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Genetic variability on leaf morpho-anatomical traits in relation to sterility mosaic disease (SMD) resistance in pigeonpea

Patil P.G*[1,2], Byregowda M[1], Vimarsha H.S[1], Keerthi C.M[1], Kundur P.J[1] and Shashidhar H. E[1]

[1]University of Agricultural Sciences, GKVK, Bangalore-560 065, India
[2]Indian Institute of Pulses Research, Kanpur-208 024, India

***Corresponding author: patilbt@gmail.com**

Abstract

Sterility mosaic disease (SMD) is a major biotic constraint in almost all pigeonpea growing areas caused by eriophyid mite transmitted pigeonpea sterility mosaic virus (PPSMV). Direct selection for resistance to SMD is expensive and laborious as it requires dependent of sick plots. Identification of easily assayable and simply inherited morphological traits such as leaf anatomical traits would enable increased efficiency of breeding pigeonpea for SMD resistance. A set of 70 pigeonpea accessions were evaluated for 12 leaf structural features such as leaf thickness (LT), upper epidermal thickness (UEPT), lower epidermal thickness (LEPT), upper cuticle cell wall complex (UCWC), lower cuticle cell wall complex (LCWC), trichome number on upper surface of leaf (TNUS), trichome number on lower surface of leaf (TNLS), trichome length on upper surface of leaf (TLUS) and on lower surface of leaf (TLLS) at experimental plots of Zonal Agricultural Research Station (ZARS), UAS, Bengaluru. The accessions differed significantly for most of the traits except for specific leaf area (SLA) and specific leaf weight (SLW). The accessions were grouped into four clusters, with significant differences in cluster means and variances. Principal component analysis (PCA) showed first three PCs explaining 69.70 % of the total variation and morpho-anatomical traits such as leaf thickness (LT), trichome length on upper (TLUS) and lower (TLLS) surface of leaf were the most important characters for disease incidence. Furthermore, correlation of all the leaf traits in relation to percent incidence (PDI) indicated only TLLS having significant negative correlation (-0.456*) with SMD incidence. While, trichome length also showed higher phenotypic (PCV) and genotypic (GCV) coefficient of variation 34.33 and 34.02, respectively and broad senesce heritability (98.2%) coupled with high genetic advance (69.45). Therefore, breeding for trichome length is very important to impart vector resistance. This may provide broad based resistance to all the isolates of SMD in pigeonpea.

Keywords: Cluster analysis, Leaf morpho-anatomical variability, Pigeonpea, SMD.
Abbreviations: SMD_sterility mosaic disease, PDI_*per cent* disease incidence.

Introduction

Pigeonpea (*Cajanus cajan* L. Millsp.) is a major grain legume crop. Grown extensively in India and other developing countries of Asia, Africa, and Latin America. Globally, it is cultivated in an area of about 4.75 million ha with annual production of 3.68 million tonnes. India accounts for 90 per cent of the global production with area 3.86 million ha and production 2.65 million tonnes (FAOSTAT, 2012). Despite of larger area under pigeonpea in India, the production levels are stagnant due to various biotic and abiotic stresses. Among the biotic stresses, sterility mosaic disease (SMD) is considered as major biotic constraint. This alone accounts for annual economic losses of over US$ 300 million (Kannaiyan et al., 1984). The disease is caused by *Pigeonpea sterility mosaic virus* (PPSMV) (Kumar et al., 2000; Jones et al., 2004) and transmitted by eriophyid mite (*Aceria cajani*) (Kulkarni et al., 2002; Kumar et al., 2003). A comprehensive study on variability for virus and vector revealed plasticity on sterility mosaic pathogen and not on mite vector. The occurrence of five different strains of PPSMV were reported in India (Reddy et al., 1993). Among them, three distinct strains have been well characterized *viz*: Bangalore, Patancheru and Coimbatore. The Patancheru and Coimbatore strains were reported as mild, while Bangalore isolate as the most virulent strain (Kulkarni et al., 2003). Control of this disease by chemical methods was found effective, but is non-economical and non-ecofriendly (Nine et al., 1989). Therefore, breeding SMD resistant varieties is considered to be most effective and economic methods for reducing crop losses. Wild relatives of pigeonpea have been shown to possess high level of resistance to several biotic constraints of pigeonpea (Remanandan, 1981). Therefore, there is need to exploit these genetic resources in disease resistance breeding. Since, no plasticity observed in virus transmitting eriophyid mite vector hence, in-order to impart broad based resistance, combining of vector resistance in addition to host resistance is a most vital. Leaf morphological characters have positive or negative influence on their herbivores (Krips et al., 1999). The type of trichomes and their orientation, density and length have been well correlated with reduced insect damage in several crops (Peter et al., 1995). In pigeonpea five types of trichomes, three glandular (Types A, B, and E) and two non-glandular (Types C and D) types were reported (Romeis et al., 1999). Bisen and Sheldrake (1981) and Navasero and Ramaswamy (1991) studied trichomes on few cultivated pigeonpea (*C. cajan*). While, Romeis et al. (1999) studied trichomes on two wild species (*C. platycarpus* and *C. scarabaeoides*). Till date, no information available for such traits in large collection of pigeonpea. In this study,

