RESEARCH ARTICLE

Genetic diversity studies and identification of SSR markers associated with *Fusarium* wilt (*Fusarium udum*) resistance in cultivated pigeonpea (*Cajanus cajan*)

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Abstract

Genetic diversity and identification of simple sequence repeat markers correlated with *Fusarium* wilt resistance was performed in a set of 36 elite cultivated pigeonpea genotypes differing in levels of resistance to *Fusarium* wilt. Twenty-four polymorphic sequence repeat markers were screened across these genotypes, and amplified a total of 59 alleles with an average high polymorphic information content value of 0.52. Cluster analysis, done by UPGMA and PCA, grouped the 36 pigeonpea genotypes into two main clusters according to their *Fusarium* wilt reaction. Based on the Kruskal–Wallis ANOVA and simple regression analysis, six simple sequence repeat markers were found to be significantly associated with *Fusarium* wilt resistance. The phenotypic variation explained by these markers ranged from 23.7 to 56.4%. The present study helps in finding out feasibility of prescreened SSR markers to be used in genetic diversity analysis and their potential association with disease resistance.

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Introduction

Pigeonpea (*Cajanus cajan* L. Millspaugh) is a major grain legume of the tropical and subtropical regions. It is a diploid species ($2n = 2x = 22$) comprising a genome of 833.1 Mbp arranged into 11 linkage groups (Varshney *et al.* 2012). India is the centre of origin and largest producer of pigeonpea in the world sharing approximately 70% of the production and covering 74% of the area (Bohra *et al.* 2012). It plays an important role in food security, balanced diet and subsistence agriculture because of its diverse usages in food, fodder, fuel, soil conservation, integrated farming systems and symbiotic nitrogen fixation (Reddy *et al.* 2005). Further, pigeonpea offers a rich source of variability in the form of wild relatives, which could be utilized for disease resistance, good agronomic traits, enhancing nutritional quality, identification and diversification of cytoplasmic base of cytoplasmic male sterility (CMS) system etc.

Pigeonpea suffers from a number of abiotic (e.g. drought, salinity and water-logging) and biotic (e.g. diseases like *Fusarium* wilt, sterility mosaic and pod borer insects) stresses. Among biotic stresses, *Fusarium* wilt (FW) caused by *Fusarium udum* Butler is an important fungal disease prevalent in the pigeonpea growing areas but it is more severe in Indian subcontinent. Wilt symptoms usually appear during flowering and podding stage of the crop. However, symptoms also appear in early developmental stages. In Indian subcontinent the crop loss ranged from 16–47% (Prasad *et al.* 2003) and wilt incidence is believe to have increased significantly over the time (Gwata *et al.* 2006). The genetics of FW resistance is still not clear, and numbers of genes postulated to be involved vary from a single dominant gene to two complementary genes and even involvement of multiple factors (Saxena and Sharma 1990; Okiror 2002). Advent of genomic tools especially molecular markers have facilitated breeding in many cereal crops leading to development of several improved cultivars/varieties with enhanced resistance/tolerance to biotic or abiotic stresses (Varshney *et al.* 2006). Discovery of molecular markers served several functions in pigeonpea including, genetic diversity analysis using restriction fragment length polymorphism (RFLP) (Nadimpalli *et al.* 1994; Sivaramakrishnan *et al.* 2002), amplified fragment length polymorphism (AFLP) (Panguluri *et al.* 2006; Wasike *et al.* 2005), random amplification of polymorphic DNA (RAPD) (Yadav *et al.* 2012),

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microsatellite markers (Odeny *et al.* 2007) and DArT (Yang *et al.* 2006), characterization of hybrid parents and purity assessment (Saxena *et al.* 2010), gene tagging for FW resistance (Kotresh *et al.* 2006), mapping for various traits viz., (drought tolerance; Saxena *et al.* 2011), (determinacy; Mir *et al.* 2012), (sterility mosaic disease; Gnanesh *et al.* 2011), (fertility restoration; Bohra *et al.* 2012). But, the low level of genetic diversity among cultivated genotypes and poor availability of DNA markers have hindered progress of development of saturated genetic maps in pigeonpea (Bohra *et al.* 2012). The present study was undertaken with the objectives to assess the level of genetic diversity within a collection of elite pigeonpea genotypes and to evaluate the recently developed SSRs associated with FW resistance.

