Original Research Article  

**Genetic Diversity Analysis and Variability Estimation in Betelvine (Piper betle L.)**

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**Abstract**

Fifteen genotypes of betelvine including 2 checks planted in Randomized block design with three replications during the year 2018-19 in two structure viz., Poly tunnel and Net tunnel. Net tunnel mean performance of genotypes are considered for further study of genetic diversity as net tunnel gives better results than poly tunnel. For diversity study biometrical techniques which are used are principle component analysis and Mahalanobis D² cluster analysis, both methods are categorized as grouping method of analyzing variability and genetic diversity.

**Keywords**

Diversity, PCA, Cluster

**Article Info**

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**Introduction**

Betelvine (Piper betle L.) belongs to family Piperaceae is a perennial, dioecious creeper (Hassan and Shahadat, 2005) originated from Malaysia are under cultivation in India and many other countries on commercial scale (Kumar et al, 2010). The betel leaves are rich in vitamins, minerals, protein and essential oil. Beside this it is also used as medicine, in different ceremony and is of religious importance in Chhattisgarh and India as well. For better growth and yield of betelvine, climatic factors like temperature, relative humidity, plays a crucial role (Walker, 1965). In general, the climate of Chhattisgarh is hot and humid because of its proximity to the tropic of cancer and it is depend on the monsoon for rains. The hot season starts from March to mid June and summer temperature reaches up to 45°C (113°F). The rainy season starts from late June to September. The average rainfall is 1292 mm (50.9 in). The winter starts from November to January and from September temperature starts decreasing causes due to rainfall and humidity. The cultivation of betelvine in Chhattisgarh is possible only under protected structure as the summer is dry with more than 45°C and winter is cold.

The betelvine plant require humidity 65% and temperature less than 35°C for its cultivation which is possible only under micro climate maintained under protected structure. Therefore experiment was conducted under
two condition Poly tunnel improved protected structure and Net tunnel improved protected structure.

Materials and Methods

The experiment was carried out during 2018-19 at different protected structures viz; Poly tunnel, and Net tunnel at research cum instructional farm of IGKV, Raipur (C.G.). The experiment was laid out in randomized complete block design with three replications. Fifteen genotypes collected from different states of India in which local genotypes Billori local and kapoori local are considered as checks.

Principal Component Analysis (PCA) is a powerful tool in modern data analysis because it is a simple, non-parametric method for extracting relevant information from confusing data sets. It reduces the dimensionality of the data while retaining most of the variation in the data set. PCA accomplishes this reduction by identifying directions, called principal components (PCs), along which the variation in the data is maximal.

In the present study, PCA was performed for 13 quantitative traits in genotypes of betelvine. The PC with eigen values (>1) and which explained that at least 5% of the variation in the data were considered in study. The PC with higher eigen values and variables which had high factor loading were considered as best representative of system attributes. Only five principal components (PCs) taken out of 13 (Table 1).

Results and Discussion

Quantitative principle component analysis

The first principle component (PC1) illustrate that leaf thickness (0.401), depth of sinus (0.439), internodal length (0.235) and leaf length (0.244) contribute mostly to the diversity between the genotypes of betelvine at 46.92% variation. (Table 2).

Similarly, for the second principle component (PC2), following traits are dominated at 18.06% viz., leaf area (0.378), width of lobe (0.345), leaf yield (0.198) and internodal length (0.395) correlated with PC1. 13% variation is contributing for principle component third (PC3) including traits which are correlated with leaf length (0.387) and leaf area (0.143). Principle component fourth (PC4) show correlation PC1 and PC2 via leaf length (0.372), depth of sinus (0.57) and width of lobe (0.514) at 8.49% variation.

Scree plot

A graph which is obtained in between eigen value and principle component numbers which explains the variation percentage which is related to each principle component. First principle component PC1 show 46.92% variation with eigen value 6.1 which gradually decreases with further principle components. From the graph it’s clear that maximum variation is in PC1 (Fig. 1). The result of PCA explains the genetic diversity of genotypes where, importance and contribution of each component measured by proper values associated with each principle component.

