittagong and Dhaka [13]. Pumpkin fruits are not perishable like other vegetables, it can be eaten fresh as well as ripen, fruits can be stored up to 6 months to eat or sell in the lean period when other vegetables are scarce. Pumpkins serve as an important supplement for the malnutrition in the developing countries as it is rich in carbohydrate and minerals and pigments containing antioxidants, pro-vitamin-A.Thus, It can contribute to meet the requirement of Vitamin-A, and other nutrient deficiency in the rural areas of the country.

Though it has numerous health and nutritional value, its cultivation in Bangladesh is usually on a small-scale and the average production is relatively poor. The statistics of pumpkin production published in Bangladesh Bureau of Statistics [13] shows a total land under pumpkin cultivation was 120993 acres with an average production of 5470 kg per acre as of 2014 data. Besides, Bangladesh also earns foreign currency by exporting it to the U.K., Pakistan and Middle East [14]. In addition to growing pumpkin in marginal fellow land and homesteads for home consumption, large-scale cultivation of this multi-purpose crop could help to eradicate the malnutrition in the country and earning foreign currency exporting the surplus. Many local germplasms are available in Bangladesh, locally they are cultivated, but production is relatively poor, in most cases quality is inferior and resistant to diseases are low. These attributes and nutritious values are largely ignored in the breeding program of the country. This loophole is met by the foreign hybrid seeds that put the local germplasm at the risk of extinction. Considering its economic, nutritional, medicinal and agroforestry value, development of a high yielding variety of pumpkin is important. As the species is cross-pollinated, it is difficult to produce sufficient hybrid seeds. Inbreeding depression is well-known in this plant that shows after two generations of selfing. Thus, population breeding is one of the suitable methods

to develop a high yielding variety for which the best parents need to be selected. Existing germplasm in the country could serve a good source to screen the good parents for improved breeding.

Therefore, present study has taken an initiative with the research grant of Education ministry, Government republic of Bangladesh to develop a high yielding variety of pumpkin for which the initial screening was started to select the potential parents based on phenotypic and genotypic evaluation.

2. Methodology

2.1 Materials

A total of 20 local germplasms were collected from Plant Genetic Resources Center, Bangladesh Agricultural Research Institute (BARI), local areas of Sylhet, Noakhali, Chittagong, Siddique bazar, Dhaka and Cumilla. The seeds were sown at the field research station located beside the IICT building of Shahjalal University of Science and Technology, Sylhet and at the forestry nursery in a randomized block design with three replications and six plants per plot.

2.2 Bed preparation and experimental procedure

Before sowing, the bed was prepared at 1 m×1 m spacing, added the fertilizer at the ratio of 50 g Urea +50 g Potash +20 g DAP +20 g oilcake per bed, and allowed 15 days before sowing the seeds. The parameters evaluated were phenotypic and genotypic variations based on first flowering to days, vine length (ft), fruit length (ft), fruit weight (kg), fruit yield (number/plant), pericarp length (ft). The seedlings were watered in very early morning and late afternoon for the first two days followed by watering every 5 days along with regular weeding. Thereafter, fertilizer was added at the amount of 5g Urea +25g Potash +25 g DAP +10g oilcake per bed.

Table-1. Collection of germplasms from different parts of the country

Germplasms code	Collection place	Number of germplasms
R1, R2, R3	BARI	3
R4, R5, R6, R7, R8, R9	Dhaka Siddique bazar	6
R10, R11, R12, R13, R,14	Kamal bazar Sylhet	5
R15, R16	Comilla	2
R17, R18	Noakhali	2
R19, R20	Chittagong	2
Total		**20**

2.3 Data analysis

The data were analyzed using IBM SPSS 22 version for the analysis of variance (ANOVA), cluster analysis and correlation to find out the extent of variation among the germplasms. The genetic and environmental factors of phenotypic variation for each trait were estimated according to [15,16]:

Phenotypic variance (σ^2_p) = MSg/r;

