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ASSESSMENT OF ANALYSIS OF VARIANCE, HERITABILITY GENETIC ADVANCE AND GENETIC DIVERSITY IN BETEL VINE (*PIPER BETLE* L.) GENOTYPES USING MORPHOLOGICAL CHARACTERS

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ABSTRACT

Betel vine (*Piper betle* L.) is one of the cash crops of country contributing maximum of export income for nation. The research study was carried out with 15 betel vine genotypes including two checks Kapoori local and billori local at research cum instructional farm if IGKV, Raipur. In season from august 2018 to February 2019 under protected structures with RBD design of 3 replications. By present study we observed that analysis of variance shows presence of high significant variation for all traits of betel vine leading to evidence for presence of variability. The slightly highest value of PCV with GCV, heritability and genetic advance is observed in leaf area. The values of PCV are slightly high indicating the variation present is not only due to genotypes but also because of environmental influence. The result of variability assessment of quality traits shows slightly higher value of PCV coupled with GCV i.e. more than 20% in oil content. Highest heritability and High genetic advance is observed in leaf yield, leaf length, leaf area, leaf weight, leaf width, leaf thickness and leaf lamina length, except for Inter-nodal length. All the traits having high values of GA with higher heritability depict that the characters are governed by additive genes and selection will be rewarded for that trait improvement. The leaf yield is positively correlated with leaf length, petiole length, leaf width, leaf area, lamina length, width of lobe, leaf thickness showing practical significance for future breeding programme. Very high direct and positive effect on leaf yield was exhibited by leaf lamina length. Positive correlation with high direct effect is observed in leaf length, petiole length, lamina length and leaf weight resulting in true relation and direct selection is employed for increasing leaf yield.

Genetic diversity as genotypes chosen from different eco-geographical regions is grouped in different clusters. The cluster I consist of largest which consists of 05 genotypes, followed by cluster II, III, and V with 03 genotypes and cluster IV with 01 genotype. The D2 analysis indicated the presence of appreciable diversity in genotypes with inter cluster distance varied from 6.261 to 2.469 demonstrating presence of genetic diversity and intra cluster ranged from 0.132 to 2.053 which indicate presence of diversity in term of genetic distance D^2. Four principal components (PCs) exhibit eigen value more than 1.0 and showed cumulative variability of 64.98 % among quantitative traits, suggesting considerable diversity among the genotypes, rest of components are not considered. PCs score is interpreted as relative weight of variables in each component based on correlation. Selection is based upon contribution of traits towards divergence.

Keywords **:** Dioecoius, Diversity, Genetic Advance, Heritability,

Introduction

Betel vine the most beneficial medicinal plant is *Piper betle* L., whose leaves have been utilized for a variety of therapeutic purposes. Pan is another name for Piper betel L., Or ‘betel qui’ the leaf of a vine that belongs to the vast plant family Piperaceae. Malaysia and Indonesia refer to it as "Sirih," while India calls it "pan." It is indigenous to eastern and central Malaysia. Betel vine is a perennial creeper that grows dioecious and evergreen. It is grown in tropical and subtropical

regions for its chewing stimulating leaves (Maiti and Shivashankara, 1998). It is a spreading vine with shiny heart-shaped leaves and white catkin that easily roots when trailing stems touch the ground. The full, alternating leaves are 05 to 10 centimeters in length and 03 to 06 centimeters in diameter. At the leaf nodes, smaller flowers bloom on pendulous spikes that are 4 to 8 cm long. As the fruit reaches maturity, the spikes' length increases to 7 to 15 cm. The betel vine's stem and leaves have a pungent, aromatic flavor. One way to get calcium from food is to chew betel leaves.

In many parts of the world, from South Asia eastward to the Pacific, the combination of arecanut and betel vine leaves has been utilized for centuries as a chewing stimulant and as part of traditional customs or rituals. There are currently 100 betel vine varieties worldwide, with 40 of those found in India and 30 in Bangladesh (Guha, 1997). It is an economically significant medicinal crop that grows on around 3,600 hectares in Andhra Pradesh, India. A total of 55,000 hectares are used for betel vine cultivation with an approximate yearly output value of Rs. 9000 million in India (Richa and Neetu Singh, 2017).