Thus, the prominent traits are congregated in different principle components contributing to explain variability. They may be considered together in utilization of these traits for breeding programmes. From the first four PCs it is clear that PC1 mostly related to leaf length, internodal length, depth of sinus and leaf thickness. PC2 relevant to leaf area, width of lobe, internodal length and leaf yield traits. PC3 relevant to yield contributing traits. So, a good breeding program can be initiated by selecting the accessions from PC2, and PC3.
### Table 1: Source of collection of genotype

| S. No. | Genotypes          | Source of collection |
|--------|--------------------|----------------------|
| 1      | Bhaichigudi        | Orissa               |
| 2      | Meetha             | Madhya Pradesh       |
| 3      | Bidhan Pan         | West Bengal          |
| 4      | Karapaku           | Andhra Pradesh       |
| 5      | Awni               | Assam                |
| 6      | Ramtek Meetha pan  | Maharashtra          |
| 7      | Ramtek Bangla      | Maharashtra          |
| 8      | Ramtek Kapoori     | Maharashtra          |
| 9      | Maghai pan         | Bihar                |
| 10     | Bali pan           | Orissa               |
| 11     | Uttkal sudama      | Orissa               |
| 12     | Meetha-cum-bangla  | Maharashtra          |
| 13     | Bangla local       | Chhattisgarh         |
| 14     | Billori local (LC) | Chhattisgarh         |
| 15     | Kapoori local (LC) | Chhattisgarh         |

### Table 2: Principle component analysis of 13 quantitative traits for 15 betelvine (*Piper betle* L.) genotypes

| TRAITS                   | PC1  | PC2  | PC3  | PC4  |
|--------------------------|-----|-----|-----|-----|
| LL(cm)                   | 0.244| 0.308| 0.387| 0.372|
| PL (cm)                  | -0.073| -0.07| 0.065| -0.018|
| LW (cm)                  | -0.508| 0.298| 0.101| 0.059|
| LA (cm²)                 | 0.013| 0.378| 0.143| -0.144|
| L/B ratio                | -0.382| -0.148| 0.03| -0.087|
| IL (cm)                  | 0.235| 0.395| -0.239| -0.335|
| LLL (cm)                 | 0.105| -0.087| -0.113| -0.214|
| DS (cm)                  | 0.439| -0.221| -0.212| 0.57|
| W. lobe (cm)             | -0.226| 0.345| -0.206| 0.514|
| D/W. lobe ratio          | -0.236| -0.523| -0.135| 0.064|
| LT(cm)                   | 0.401| -0.041| 0.044| -0.274|
| L.WGT                    | -0.057| -0.037| -0.083| 0.045|
| LY(g)                    | -0.039| 0.198| -0.794| -0.027|
| EIGEN ROOT               | 6.1| 2.348| 1.69| 1.104|
| % VARIATION              | 46.92| 18.06| 13| 8.49|
| CUMMULATIVE %            | 46.92| 64.98| 77.98| 86.47|

Leaf length (LL); petiole length(PL); leaf width(LW); leaf area(LA); length: breadth ratio(L/B); Internodal length (IL); leaf lamina length(LLL); depth of sinus (DS); width of lobe(WL); leaf thickness(LT); 100 leaf weight(L.WGT.); leaf yield (LY)
Table 3 Principle component score of different genotypes of betelvine (*Piper betle* L.) for 13 quantitative traits

| Genotypes                          | PC1   | PC2    | PC3    | PC4    |
|------------------------------------|-------|--------|--------|--------|
| Baichigudi                        | 5.325 | -0.932 | -1.275 | -0.939 |
| Meetha local                      | -1.588| 0.485  | -0.083 | -2.145 |
| Ghanaghatte (Bidhan pan)          | 2.919 | -1.393 | -0.436 | 1.166  |
| Karapaku                           | 3.828 | 0.295  | -0.455 | -0.822 |
| Assam kapoori (awni)              | 2.181 | 4.468  | 0.436  | 1.122  |
| Ramtek kapoori                    | -2.096| -1.474 | -1.56  | 0.506  |
| Maghai pan                        | -1.168| -0.532 | -1.421 | 1.448  |
| Bali pan                          | -1.618| 0.389  | 0.632  | -1.496 |
| Uttkal sudama                     | -2.329| 0.082  | -0.256 | 0.785  |
| Meetha cum bangla                 | -2.04 | 0.413  | -0.631 | -0.914 |
| Ramtek bangla                     | -1.707| 0.641  | 0.357  | 0.672  |
| Ramtek meetha pan                 | -2.151| 0.835  | -1.485 | -0.085 |
| Bangla local                      | 0.029 | 0.088  | 2.833  | 0.137  |
| Billourli (LC.)                   | 0.916 | -1.354 | 1.367  | 0.302  |
| Kapoori (LC.)                     | -0.5  | -2.011 | 1.976  | 0.263  |

