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DISEASE CONTROL

Multi-environment field testing to identify broad, stable resistance to sterility mosaic disease of pigeonpea

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Abstract Sterility mosaic disease (SMD) caused by *Pigeonpea sterility mosaic virus* and vectored by the eriophyid mite is a serious disease of pigeonpea in almost all pigeonpea-growing areas. Managing the disease with chemicals such as acaricides is very difficult, non-eco-friendly and costly; hence, host plant resistance is the best strategy implemented to manage this disease. In this context, 28 pigeonpea genotypes identified as resistant from preliminary screening of 976 pigeonpea accessions were evaluated in field at eight different agro-ecological locations in India for the stability of their resistance against SMD during 2007/2008 and 2008/2009. Genotype plus genotype × environment (GGE) analysis partitioned main effects into genotype, environments and G × E interactions and showed significant effects ($P < 0.001$) for SMD percentage incidence. Environment variance had the greatest effect (76.68 %), indicating the maximum variation in the disease due to the environment. At Bangalore, Dholi and Rahuri locations, all genotypes were susceptible to SMD with mean disease incidence of 71.1, 50.4 and 32.6 % respectively. However, most of the genotypes were resistant at four locations, Akola, Badnapur, Patancheru, and Vamban, and moderately resistant at Coimbatore. The GGE biplot analysis explained about 67.26 % of total variation and identified four genotypes (ICPLs 20094, 20106, 20098, 20115) as the most stable and resistant to SMD. Three genotypes (ICPLs 20096, 20107, 20110) showed moderately stable performance against SMD. These genotypes should be included in pigeonpea breeding programs as additional sources of resistance to SMD.

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Introduction

Pigeonpea [*Cajanus cajan* (L.) Millisp.], a major legume crop, supplies dietary protein requirements to large populations of people living in the semi-arid tropics of the Indian subcontinent. Although India leads the world in area and production of pigeonpea, its productivity is lower than the world's average (FAOSTAT 2013). This deficit can be attributed to various abiotic stresses (e.g., drought, salinity and water-logging) and biotic factors (e.g., wilt, sterility mosaic, phytophthora blight and pod borers) encountered by the crop at different growth stages. Among the diseases, sterility mosaic disease (SMD), initially discovered in Pusa in 1931 (Mitra 1931), is a major constraint throughout the world. This disease occurs with regularity, with an annual incidence between 10 and 100 % (Nene et al. 1981). Estimated losses caused by SMD were over US$ 300 million (Kannaiyan et al. 1984). This disease is characterized by sterility (complete loss of flower production), mosaic pattern on leaves, and excessive vegetative growth of the plant, severe stunting and reduced leaf size (Fig. 1) (Pande et al. 2012). The disease is caused by *Pigeonpea sterility mosaic virus* (PPSMV) (Jones et al. 2004; Kumar et al. 2000), a putative virus transmitted in a semi-persistent manner by the eriophyid mite *Aceria cajani* (Kulkarni et al. 2002; Seth 1962). The PPSMV was hypothesized as belonging to the same group of other mite-borne viruses having double-membraned bodies (Kumar et al. 2003). Recently, based on the molecular, morphological and epidemiological features, PPSMV was listed as the seventh species of emaraviruses (Elbeaino et al. 2014). It consists

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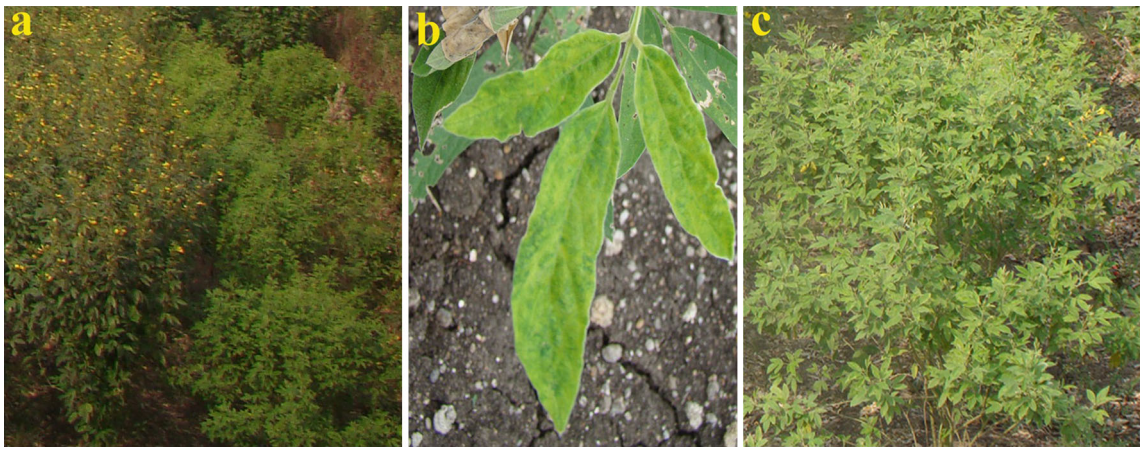

Fig. 1 Symptoms of sterility mosaic disease (SMD) on infected pigeonpea. **a** No flowers produced (sterility), **b** leaves with mosaic pattern and **c** excessive vegetative growth

of five large single-stranded RNA genomes of negative orientation (RNA 1, RNA 2, RNA 3, RNA 4 and RNA 5) with a length of ca. 7022, 2223, 1442, 1563 and 1801 nts (Elbeaino et al. 2014).