comprehensive variability for 12 leaf morpho-anatomical traits including trichomes were studied in 70 pigeonpea genotypes. The traits associated with PDI in extreme resistant and susceptible lines were discussed in relation to vector mediated resistance. Hence, identification of easily assayable and simply inherited morphological traits such as leaf anatomical traits would enable increased efficiency of breeding pigeonpea for SMD resistance. The information generated will be useful for selection of suitable genotypes in broad based resistance breeding for SMD in pigeonpea.

Results

Screening for SMD resistance

Based on SMD screening results, all the pigeonpea genotypes were grouped into different disease response groups. Thirteen genotypes were found resistant with disease score (0-10% PDI), 7 genotypes moderately resistant (10.1-30% PDI) and 50 genotypes were susceptible (30.1-100% PDI) (Supplementary Table 1).

Analysis of variance for leaf morph-anatomical traits

Analysis of variance revealed highly significant mean sum of squares due to genotypes for all the traits except SLA and SLW (Table 1). But, mean squares due to checks were non-significant for all traits except leaf chlorophyll content (LCC), while those due to 'genotypes *vs* checks' were significant for all the traits except SLA and SLW.

Correlation of leaf morpho-anatomical traits with PDI

For correlation study, 23 extreme pigeonpea genotypes with resistant and susceptible response to SMD were selected. Trichome length on lower surface of leaf (TLLS) exhibited significant and negative association with PDI, while other traits showed non-significant associations with PDI (Table 7)

Components of variability, heritability and genetic advance

The estimates of mean values with wider range were noticed for all the traits across 70 genotypes (Table 2). The genetic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were highest (>20 %) for TNUS, TNLS, TLUS, TLLS and LCC. Moderate GCV and PCV (10-20%) were noticed for LT, UEPT, LEPT, UCWC and LCWC. But, SLA and SLW exhibited higher PCV and moderate GCV. High heritability (>60 %) estimates were recorded for all the traits except SLA and SLW. High heritability coupled with high genetic advance as percent mean (>20%) were recorded for most of the traits except SLW. However, low heritability coupled with moderate genetic advance was noticed for SLW. The traits like TNUS, TNLS, TLUS, TLLS and LCC were having highest GCV, PCV, broad sense heritability and genetic advance.

Cluster analysis

Seventy genotypes were grouped into 4 clusters (Table 3 and Fig.2). The cluster 1 with 17 genotypes had only one resistant genotype ICP 15770. Similarly, cluster 2 comprised 18 genotypes, including 3 resistant genotypes ICP 817, BDNP 1 and BDNP 2. Whereas, cluster 3 constituted 14 cultivated pigeonpea and has two resistant genotypes ICP7035 and BRG 3. Cluster 4 constituted 21 genotypes, including 7 resistant genotypes *viz*., ICP 15815, ICP 15853, ICP 15890, BDNP 3, ICP 15681, BNG 1, BNG 2, BNG 3 and BDNP 4. The traits mean differences between clusters were found significant for most of the traits except LCC (Table 4). The trait variances among four clusters were found significant for 7 traits such as TNUS, TNLS, TLUS, TLLS, LEPT, SLA and LCC (Table 5).