Materials and methods

Plant material and disease screening

A set of 36 elite pigeonpea genotypes, that are adapted to different climatic zones with good agronomic performance, were evaluated for FW resistance during 2009–10 and 2010–11. This set includes 22 FW resistant and moderately resistant genotypes and, 14 FW susceptible and moderately susceptible pigeonpea genotypes (table 1).

Test genotypes were screened in a wilt-sick plot at Agriculture Research Farm, Banaras Hindu University, Varanasi, India. Chopped wilted pigeonpea plant sticks (5–8-cm long) were uniformly buried into the soil across the field every year to artificially enhance and maintain the *F. udum* inoculum

Table 1. List of pigeonpea genotypes used for screening of SSR markers.

	Genotypes	Wilt reaction*	Characteristic features
1.	Bahar	S	Medium, compact, yellow flower, purple pod containing medium brown seed (10.5 g per100 seeds)
2.	IPA-204	MR	Tall, semi compact, yellow flower, pod green with streaks
3.	KPL-43	R	Compact, yellow flower, purple pod
4.	BDN-2010	R	Semi compact, reddish yellow flower with red base, purple pod
5.	BDN-2029	R	Semi compact, dark red flower, pod green with purplish streaks
6.	IPA-8F	R	Semi spreading, dark red flower, purple pod
7.	BDN-2001-9	R	Spreading, yellow flower, pod green with streaks
8.	IPA-234	R	Compact, yellow flower, pod green with streaks
9.	ICP-9174	MR	Semi spreading, yellow flower, pod green with streaks
10.	BWR-23	R	Flower red, pod green with streaks
11.	BSMR-846	R	Semi compact, yellow flower with red streaks, pod green with streaks
12.	IPA-9F	R	Compact, light yellow flower with light red base, pod green with streaks
13.	IPA-16F	R	Spreading yellow flower, tall, pod green with streaks
14.	BDN-2004-1	R	Semi spreading, medium dwarf, purple flower, pod green with streaks, purple stem
15.	BWR-133	R	Semi spreading, yellow flower, pod green with streaks
16.	MAL-31	S	Semi spreading, light yellow flower, pod green with streaks, brown bold seed (14 g/100 seeds)
17.	NDA-1	MS	Compact, yellow flower with purple veins, green pod
18.	ICP-9150	MR	Compact, purple stem, yellow flower, pod green with streaks
19.	Amar	MR	Compact, yellow flower, purple pod with constricted locule
20.	MAL-13	MR	Spreading, light yellow flower, pod large green with purplish black streaks and constricted locule, resistant to sterility mosaic virus
21.	MA-6	MS	Semi spreading, yellow flower, purple pod, highly resistant to sterility mosaic virus
22.	MAL-18	S	Spreading, yellow flower, purple pod, highly resistant to sterility mosaic virus
23.	ICP-2376	S	Semi spreading, yellow flower, pod green with streaks
24.	LRG-41	MS	Semi spreading, purple pod
25.	BSMR-301	MR	Semi compact, yellow flower, pod green with purplish streaks
26.	MAL-23	MS	Spreading, yellow flower, purple pod, susceptible to sterility mosaic virus
27.	MA-3	MS	Semi spreading, yellow flower, small green pod with streaks and constricted locules, small seeds (9 g per 100 seeds)
28.	MAL-34	MS	Spreading, red flower, pod green with streaks
29.	MA-23	MS	Spreading, yellow flower, purple pod with green splashes
30.	MA Deo-89	S	Compact, flower yellow with purple streaks
31.	MA PTH-2	S	Compact, red flower and pod, red large seeds, highly resistant to sterility mosaic virus
32.	JKM-7	S	Compact, flower yellow with purple streaks
33.	ICP-11887	S	Spreading, yellow flower
34.	ICP-7200	MR	Semi spreading, yellow flower
35.	ICP-8862	MR	Highly resistant to sterility mosaic virus
36.	KPBR-80-2-1	MR	Semi compact, yellow flower