Genotypic variance (σ^2_g) = (MSg-MSe)/r; and

Environmental variance (σ^2_e) = MSe/r,

Where,
MS are mean squares, g, and e stand for genotypes and environmental variables respectively, r for replications.
For the comparison of variations between the traits phenotypic, genotypic and environmental coefficient of variations PCV, GCV and ECV respectively were computed according to [17]:

$$PCV = \frac{\sigma^2_p}{X} \times 100$$

$$GCV = \frac{\sigma^2_g}{X} \times 100$$

$$ECV = \frac{\sigma^2_e}{X} \times 100$$

Where, PCV, GCV and ECV stand for phenotypic, genotypic and environmental coefficient of variations respectively. $\overline{X}$ is the grand mean for each of the traits measured.

3. Results and discussion

3.1 Diversity of collected germplasms

The ANOVA showed existence of significant differences in all the experimental traits of 20 germplasms except the number of fruits per plant at $p<0.01$ (data not shown). The observed significant variations suggest the presence of genetic variability in all the germplasms collected for the study (Table-2). Existence of such Genetic variations is indicative of rich gene pool needed for effective and successful selection program [18] in the improvement breeding.

3.2 Distribution of land races of pumpkins in different clusters

The twenty germplasms were clustered into five groups (Table -2). Of them, the highest number (10) were grouped in Cluster IV followed by the cluster I and II, both had four germplasm while only one germplasm was found to group in each of the cluster III and V. Clustering different germplasms in one group signposted inherent variation in them irrespective of their source of collection. This might be due to usual exchange of pollens among them in different locations of the country. The result is in conformity with the earlier findings of [19] who collected pumpkin germplasms from different sources across he world but found the pattern of clustering did not follow the geographical origin.

Table-2. Cluster analysis of 20 germplasms collected from different locations of the country

Cluster No.	No. of accessions	No. of populations
I	R15, R16, R17, R19	4
II	R8, R11, R13, R14	4
III	R18	1
IV	R1, R2, R3, R4, R5, R6, R7, R9, R10, R12	10
V	R20	1

3.3 Correlations among the germplasmsof pumpkins

The result of correlation coefficient (Table-3) shows the days to first flowering are correlated positively with pericarp length (r = 0.48) and vine length (r = 0.153). This suggests that increasing the time of flowering would increase the pericarp length. Fruit length is positively correlated with the fruit weight (r = 0.206), fruit yield (r = 0.165) and pericarp length (r = 0.242) (Table-3). This result indicates that weight, length, and pericarp length are influenced by each other. In the selection of the plant based on one or two of the three attributes of weight, yield and pericarp is enough. Vine length was found to be negatively correlated with the fruit yield per plant (Table-3). This could be due to decrease of vine length means less internode number which ultimately causes less fruit-set per plant.

Table-3. Pearson Correlations for the six different agronomic traits evaluated in the study

	Days to first Flowering	Vine length (cm)	Fruit length (cm)	Fruit weight (kg)	Fruit yield (number/plant)	Pericarp length (cm)
Days to first Flowering	0.1	0.153*	0.110	-0.062	-0.084	0.487**
Vine length (cm)		1	-0.113	-0.022	-0.235**	0.100
Fruit length (cm)			1	0.206**	0.165*	0.242**
Fruit weight (kg)				1	0.064	0.114
Fruit yield (number/plant)					1	-0.081
Pericarp length (cm)						1

3.4 Quantitative variation

Days required to the appearance of flowering were recorded as shown in (Table 4). It was found that R11 required significantly ($p< 0.01$) more days to flower (83 days) while R7, R19, R5, R17, R18 and R1 required short time (less than 60 days). The other germplasms flowered in between 61 and 75 days (Table 4). There was a huge significant variation in the vine length among the germplasms ranging from 11to 23 ft, R16 had the shortest vine while R11 had the longest vine length (Table- 4). The majority of thegermplasms; R2, R4, R6, R8, R11, R12, R13, R14 had the vine length more than 20 ft. The number of fruits in each accession were counted and compared statically to find out significant differences among the germplasms. The result shows that germplasms R17, R18, R19 and R20 produced on an average more than 3 fruits and R11, R12, R13 produced less than 2 fruit per plant. The length of fruits was found to vary, the smallest size, just above 10 cm was found for the fruits of 7 accessions namely R15, R16, R17, R18, R19 and R20 (Table 4). The other accessions were in between 40-53 cm in length. Similarly, the fruit weight