Principal component analysis

Twelve principal components (PCs) were extracted, out of which 3 components having eigen value >1. These three PCs contributed 69.70 % of the total variability among pigeonpea genotypes (Table 6). The PC I contributed maximum towards the variability (34.49 %), followed by PC II (21.84 %) and PC III (13.36 %). The traits like TLUS, TLLS and LT showed considerable positive factor loadings on PC I, while TNUS, TNLS and LCC had maximum negative factor loadings. Similarly, for PC 2 traits like UCWC, LCWC, UEPT, LEPT and LCC, and for PC III traits like TLLS and SLW showed maximum positive factor loadings.

Discussion

Although host plant resistance is important for minimizing crop losses due to diseases and insect pests, development of novel breeding strategy by combining vector mediated resistance along with host plant resistance is necessary. In-order to develop new breeding strategies for resistance to disease and insect pests, an understanding of different morphological and biochemical components of resistance is also important (Sharma et al., 2009). In the present study, disease screening results indicated majority (71.42 %) of genotypes were susceptible to Bangalore isolate of SMD. This confirmed the earlier finding that Bangalore isolated of PPSMV is highly virulent strain (Kulkarni et al., 2003). In cultivars group, only two genotypes were found resistant such as ICPL 7035 and BRG 3. But, in wild accessions, 11 genotypes were found resistant *viz*., ICP 15770, ICP 817, ICP 15815, ICP 15853, ICP 15890, Badnapur-1, Badnapur-2, Badnapur-3, BNG 1, BNG 3 and Badnapur-4. Kumar et al. (2005) identified 15 wild SMD resistant accessions after screening 150 wild *Cajanus* accessions, through graft inoculation method confirmed wild accessions did not support mite multiplication and governing vector resistance. Therefore, breeding for vector resistance in addition to host resistance may impart broad-based resistance to SMD. ANOVA and descriptive statistics revealed, significant mean sum of squares and wider range of mean values for ten traits i.e. LT, UEPT, LEPT, UCWC, LCWC, TNUS, TNLS, TLUS, TLLS and LCC reflected presence of higher variability for the traits studied. But, presence of phenotypic variability *per se* is of less significance in crop breeding programmes. However, knowledge on relative contribution of genetic and non-genetic sources for the trait expression is most important in formulating appropriate selection strategies to breed new varieties. Therefore, GCV and PCV are the most important estimates for understanding genetic variability and trait expression among the genotypes. Higher GCV and PCV values with less differences for TNUS, TNLS, TLUS, TLLS and LCC suggested, these characters were mainly under the influence of genetic control and not by the environment. The higher heritability and genetic advance estimates for the same traits also revealed traits are under control of genetic factors and can respond for direct selection in trait improvement.

Table 1. Analysis of variance for leaf morph-anatomical traits in pigeonpea germplasm.

Sources of variation	d.f.	Mean sum of squares											
		[a]LT	[b]UEPT	[c]LEPT	[d]UCWC	[e]LCWC	[f]TNUS	[g]TNLS	[h]TLUS	[i]TLLS	[j]SLA	[k]SLW	[l]LCC
Blocks	7	829.0	15.04	7.55	0.29*	0.05	0.12	0.05	162.8	259.9	0.003	0.92	121.8**
Entries (Genotypes+ Checks)	69	12978.0**	82.90**	62.63**	1.24**	0.88**	1.86**	2.03**	6457.5**	5836.4**	0.003	1.98	103.6**
Genotypes	67	7419.9*	64.98**	52.50**	0.84**	0.58**	1.47**	1.53**	4818.8**	4706.1**	0.003	1.91	101.07**
Checks	01	0.324	1.56	0.04	0.04	0.04	0.25	0.01	152.5	146.4	0.006	5.29	152.5*
Genotypes *vs* Checks	01	398341.8**	1364.3**	804.3**	29.14**	22.03**	30.09**	38.00**	122555.4**	87254.3**	0.007	3.52	227.6**
Error	7	1548.7	6.67	5.09	0.07	0.05	0.08	0.03	76.68	167.5	0.001	1.30	14.12