*Wilt reaction based on two years (2009–10 and 2010–11) of FW screening. R, resistant; MR, moderately resistant; S, susceptible; MR, moderately susceptible.

load (5×10^6 spores m^{-2}). The test genotypes were sown in three replications using 3-m long ridges. The distance between two ridges was kept 75 cm and plant-to-plant distance was 25 cm. Recommended agronomic practices 20 kg nitrogen, 60 kg P_2O_5 and 60 Kg K_2O were given as basal placement during land preparation. To access the uniformity of disease incidence a row of susceptible check 'Bahar' was planted after every 10 rows. The scoring for FW reaction were done according to Nene and Kannnaiyan (1982) as follows: 0–20% infection; resistant (R); 21–40% infection; moderately resistant (MR); 41–60% infection; moderately susceptible (MS); above 60% infection; susceptible (S).

DNA extraction and PCR

Total genomic DNA was isolated from about 100 mg young (30 days old) and healthy leaf samples collected from an individual plant of each genotype and stored at −80°C. DNA extraction was carried out using DNA extraction kit (Quiagen, Hilden, Germany) following manufacturer's instructions. DNA quality was visualized by using 0.8% agarose gel electrophoresis and its quantity was determined using a Biophotometer plus spectrophotometer (Eppendorf, Hamburg, Germany).

Four pigeonpea genotypes differing in their FW reactions viz., BWR-23 (resistant), ICP-9174 (moderately resistant), MA-6 (moderately susceptible) and Bahar (susceptible) were initially screened for polymorphism with 60 pigeonpea SSR markers (Dutta *et al.* 2011); finally, 24 SSR markers found polymorphic among these four lines were used for diversity analysis across 36 genotypes (table 2). Polymerase chain reaction (PCR) reaction mixture (15 μL) consisted of 20–25 ng of genomic DNA, 200 μM dNTPs, 2 mM $MgCl_2$, 1 unit *Taq* DNA polymerase (MBI Fermentas, Hanover, USA), 1× PCR buffer and 0.6 mM reverse and forward primers. DNA amplification was carried out in a Thermal Cycler (Mastercycler gradient, Eppendorf, Hamburg, Germany) with a PCR profile which included an initial denaturation step at 94°C for 3 min followed by 35 cycles with a denaturing step at 94°C for 30 s, a primer annealing step at optimum annealing temperature for 30 s and an extension step at 72°C for 1 min. After the last cycle, samples were kept at 72°C for 5 min for final extension. The amplification products were separated electrophoretically in 2.5% agarose gels containing 0.05 μg/mL ethidium bromide and prepared in 1x TAE buffer (40.0 mM Tris-base, 16.65 M acetic acid, 0.5 M EDTA (pH 8.0)). The amplification products were examined under UV light and photographed using a gel documentation system (Gel Doc™ XR+, Biorad Laboratories, Hercules, USA).

Statistical analyses

SSR markers generated clear and unambiguous bands of various molecular weight sizes and were scored for the presence (1) and absence (0) of the corresponding band among the genotypes and a binary data matrix was prepared for further analysis using NTSYS-pc ver. 2.11W (Rohlf 1997).

Table 2. Details of the polymorphic SSR markers used in the study.