Table 4 List of selected genotypes in each principle component on the basis of to 05 PC score (13 quantitative traits)

| PC1       | PC2       | PC3       | PC4       |
|-----------|-----------|-----------|-----------|
| Bhaichigudi | Assam kapoori (Awni) | Bangla local | Ghanaghatte (Bidhan pan) |
| Karapaku   | Ramtek Meetha pan | Kapoori (LC) | Assam kapoori (Awni) |
| Ghanaghatte (Bidhan pan) | Ramtek bangla | Billori (LC) | Maghai pan |
| Assam kapoori (Awni) | Meetha local | Bali pan | Uttkal sudama |
| Billori (LC) | Meetha cum bangla | Assam kapoori (Awni) | Ramtek bangla |

Table 5 Showing 05 clusters of 15 betelvine (*Piper betle* L.) cultivars based on Euclidean distance as dissimilarity

| S. No. | Clusters | Number | Cultivars                                                                 |
|--------|----------|--------|---------------------------------------------------------------------------|
| 1      | I        | 5      | Ramtek kapoori, Maghai pan, Uttkal sudama, Ramtek bangla, Ramtek Meetha pan |
| 2      | II       | 3      | Bhaichigudi, Ghanaghatte (Bidhan pan), Karapaku                            |
| 3      | III      | 3      | Meetha local, Balipan, Meetha cum bangla                                   |
| 4      | IV       | 1      | Assam kapoori (awni)                                                      |
| 5      | V        | 3      | Bangla local, Billori (LC), Kapoori (LC)                                   |
Table 4.17 Intra(diagonal and bold) and inter cluster distance among 5 clusters of 15 betelvine (Piper betle L.)

| Cluster | I     | II    | III   | IV    | V     |
|---------|-------|-------|-------|-------|-------|
| I       | 1.428 |       |       |       |       |
| II      | 6.016 | 2.469 | 6.273 | 3.742 |
| III     | 1.710 | 6.071 | 5.755 | 4.813 |
| IV      | 2.053 |       | 6.261 | 3.67  |
| V       |       | 0.132 | 6.206 |

Table 4.18 Mean value of five clusters for 13 quantitative traits which are yield contributing traits in 15 genotypes betelvine (Piper betle L.)

| S. NO. | Characters          | I     | II    | III   | IV    | V     |
|--------|--------------------|-------|-------|-------|-------|-------|
| 1      | Leaf length (cm)   | 9.06  | 11.77 | 7.25  | 8.94  | 7.05  |
| 2      | Petiole length (cm)| 4.90  | 6.11  | 4.27  |       | 6.29  |
| 3      | Leaf width (cm)    | 4.46  | 7.72  | 4.49  | 7.57  | 5.85  |
| 4      | Leaf area (cm²)    | 28.64 | 87.19 | 33.32 | 57.45 | 50.80 |
| 5      | L/B ratio          | 1.39  | 1.41  | 1.68  | 1.23  | 1.74  |
| 6      | Internodal length (cm)| 7.31| 6.52  | 6.97  |       | 8.13  |
| 7      | Lamina length (cm) | 5.75  | 10.91 | 7.61  | 9.34  | 10.16 |
| 8      | Depth of sinus (cm)| 0.86  | 0.97  | 0.97  | 1.75  | 0.84  |
| 9      | Width of lobe (cm)| 2.24  | 3.84  | 2.23  | 3.11  | 2.92  |
| 10     | Depth / lobe width ratio | 0.38 | 0.25  | 0.44  |       | 0.61  |
| 11     | Leaf thickness (mm)| 0.34  | 0.40  | 0.34  |       | 0.43  |
| 12     | Leaf weight (100 leaves)| 112.53| 164.02| 166.07| 219.23| 95.41 |
| 13     | Leaf yield (g)     | 73.98 | 108.79| 77.68 | 91.06 | 86.93 |

Fig.1
Scatter plot matrix

The two components PC1 and PC2, from quantitative data explains cumulative variability of 64.98%. Based on the distribution of genotypes, Bhaichigudi and Assam kapoori (Awni) were the most distantly related to the group.