* Significant at P= 0.05 level, ** Significant at P= 0.01 level

Note* [a]Leaf thickness (µm); [b]Upper epidermal thickness (µm); [c]Lower epidermal thickness (µm); [d]Upper cuticle cell wall complex (µm); [e]Lower cuticle cell wall complex (µm); [f]Trichome number on upper surface of leaf; [g]Trichome number on lower surface of leaf; [h]Trichome length on upper surface (µm); [i]Trichome length on lower surface (µm); [j]Specific leaf area (cm^2/mg); [k]Specific leaf weight (mg/cm^2); [l]Leaf chlorophyll content

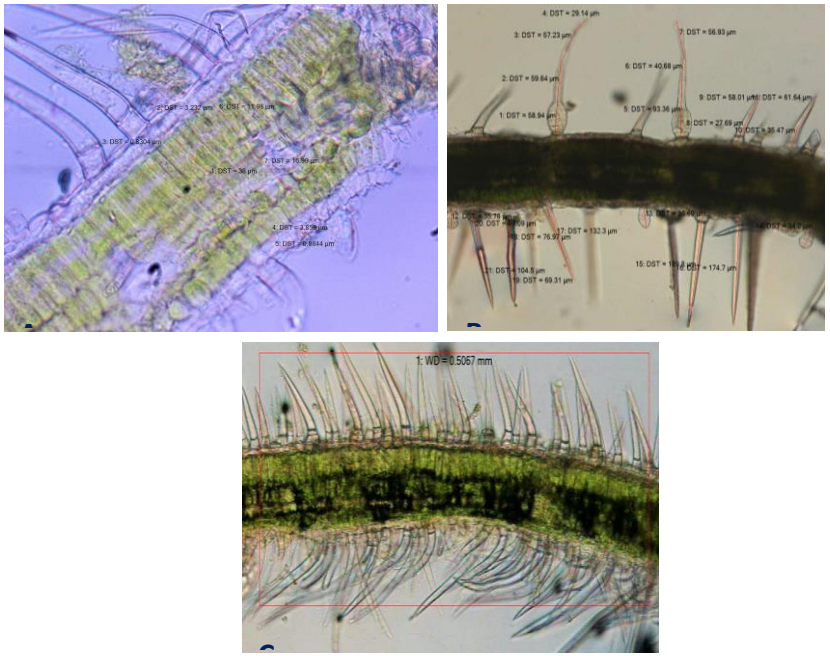

Fig 1. Leaf cross sections showing various morpho-anatomical features A. thickness of leaf, epidermis and cell wall; B. type and length of trichomes; C. number of trichomes on 0.5 mm cross-section length.

Table 2. Descriptive statistics for 12 leaf morpho-anatomical traits in pigeonpea.

Sl. No.	Traits	Mean ± SE	Coefficient of variability		Broad-sense h^2 (%)	GAM (%)
			PCV (%)	GCV (%)		
1	LT	522.90 ± 10.73	15.53	13.62	76.9	24.62
2	UEPT	43.22 ± 0.97	17.51	16.47	88.5	31.93
3	LEPT	38.46 ± 0.86	17.69	16.70	89.1	32.49
4	UCWC	5.88 ± 0.11	14.68	13.97	90.5	27.40
5	LCWC	5.61 ± 0.09	12.76	12.11	90.1	23.69
6	TNUS	3.35 ± 0.14 (12.24±1.10)	34.52	33.35	93.3	66.37
7	TNLS	3.58 ± 0.14 (13.84±1.13)	32.93	32.56	97.7	66.34
8	TLUS	187.25 ± 8.40	34.33	34.02	98.2	69.45
9	TLLS	212.18 ± 8.24	30.09	29.48	95.9	59.50
10	SLA	0.178 ± 0.006	27.36	18.24	44.4	25.05
11	SLW	6.005 ± 0.16	22.66	12.22	29.1	13.58
12	LCC	39.29 ± 1.18	24.32	22.35	84.4	42.30

Note* Mean values mentioned in parenthesis for TNUS, TNLS are actual trichrome numbers observed without transformation.