SSR marker	SSR motif	Expected size of PCR product (bp)	Observed size range (bp)	No. of alleles	PIC
ASSR-1	$(GA)_{10}$	100	100–120	2	0.49
ASSR-3	$(AGAAAG)_5$	145	140–175	2	0.45
ASSR-8	$(AGA)_9$	140	120–140	3	0.40
ASSR-23	$(CCTTCT)_5$	150	120–160	2	0.42
ASSR-66	(CT)12	180	180–210	3	0.65
ASSR-70	$(GGTAGA)_6$	170	170–200	2	0.50
ASSR-77	$(CT)_{10}$	140	120–140	2	0.32
ASSR-93	$(CATTTG)_5$	170	110–160	2	0.30
ASSR-97	$(ATGGAC)_8$	150	110–200	4	0.55
ASSR-148	(CAA)7	110	140–160	2	0.49
ASSR-206	(GTAATA)6	150	150–170	3	0.50
ASSR-228	(CTAAGG)5	140	100–140	3	0.42
ASSR-229	(TAAGGG)5	160	150–160	2	0.60
ASSR-277	(TCCTGT)5	130	110–150	3	0.44
ASSR-281	(CAAATG)6	220	200–250	2	0.70
ASSR-304	(GTT)7	110	110–140	2	0.51
ASSR-317	$(GAGCAT)_9$	150	130–170	2	0.46
ASSR-352	$(TTTAA)_5$	130	100–130	3	0.48
ASSR-363	$(GCATCA)_5$	190	190–210	2	0.76
ASSR-366	$(CGT)_8$	140	140–180	3	0.70
ASSR-379	$(TTCATG)_5$	140	140–170	3	0.62
ASSR-390	$(GAGCAA)_6$	190	190–210	2	0.60
ASSR-495	(CT)9	200	130–160	3	0.50
ASSR-610	(GTG)6	150	140–150	2	0.49
	Average	150	137–169	2.46	0.52

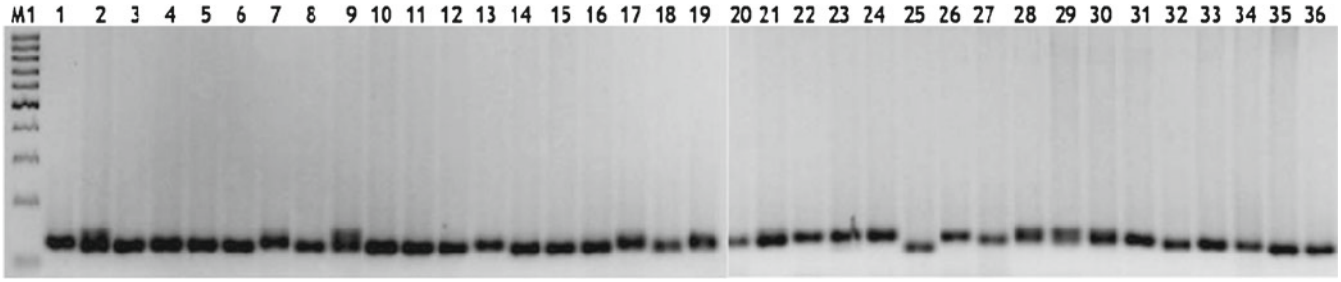

Figure 1. SSR banding profile obtained by ASSR-352 marker. Lanes 1–36 are different pigeonpea genotypes including wild relatives; M1 = 100 bp DNA size marker.

The SimQual program was used to calculate the Jaccard's similarity coefficients (Jaccard 1908). The resulting similarity matrix was used for unweighted pair group method with arithmetic averages (UPGMA) based dendrogram (Sneath and Sokal 1973) using the sequential agglomerative hierarchical nested cluster analysis (SAHN) module of NTSYSpc. Polymorphic information content (PIC) for SSR markers was calculated as $PIC_i = 1 - \Sigma P_{ij}^2$, where PIC_i is the PIC of a marker i; P_{ij} is the frequency of the jth pattern for marker i and the summation extends over n patterns. Principal component analysis (PCA) was also done to check the results of UPGMA based clustering using EIGEN module of NTSYSpc.