Pigeonpea is grown with marginal input; hence, although chemical management of disease is effective it is not economical. The most reliable option to manage disease is the cultivation of resistant varieties. Developing resistant varieties of pigeonpea, however, is complicated by the genetic plasticity of the pathogen, which is affected by location-specific environments (Amin et al. 1993; Nene et al. 1989; Sharma and Pande 2011; Sharma et al. 2012b). SMD incidence also differs from plant to plant due to variability in the pathogen (Kulkarni et al. 2003; Nene et al. 1989; Reddy et al. 1993); one report on this variability revealed five strains of PPSMV in India (Reddy et al. 1993). Among these five, three distinct strains have been characterised—Bangalore, Patancheru and Coimbatore. The Patancheru and Coimbatore strains are mild strains, and the Bangalore strains are the most severe (Kulkarni et al. 2003).

Adequate understanding of the genotype × environment (G × E) interaction of any pathosystem is required in order to maximise the use of host plant resistance to manage a disease. A GGE biplot is a method of graphical analysis of multi-environment data, displaying the main genotype effect (G) and the genotype × environment (G × E) interaction in multi-environment tests. GGE biplot analysis has also enabled the selection of more stable genotypes for crops such as chickpea against Fusarium wilt and Ascochyta blight diseases (Pande et al. 2013; Sharma et al. 2012a), cassava against cassava mosaic disease (Egesi et al. 2007), wheat against powdery mildew (Lillemo et al. 2010), faba bean against Ascochyta blight (Rubiales et al. 2012), mungbean against multiple diseases (Kaur et al. 2011), and maize against downy mildew (Rashid et al. 2013). GGE biplot analysis has been widely used in recent years to determine the stability of resistance through multi-location trials and thus identify stably resistant genotypes (Egesi et al. 2009; Sharma et al. 2012a).

Genotypic stability has often been used to describe how consistently a genotype performs against different pathogen variants across environments. Understanding the effect of changing environmental conditions on the resistance of crops to a particular disease will facilitate the identification of germplasm that is stable across environments and enhance the efficiency of breeding and use of resistant cultivars to manage disease. The present investigation was thus undertaken with three objectives: (1) to identify stable sources of SMD resistance in pigeonpea germplasm accessions and breeding lines, (2) to validate the stability of resistance through multi-year and multi-location field experiments in India, and (3) to identify strain-specific resistant sources for different isolates of SMD.

Materials and methods

Plant material and locations

A collection of 976 pigeonpea genotypes including germplasm accessions and breeding lines was evaluated for SMD resistance under artificial epiphytotic conditions in a disease nursery at ICRISAT, Patancheru during 2003/2004. Based on this evaluation, a set of 166 pigeonpea genotypes with high levels of SMD resistance was selected and again evaluated for 3 years during 2004/2005–2006/2007 in a disease nursery at Patancheru. Finally, a Pigeonpea Sterility Mosaic Disease Nursery (PSMDN) of the 28 genotypes with the most resistance against SMD was established for

multi-environment evaluation. The pedigree, days to 50 % flowering and maturity of the 28 selected genotypes are summarized in Table 1.

The nursery plants were evaluated for SMD resistance at eight locations (Akola, Badnapur, Bangalore, Coimbatore, Dholi, Patancheru, Rahuri and Vamban) in India during two crop seasons (2007/2008 and 2008/2009). These sites encompassed a wide diversity of agro-climatic zones, with latitudes from 10°25′ at Vamban to 25°59′ at Dholi, longitudes from 74°42′ at Rahuri to 85°35′ at Dholi, and altitudes from 52.2 m of Dholi to 920 m of Bangalore. The tested environments (total 16 environments during two cropping seasons) are detailed in Table 2.

Field trials

The 4-year screening and selection process (2003/2004–2006/2007) included preliminary screening to identify genotypes with resistance to SMD at ICRISAT, Patancheru. The PSMDN was established and screened at eight locations for 2 years (2007/2008 and 2008/2009). The scheme of this process is described next.

Identification of genotypes for multi-environment screening

As a preliminary screen, 976 genotypes were evaluated in a randomized complete block design (RCBD) with two

Table 1 Pedigrees and agronomic traits of the pigeonpea genotypes used in the pigeonpea sterility mosaic disease nursery during 2007/2008 and 2008/2009

