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Estimation of Genetic Diversity in Onion (Allium cepa L.)

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Abstract

The experiment was conducted at the field laboratory of the Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during November 2008 to March 2009 to estimate the genetic diversity of exotic and local onion. Ten genotypes of onion were evaluated for 16 characters in a randomized complete block design to study the genetic divergence through multivariate analysis. Ten genotypes formed three clusters. Cluster III contained maximum number of five genotypes. Cluster I and II contained three and two genotypes, respectively. The inter cluster distance was larger than the intra cluster distances. The inter cluster distance was maximum between the cluster II and III (6.336) and minimum between the cluster I and II (3.876). The intra cluster distance in the entire three clusters was more or less low, indicating that genotypes within the same cluster were closely related. Considering clustered distance and cluster mean, the intra cluster distance revealed that the genotypes Indian big (G₁₎ and Patnai pink (G₅) from the cluster II and genotypes Taherpuri, Indian medium (G₁), Big single bulb (G₇), Big double bulb (G₈), Small single bulb (G₉) and Small double bulb (G₁₀) from the cluster III may be selected as parents for future breeding program.

Keywords: Cluster, principal component analysis (PCA), non-hierarchical clustering, Principal coordinate analysis (PCO), canonical variate analysis, intra-cluster mean

1. Introduction

Onion (*Allium cepa* L.) is a cross pollinated and biennial important spices as well as vegetable crops throughout the world. The genus *Allium* consists of 500 species where onion (*Allium cepa* L.) is the only cultivated species in this genus. It is an asexually propagated crop and displays great morphological diversity in terms of bulb and leaf size, color and shape, presence of scale, plant height, flower color, fertility and bulbil (top set) development (Pooler and Simon, 1993).

The main onion producing countries are China, India, USA, Russia, Japan, Turkey, Netherlands and America (FAO, 1997).

In Bangladesh it is widely cultivated in the greater districts of Faridpur, Pabna, Rangpur, Rajshshi, Dinajpur, Jessore, Dhaka, Comilla, Mymensingh and Barisal (Anonymous, 1998). Central Asia is the primary center of its origin and the Mediterranean area is the secondary center for large type of onion (Mc. Collum, 1976). The annual onion requirement of

Bangladesh is 15,75,000 metric tons and the total onion production is 9,00,000 metric tons, therefore, a shortage of 6,75,000 metric tons per year has been prevailing in the country (Anonymous, 2008). Increased onion production largely depends on good variety, modern production technology and good quality seeds. Inferior seeds may decrease production by 15-25%. Yield could be regarded as a complex character, which is dependent on a number of agronomic characters and is influenced by many factors, which could be of genetic or environmental (Uddin et al., 1985). Evaluation of genetic diversity is, therefore important to know the source of genes for a particular trait within the available germplasm (Tomooka, 1991).

The progress of breeding is conditioned by the magnitude of nature and interrelationship of genotypic and environmental variation in different characters. It is also beneficial to make a comparative study of genetic diversity to select the desirable ones in different genotypes As a result various types of onion are available in the market. Genetic diversity of those of various onion types under Bangladesh conditions are not yet known. Therefore, the present study has been undertaken to estimate the genetic diversity of exotic and local genotypes of onion.

2. Materials and Methods

2.1. Experimental site and season

The research work was carried out at the experimental field of the Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during rabi season of 2008-2009. The experimental site was situated in the tropical climate zone. The location of the site was at 24.00 ^oN latitude and 90.25 ^oE longitudes with and elevation of 8.4 meter from sea level. The soil of the experimental field was sandy loam in texture having a pH around 6.5. The land was high with uniform topography and almost homogenous in respect to soil fertility.

2.2. Design and treatments

The material comprised of ten genotypes of onion (Allium cepa L.) including one standard variety Taherpuri. Genetically pure and physically healthy bulb of Indian big (G_1) , Indian medium (G_2), Indian small (G_3), Taherpuri (G_4), Patnai pink (G₅), Patnai small (G₆), Big single bulb (G_7), Big double bulb (G_8), Small single bulb (G_9), Small double bulb (G_{10}) genotypes were obtained from the BSMRAU germplasm Karwan bazar and Dhaka collections. Newmarket. The experiment was laid out in a randomized complete block design (RCBD) with three replications. The blocks were subdivided into ten plots, where the genotypes were randomly assigned. The unit plot size was 4 m x 2 m consisting of four rows of each genotype. Row to row distance was 50 cm and plant to plant distance was 20 cm.