Around the world, betel vine grows vigorously in humid climates. It has been cultivated in two different environments: naturally occurring and intentionally cultivated. Under normal circumstances, plants grow taller than trees in the tropical rain forest region (Western and northeastern parts) in the south, where temperatures and humidity don't fluctuate much and high humidity and moderate sunshine prevail all year long. The cultivation of betel vines is performed. Betel vine is a tropical plant, so it needs special care and attention to grow in India's subtropical climate. Cultivation is carried out in artificially generated regulated conditions in areas where the climate is unsuitable for natural cultivation. Stated differently, the optimal conditions are achieved by keeping the temperature between 15°C and 40˚C and the humidity between 40 and 80 percent. The performance of various betel vine cultivars under two distinct sets of protected structures (microclimates) is thus examined in the current study. It gives the crop the shade and high humidity it needs for a successful harvest (Khatua, 2013). However, the pungency of leaves can occasionally become unfit for human consumption if the light intensity is not well controlled.

Material and Methods

The present investigation is entitled with "study the variability parameters in betel vine (*Piper betle* L.)." was conducted during 2018-19 at two different protected structures at research cum instructional farm of Indira Gandhi Krishi Viswavidhyalaya, Raipur (C.G.). Average Temperature and Relative Humidity prevailed during the period of study (2018 & 2019) in two different protected structures. Environment of Chhattisgarh area comes under dry and semi-arid condition. In such climatic condition the cultivation of betel vine is not possible in an open area. Therefore, experiment was conducted under two sets of condition. Total 15 genotypes along with 2 checks (Kapoori local and Billori local) of betel vine were grown in 2 different protected structures i.e., Poly tunnel and Net tunnel.

Table 1 : List of cultivars and their source of collection.

S.No.	Cultivar	Source of Collection
1.	Bhaichigudi	Orissa
2.	Meetha	Madhya Pradesh
3.	Ghanaghati (Bidhan pan)	West Bengal
4.	Karapaku	Andhra Pradesh
5.	Assam Kapoori (Awni)	Maharashtra
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 (L.C.)	Chhattisgarh
14.	Kapoori	Chhattisgarh
15.	Billori	Chhattisgarh

Table 2 : Details of experiment.

No. of Treatments	:	15 Betel vine Cultivars
Design	:	Randomized Block Design (RBD)
Year of Planting	:	July 2017
System of Cultivation	:	Protected Cultivation (Poly Tunnel & Net Tunnel)
Method of planting	:	Row Planting
RxP spacing	:	85cm x 20cm
Month of Lowering	:	July 2018
Local Checks	:	Kapoori , Billori & Bangla

As per the recommendations adequate quantity of farmyard manure (40 t/ha) was applied. A total of 200 kg nitrogen, 100 kg P_2O_5 and 100 kg K_2O per hectare was applied through different organic manures viz., FYM, neem cake groundnut cake and vermi-compost along with chemical fertilizers. Fifty percent of nitrogen was applied at the time of lowering, twenty five percent at four months after lowering and remaining twenty five percent at six months after lowering (Anon., 2007). Observations were recorded at monthly intervals on different genotypes of betel vine for various morphological and Quantitative parameters.

Five vines per plant were selected randomly, tagged for monthly observations in each genotype starting from one month after lowering in all the three different structures.

Result and Discussion

The research was conducted under two different protected structures at research cum instructional farm of Indira Gandhi Krishi Viswavidhyalaya, Raipur (C.G.). The result presented below are the interpretation of analysis done on average performances of different characters. The results obtained by experiments performed under the study are described below:

Quantitative Traits

Mean performance of 13 quantitative yield contributing traits with leaf yield are studied for 15 cultivars of betel vine under controlled conditions of net tunnel and poly tunnel. The breeding programme depends largely on the presence of significant genetic variability to permit effective selection. Results revealed that high degree of variability was present in the cultivar for all the characters under study.

Petiole length

Petiole length is one of the economically important traits of betel vine as larger sized leaf with long petiole is in demand in export markets. The data recorded related to petiole length of betel vine genotype at monthly interval for 7 months i.e. from august to February. Genotypes show significant variations amongst themselves with respect to petiole length under different micro-climatic conditions.

The net tunnel recorded the longest petiole length (9.82cm) with medium light intensity and it was recorded statistically significant over both structures. Amongst the 15genotypes with2checks (cultivar) maximum petiole length was observed in Assam kapoori (Awni) (8.61cm) followed by karapaku (7.14 cm), Bangla local (7.08cm) and kapoori local (check) (6.42cm) comparatively minimum petiole length was observed in Meetha local (4.34cm).

Leaf length

Leaf length is important contributing character of yield governing quantitative traits amongst all character's longest leaf length fetch greater prize on market compared to medium and smaller leaf length of leaves. As per the data recorded net tunnel shows the longest leaf length (12.91cm) followed by poly tunnel (11.55 cm) on an average.