Serial no.	Genotype	Type	Pedigree	Days to 50 % flowering	Days to maturity
1	ICP 9174	Germplasm	ICRISAT-COOP-N/A	161	252
2	ICP 12749	Germplasm	ICP 7065 × 7035-F4B-S218X	138	218
3	ICP 14819	Germplasm	ICRISAT-COOP-0624	158	210
4	ICPL 20093	Breeding line	ICPX 900148-7[a]	127	183
5	ICPL 20094	Breeding line	ICPX 900152-[a]	129	185
6	ICPL 20096	Breeding line	ICPX 900146-[a]	127	185
7	ICPL 20097	Breeding line	ICPX 900146-[a]	131	187
8	ICPL 20098	Breeding line	ICPX 900146-[a]	128	184
9	ICPL 20099	Breeding line	ICPX 900155-[a]	127	184
10	ICPL 20100	Breeding line	ICPX 900148-[a]	127	183
11	ICPL 20101	Breeding line	ICPX 900147-[a]	128	185
12	ICPL 20102	Breeding line	ICPX 900148-9[a]	126	181
13	ICPL 20103	Breeding line	ICPX 900150-[a]	131	186
14	ICPL 20106	Breeding line	IPH487 Inbred-12[a]	127	182
15	ICPL 20107	Breeding line	IPH487 Inbred-2[a]	130	185
16	ICPL 20109	Breeding line	IPH487 Inbred-9[a]	131	187
17	ICPL 20110	Breeding line	IPH487 Inbred-7[a]	130	186
18	ICPL 20113	Breeding line	IPH487 Inbred-1[a]	129	185
19	ICPL 20114	Breeding line	IPH487 Inbred-11[a]	129	184
20	ICPL 20115	Breeding line	IPH487 Inbred-14[a]	125	181
21	ICPL 20116	Breeding line	IPH487 Inbred-4[a]	125	181
22	ICPL 20120	Breeding line	IPH487 Inbred-17[a]	131	186
23	ICPL 20126	Breeding line	GUPH 1126 Inbred-3[a]	128	183
24	ICPL 20128	Breeding line	GUPH 1126 Inbred-11[a]	126	182
25	ICPL 20129	Breeding line	GUPH 1126 Inbred-10[a]	131	185
26	ICPL 20132	Breeding line	GUPH 1126 Inbred-1[a]	129	184
27	ICPL 20134	Breeding line	GUPH 1126 Inbred-7[a]	129	183
28	KPBR 80-2-4	Germplasm	Gene bank accession	165	215
29	ICP 8863[b]	Germplasm	ICRISAT-COOP-0436	–	–
30	Local SM susceptible Check	–	–	–	–

[a] Selfed population

[b] Susceptible check

Table 2 Test environments for evaluating pigeonpea cultivars against sterility mosaic disease

Location	State	Environment[a]	Latitude (N)	Longitude (E)	Altitude (m)
Akola	Maharashtra	Ak-07, Ak-08	20°42′	76°59′	282
Badnapur	Maharashtra	Bd-07, Bd-08	19°23′	75°43′	582
Bangalore	Karnataka	Bn-07, Bn-08	12°58′	77°35′	920
Coimbatore	Tamil Nadu	Co-07, Co-08	10°59′	76°57′	411
Dholi	Bihar	Dh-07, Dh-08	25°59′	85°35′	52.2
Patancheru	Andhra Pradesh	Pa-07, Pa-08	17°31′	78°15′	545
Rahuri	Maharashtra	Ra-07, Ra-08	19°23′	74°42′	511
Vamban	Tamil Nadu	Va-07, Va-08	10°25′	76°49′	90.0

[a] Environment is denoted as first two letters of each location followed by year of screening

replications. Each genotype was sown in a 4 m long row with rows 75 cm apart, plant to plant spacing of 10 cm and susceptible genotype ICP 8863 was sown after every 4 test rows. To inoculate each plant at the third leaf stage (Nene et al. 1981), we detached an SMD-infected leaflet, folded it around the edge of a primary leaf of the test seedling so that the abaxial surface of the leaflet was in contact with the adaxial and abaxial surfaces of the primary leaf of the test seedling, then stapled it in place (Fig. 2). Infected leaves were checked for mite presence before inoculation using a light microscope. Each year, any accessions that were resistance (SMD incidence <10 %) in the previous season of screening were revalidated in a disease nursery at Patancheru.

Multi-environment evaluation

The PSMDN consisted of 28 genotypes (4 germplasm accessions and 24 breeding lines) with days to maturity ranging from 181 to 252 days. Two susceptible checks, ICP 8863 and a local susceptible cultivar for each location were included to evaluate the disease. Seed stocks of test genotypes were increased and maintained at ICRISAT, Patancheru and subsampled to supply the collaborators at eight locations in the major pigeonpea-growing areas. These locations had sufficient inoculum maintained during the off-season to screen material against SMD. Genotypes were evaluated in the field at all locations during the 2007/2008 and 2008/2009 crop seasons. The nursery was laid out in a RCBD with two replications with row and plant spacing as mentioned already. At the time of inoculation, each plant was inoculated at the third-leaf stage using the leaf stapling technique already described. To increase the disease pressure and for comparison, the local susceptible check was planted after 4 test rows. Disease pressure in nurseries was considered adequate for sterility mosaic evaluation when a susceptible check had >80 % disease incidence.

Data collection and analysis

Data on SMD incidence were recorded from each replication at seedling and flowering stage (i.e., 30 and 150 days after inoculation). Percentage disease incidence was calculated as:

$$\%\,\text{SMD incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

Fig. 2 Field screening of pigeonpea for sterility mosaic disease. **a** Leaf stapling technique for inoculation and **b** field plot with pigeonpea material

Depending on the SMD incidence, the test genotypes were categorized as resistant (≤10.0 % incidence), moderately resistant (10.1–20.0 % incidence), susceptible (20.1–40.0 % incidence) and highly susceptible (>40 % incidence).