2.3. Crop management

The experimental plots were prepared by deep ploughing followed by harrowing and laddering. Recommended doses of fertilizers such as cow dung, Urea, TSP and MP were applied @ 10 t, 130 kg, 200 kg, 75 kg per ha, respectively. Cowdung, TSP, half of urea and half of MP were applied at the time of final land preparation. The remaining urea and MP were applied as top dressing in two instalments. Immediately after germination, the straws used for mulching to enhance germination were removed and weeding was done as needed. Frequent irrigation was applied in plot as per requirements of the bulb. Fungicide (Rovral) was applied two times at seven days interval after bulb sowing to control Alternaria leaf blight. No insecticides were used in the experimental plot.

2.4. Data collection and analysis

Data were recorded on days to 100% sprouting, days to1st bolting, days to 50% bolting, number of leaves per plant, days to anthesis, style length, style breadth, filament length, anther length, anther breadth, number of stalks per plant, length of stalk, stalk diameter, percent of fertile pollens and umbel diameter. Onion seeds were harvested when the umbels or flowering heads turning brown. Genetic diversity was estimated using Mahalanabis generalized distance (D^2) extended by Rao (1952). Tochers method was followed to determine the group constellation. Canonical variate analysis was performed as per Rao (1964) to confirm the results of cluster D^2 analysis. Mean data for each character was subjected to both univariate and multivariate analysis. Univariate analysis of the individual character (analysis of variance) was done by computer using MSTAT-C software. Genetic diversity of ten genotypes was analyzed using GENSTAT 5.13 software program (copyright 1987, Lawes Agricultural Trust, Rothamasted Station, UK).

3. Results and Discussion

3.1. Flowering behaviours seed related characters

The maximum days to 100% sprouting was observed in the genotype G_{10} (18.00) and the minimum (10.66) days to 100% sprouting was found in the genotype G_4 . The minimum duration (37.00) for days to 1st bolting was required by the genotype G_{10} and the maximum duration was required by (56.33) in the genotype G_5 . Maximum days to 50% bolting was found in the genotype G_7 (43.66). The maximum number of leaves per plant was observed in the genotype G_1 (59.53) and minimum number of leaves per plant was found in the genotype G_6 (18.00) (Table 1).

The highest days to anthesis was found in the genotypes G_4 (83.67) and it was the lowest (46.00) in the genotype G_7 . The maximum style length and style breadth were found in the genotypes G_2 (97.9) and G_{10} (21.86), respectively. On the other hand, the lowest mean style length and style breadth were found in the genotypes G_5 (55.91) and G_1 (13.62), respectively. The maximum filament length was found in the genotype G_{10} (197.00) and minimum was in G_1 (160.00). Maximum anther length breadth were observed in the genotypes G_2 (66.22) and G_{10} (39.53), respectively. On the other hand, the lowest mean anther length and G_1 (100).

anther breadth were found in the genotypes G4 (77.80) and G₂ (32.76), respectively. The highest number of stalks per plant was found in the genotypes $G_{10}(5.06)$ and it was the lowest (2.66) in G₆. The maximum length of stalk was found in the genotype G_4 (66.48) and minimum in the genotype G_1 (56.08). The Maximum stalk diameter was observed in the genotype G_5 (14.13) and minimum was in the genotype G_1 (10.97). The maximum number of flower per umbel was found in the genotype G_6 (287.4) and minimum was found in the genotype G_4 (141.5). The highest umbel diameter was found in the genotype G_4 (41.44) and it was the lowest in the genotype G_1 (33.39). The highest percent of fertile pollen was observed in the genotype G_2 (98.08) and the lowest was observed in the genotype G₅ (83.62).

3.2. Principal component analysis (PCA)

Among the ten genotypes, sixteen characters were considered for genetic diversity analysis. So, sixteen eigen values of sixteen principal component axes and percentage of total variation accounted for them obtained from the principal component were presented in Table 2. The results revealed that the first axes largely accounted for 37.1% of the total variation among the genotypes, while sixteen of these with eigen values accounted for 100%. The first five axes accounted for 91.25% of the total variation among the 16 characters.

3.3. Construction of scatter diagram

On the basis of principal axes I and II from the principal component analysis, a two dimensional scatter diagram (Z_1Z_2) using component score I as X- axis and component score II as Y- axis was constructed (Figure 1). The distribution of genotypes in scattered diagram (Figure 1) was apparently distributed into three groups per clusters, which revealed that there exists considerable diversity among the genotypes.