The data on each marker was subjected to the nonparametric Kruskal–Wallis one-way analysis of variance (K–W ANOVA) to identify markers potentially linked to FW resistance using the software PAST ver. 2.15 (Hammer *et al.* 2001). We chose this nonparametric method instead of the usually adopted parametric ANOVA in view of the ordinal nature of the disease phenotyping data (Mace *et al.* 2006). Single-marker analysis was also done to detect potential associations between individual markers (genotypic data) and FW score (phenotypic data) using simple regression analysis by PROC REG of SAS (SAS Institute 1998).

Results

SSR marker polymorphism

Twenty-four polymorphic SSR markers used in the present study yielded a total of 59 polymorphic bands, the number of polymorphic bands per primer ranged from 2 to 4, the average being 2.46 (table 2). Markers ASSR-363, ASSR-281 and ASSR-366 were the most informative primers on the basis of highest PIC of 0.76, 0.70 and 0.70, respectively. SSR marker ASSR-93 showed least PIC value of 0.30 (table 2). Gel image obtained from SSR banding profile of marker ASSR-352 is given in figure 1. These 24 polymorphic markers produced on an average of 0.52 PIC (table 3). The average expected size of amplification product was 150 bp whereas the observed amplification ranged from 100 to 250 bp (table 2).

Genetic similarity

The genetic coefficients measured through molecular data on 24 polymorphic SSR markers revealed varying degree of genetic relatedness among the pigeonpea genotypes. Jaccard's similarity coefficient ranged from 0.10 to 0.85 due to diversification in morphology and pedigree among the genotypes. A high genetic similarity coefficient of 0.85 was found between six pairs of genotypes viz., BDN 2004-1 and BDN-2010, Amar and NDA-1, MAL-13 and BWR-133, LRG-41 and BSMR-846, MAL-34 and MA-6, MA Deo-89 and MAL-34. Whereas, 10 pairs of genotypes viz., BWR-23 and Bahar, LRG-41 and IPA 16-F, LRG-41 and NDA-1, ICP-7200 and IPA 16-F, BSMR-301 and Amar, MA PTH-2 and MAL-23, ICP-9150 and MA-3, ICP-9150 and MAL-34, ICP-9150 and MA-23, ICP-9150 and MA Deo-89, showed the least

Table 3. Association of SSR markers with FW resistance based on Kruskal–Wallis one-way ANOVA and simple regression analysis.

	Kruskal–Wallis ANOVA		Simple regression analysis	
SSR markers	HC value	P	R^2	b
ASSR-1	8.19	0.042	37.5	1.47**
ASSR-23	8.11	0.044	34.3	1.28*
ASSR-148	7.87	0.50	23.7	1.12*
ASSR-229	9.91	0.98	35.2	1.30*
ASSR-363	11.54	0.991	56.4	1.86**
ASSR-366	10.75	0.99	41.1	1.58**

*, ** Significant at 5 and 1% level, respectively.