To test for any G × E interaction, data across 16 environments and 29 genotypes were first arc-sine transformed to attain normality of residuals, then an analysis of variance was carried out using the mixed model procedure of GenStat software, 14th edition (VSN International, Hemel Hempstead, UK) to model environment error variances. Genotypes, environments and G × E interactions were declared significant at 5 % ($P < 0.05$) level.

Stability of genotypes across environments was determined numerically and graphically using a GGE biplot analysis (Yan 2001), a method of graphical analysis of multi-environment data. It displays the main genotype effect (G) and the genotype × environment (G × E) interaction of multi-environment tests. The following GGE model was used to determine the stability of genotypes across 16 environments:

$$Yij - \mu - \beta j = \sum_{i=1}^{k} \lambda l \xi i l \eta l j + \varepsilon ij,$$

where Yij is the mean genotype incidence i in environment j, μ is the grand mean, βj is the environment j main effect, n is the singular value, λ and ζ are the singular vectors for genotype and environment for $n = 1, 2,\ldots$, respectively, and εij is the residual effect. GGE biplots were generated using the first two symmetrically scaled principal components (PC) for an average tester coordinate and polygon view biplots. To visualize correlations between locations, we generated a vector view biplot by plotting the first two components (PC1 and PC2) derived from single value decomposition of the environment centered data. Genotypes and environments were displayed in the same plot. Each genotype and environment was defined by their respective scores on the two PCs. Angles between the various environment vectors were used to judge the correlation between the environments (Yan and Kang 2003). The length of the vector represents the genotypic variability in the respective environment. To assess the stability of genotypes, we plotted the average environment coordinate by taking the mean of the PC1 and PC2 scores for environments. A performance line passing through the origin of the biplot was used to determine the mean performance of the genotype. The arrow on the performance line represents a decrease in stability of the genotype, i.e., higher susceptibility (Yan and Falk 2002).

To identify the relationship between environments, Spearman's rank correlation was calculated by comparing disease incidence of genotypes across locations.

Results

Preliminary field screening

The preliminary screening of the 976 pigeonpea genotypes in the disease nursery during 2003/2004 at Patancheru, India revealed a broad range of response to SMD among the tested material and allowed the selection of 166 promising genotypes (≤10 % incidence) for further confirmation (data not shown). Of these 166 genotypes, 28 highly resistant genotypes were selected for the nursery to determine the stability of resistance across 8 locations over 2 years (2007/2008 and 2008/2009) in India.

Multi-environment evaluation of PSMDN

The SMD incidence in 28 pigeonpea genotypes varied greatly between 8 locations and 2 years (Table 3). The variability in disease incidence is also shown by the frequency distributions for the four levels of genotype response in each location over the 2 years suggesting a genotype × environment interaction (Fig. 3). A subsequent analysis of variance of SMD incidence showed that the effect of genotype, environment and the genotype × environment interactions were significant ($P < 0.001$) (Table 4). The environment effect contributed the most (76.68 %) to total variation; the genotype and genotype × environment interaction contributed 9.62 and 13.69 %, respectively. Mean SMD incidence of the local susceptible check ranged between 42.8 and 100 % at the test locations. Highest (mean for 29 genotypes) SMD incidence (71.1 %) over 2 years was recorded at Bangalore followed by Dholi (50.4 %) and Rahuri (32.6 %), while incidence was lowest (4.3 %) at Patancheru followed by Akola (7.2 %) and Vamban (9.7 %) (Table 3).

Many genotypes differed in their individual reactions across locations (Table 3). Genotypes ICPL 20094, ICPL 20106, ICPL 20098 and ICPL 20115 were moderately resistant with a mean incidence of 18.1, 18.2, 19.3 and 19.9 %, respectively, although the incidence of SMD on the genotype varied depending on the location (Table 3). Although 27 genotypes at Patancheru, 19 at Vamban, 26 at Akola and 16 at Badnapur were resistant (<10 % incidence), no genotypes were resistant at Bangalore, Rahuri or Dholi (Fig. 3, Table 3).

A significant positive correlation (disease incidence) was found in some of the test environments using Spearman's correlation analysis ($P < 0.0001$). For instance, a positive correlation was found for the levels of SMD incidence between locations Ak-08 and Bd-07, however, the correlation was negative for other locations such as Co-08 and Ra-08 (Table 5).

Table 3 Sterility mosaic disease incidence in 30 genotypes of pigeonpea at eight locations during 2007/2008 and 2008/2009