3.4. Non-hierarchical clustering

Non-hierarchical clustering using co-variance matrix among 10 genotypes of onion were grouped them into three clusters (Table 3).

| Genotypes | Accession No. | Days to 100% sprouting | Days to 1st bolting | Days to 50% bolting | No. leaf/plant | Days to Anthesis | Style length | style breadth | Filament length | Anther length | Anther breadth | No. stalk/plant | length of stalk | Stalk diameter | No. of flower/umbel | Umbel diameter | Percent fertile pollens |
|-------------------|-----------------------|---------------------------|------------------------|------------------------|----------------|---------------------|--------------|---------------|--------------------|---------------|-------------------|-----------------|-----------------|----------------|------------------------|-------------------|----------------------------|
| Indian big | G_1 | 13.33 | 52 | 72.66 | 59.53 | 72.67 | 80.31 | 13.62 | 160.0 | 71.97 | 34.42 | 4.86 | 56.08 | 10.97 | 203.7 | 33.39 | 93.55 |
| Indian medium | G_2 | 16.66 | 49 | 77.33 | 30.2 | 79.67 | 97.9 | 14.72 | 165.1 | 66.22 | 32.76 | 4.93 | 58.2 | 13.32 | 248.8 | 37.71 | 98.08 |
| Indian small | G_3 | 13.33 | 44.66 | 65 | 22.86 | 68.33 | 69.46 | 16.13 | 185.2 | 74.26 | 37.86 | 4.26 | 63.7 | 13.11 | 243.9 | 38.45 | 94.62 |
| Taherpuri | G_4 | 10.66 | 37.66 | 63.66 | 35.2 | 83.67 | 87.13 | 18.26 | 189.6 | 77.80 | 37.14 | 4.13 | 66.48 | 14.09 | 141.5 | 41.44 | 91.93 |
| Patnai pink | G_5 | 14.33 | 56.33 | 80.33 | 35.66 | 80.00 | 55.91 | 15.24 | 168.9 | 69.77 | 35.46 | 3.33 | 60.23 | 14.13 | 164.6 | 35.04 | 83.62 |
| Patnai small | G_6 | 15.66 | 46.33 | 65.6 | 18 | 58.67 | 74.46 | 18.26 | 162.6 | 72.53 | 37.13 | 2.66 | 56.88 | 13.23 | 287.4 | 39.47 | 86.62 |
| Big single bulb | G_7 | 13.33 | 36.66 | 43.66 | 28.06 | 46.00 | 79.93 | 17.6 | 172.3 | 75.86 | 39.06 | 3.8 | 61.90 | 11.93 | 153.2 | 38.48 | 90.56 |
| Big double bulb | G_8 | 17.33 | 39 | 45.66 | 35.46 | 47.33 | 80.5 | 16.6 | 181.7 | 77.53 | 37.64 | 4.26 | 59.31 | 11.53 | 151.9 | 38.75 | 94.97 |
| Small single bulb | G ₉ | 17.33 | 48.66 | 63 | 30.6 | 69.33 | 75.8 | 17.33 | 187.9 | 77.2 | 38.26 | 3.2 | 60.33 | 12.18 | 153.3 | 37.09 | 95.17 |
| Small double bulb | G_{10} | 18.00 | 37 | 61 | 31.26 | 66.00 | 86.6 | 21.86 | 197.0 | 76.6 | 39.53 | 5.06 | 62.68 | 12.25 | 152.4 | 37.35 | 96.68 |

| Table 1. Mean performance of ten onion gen | otypes for flowering behaviour and s | eed yield related characters |
|--|--------------------------------------|------------------------------|
|--|--------------------------------------|------------------------------|

| Principle component axis | Eigen value | Percentage of total variation | Percentage of cumulative |
|--------------------------|-------------|----------------------------------|--------------------------|
| Days to 100% sprouting | 5.936 | 37.1 | 37.1 |
| Days to 1st bolting | 2.931 | 18.32 | 55.42 |
| Days to 50% bolting | 2.672 | 16.7 | 72.12 |
| No. of leaves per plant | 1.74 | 10.87 | 82.99 |
| Days to anthesis | 1.321 | 8.26 | 91.25 |
| Style length | 0.561 | 3.51 | 94.76 |
| Style breadth | 0.403 | 2.52 | 97.28 |
| Filament length | 0.334 | 2.09 | 99.37 |
| Anther length | 0.102 | 0.64 | 100.0 |
| Anther breadth | 0 | 0 | 100.0 |
| No. of stalks per plant | 0 | 0 | 100.0 |
| length of stalk | 0 | 0 | 100.0 |
| Stalk diameter | 0 | 0 | 100.0 |
| No. flowers per umbel | 0 | 0 | 100.0 |
| Percent fertile pollens | 0 | 0 | 100.0 |
| Umbel diameter | 0 | 0 | 100.0 |