Identification of genotypes contrasting for traits of economic importance is a prerequisite for developing novel varieties. Cluster analysis helps in grouping of genotypes into different clusters based on number of characters shared and to identify genetically diverse and desirable genotypes. K-mean clustering grouped all the genotypes into 4 clusters. All the cultivated genotypes were grouped into cluster 3 and wild genotypes distributed in different clusters. Seven out of 12 traits such as TNUS, TNLS, TLUS, TLLS, LEPT, SLA and LCC were found significant for differences in trait mean and variance between clusters based on 'F' and Leven's test. These results suggested K-means clustering approach was efficient to minimise within cluster variance and maximise between cluster variance as a result of inclusion of diverse genotypes into different clusters. The wild genotypes distributed into cluster 1, 2 and 4 with wide range of variability for all mean trait values compared to cultivars (cluster 3). This, indicated wild genotypes with wide variability for leaf traits compared to cultivars. Such leaf features may be providing vector resistance in most of the wild species (Kumar et al., 2005). The cluster 2 and 4 constituted maximum number of resistant genotypes.

PCA analysis extracted three PCs explaining 69.70 % of the total variability among the pigeonpea genotypes. This indicated considerable variability existed for traits studied. However, PC 1 contributed maximum variability (34.49 %) with positive factor loading of TLUS, TLLS and LT. This suggested considerable variability in pigeonpea may be due to major contribution of these three traits. Correlation of leaf traits in relation to PDI was analysed in extreme resistant and susceptible genotypes. Significant and negative correlation was observed for TLLS with the PDI, suggested trichome length may be significantly influencing mite population. Shakoor et al. (2010) reported length of leaf hair has negative correlation with tomato phytophagous mites (*Acari*) population. Since, long hairs are not preferred by mites because these produces hindrance in mite movement and impairing mite pest population as they become unable to take their mouth parts at the feeding sites (Pavlova and Egamberdiev, 1990). Afzal and Bashir (2007) in cucumber reported predatory mites (*cunaxidae*) population decreases with increase in hair length (300.54 µm) compared to brinjal (150.94 µm). Based on TLLS, three genotypes BNG 1 (*C. scarabaeoides*), BNG 3 (*C. scarabaeoides*) and BDNP 4 (*C. albicans*) were selected. Since, *C. scarabaeoides* (Pundir and Singh, 1987; Rupakula et al., 2005) and *C. albicans* (Sharma et al., 2003; Mallikarjuna et al., 2007) were easily crossable with cultivated pigeonpea *C. cajan.* These genotypes will be