The cultivar Bhaichigudi bears the longest leaf length (14.05 cm) under net tunnel. Among all the cultivars maximum leaf length was recorded by Bhaichigudi (11.55cm, 12.91cm) followed by Karapaku (11.28cm, 10.31cm) and compared to the minimum in Ramtek kapoori (5.63cm, 6.18cm) and Ramtek Meetha pan (5.98 cm) in poly tunnel and net tunnel, respectively. Similar finding are observed in experiment of Kumar *et al*. (2013).

Leaf area

The leaf area of 15 cultivars shows variable differences and high production of leaves are observed most in net tunnel which show favorable effect on yield of betel vine leaves. Net tunnel show significant variation among leaf area with highest area 139.42 cm^2 in net tunnel whereas, Bhaichigudi cultivar show significantly highest leaf area (116.2cm^2) amongst all 15 cultivars under net tunnel followed by karapaku (86.08cm^2), Billori local (check) (78.70cm^2) Ghanaghatte (Bidhan pan) with area (77.66cm^2), comparatively minimum leaf area of Ramtek kapoori (32.71cm^2) and Ramtek Meetha pan (33.14cm^2). Similar result is found with finding of Sridevi *et al.* (1992).

Leaf width

Leaf width is characteristic data which show significant difference amongst all 15 genotypes for increasing yield contribution towards market demand. The widest leaves are observed with width of (8.38 cm) in Bhaichigudi cultivar in net tunnel, which is significant over all in different microclimatic structures. Amongst all the genotypes maximum leaf width with an average value was noted in Bhaichigudi (8.22cm) followed by Assam kapoori/Awni (7.31 cm) and karapaku (6.82 cm) and Ghanaghatte pan (6.81 cm) comparatively minimum leaf width recorded in Ramtek kapoori (3.83 cm).

Inter-nodal length

A genotype having shorter inter-nodal length with respect to moderate leaf production is considered to be good genotype. On comparison study genotypes of net tunnel having microclimatic condition with high intensity of light shows longer inter- nodal lengths which is significant over mean value of polytunnel. Accordingly, Billori local (Check) observed to be with longest inter-nodal length (9.41 cm) and equalized with Assam Kapoori (Awni) pan (7.91 cm), Meetha-cum-Bangla (7.57 cm). The shortest inter-nodal length is observed in genotype Bhaichigudi (5.82 cm) in net tunnel.

Leaf lamina length

According to the data observed net house having medium light intensity recorded the longest leaf lamina

length in genotype bhaichigudi (12.91cm) which is significant over all other genotypes at different micro-climatic structures. Amongst all the genotypes maximum leaf lamina length was noted in bhaichigudi (12.23cm) followed by karapaku (10.79 cm) and Billori local (check) (10.26cm) comparatively minimum leaf lamina length recorded in Ramtek kapoori (5.90cm). Similar results are found with Rahman *et al*. (1997).

Depth of sinus and width of lobe(cm)

The observations show statistically significant variation amongst all 15 genotypes with maximum depth shown in net tunnel with medium light intensity on genotype Assam kapoori/Awni (2.19). The depth varies from maximum to minimum in all genotypes, on an average maximum depth of sinus is in Assam kapoori/ Awni (2.10) followed by karapaku (1.19) and Meetha-cum-Bangla (1.14) comparatively minimum depth is in Ramtek kapoori (0.68) and kapoori local (check) (0.81). The observations show statistically significant variation amongst all 15 genotypes with maximum width of lobe shown in net tunnel with medium light intensity on genotype Bhaichigudi (4.02cm). The width of lobe varies from maximum to minimum in all genotypes, on an average maximum width of lobe is seen in Bhaichigudi (4.02cm) followed by Assam kapoori/Awni (3.65cm) and Ghanaghatte Pan (3.62 cm) comparatively minimum width of lobe is seen in Meetha-cum-Bangla (2.05cm) and Ramtek kapoori (1.83cm).

Ratio of depth of sinus and width of lobe.

Ratio between the depth of sinus and width of lobe is to be calculated in centimeters. The result obtained by ratio of both will shows that the leaves can be categorized as follows:

1. Entire/ slightly lobed which shows the ratio less the 0.15 (<0.15).
2. Moderately lobed which shows the ratio value between 0.15– 0.25.
3. Deeply lobed shows the ratio of more than 0.25 (>0.25).