 Table 2. Eigen values and percentage of variation for corresponding 16 component characters in ten genotypes of onion

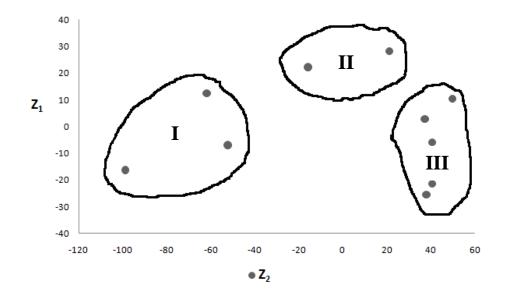


Figure1. Scatter diagram of 10 genotypes of onion based on their principal component scores

| Cluster number | Number of genotypes | Genotypes with accession number |
|----------------|---------------------|--|
| Ι | 3 | Indian medium (G_2), Indian small (G_3) and Patnai small (G_6). |
| II | 2 | Indian big (G_1) and Patnai pink (G_5) Taherpuri (G_4) , Big single bulb (G_7) , Big double |
| III | 5 | bulb(G_8), Small single bulb(G_9) and Small double bulb(G_{10}). |

Table 3. Distribution of ten genotypes of onion in different clusters

Table 4. Intra (bold) and inter cluster distance among 10 genotypes of onion

| | Ι | II | III | |
|-----|-------|-------|-------|--|
| Ι | 0.688 | | | |
| II | 3.876 | 0.789 | | |
| III | 4.980 | 6.336 | 0.638 | |

The clustering pattern obtained coincided with the apparent grouping patterns performed by PCA. So, the results obtained through PCA were confirmed by non-hierarchical clustering. The distribution pattern indicated that the maximum number of genotypes (five) were included in cluster III and minimum number of genotypes (two) were included in cluster II. Cluster I composed of three genotypes.

3.5. Canonical variate analysis

Canonical variate (vector) analysis was performed to obtain inter cluster distances (Mahalanobis's D^2 values). The values of inter cluster distances (D²) are presented in Table 4. The highest inter-cluster distance was observed (Table 3) between cluster II and III (6.336), followed by cluster I and III (4.980) and the lowest inter-cluster distance was observed between I and II (3.876). The intra cluster distance was the highest (0.789) in cluster II followed by cluster 1 (0.688). Moderate intermediate distances were found between clusters III and I (4.980). It was favored to decide that intra-cluster distance was highest in cluster II i.e. more heterogeneous whereas, intracluster distance was lowest in cluster III i.e. comparatively homogenous. Similar supportive

reports were made by Mohanty and Prusti, 2002 and Mohanty (2001) in winter onion and Mohanty (1999) in kharif. There was evidence from Shanmugam and Rangasamy (1982) that materials from same origin distributed in different clusters is an indication of broad genetic base of the genotypes belonging to that origin.

3.6. Intra-cluster mean

Intra cluster mean for 16 characters were presented in Table 5. Among 16 characters cluster I had the highest estimates for three characters stalk diameter (13.22), number of flowers per umbel (260.03) and percent fertile pollens (92.79). On the other hand, cluster I had two characters viz. number of leaves per plant (23.69) and number of stalks per plant (3.95) having the lowest mean cluster value among 16 characters. In cluster II, the highest cluster mean value was achieved for five characters days to 1st bolting (54.17), days to 50% bolting (76.5), number of leaves per plant (47.6), days to anthesis (76.34) and number stalks per plant (4.1). Among 16 characters cluster II had the lowest cluster mean value for days to 100% sprouting (13.83), style length (68.11), style breadth (14.43), filament length (164.45), anther length (70.87), anther breadth (34.94), length of stalk (58.16) and umbel diameter (34.22). The highest cluster mean values were observed in cluster III for only eight characters viz. days to 100% sprouting (15.33), style length (81.99), style breadth (18.33), filament length (185.7), anther length (77), anther breadth (38.33), length of stalk (62.14) and umbel diameter (38.62). Among 16 characters, cluster III showed lowest cluster mean value for days to 1st bolting (39.8), days to 50% bolting (55.4), days to anthesis (62.47), stalk diameter (12.4), percent fertile pollens (92.39) and number of flowers per umbel (150.46). Similar results were also reported by Mohanty (1999) for number of leaves per plant and Mohanty and Prusti (2001) for weight of bulb and neck thickness in onion.