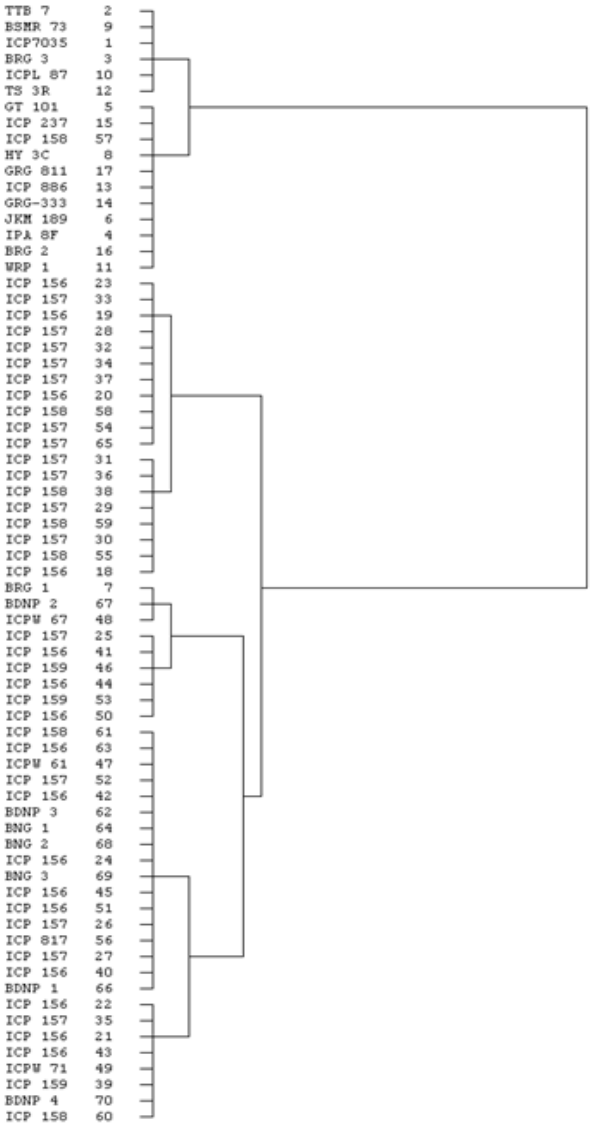

Fig 2. Dendrogram using Ward linkage method.

Table 3. Grouping of genotypes into different cluster based on K-mean clustering.

Clusters	Number of genotypes	Name of genotypes
Cluster 1	17	ICP 15685, ICP 15687, ICP 15688, ICP 15692, ICP 15711, ICP 15718, ICP 15722, ICP 15724, ICP 15725, ICP 15727, ICP15739, ICP 15748, ICP 15882, ICP 15770, ICP 15855, ICP 15857 and ICP 15799
Cluster 2	18	ICP 15683, ICP 15698, ICP 15701, ICP 15703, ICP 15710, ICP 15719, ICP 15661, ICP 15662, ICP 15666, ICP 15921, ICPW 67, ICP 15621, ICP 15900, ICP 15817, ICP 817, BDNP 1, BDNP 2 and BRG 1
Cluster 3	14	ICP7035, TTB 7, BRG 3, IPA 8F, JKM 189, HY 3C, BSMR 736, ICPL 87119, WRP 1, TS 3R, ICP 8863, GRG 333, BRG 2 and GRG 811
Cluster 4	21	ICP 2376, GT 101, ICP 15689, ICP 15731, ICP 15922, ICP 15663, ICP 15664, ICP 15667, ICPW 61, ICPW 71, ICP 15642, ICP 15761, ICP 15815, ICP 15853, ICP 15890, BDNP 3, ICP 15681, BNG 1, BNG 2, BNG 3 and BDNP 4

Table 4. Estimates of leaf morpho-anatomical trait means for genotypes belonging to different clusters.

Sl. No	Traits	Means of clusters				'F' Statistic	Probability
		C1 (17)$^{+}$	C2 (18)$^{+}$	C3 (14)$^{+}$	C4 (21)$^{+}$		
1	LT	559.06	613.97	393.63	501.77	64.25	0
2	UEPT	41.21	48.59	37.71	43.92	6.37	0.001
3	LEPT	35.91	41.28	33.85	41.21	5.33	0.002
4	UECWT	6.13	6.02	5.23	6	3.15	0.031
5	LECWT	5.77	5.7	5.04	5.8	3.53	0.019
6	TNUS*	3.18 (9.72)	2.51 (6.40)	5.01 (24.92)	3.12 (10.83)	23.21	0
7	TNLS*	3.53 (12.00)	2.63 (7.22)	5.36 (28.42)	3.25 (11.29)	30.62	0
8	TLUS	265.14	194.99	88.94	183.12	52.05	0
9	TLLS	304.02	185.98	125.86	217.83	84.73	0
10	SLA	0.14	0.2	0.16	0.2	10.47	0
11	SLW	7.18	5.3	6.16	5.56	8.80	0
12	LCC	35.36	42.29	41.19	38.63	1.67	0.181

Note $^{+}$ Values in parenthesis indicates number of individuals in each cluster.
*****Indicates mean values for each clusters mentioned in parenthesis for TNUS and TNLS are actual values observed without transformation.