According to data pertaining to betel vine it is observed that poly tunnel having medium light intensity under its micro climatic condition shows the maximum ratio of depth of sinus (cm) and width of lobe (cm) i.e. 0.673. The genotype having maximum average ratio is in Assam kapoori (Awni) (0.570) followed by Meetha-cum-Bangla (0.536), and Meetha local (0.482) with comparatively minimum ratio of 0.254 in genotype Bhaichigudi.

Leaf yield (g)

Leaf yield (weight of harvested leaves per vein) of different cultivars varied under different structures. Net tunnel had a favorable effect on leaf yield of different cultivars of betel vine. It is evident from the data presented that Net tunnel produced maximum leaf yield (118 gm). Karapaku recorded the largest leaf yield (118.73 gm) in Net tunnel. Among the genotypes maximum leaf yield was recorded by Karapaku followed by Bhaichigudi (114 gm) and kapoori local (check) (95.66 gm) compared to the minimum in Ramtek Bangla (69.94 gm) and Uttkal sudama (56.56 gm).

Estimation of genetic variance

Analysis for variance for different conditions (ANOVA)

The statistical procedure which separates or splits the total variation into different components is known as analysis of variation. Different components of variance are estimated through ANOVA. The result obtained by data presented below shows the analysis of variance for different microclimatic conditions through mean sum of square of all characters estimated which shows the significant differences depicting that the adequate variability is present among all fifteen cultivars. In poly tunnel, all dominant characters show significant relation due to genotype/ treatments was found to be highly significant for all the traits with leaf yield. The significant relationship of traits is present in all traits which are studied. Hence, depict the result that in all cultivars abundant variability is present within genotypes and in between both protected structures for all the traits. The significant and relatively large percentage of the total variation attributable to GxE interaction suggests that genotypes responded differently. The result of analysis of variance (ANOVA) of net tunnel shows that the mean sum of square of genotype/ treatment shows high significance towards all characters This shows the presence of abundant variability among all 15 genotype relevant to all traits which are estimated. Further, following result shows non- significant variations in mean sum of square of replication which shows homogenized environment towards genotypes. Whereas, the result of analysis of variance (ANOVA) of poly tunnel shows that mean sum of square of replication of shows homogeneous environment by showing no significant differences with the estimated traits. The result of mean sum of square of treatments which denotes genotypes in net tunnel, show highly significant relation with the traits which are estimated. As it shows significant differences resulting in abundant variability

amongst all the 15 genotypes by following traits. Hence, this proves that sufficient variability amongst the genotypes is present in both the tunnels which can further be utilized for analysis of variability.

The result of analysis of variation (ANOVA) of qualitative traits of genotypes also shows significant differences in outcome showed sufficient variability among all15 cultivars of betel vine with the traits which are estimated. The significant outcomes of all quantitative and qualitative traits are the evidence for genotypes that abundant variation is present in them for variability analysis.

Table 3 : Analysis of variance (ANOVA) of quantitative traits of net tunnel

SN	Source of Variation DF	Replication (2)	Genotype (14)	Error (28)
		Mean sum of Squares		
1.	Leaf length (cm)	1.676	14.391**	0.013
2.	Petiole length (cm)	0.242	2.292**	0.204
3.	Leaf width (cm)	2.352	6.25**	0.069
4.	Leaf area (cm²)	2.228	1,956.90**	2.105
5.	L/B ratio	0.103	0.12**	0.015
6.	Inter-nodal length (cm)	0.111	0.872**	0.419
7.	Lamina length (cm)	1.191	12.829**	0.294
8.	Depth of sinus (cm)	0.115	0.255**	0.015
9.	Width of lobe (cm)	0.355	1.349**	0.091
10.	Depth/ lobe width ratio	0.036	0.044**	0.007
11.	Leaf thickness (mm)	0.05	0.004**	0.019
12.	Leaf weight (100 leaves.)	9.0	7, 561.84**	60.577
13.	Leaf yield (g)	3.911	788.263**	0.131

* and ** Significant at 5% and 1% probability level

Table 4 : Analysis of variance (ANOVA)of quantitative traits of poly tunnel

SN	Source of Variation DF	Replication (2)	Genotype (14)	Error (28)
		Mean sum of Squares		
1	Leaf length(cm)	0.269	14.707**	0.012
2	Petiole length(cm)	2.809	2.233**	0.258
3	Leaf width(cm)	0.097	4.869**	0.07
4	Leaf area (cm²)	0.761	1,688.58**	3.084
5	L/B ratio	0.019	0.175**	0.005
6	Inter-nodal length(cm)	0.082	0.729**	0.151
7	Lamina length(cm)	0.724	13.378**	0.118
8	Depth of sinus (cm)	0.114	0.35**	0.015
9	Width of lobe(cm)	0.018	1.282**	0.016
10	Depth/ lobe width ratio	0.033	0.034**	0.005
11	Leaf thickness(mm)	0.01	0.005**	0.012
12	Leaf weight (100 leaves)	4.436	6,146.54**	35.546
13	Leaf yield(g)	2.626	686.583**	0.41

*and** Significant at 5% and 1 % probability level.