Entry	Genotype	Sterility mosaic disease incidence (%)[a]								
		Akola	Badnapur	Bangalore	Coimbatore	Dholi	Patancheru	Rahuri	Vamban	Mean
1	ICP 9174	11.8	9.5	84.7	21.4	49.9	0.0	39.9	4.3	27.7
2	ICP 12749	6.8	2.3	56.0	17.9	64.5	0.0	30.7	6.6	23.1
3	ICP 14819	2.3	2.8	76.4	19.4	51.5	0.0	42.1	0.0	24.3
4	ICPL 20093	4.1	22.6	57.2	23.1	54.5	3.8	18.1	16.6	25.0
5	ICPL 20094	2.8	4.5	70.2	28.4	19.0	0.0	19.0	1.2	18.1
6	ICPL 20096	5.3	6.0	60.7	30.9	42.1	0.0	13.9	4.3	20.4
7	ICPL 20097	5.1	16.1	93.0	15.5	47.5	0.0	23.1	4.1	25.6
8	ICPL 20098	5.3	4.7	66.0	9.0	44.5	0.0	22.1	2.8	19.3
9	ICPL 20099	5.3	13.0	55.5	17.3	51.1	0.0	48.5	5.2	24.5
10	ICPL 20100	5.5	17.9	72.5	18.7	58.4	0.0	39.6	15.3	28.5
11	ICPL 20101	3.8	5.7	82.0	14.9	38.5	2.2	33.1	16.8	24.6
12	ICPL 20102	2.2	7.7	86.4	19.7	58.9	3.5	23.4	5.0	25.9
13	ICPL 20103	4.0	7.9	83.5	6.2	55.9	3.2	20.5	15.2	24.6
14	ICPL 20106	0.0	3.7	74.2	24.2	20.6	0.0	11.5	11.7	18.2
15	ICPL 20107	8.8	10.8	74.8	19.1	27.0	0.0	24.5	5.3	21.3
16	ICPL 20109	3.3	16.8	66.8	20.9	49.8	0.7	29.0	4.8	24.0
17	ICPL 20110	3.8	8.2	70.3	25.6	30.9	0.0	33.5	12.8	23.1
18	ICPL 20113	7.1	14.9	71.3	27.9	52.3	0.0	28.0	6.3	26.0
19	ICPL 20114	4.8	26.5	84.3	21.8	56.0	4.4	27.4	0.0	28.1
20	ICPL 20115	1.5	6.1	62.5	27.4	32.6	0.0	24.5	4.4	19.9
21	ICPL 20116	5.8	8.0	54.9	19.0	59.0	0.7	33.6	6.6	23.5
22	ICPL 20120	10.3	7.7	73.8	16.2	56.0	0.7	32.1	11.0	26.0
23	ICPL 20126	2.9	6.0	71.6	15.8	64.8	0.0	42.9	5.8	26.2
24	ICPL 20128	4.4	10.1	60.7	18.5	52.0	0.0	29.9	8.5	23.0
25	ICPL 20129	8.8	11.2	61.3	24.9	56.9	0.0	32.9	3.3	24.9
26	ICPL 20132	5.0	22.3	63.7	13.5	64.8	12.3	47.9	13.3	30.3
27	ICPL 20134	8.5	28.5	71.2	14.4	57.9	2.5	47.9	13.6	30.6
28	KPBR 80-2-4	0.0	0.0	55.5	19.5	46.1	0.8	42.5	0.0	20.5
29	ICP 8863[b]	70.3	74.3	100.0	15.0	100.0	90.3	82.0	76.5	76.0
30	Local SM susceptible check	72.0	85.8	88.8	54.4	100.0	87.3	100.0	42.8	78.9
	Mean	7.2	12.9	71.1	19.5	50.4	4.3	32.6	9.7	

[a] Percentage disease incidence based on the mean of two replications for 2 years

[b] Susceptible check

Stability of genotypes and environment

According to the GGE biplot analysis, 67.26 % of the total variation was explained by principal components PC1 (SMD incidence) and PC2 (resistance stability), which accounted for 54.41 and 12.85 % of the total variation, respectively. Environment Dh-07, Bn-08 and Pa-07 had longer vectors than other environments, indicating that these locations were most discriminating for genetic differentiation of genotypes. Locations Bn-07 and Co-07, with the shortest vectors, were the least discriminatory. Negative correlations were found for some environments (e.g., Bn-08 and Dh-07, Co-08 and Bn-08 as indicated by obtuse angles between them. Dh-07, Ra-07 and Ra-08 had the higher PC1 scores and lower PC2 scores, which indicated greater discriminating ability of these environments (Fig. 4).

The polygon was drawn on genotype groups in that biplot that were located farthest from the origin (Fig. 5). Genotypes located at the vertices of polygon contributed the most to the interaction, i.e., those with the highest or the lowest disease incidence. Three groups of mega-environments were formed in this biplot, indicating the variability of the environments. Co-08 and Bn-07 formed one group, Co-07 formed an individual group, and the remaining environments formed one mega-environment.