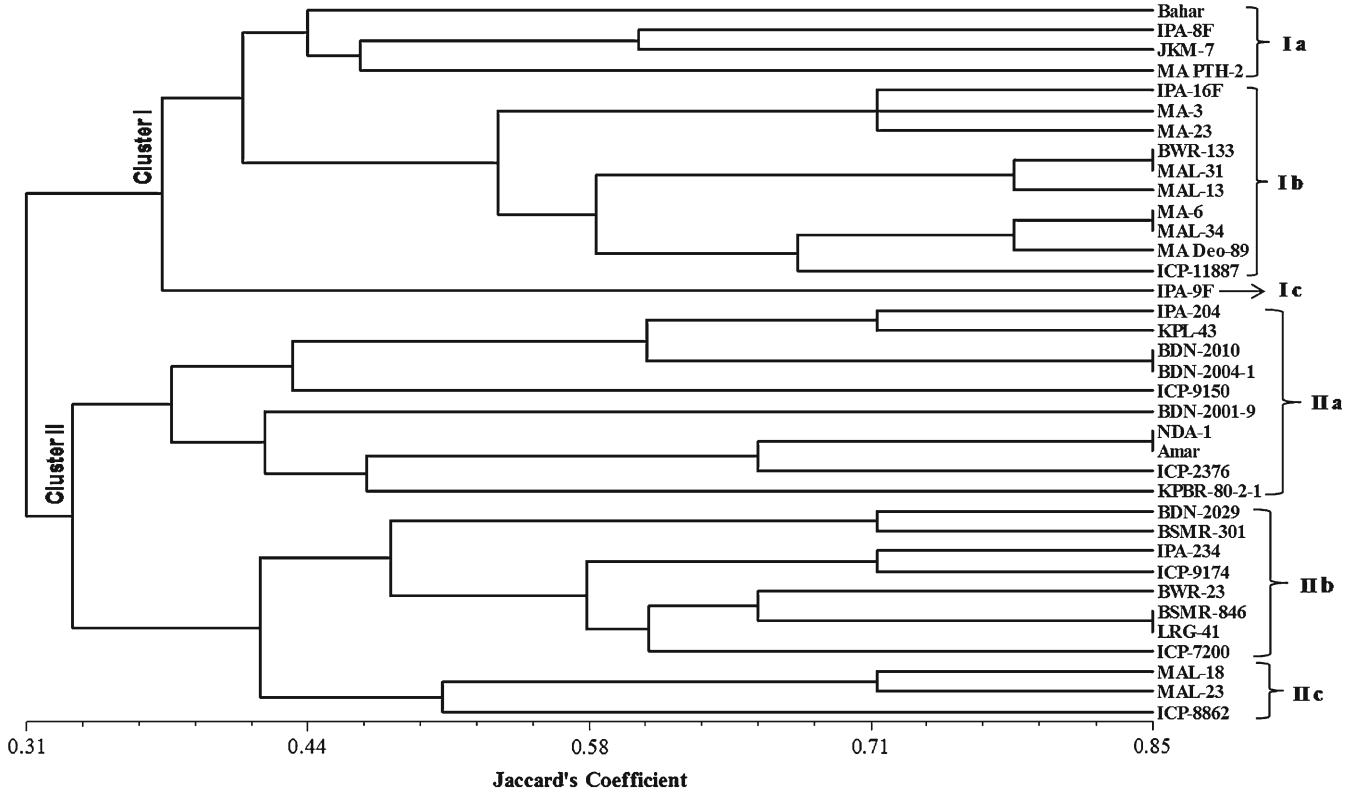

Figure 2. Dendrogram of 36 pigeonpea genotypes based on Jaccard's similarity coefficients of 24 polymorphic SSR markers.

genetic similarity of 0.10 (table 1 in electronic supplementary material at http://ias.ac.in/jgenet/).

Cluster analysis

In the UPGMA dendrogram 36 pigeonpea genotypes were grouped into two main clusters consisting of 15 and 21 genotypes, respectively (figure 2). Cluster I was further divided into three sub-clusters viz., Ia (four genotypes: Bahar, IPA-8F, JKM-7, MA PHT-2), Ib (10 genotypes: IPA-16F, MA-3, MAL-23, BWR-133, MAL-31, MAL-13, MA-6, MAL-34, MAL Deo-89 and ICP-11887) and Ic consisted of only one pigeonpea genotype i.e., IPA-9F. Cluster II was also divided into three sub-clusters, sub-cluster IIa consisted of 10 genotypes viz., IPA-204, KPL-43, BDN-2010, BDN-2004-1, ICP-9150, BDN-2001-9, NDA-1, Amar, ICP-2376, KPBR-80-2-1. While, sub-cluster IIb consisted of eight genotypes (BDN-2029, BSMR-301, IPA-234, ICP-9174, BWR-23, BSMR-846, LRG-41 and ICP-7200) and sub-cluster IIc contains only three genotypes, namely, MAL-18, MAL-23 and ICP-8862 (figure 2). PCA revealed that PC 1, PC 2 and PC 3 accounted for 23.41%, 16.0% and 13.0% of the total variation, respectively. Together, the first three PCs accounted for 52.41% of the total variation. Two-dimensional plot was prepared by using the first two principal components. In 2-D plot, the genotypes were also seems to be grouped in two main clusters consisted of 18 genotypes in each cluster (figure 3).