Mean and variability parameters for 15 cultivars of betel vine for 23 quantitative traits.

Estimation of variability parameters: Mean, range and coefficient of variation as variability parameter for 23 quantity traits of 15 cultivars of betel vine (*Piper betle *L.)

The mean average differences and genetic variability in any breeding material is a pre-requisite as it does not only provide a basis for selection but also provide some valuable information regarding selection of diverse parents for use in hybridization programme. Further analysis was carried out with the observation taken in net tunnel as it showed good result for quantitative traits studied than poly tunnel.

Poly tunnel temperature during summer rises very high and it became difficult to maintaining the

humidity and temperature inside it, which as a result not only reduces leaf size, inter-nodal length but also increases maturity of plant. Therefore, the analysis was carried out only of the data of net tunnel.

Leaf length (cm) – The mean value of leaf length is observed 8.83 with leaf length having maximum value of mean 12.71 (Bhaichigudi) and minimum mean 6.02 (Meetha cum Bangla).

Petiole length (cm) – The mean value of petiole length is observed 5.35 with maximum petiole length in 6.61(kapoori LC) and minimum petiole length in 3.72 (Meetha cum-Bangla).

Leaf width (cm) – The mean value of leaf width is 5.60 With maximum leaf width 11.62 (Ramtek kapoori) and minimum leaf width 4.19(Ramtek Meetha pan).

Leaf area (cm^2) – The mean value of leaf area is 47.64 with maximum leaf area of 113.78(bhaichigudi) and minimum leaf area of 25.13(Maghai).

Length: breadth ratio – The mean value of L/B ratio is 1.51 with maximum L/B ratio of 1.79 (Bali pan) and minimum L/B ratio of 1.18 (Maghai).

Inter-nodal length(cm)–The mean value of internodal length is observed 7.19 with maximum length of internodes 8.12 (Assam kapoori /Awni) and minimum 6.04 (Bhaichigudi).

Lamina length (cm) – The mean value of lamina length is observed to be 8.40 with maximum value of lamina length 11.49(Bhaichigudi) and minimum lamina length is observed (Ramtek kapoori) 5.40.

Depth of sinus (cm) – The mean value 0.96 is observed in depth of sinus of cultivars with maximum 1.75(Assam kapoori/Awni) and minimum 0.60(Ramtek kapoori).

Width of lobe (cm) – The mean value of width of lobe is observed 2.75 with maximum width of lobe 4.03(Bhaichigudi) and minimum width of lobe 1.93(Ramtek kapoori).

Ratio of depth of sinus and width of lobe – The mean value of ratio of depth of sinus to width of lobe is 0.37 with maximum ratio observed 0.61 (Assam kapoori/Awni) and minimum ratio 0.21(Ghanaghatte/ Bidhan pan).

Leaf thickness (mm) – The mean value of leaf thickness is 0.36 with maximum leaf thickness in 0.425 (Assam kapoori/ Awni) and minimum leaf thickness with 0.305(Meetha local).

Leaf weight (100 leaf weight) – The mean value of 100 leaf weight is 137.24 with maximum leaf weight of 100 leaves 222.53 (Meetha cum bangla) and minimum 100 leaf weight 73.95(Bangla local).

Leaf yield (gm)–The mean value performance of leaf yield shows value of 85.41 with maximum leaf yield is observed in karapaku 118.23 and minimum leaf yield of 57.59 (Uttkal sudama).

Phenotypic and genotypic coefficient of variation

Coefficient of variation was evolved by Karl Pearson. It is very useful for the study of variation. It indicates that when the C.V. is high the sample is less consistent or more variable. Phenotypic coefficient of variation and genotypic coefficient of variation i.e. PCV & GCV of any traits show the magnitude of variation in any genotype. The PCV and GCV are classified as follows as suggested by Preethy *et. al.* (2016) (low < 10%; moderate 10-20% and high > 20%). The slightly high value of PCV coupled with GCV and GA is observed in leaf area in net tunnel (53.67%- 53.58%) followed by ratio of depth of sinus and width of lobe(38.08-30.32%).The values of PCV are slightly higher than GCV indicating the apparent variation is not only due to genotypes but also due to the influence of environment. In the present investigation phenotypic coefficient of variation was recorded slightly higher than genotypic coefficient of variation i.e. traits recorded high to moderate PCV in association with GCV.