Table-4. Quantitative variation of 20 germplasms with respect to flowering days, vine length, fruit weight, fruit yield and pericarp length

Germplasms	Days to first flowering	Vine length (ft)	Fruit length (cm)	Fruit weight (kg)	Fruit yield (no./plant)	Pericarp length (cm)
R1	58.5 ab	16.2abc	40.87 b	3.86 cde	2.3 ab	3.25 ab
R2	61.1 cd	21.3 fgh	46.8 c	3.2 bcd	2 ab	3.52 ab
R3	62.6 de	17.1 cde	48.73 cd	2.76 bc	2.3 ab	2.76 b
R4	63.1 de	22.88 h	51.47 cd	2.28 b	2 ab	2.82 b
R5	58.4 ab	17.21 cde	50.97cd	3.23 bcd	2.45 abc	3.79 b
R6	66.8 f	22.97 h	48.24 cd	2.97 bc	2.1 ab	4.1 d
R7	57 a	19.43 def	47.42 cd	3.87 cde	2.3 ab	3.67 bc
R8	72.7 g	22 gh	41.21 b	3.87 cde	2.3 ab	3.93 cd
R9	64.4 e	18.36 def	49.46 cd	4.14 de	2.5 ab	4.42 d
R10	67.1 f	19.85 efg	47.11 cd	4.35 e	2.2 ab	4.52 d
R11	83 i	23 h	46.48 c	3.52 cde	1.7 a	5.44 d
R12	67.4 f	22.12 gh	50.24 cd	3.69 cde	1.8 a	5.53 d
R13	74.6 h	20.96 fgh	52.76 d	3.7 cde	1.83 a	4.77 d
R14	70.7 g	21.61 fgh	51.47 cd	3.75 cde	2.3 ab	4.53 d
R15	63.8 f	14.2 abc	16.43 a	2.33 b	2.3 ab	3.79 bc
R16	63.8 f	11.02 a	14.6 a	1.2 a	2.6 abc	4.27 d
R17	57.3 ab	13.77 ab	13.64 a	1.57 a	3.2 bc	5.53 d
R18	59.5 bc	13.23 ab	15.4 a	1.25 a	3 bc	5.64 d
R19	56.4 a	13.3 ab	12.45 a	1.2 a	3 bc	3.85 cd
R20	64 e	17.75 bc	11.16 a	0.90 a	3.3 c	2.77 a

Values followed by thesame letters are not significantly different according to Duncan Multiple Range Test (DMRT) at *p* values ($p< 0.01$).

varied significantly ($p<0.01$) from 0.90 to 4.35 kg, minimum weight was found for R20 while the highest weight was found for R10 (Table 4). The length of the pulp (pericarp) is an important agronomic trait that represents the actual edible portion of the fruit. It also varied among the germplasms, with the lowest pericarp (less than 3cm) in R3, Followed by R4 (Table 4). The highest pericarp was found in R18 (5.64 cm) followed by R 12 and R17 (5.53 cm) and R11 (5.44 cm) respectively (Table 4).