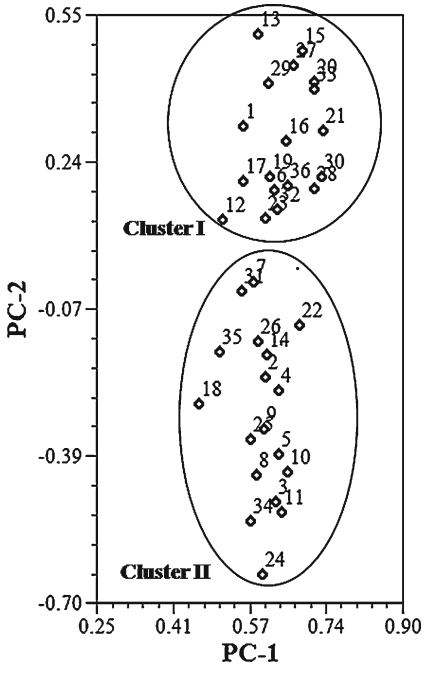

Figure 3. Two-dimensional plot of principal components 1 and 2 based on SSR markers of pigeonpea genotypes. Names of the 36 genotypes are listed in table 1.

SSR markers association with FW resistance

To determine the association of a particular SSR marker to the respective phenotype, 36 pigeonpea genotypes were scored for FW reaction in a wilt sick plot. The genotypic data generated by 24 polymorphic SSR markers were subjected to K–W ANOVA by using the marker and the respective phenotypic data. K–W ANOVA detected the significant association of six SSR markers viz., ASSR-1, ASSR-23, ASSR-148, ASSR-229, ASSR-363 and ASSR-366 with *Fusarium* wilt resistance (table 3). The phenotypic variation (R^2) and regression values (b) of these six SSR primers are also summarised in table 3. The same six markers also showed significant association in simple regression analysis owing to higher R^2 values and significant deviation of b value from zero. Among the six markers, ASSR-363 explained a maximum of 56.4% (b value = 1.86; $P < 0.01$) of phenotypic variation due to FW resistance. The phenotypic variation explained by these markers ranged from 23.7 to 56.4%.

Discussion

In pigeonpea a fairly enough genomic resources are now being available including, a consensus genetic map comprising 339 loci spanning a distance of 1059 cM with an average marker density of 3.1 cM (Bohra *et al.* 2012). A total of 156 SSR markers information (Burns *et al.* 2001; Odeny *et al.* 2007, 2009; Saxena *et al.* 2010) was used until development of intraspecific consensus genetic map of Bohra *et al.* (2012). In the present study, the reason of getting high polymorphism rate (average PIC of 0.515 with a maximum of 0.76) underneath the efficiency of the prescreened SSR markers. The finally used 24 polymorphic SSR markers were selected on the basis of their banding pattern, to differentiate the FW resistant genotypes (BWR-23 and ICP-9174) from the FW susceptible genotypes (MA-6 and Bahar), during initial screening. The coefficient of genetic similarity obtained in the present study ranged from 0.10 to 0.85, indicating that a high level of genetic diversity existed among the 36 pigeonpea genotypes. However, several other workers reported a quite narrow genetic diversity (0.82–1.0; Panguluri *et al.* 2006), (0.67–1.0; Yang *et al.* 2006), (0.34–0.98; Odeny *et al.* 2007) in pigeonpea. The higher estimated genetic distance could be ascribed to differences between genotypes due to diversification in the pedigree. Narrow genetic base has been reported by a number of workers in pigeonpea due to use of only few genotypes with high degree of relatedness in crossing programmes for the development of new cultivars (Kumar *et al.* 2004; Yang *et al.* 2006). Kumar *et al.* (2004) observed that 16 (34%) pigeonpea cultivars released in India involved only one or two genotypes as one of the ancestors in their pedigree.