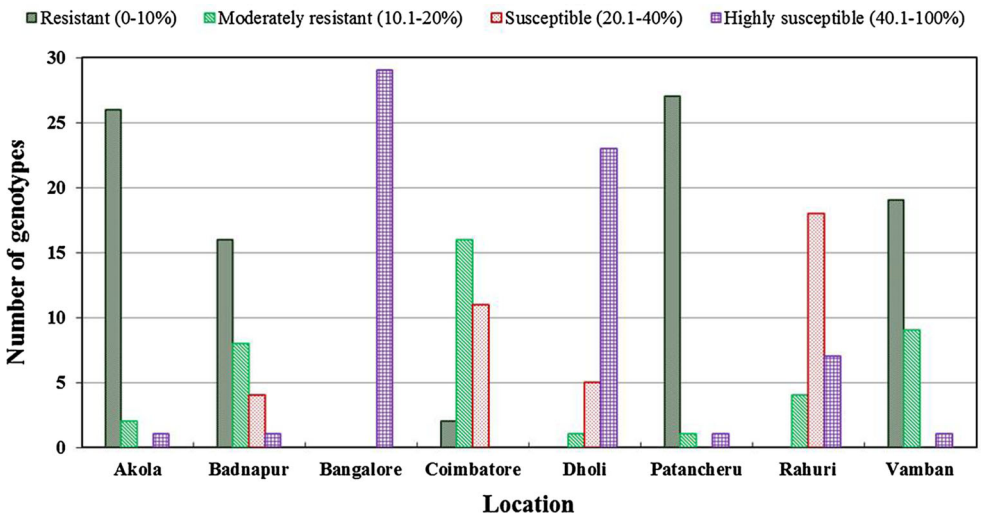

Fig. 3 Frequency distribution of 29 pigeonpea genotypes for levels of of sterility mosaic disease (SMD) of at 8 locations of India over 2 years (2007/2008 and 2008/2009). Rating of genotype reaction: resistant = 0–10 % SMD incidence; moderately resistant = 10.1–20 % SMD incidence; susceptible = 20.1–40 % and highly susceptible = 40.1–100 %

Table 4 Analysis of variance with percentage variation for incidence of sterility mosaic disease on 29 pigeonpea genotypes evaluated at 8 locations in India during 2007/2008 and 2008/2009

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	*P* value	Variation (%)[a]
Genotype (G)	28	54275.12	28862.62	<0.001	9.62
Environment (E)	15	432607.38	1939.41	<0.001	76.68
G × E	420	77270.71	184.17	<0.001	13.69
Error	464	463.71			
Total	927	564207.48			

[a] Relative percentage contribution of each source of variation to the total variance

In the GGE biplot, the genotypes were distributed on all sides of the axis as per the stability and resistance as indicated in Figs. 4 and 5. Genotypes at the right side of the *y*-axis had susceptible reactions in all the environments, while those on the left side had stable resistance across location except for locations Co-07, Co-08 and Bn-07. The GGE biplot analysis of the 28 genotypes revealed that 8 genotypes with low SMD incidence [ICPL 20094 (5), ICPL 20106 (14), ICPL 20115 (20), ICPL 20096 (6), ICPL 20107 (15), ICPL 20098 (8), ICPL 20110 (17) and KPBR-80-2-4 (28)] had high to moderate level of resistance stability. The susceptible check (ICP 8863) was consistently the most susceptible as seen by its placement farthest to the right of the origin of the biplot.

Discussion

Host plant resistance as a part of an integrated disease management is an effective strategy to manage SMD of pigeonpea. Large-scale evaluation of a genetically diverse germplasm collection and breeding lines against diseases in multi-locations is an expensive process, which can impede effective use of resources. Therefore, a large collection needs to be reduced to a minimal and manageable number for evaluation in multi-environments. Screening of pigeonpea for resistance to PPSMV is complicated further by the fact that the causal virus is transmitted by a vector, an eriophyid mite (Jones et al. 2004; Kulkarni et al. 2002; Kumar et al. 2000, 2003). In the present study, 976 germplasm and breeding lines were screened under artificial epiphytotic conditions at ICRISAT, Patancheru during 2003/2004 to eliminate genotypes that are ultra-susceptible to SMD. Further selection of SMD-resistant genotypes during 2004/2005–2006/2007 in a disease nursery at Patancheru helped us set up the PSMDN comprising 28 highly resistant genotypes.

A multi-environment evaluation revealed significant differences in genotypes, environments, and genotype × environment interactions. Differential reactions of the pigeonpea genotypes to SMD in multi-environment can be attributed to variations in virulence in the pathogen population (Kulkarni et al. 2003; Nagaraj et al. 2006). Some genotypes were resistant at a few locations, but were susceptible at other locations, suggesting variability either in genotypes or in environments or in the pathogen.

Table 5 Spearman's rank correlation between each of the 16 environments for incidence of sterility mosaic disease

Environment[a]	Ak-07	Ak-08	Bd-07	Bd-08	Bn-07	Bn-08	Co-07	Co-08	Dh-07	Dh-08	Pa-07	Pa-08	Ra-07	Ra-08	Va-07	Va-08
Ak-07	1.000															
Ak-08	0.421	1.000														
Bd-07	0.312	0.718	1.000													
Bd-08	0.315	0.166	0.336	1.000												
Bn-07	−0.192	0.050	−0.043	−0.026	1.000											
Bn-08	0.142	0.332	0.289	0.278	0.227	1.000										
Co-07	0.336	0.005	−0.043	0.077	0.146	−0.193	1.000									
Co-08	−0.128	−0.051	−0.098	0.219	0.033	−0.040	0.200	1.000								
Dh-07	0.416	0.321	0.301	0.295	−0.089	0.135	−0.018	−0.198	1.000							
Dh-08	0.219	0.394	0.419	0.243	−0.029	0.088	−0.259	−0.232	0.374	1.000						
Pa-07	0.266	0.319	0.497	0.499	−0.112	0.376	−0.238	0.006	0.433	0.591	1.000					
Pa-08	0.052	0.231	0.360	0.395	0.209	0.282	−0.083	−0.246	0.363	0.335	0.641	1.000				
Ra-07	0.242	0.222	0.263	0.171	0.078	−0.013	0.160	0.067	0.561	0.245	0.219	0.236	1.000			
Ra-08	0.498	0.466	0.596	0.175	−0.275	0.204	0.101	−0.408	0.470	0.428	0.423	0.250	0.113	1.000		
Va-07	0.549	0.393	0.474	0.234	−0.074	0.063	0.066	−0.190	0.346	0.290	0.403	0.303	0.243	0.572	1.000	
Va-08	0.161	0.318	0.455	0.149	0.078	0.296	0.027	−0.150	0.335	0.292	0.317	0.482	0.205	0.309	0.234	1.000