Top 05 principle components scores (PC score) for all the genotypes were estimated in five principle components and presented in Table 3. These scores are used for precise selection indices whose intensity is explained by variability explained by each of the PC. High value of variability is explained in a particular genotypes is denoted by high value of PC score for that particular genotypes in particular principle component.

Result explains that the “Bhaichigudi” had highest score in PC1 followed by karapaku, Ghanaghatte (Bidhan pan), Assam kapoori/Awni, and Billori (LC), indicating that they had high or greater leaf length, internodal length, depth of sinus and leaf thickness. In PC2, Assam kapoori/Awni, followed by Ramtek Meetha pan, Ramtek bangla, Meetha local and Meetha cum bangla have high value for leaf area, width of lobe, internodal length and leaf yield traits. PC3 have highest scored genotypes are as follows: Bangla (LC), Kapoori (LC), Billori (LC), Bali pan, Assam kapoori/Awni exhibited high for leaf length and leaf area (Table 4).

High principle component score for particular accession in a particular component denotes high value for the variables in that particular accession. Thus, it clearly denotes that analysis of principle components highlights the characters with maximum variability. So, intensive selection procedures can be designed to bring improvement of traits of betelvine (Piper betle L.).

Non hierarchical cluster analysis

Cluster analysis is an multivariate cluster analysis which classify the variables into number of different groups on basis of trait which are to be studied. Clustering pattern is estimated by method of non hierarchical Euclidean cluster analysis through software SPARK using Euclidean distance as dissimilarity measure which further classify 15 betelvine (Piper betle L.) genotypes into 05 clusters randomly and they did not follow (Table 5).

The computed intra and inter cluster distance shows the average distances of cluster members from cluster centroids which is given in Table 6. The intra cluster distance ranged from 0.132 (cluster IV) to 2.053 (cluster III) and inter cluster average distance with maximum magnitude is observed in between cluster I and cluster IV (6.273) followed by minimum inter cluster distance between cluster I and cluster III (2.469). Maximum intra cluster distance shown by cluster III (2.053) which produce desirable breeding material for maximum genetic advance. Minimum intra cluster distance is shown in cluster IV (0.132) indicate unidirectional selection lead to uniformity with less deviation. The mean values of clusters show wide range of variation for all 13 traits under this study (Table 7). Cluster II exhibit highest mean value for leaf length (11.77), leaf width (7.72), leaf area (87.19), leaf lamina length (10.91), depth of sinus (0.97), width of lobe (3.84), and leaf yield (108.79). While, cluster III contained genotypes with higher mean value for length: breadth ratio (1.68), depth of sinus (0.97). Cluster IV contain highest mean value for traits petiole length (6.29), internodal length (8.13), ratio for depth of sinus and width of lobe (0.61), leaf thickness (0.43), and leaf weight (219.23).
In the present study highest inter cluster distance is found between cluster I and cluster IV (6.273) which followed by in between cluster III and cluster IV (6.261). Lowest inter cluster distance in between cluster I and cluster III (2.469) indicate close relationship between these two clusters therefore, hybridization among these genotypes of these clusters would not provide the encouraging results. In all cases inter cluster distance is higher than the intra cluster distances reflecting wider diversity among the breeding lines of distance groups. To check much variability, genotypes must be selected from two clusters having wider inter cluster distance (cluster I and cluster IV) reveal highly divergent group and cross between them produce more variability. Thus, selections of parents from the clusters with high inter and intra cluster distance will help to obtain substantial heterosis in respect of economic traits during hybridization programme. (priya et al.,).

In conclusion out of 13 only 4 principle components exhibit eigen value more than 1 and showed 86.47 % cumulative variation among quantitative characters. So, these four PCs explain further diversity.

On the basis of cluster analysis 15 genotypes were grouped into 05 clusters. The highest numbers of genotypes were in cluster I. The highest cluster mean value was obtained in cluster IV for leaf weight and lowest in cluster I and V for leaf thickness. The highest inter cluster distance between cluster I and cluster IV depict that cross between them produce more variability and The lowest inter cluster distance was found between cluster I and cluster III is evidence for close relationship between the genotypes and maximum intra cluster distance shown by cluster III produce desirable breeding material for maximum genetic advance.

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