Contribution of characters towards the divergence obtained from canonical variate

analysis is presented in Table 6. In this method vectors were calculated to represent the varieties in the graphical form (Rao et al., 1952). This is helpful in cluster analysis as it facilities the study of group constellations and also serves as a pictorial representation of the configuration of various groups. The absolute magnitude of the coefficients in the first two canonical vectors also reflects to a great extent, the importance of the characters for primary and secondary differentiation. The character which gives high absolute magnitude for vector II, is considered to be responsible for secondary differentiation. Likewise, the characters which give higher absolute magnitude for vector I is considered to be responsible for primary differentiation. If the same character gives equal magnitude for both the vectors then the character is considered responsible for primary as well as secondary differentiation.

| | Cluster | | | | | |
|-------------------------|---------|---------|---------|--|--|--|
| Characters | Ι | Π | III | | | |
| Days to 100% sprouting | 15.22 | 13.83L | 15.33H | | | |
| Days to 1st bolting | 46.66 | 54.17H | 39.8L | | | |
| Days to 50% bolting | 69.31 | 76.5H | 55.4L | | | |
| No. Leaf per plant | 23.69L | 47.6H | 32.12 | | | |
| Days to an thesis | 68.89 | 76.34H | 62.47L | | | |
| Style length | 80.61 | 68.11L | 81.99H | | | |
| Style breadth | 16.37 | 14.43L | 18.33H | | | |
| Filament length | 170.97 | 164.451 | 185.7H | | | |
| Anther length | 71 | 70.87L | 77H | | | |
| Anther breadth | 35.92 | 34.94L | 38.33H | | | |
| No. Stalk per plant | 3.95L | 4.1H | 4.09 | | | |
| Length of stalk | 59.59 | 58.16L | 62.14H | | | |
| Stalk diameter | 13.22H | 12.55 | 12.4L | | | |
| No.of flowers per umbel | 260.03H | 184.15 | 150.46L | | | |
| Percent fertile pollens | 92.79H | 92.74 | 92.39L | | | |
| Umbel diameter | 38.54 | 34.22L | 38.62H | | | |

Table 5. Cluster mean values of 16 characters of 10 genotypes of onion

 Table 6. Latent vectors for 16 component characters of ten genotypes of onion

| Characters | Vector1 | Vector 2 |
|------------------------|----------|----------|
| Days to 100% sprouting | 0.28304 | 0.08550 |
| Days to 1st bolting | -0.09705 | -0.04393 |
| Days to 50% bolting | -0.03673 | 0.02042 |
| No. Leaf/plant | 0.00864 | 0.00967 |
| Days to anthesis | -0.02443 | 0.00674 |
| Style length | -0.01877 | 0.01429 |
| Style breadth | -0.00009 | 0.23311 |
| Filament length | 0.04616 | 0.00303 |
| Anther length | 0.10549 | 0.05687 |
| Anther breadth | -0.17639 | -0.07516 |
| No. Stalk/plant | 0.35566 | -0.04770 |
| Length of stalk | -0.06343 | -0.07216 |
| Stalk diameter | 0.17080 | 0.15843 |
| No. Of flower/umbel | -0.02287 | 0.02188 |
| % Fertile pollens | -0.00107 | -0.05276 |
| Umbel diameter | 0.46822 | 0.29802 |

In vector I (Z_1) and vector II (Z_2) obtained from PCA, days to 100% sprouting, number of leaves per plant, filament length, anther length, stalk diameter and umbel diameter were important because all these characters had positive values. Days to 1st bolting, anther breadth, length of stalk and percent fertile pollen in both the vectors, which indicated that they were the less important characters having lower contribution to the genetic divergence among the materials studied. Similar results were also found by Mohanty (1999) for number of leaves per plant and Mohanty and Prusti (2001) for weight of bulb and neck thickness in onion.

4. Conclusions

Considering clustered distance and cluster mean, the intra cluster distance revealed that the genotypes Indian big (G_1) and Patnai pink (G_5) from the cluster II and genotypes Taherpuri, Indian medium (G_1), Big single bulb (G_7), Big double bulb (G_8), Small single bulb (G_9) and Small double bulb (G_{10}) from the cluster III may be selected as parents for future breeding program. The characters days to 100% sprouting, number of leaves per plant, filament length, anther length, stalk diameter and umbel diameter contributed maximum towards divergence. Hence major emphasis should be given on them for selecting parents for hybridization in onion.

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