Table 5. Estimates of leaf morpho-anatomical trait variances for genotypes belonging to different clusters.

Sl. No	Traits	Variance of clusters				'F' Statistic	Probability
		C1 (17)$^{+}$	C2 (18)$^{+}$	C3 (14)$^{+}$	C4 (21)$^{+}$		
1	LT	1882.03	2218.67	2872.35	1843.06	0.69	0.56
2	UEPT	22.76	102.54	35.15	48.50	1.40	0.24
3	LEPT	38.54	47.74	13.88	65.92	2.51	0.06
4	UCWC	0.57	1.30	0.59	0.72	0.93	0.42
5	LCWC	0.63	0.42	0.46	0.66	0.41	0.74
6	TNUS*	0.11 (4.16)	0.62 (21.94)	0.45 (52.28)	1.59 (88.87)	7.7	0
7	TNLS*	0.07 (3.41)	0.92 (40.97)	0.13 (15.81)	1.33 (64.59)	10.57	0
8	TLUS	1845.32	2639.52	45.45	1319.99	5.68	0.002
9	TLLS	847.21	1479.18	248.53	1287.90	2.90	0.041
10	SLA	0.002	0.001	0.002	0.001	8.04	0
11	SLW	1.29	0.88	1.52	1.84	0.61	0.61
12	LCC	47.05	141.94	32.41	137.70	5.41	0.002

Note $^{+}$ Values in parenthesis indicates number of individuals in each cluster.
***** Indicates variance for each clusters mentioned in parenthesis for TNUS and TNLS are actual values observed without transformation.

Table 6. Principle component analysis based on leaf morpho anatomical traits of pigeonpea.

	PC I	PC II	PC III
Eigen value	4.139	2.621	1.604
% of total variance	34.49	21.84	13.36
Cumulative variance %	34.49	56.33	69.70
Factor loadings by various traits			
Variable	PC I	PC II	PC III
LT	0.220	0.006	-0.040
UEPT	-0.045	0.277	-0.078
LEPT	-0.054	0.287	-0.046
UECWT	-0.053	0.337	0.103
LECWT	-0.089	0.390	0.171
TNUS	-0.246	0.016	0.107
TNLS	-0.243	0.010	0.133
TLUS	0.305	-0.100	0.074
TLLS	0.237	-0.038	0.212
SLA	0.041	-0.075	-0.379
SLW	-0.004	0.053	0.372
LCC	-0.159	0.172	-0.032

Table 7. Estimates of correlation coefficients of leaf morph anatomical traits with the PDI.

Traits	r	Pr>r
LT	-0.17	>0.05
UEPT	0.073	>0.05
LEPT	0.044	>0.05
UCWC	0.159	>0.05
LCWC	-0.031	>0.05
TNUS	0.068	>0.05
TNLS	0.086	>0.05
TLUS	-0.251	>0.05
TLLS	-.456	<0.05
SLA	0.328	>0.05
SLW	-0.341	>0.05
LCC	0.367	>0.05

useful in broad-based resistance breeding against three isolates of PPSMV.

Materials and Methods

Plant materials

Plant material constituted 70 pigeonpea genotypes, belonging to 3 genus and 12 different species *viz., Cajanus cajan, C. volublis, C. scarabaeoides, C. albicans, C.lineatus, C. sericeus, C. viscida, C. platycarpus, Ryncosia bracteata, R. rothi, R. minima* and *Flemingia macrophylla* (Supplementary Table 1). The seeds of these genotypes were obtained from Indian Institute of Pulses Research, Kanpur and All Indian Coordinated Research Project (AICRP) on pigeonpea, UAS, Bangalore.

Field screening for SMD resistance

Fifteen seedlings for the each entry were raised in plastic covers and evaluated for SMD reaction during 2013 rainy season at experimental plots of ZARS, UAS, Bangalore. For inoculation of virus, leaf stapling method was followed as described by Nene et al. (1981). Fifteen days old young seedlings were selected to staple SMD infected leaves carrying sufficient number of mites, as confirmed from microscopic observations. Disease incidence was scored visually by counting healthy and diseased plants for each genotype at 15 days intervals up to 60 days after first inoculation. Percent disease incidence (PDI) was calculated by using formula as described by Singh et al. (2003).

$$\%\,\text{Disease incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants screened}} \times 100$$

Finally, all genotypes were classified into resistant (0-10% of plants infected), moderately resistant (10.1-30%) and susceptible (30.1-100%) groups (Supplementary Table 1).