Heritability and genetic advance as mean percentage

Heritability and genetic advance are important selection parameters. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone. Improvement in the mean genotypic value of selected plants over the parental population is known as genetic advance. It is the measure of genetic gain under selection. The success of genetic advance under selection depends on genetic variability, heritability and selection intensity. In the present study of genetic variation in betel vine cultivars heritability in broad sense and genetic advance as percentage mean is calculated using software opstat.

High estimation of heritability was found for all characters/traits in net tunnel with highest heritability is observed in leaf yield (99.95%), followed by leaf length (99.74%), leaf area (99.68%) followed by leaf weight (97.64%), leaf width (96.76%) , leaf thickness (95.29%) and leaf lamina length (93.43%), except for Inter-nodal length with low heritability (26.47%). High heritability indicates good index of transmission of all these traits under study.

Table 5 : Net tunnel: mean and genetic variability parameters of 13 yield and yield contributing traits during 2018-19.

Characters	Mean	Range		CV		h2	GA%
		Min.	Max.	PCV%	GCV%	%(BS)	
Leaf length(cm)	8.83	6.02	12.71	24.83	24.79	**99.74**	51.008
Petiole length (cm)	5.35	3.72	6.61	17.75	15.61	77.31	28.266
Leaf width(cm)	5.60	4.19	11.62	26.04	25.62	96.76	51.91
Leaf area (cm^2)	47.6	25.1	113.7	**53.67**	**53.58**	**99.68**	**99.8**
L/B ratio	1.51	1.18	1.79	14.77	12.33	69.75	21.22
Inter-nodal length (cm)	7.19	6.04	8.12	10.51	5.41	26.47	5.73
Lamina length (cm)	8.40	5.40	11.49	25.18	24.33	93.43	48.45
Depth of sinus (cm)	0.96	0.60	1.75	32.11	29.52	84.52	55.91
Width of lobe (cm)	2.75	1.93	4.03	25.96	23.54	82.23	43.967
Depth/lobe width ratio	0.37	0.21	0.61	38.08	30.32	63.39	49.724
Leaf thickness (mm)	0.36	0.305	0.425	10.97	10.70	95.29	21.525
Leaf weight (100 leaves) (g)	137.2	73.95	222.53	36.87	36.44	97.64	74.164
Leaf yield(g)	85.41	57.59	118.23	18.98	18.98	**99.95**	39.08

Genetic advance is the way of selection under measure of genetic gain. The successful selection depends on heritability of the character under this study. This shows that though the traits is less influenced by environmental effects, the selection for improvement of such trait may not be useful because, heritability is based on total genetic variance which includes fixable (additive) and non-fixable (dominance and epistatic) variance.

The magnitude of genetic advance as mean percentage was observed high for all traits. Highest genetic advance is observed in leaf area (99.8%), leaf weight (74.164%), leaf length (51.008%), depth of sinus (55.91%), leaf lamina length (48.45%), width of lobe (43.96 %) GA is seen. Low genetic advance is seen in Inter-nodal length of both net tunnel (5.73%) and poly tunnel (9.37%). All the traits possessing high values of genetic advance indicate that the characters are governed by additive genes and selection will be rewarding for improvement of such trait. Slightly High PCV value than GCV values indicate variability exist among the genotypes at genotypic and phenotypic level and influenced by environment.

Amongst all traits either quantitative or qualitative of 15 betel vine cultivars in net tunnel leaf yield (g) (99.95- 39.08), leaf length (cm) (99.74-51.008), leaf area(cm^2) (99.68- 110.2), leaf weight(g) (97.64-74.16), leaf width (cm) (96.76 – 51.91), leaf thickness(mm) (97.64- 74.16), leaf lamina length (cm) (93.43- 48.45), depth of sinus(cm) (84.52- 43.96), width of lobe(cm) (82.23 – 43.96), and L/B ratio (69.75- 21.22. %) show high heritability coupled with high genetic advance mean per cent for all traits except inter-nodal length shows that heritability is due to additive gene action which led to effective selection. Similar results are found in experiments of S. *Gopi priya* (2011). Low heritability coupled with low GA is observed in trait inter-nodal length in net tunnel (26.47- 5.73), resulting in evidence for non-additive (dominance and *epistasis*) gene action.