[a] Environment is denoted as first two letters of each location followed by year of screening

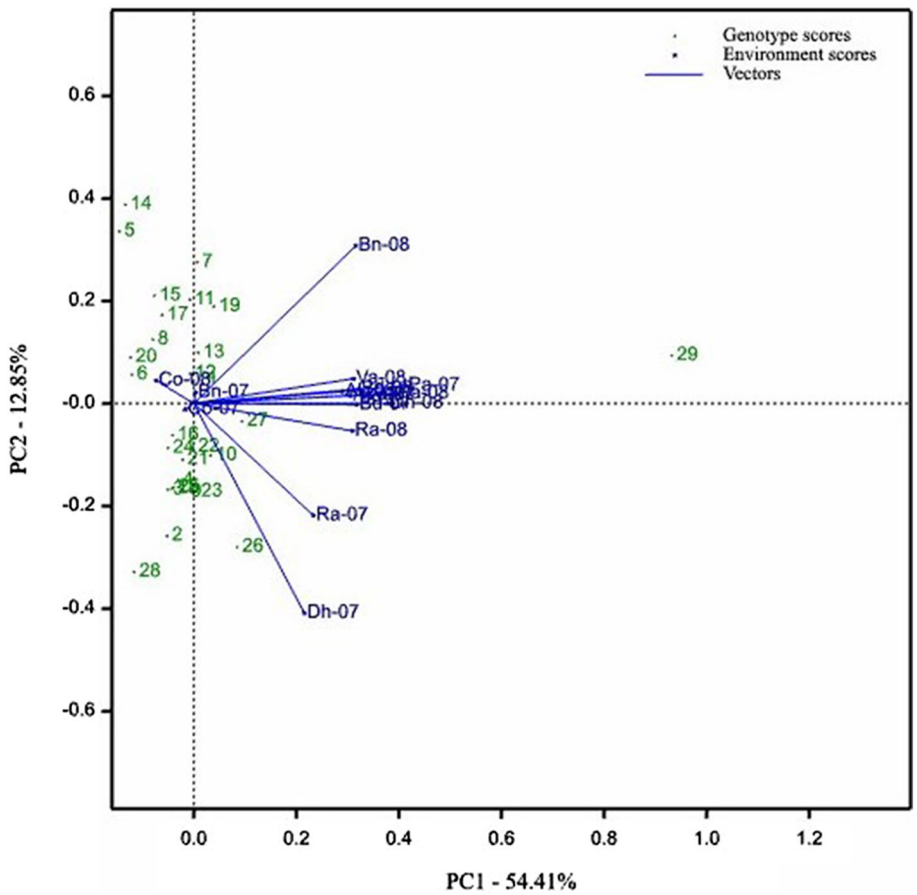

Fig. 4 GGE biplot showing the relationship among 16 environments based on sterility mosaic disease incidence of 29 pigeonpea genotypes evaluated across 8 locations in India. First and second principal components PC1 (SMD incidence) and PC2 (resistance stability) explained 54.41 and 12.85 % of total variation. The environments are denoted by first two letters of the location followed by year (2007 = 07, 2008 = 08); vectors are as *solid lines*

Environment variance contributed the most (76.68 %) to the total variance and was mainly responsible for variation in disease incidence, indirectly by favouring the mite population, its multiplication, survival, and spread. Higher G × E variation indicated the need for evaluating the genotypes at different environments. The 28 genotypes used in this study differed considerably in resistance to SMD. These genotypes had shown resistance at Akola, Badnapur, Patancheru and Vamban but were susceptible at Bangalore, Dholi and Rahuri, and had intermediate responses at Coimbatore.

Multi-environment screening of the 28 pigeonpea genotypes demonstrated significant differences among the genotypes against 16 environments for average disease incidence. Incidence of SMD on the local susceptible cultivar was high at all the locations, indicating adequate disease pressure. Average disease incidence at some locations, such as Bangalore, Rahuri and Dholi, was much higher, where almost all lines were susceptible over 2 years in contrast to other locations. Average SMD incidence was lower at Patancheru. The difference in SMD incidence among the locations might be due to differences in the virulence of the pathogen populations or differences among the dominant genotypes or a combination of both. The higher incidences at Bangalore, Rahuri and Dholi confirm that the strains from that location are more virulent (Ganapathy et al. 2012; Kumar et al. 2003).