Experimental design

Sixty eight pigeonpea genotypes and two resistant genotypes (ICP 7035 and TTB 7) designated as checks were sown during 2013 rainy season in augmented design (Federer, 1956), with 8 compact blocks. The experiment was laid out at department of plant biotechnology, UAS, Bangalore. Experimental location was located at 12˚ 58' north latitude and 77˚35' east longitude; 930 m above mean sea level. Each genotype along with check was planted in a single row of 4 m length with a spacing of 0.9 m between rows and 0.6 m between plants within a row. Recommended agronomic practices were followed to raise healthy crop.

Leaf morpho-anatomical study

Three month old healthy plants were selected for leaf morpho-anatomical study. The second top most leaf from two randomly selected healthy plants for each entry and check were selected. For leaf cross sectioning, free-hand sectioning technique was followed using double-sided razor blade (Ruzin, 1999). The fine vertical leaf sections obtained after each cutting were bleached with water and sodium hypochlorite 5% mixture (1:1) for 15 min and rinsed with sterile distilled water for 10 min on glass slide. Finally, mounted into 1:1 mixture of water and glycerine for microscopic observation. The images and measurements were taken after observing sections in stereo binocular microscope at 10X and 40X magnification attached with Progress@capturePro 2.8.8-JENOPTIK/Optical system (Fig. 1). Microscopic observations on leaf thickness (LT), upper epidermal thickness (UEPT), lower epidermal thickness (LEPT), upper cuticle cell wall complex (UCWC), lower cuticle cell wall complex (LCWC), trichome number on upper surface of leaf (TNUS), trichome number on lower surface of leaf (TNLS), C and D type of trichome length on upper surface of leaf (TLUS) and on lower surface of leaf (TLLS) were recorded. Apart from this, other important leaf parameters like specific leaf area (SLA), specific leaf weight (SLW) and leaf chlorophyll content (LCC) were also recorded from second top-most leaf in four of the each representative genotypes. Leaf area was measured by millimetre graph paper method (Sestak et al., 1971). The same leaves were oven dried at 70 °C for 48 hr to determine leaf dry weight. SLA was calculated by taking ratio of leaf area/leaf dry weight and expressed as cm^2/mg (Kvet et al., 1971). SLW was calculated by dividing leaf dry weight/ leaf area and expressed as mg/cm^2 (Pearce et al., 1968). LCC was measured using SPAD chlorophyll meter (Minolta SPAD-502 meter, Tokyo, Japan).

Statistical analysis

Data were recorded and curated with appropriate transformations such as arcsine and square root transformation for traits like percent disease incidence (PDI) and trichome numbers, respectively. Average mean values of all 12 leaf morpho-anatomical traits were subjected to analysis of variance (ANOVA) using statistical software Windostat version 8.5. The basic descriptive statistics, Pearson's correlation, cluster analysis based on K-means clustering (Mac Queen, 1967) and dendrogram using Ward linkage method, 'F' and Levene's (Levene, 1960) significance test at P=0.05 for difference among the cluster means and variances for twelve traits and principal component analysis (PCA) were performed using statistical packages SPSS version 16.

Conclusion

Exploitation of natural genetic variability will helps in breeding new varieties with desired traits. Evaluation of germplasm provides information about the genotypes with desired combination of traits. Since, the main objective of this study was to identify pigeonpea genotypes with vector resistance traits based on leaf morpho-anatomical traits. TLLS with significant and negative correlation with the PDI, indicated breeding for this trait may enhance mite vector resistance. This study represents first effort for preliminary understanding of comprehensive variability of leaf morpho-anatomical traits in large collection of pigeonpea in relation to SMD resistance.

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