High heritability with moderate genetic advance mean percent observed in petiole length (77.31-28.26) and leaf thickness (95.29- 21.52) which include additive and non-additive effect of traits and separate breeding procedure is employed for improving these traits.

Genetic Diversity

Genetic divergence/diversity is assessed in 15 betel vine cultivars for13 quantitative traits i.e yield attributing characters using below techniques.

PCA (Principal component analysis)

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. By using a few components, each sample can be represented by relatively few numbers instead of by values for thousands of variables. Thus, the primary benefit of PCA arises from quantifying the importance of each dimension for describing the variability of a data set in more interpretable and more visualized dimensions through linear combinations of variables that accounts for most of the variation present in the original set of variables. Therefore, principal component analysis is a variable reduction procedure.

In the present study PCA was performed for 13 quantitative traits in genotypes of betel vine. 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.

Quantitative principal component analysis

The first principal component (PC1) illustrates that leaf thickness (0.401), depth of sinus (0.439), inter-nodal length (0.235) and leaf length (0.244) contribute mostly to the diversity between the genotypes of betel vine at 46.92% variation. Similarly, for the second Principal component (PC2), following traits are dominated at 18.06% viz., leaf area (0.378), width of lobe (0.345), leaf yield (0.198) and inter nodal length (0.395) correlated with PC1.13% Variation is contributing for Principal component third (PC3) including traits which are correlated with leaf length (0.387) and leaf area (0.143). Principal 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. Principal component (PC5) show that leaf width (0.52) and leaf thickness (0.416) contribute to divergence at 6.79%. At 2.76% variation principal component (PC6) is with leaf lamina length (0.434), correlated leaf area (0.49) and depth of sinus (0.465) with PC1 and PC2 At 1.79%, leaf width is considered as important trait for principal component (PC7) as petiole length (0.567) for principal component (PC8) at 1.08%.

Table 6 : Principal component analysis of 13 quantitative traits for 15 betel vine (*Piper betle* L.) genotypes.

	Principal Component (PC)			
Traits	**PC1**	**PC2**	**PC3**	**PC4**
Leaf Length (cm)	**0.244**	0.308	**0.387**	**0.372**
Petiole Length (cm)	-0.073	-0.07	0.065	-0.018
Leaf Width(cm)	-0.508	0.298	0.101	0.059
Leaf area(cm²)	0.013	**0.378**	**0.143**	-0.144
L/B ratio	-0.382	-0.148	0.03	-0.087
Inter nodal Length(cm)	**0.235**	**0.395**	-0.239	-0.335
Lamina length (cm)	0.105	-0.087	-0.113	-0.214
Depth of Sinus(cm)	**0.439**	-0.221	-0.212	**0.57**
Width of lobe (cm)	-0.226	**0.345**	-0.206	**0.514**
Depth/width of lobe ratio	-0.236	-0.523	-0.135	0.064
Leaf thickness(mm)	**0.401**	-0.041	0.044	-0.274
Leaf weight (100 leaves) (g)	-0.057	-0.037	-0.083	0.045
Leaf yield (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
Cumulative%	46.92	64.98	77.98	86.47

Scree plot

A graph which is obtained in between eigen value and principal component numbers which explains the variation percentage which is related to each principal component. First principal component PC1 show 46.92% variation with eigen vale 6.1 which gradually decreases with further principal 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 principal component.

Thus, the prominent traits are congregated in different principal 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, inter-nodal length, depth of sinus and leaf thickness. PC2 relevant to leaf area, width of lobe, inter-nodal 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.

Scatter plot matrix

The two components PC1 and PC2, from quantitative data explain 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 principal components scores (PC score) for all the genotypes

were estimated in five principal components and presented in Table 4.16. 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 genotype is denoted by high value of PC score for that particular genotype in particular Principal component.

Result explains that the "Bhaichigudi" had highest score in PC1 followed by karapaku, Ghanaghatte (Bidhan pan), Assam kapoori/Awni, and Billori local (Check), 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, inter-nodal length and leaf yield traits. PC3 have highest scored genotypes are as follows: Bangla local, Kapoori local(check), Billori local (check), Bali pan, Assam kapoori/Awni exhibited high for leaf length and leaf area.

Similarly, PC4 include Ghanaghatte (Bidhan pan), Assam kapoori/Awni, Maghai, Uttkal sudama, Ramtek Bangla have high score for leaf length, depth of sinus and width of lobe. PC5 include Meetha cum Bangla, Bali pan, Billori local (check), Uttkal sudama, and Bhaichigudi including traits leaf width and leaf thickness. On the basis of top 05 PC score in each Principal components, genotypes are selected are summarized and presented in table 8. High Principal 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 Principal components highlights the characters with maximum variability. So, intensive selection procedures can be designed to bring improvement of traits of betel vine (*Piper betle* L.).