The GGE biplot analysis is a useful tool for understanding the genotype × environment interaction (GEI), and thus avoiding GEI by selecting cultivars that are widely adapted to the entire range of environments or exploiting the GEI by selecting cultivars that are specially adapted to a subset of target environments. The GGE biplot analysis showed that seven breeding lines (ICPL 20094, ICPL 20106, ICPL 20115, ICPL 20096, ICPL 20107, ICPL 20098 and ICPL 20110) and one germplasm accession (KPBR 80-2-4) were farthest to the left of the biplot origin and could thus be considered stable to moderately stable for SMD resistance across the environments. In addition, these genotypes had very low PC1 scores (low disease incidence) and low absolute PC2 scores (high stability) in accordance with biplot analysis and use explained by Yan et al. (2007). Among these genotypes, ICPL 20094, ICPL 20106, ICPL 20098 and ICPL 20115 were moderately resistant (<20 % incidence) to SMD. These genotypes were also resistant to Fusarium wilt disease (M. Sharma,

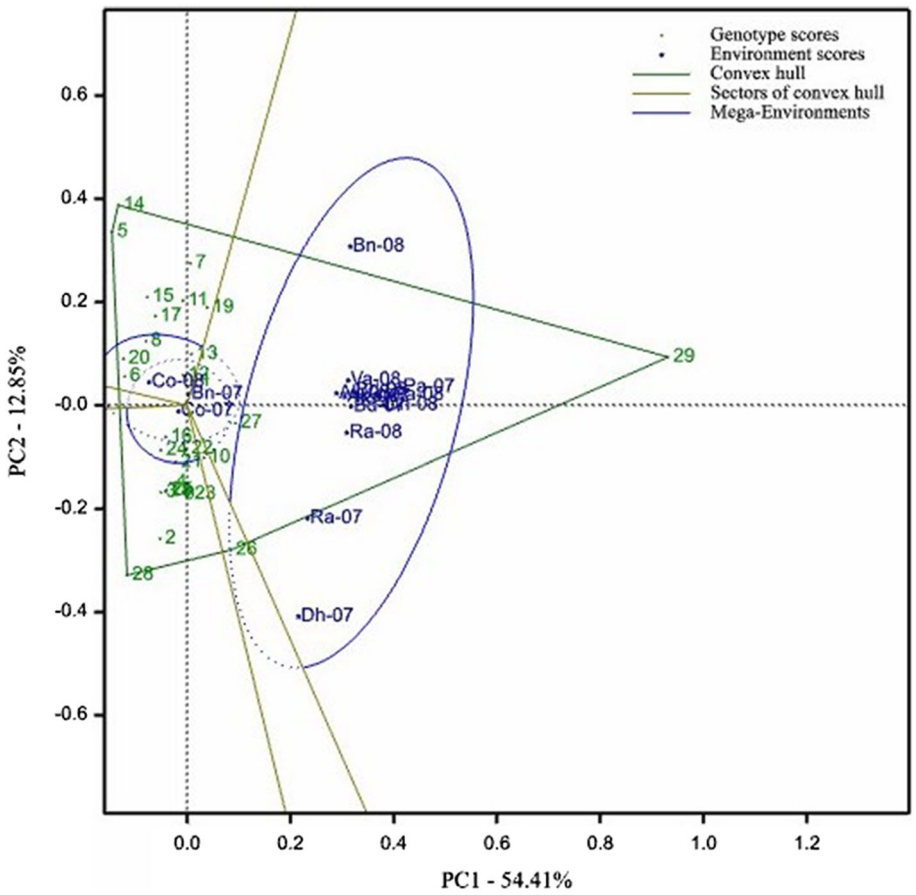

Fig. 5 GGE biplot of PC1 (SMD incidence) and PC2 (resistance stability) based on sterility mosaic disease incidence on 29 genotypes of pigeonpea in 16 environments. *Star* symbol represents the environments. The percentage of GGE variation explained by each PC is shown at *bottom left* corner (54.41 and 12.85 %). Those genotypes contributing the most to the interaction delimit the vertices of a polygon (*small dotted lines*) comprising the rest of accessions. A perpendicular line was drawn to each side of the polygon, forming seven individual sectors. *Blue lines* mark the three mega-environments found during the screening

unpublished data) in pigeonpea. Of these four moderately resistant, only ICPL 20106 and ICPL 20115 shared the same parent (IPH 487 (Table 1).

Using the GGE biplot, we found that the environments we used in India to test pigeonpea germplasm for SMD can be divided into three mega-environments having distinct incidences of SMD. These environments had a near-right angle in the GGE biplot, suggesting a more or less independent genotype response. Thus, different pigeonpea genotypes should be selected and different selection strategies should be used for environments that are conducive to susceptible vs. moderately susceptible vs. less susceptible responses. Patancheru, Bangalore and Coimbatore are representative of the three mega-environments, indicating variability of PPSMV pathogen which is in accordance with Jones et al. (2004). The genotype performance at Akola and Badnapur was actually more similar to that at Patancheru and Vamban, and the angles between the corresponding environments were less than 90°, indicating that they were positively correlated. Reddy et al. (1993) also reported that an isolate of SMD from Patancheru and one from Badnapur were variant 2 and another from of Bangalore and from Dholi represented variant 4.

Identification of genotypes that are highly stable and have low disease incidence is a key component to ensure that useful sources of high resistance are selected (Sharma and Duveiller 2007). The present study has enabled us to identify four breeding lines with stable resistance to SMD (ICPL 20094, ICPL 20106, ICPL 20098, ICPL 20115) at four locations (Akola, Badnapur, Patancheru and Vamban). All these breeding lines have a medium time to maturity and could be valuable for a breeding programme to improve SMD resistance in pigeonpea. Such resistance in pigeonpea could contribute toward the global security of food and nutrition, a major concern in the present era.

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