Figure 1: Collected pumpkin in the field (left) and the pericarp length of the sample pumpkin (right)

Table-5. The Mahalanobis distance (D^2) of each of the 20 germplasms collected from different parts of the country

Germplasms	Mahalanobis distance	Germplasms	Mahalanobis distance
R 1	1.550474121	R 11	1.8558
R 2	0.3514	R 12	2.0120
R 3	1.320503692	R 13	0.5348
R 4	1.412149727	R 14	2.3169
R 5	1.210619676	R 15	1.5932
R 6	1.765522586	R 16	1.9743
R 7	1.4206	R 17	1.8929
R 8	2.5839	R 18	1.0808
R 9	2.3149	R 19	0.2557
R 10	1.9579	R 20	1.8680

3.5 Inter genotypic distance

The D2 test computed according to [20] showed higher genotypic distance in R14 (2.32), R8 (2.58) and R9 (2.31) while the lowest were found in R19 (0.26), R2 (0.35) and R13 (0.53) (Table-5). The magnitude of the genetic distances was not found to be inconsistent with the clusters. For example, the highest value of genetic distances found in R14, R9 and R8 belongs to three different clusters, Cluster I, II and IV respectively (Table-2). Similarly, the same cluster II also contained the germplasms that showed lower genetic distances. Such inconsistencies among the clusters were also reported elsewhere [21]. However, genetic distances in individual germplasm certainly help to select the divergent accessions for the combination breeding to improve the desired traits.

Phenotypic variation contributed by genotypes and environment has been measured for the six different traits as shown in Table-6. The phenotypic coefficient of variation (PCV) was highest for fruits length (172%) followed by days to first flowering (76%) and vine length (28%) respectively (Table-6). Phenotypic variations mainly governed by inherent genetic variation and environmental factors, thus, when it is separated, the result shows genotypic variance exceeded the

environmental variance for five of the six traits, the only exception was the yield, which was found to be affected slightly more by environment than the genotype (Table-6). The Genetic coefficient of variation (GCV) was low

Table-6. The Phenotype coefficient of variation (PCV), Genetic coefficient of variation (GCV), Environment coefficient of variation (ECV) of six different attributes of pumpkins seedlings.

Attributes	PCV	GCV	ECV
First Flowering to days	76.39725	75.88122	8.864536
Vine length (ft)	28.14321	26.94216	8.13388
Fruit length (ft)	171.9913	172.2316	13.68539
Fruit weight (kg)	7.157514	6.596211	2.778489
Fruit yield (number/plant)	6.146544	3.613862	4.971921
Pericarp length (ft)	7.239475	7.05762	1.612452

for fruits yield (3.61%) compared to environmental variance (5%). The overall results indicate genotypic variation contributed to the total variations which could be used for the selection of the improved traits [22]. However, the degree of genetic variation cannot be fully explained by GCV only [23,24], it is suggested [3] to estimate heritability with GCV for exploiting the full potential of genetic variations in the improvement breeding.

4. Conclusion

Superior parent selection is a paramount important before taking any improvement breeding attempts of important agronomic traits of pumpkin. Estimating the genetic diversity on germplasm collections is equally important for conservation and breeding purposes. The study revealed the existence of a wide variation among the 20 germplasms collected from different parts of the country. Almost all the agronomic traits showed higher proportion of genotypic differences than the environmental factors that might give an ample scope for genetic manipulation to improve the crop for a single trait or a combination of multiple traits of yield, fruiting and flesh (pericarp). Based on the study of genetic diversity, fruit yield and vine length, R1 and R9 accessions were found promising. R1 had the shortest vine, early flowering accessions and larger pericarp length among the samples studied. Similarly, R9 was found good in production, producing on an average 2.5 fruit per plant having very good amount of pulp (4.42 cm) and one of the shortest plants with respect to vine length (18.36 cm) even though it showed slightly longer days to flower (64 days) compared to the others. Both the accessions are diverse with respect to genotypic distances, 1.55 and 2.31 respectively and both of them are in cluster IV. Thus, considering all the attributes, R1 and R9 had been selected for the advance breeding through population breeding and forward genetic approaches for a higher yield. Besides R1 and R9, others also showed inherent genetic variations for vine length, fruits weight, yield and pulp. Existence of such variation usually shows a rich gene pool of our local pumpkin varieties offering selection of preferable traits like more yield in short vine within a comparatively short period, thereby, maximizing the production in unit area.

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