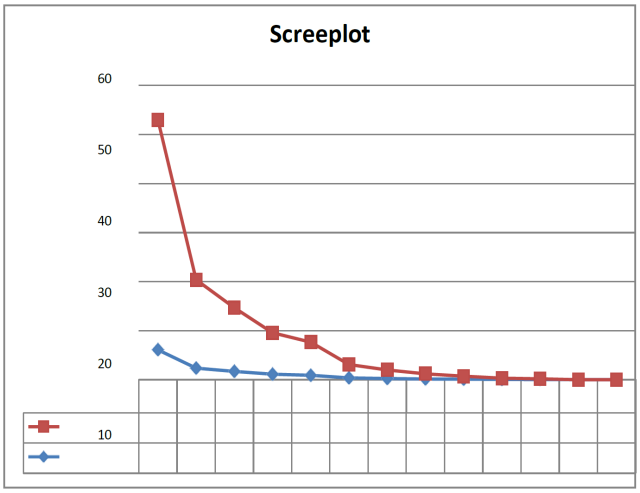

Fig. 1 : Scree plot to explain variation percentage by Principal component and eigen value on quantitative traits of betel vine (*Piper betle* L.)

Table 7: Principal component score of different genotypes of betel vine (*Piper betle* L.) for 13 quantitative traits.

Genotypes	PC1	PC2	PC3	PC4
Bhaichigudi	**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**
Balipan	-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
Billori local (check)	**0.916**	-1.354	**1.367**	0.302
Kapoori local (check)	-0.5	-2.011	**1.976**	0.263

Table 8: list of selected genotypes in each Principal component on the basis of to 05 PC score (13 quantitative traits).

PC1	PC2	PC3	PC4
Bhaichigudi	Assam kapoori (Awni)	Bangla local	Ghanaghatte (Bidhanpan)
Karapaku	Ramtek Meetha pan	Kapoori local (check)	Assam kapoori (Awni)
Ghanaghatte (Bidhan pan)	Ramtek bangla	Billori local (Check)	Maghai pan
Assam kapoori (Awni)	Meetha local	Balipan	Uttkal sudama
Billori local (check)	Meetha-cum bangla	Assam kapoori (Awni)	Ramtek bangla

Nonhierarchical cluster analysis

Cluster analysis is a 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 nonhierarchical Euclidean cluster analysis through software SPAR using Euclidean distance as dissimilarity measure which further classifies 15 betel vine (*Piper betle* L.) genotypes into 05 clusters randomly and they did not follow.

The computed intra and inter cluster distance shows the average distances of cluster members from cluster centroids which is given in table. 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) indicates unidirectional selection led to uniformity with less deviation.

Table 9: Showing 05 clusters of 15 betel vine (*Piper betle* L.) cultivars based on Euclidean distance as dissimilarity.

S. No.	Clusters	Number	Cultivars
1	I	5	Ramtek kapoori, Maghaipan,Uttkal sudama, Ramtek bangla, Ramtek Meetha pan
2	II	3	Bhaichigudi, Ghanaghatte (Bidhanpan), Karapaku
3	III	3	Meetha local, Balipan, Meetha cum bangla
4	IV	1	Assam kapoori(awni)
5	V	3	Bangla local, Billori local(Check), Kapoori local (check)

Table 10: intra (diagonal and bold) and inter cluster distance among 5 clusters of 15 betel vine (*Piper betle* L.).

Cluster	I	II	III	IV	V
I	**1.428**	6.016	2.469	6.273	3.742
II		**1.710**	6.071	5.755	4.813
III			**2.053**	6.261	3.67
IV				**0.132**	6.206
V					**1.620**

Table 11: Mean value of five clusters for 13 quantitative traits which are yield contributing traits in 15 genotypes betel vine (*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**	6.09
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	Inter nodal length (cm)	7.31	6.52	6.97	**8.13**	7.58
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	0.28
11	Leaf thickness(mm)	0.34	0.40	0.34	0.43	0.34
12	Leaf weight(100 leaf)(g)	112.53	164.02	166.07	219.23	95.41
13	Leaf yield(g)	73.98	**108.79**	77.68	91.06	86.93

The mean values of clusters show wide range of variation for all 13 traits under this study. 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), inter nodal 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.

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