In the present study, the result of PCA was almost similar to that of UPGMA cluster analysis, cluster I consisted of most of the FW susceptible genotypes while cluster II grouped most of the FW resistant genotypes. Grouping of three highly resistant FW genotypes i.e., BSMR-846, BWR-23 and IPA-234 in one sub-cluster (UPGMA dendrogram sub-cluster IIb and PCA cluster II) indicates introgression of FW resistance in these genotypes from the same or similar ancestor (Saxena *et al.* 2010). Several sub-clusters on the other hand contained both resistant and susceptible genotypes; however, the proportion of FW resistant and moderately resistant genotypes was more in cluster II (17 and 15 out of 22 genotypes in UPGMA and PCA clustering, respectively). Similarly, the proportion of FW susceptible and moderately susceptible genotypes was more in cluster I (i.e., 10 out of 14 genotypes in each of the UPGMA and PCA clustering). Mischaracterization of some of the resistant/moderately resistant genotypes in cluster I and vice-versa may be assigned to nonuniform inoculum density and pathotype variation. Therefore, it is essential to understand the genetics of FW resistance for concise mapping of genomic region(s) associated with FW resistance in pigeonpea. The clustering patterns generated by UPGMA and PCA were mostly congruent and 36 pigeonpea genotypes were mainly grouped into two clusters according to their FW reaction. In a previous study, Singh *et al.* (2012) analysed genetic diversity among forty pigeonpea genotypes including four wild species viz., *C. scarabaeoides*, *C. cajanifolius*, *C. volubilis* and *Rhynchosia rothii* using 12 polymorphic SSR markers. The PIC ranged from 0.434 to 0.594 with an average of 0.523 and the genetic diversity ranged from 0.0 to 0.85. They found that most of the *Fusarium* wilt resistant genotypes namely, MA-3, MA-6, MAL-23, MAL-13, MAL-31 and MAL-34 were grouped together in cluster I, except MAL-18 and MAL-23. Messmer *et al.* (1993) suggested that for extraction of the maximum information from the molecular marker data, ordination methods (PCA and PCoA) can be used in combination with cluster analysis, particularly when the first two or three PCs explain >25% of the original variation. In this study, the first three PCs accounted for 52.41% of the total variation.

K–W ANOVA and simple regression analysis detected the significant association of six SSR markers (ASSR-1, ASSR-23, ASSR-148, ASSR-229, ASSR-363 and ASSR-366) with FW resistance. These markers are having high phenotypic variation ranged from 23.7 to 56.4% and significant b value. Earlier, Mace *et al.* (2006) identified association of eight SSR markers with rust and late leaf spot (LLS) using K–W ANOVA in groundnut. While, Mondal and Badigannavar (2010) found three and four SSR markers associated with rust and LLS resistance, respectively based on the K–W one-way ANOVA and simple regression analysis. The markers identified through K–W ANOVA were also confirmed with simple regression analysis and therefore could be utilized in the marker assisted breeding programme for *Fusarium* wilt resistance in pigeonpea. The present study would be able to select some diverse pigeonpea genotypes e.g., BSMR-846, ICP-9174, BWR-23, BDN-2029 (FW resistant) and Bahar, MAL-18, MAL-31, MAL Deo-89 (FW

susceptible) on the basis of phenotypic data and marker polymorphism. These selected genotypes upon hybridization can be used to develop diverse mapping populations to map FW resistance. Three parental genotype combinations viz., Bahar × BSMR-846, MAL-18 × BSMR-846, MAL-18 × BDN-2029 (in F_2 generation) have been sown during crop season 2012–13, for FW mapping.

In conclusion, the present study reports genetic diversity of 36 elite pigeonpea genotypes, resistant/susceptible to FW. These genotypes were broadly grouped into two groups comprising most of the FW resistant genotypes into one group and susceptible genotypes in other group. Based on K–W ANOVA and simple regression analysis, the present study revealed feasibility of prescreened SSR markers to be used in genetic diversity analysis and their potential association with FW resistance. Diverse pigeonpea FW resistant/susceptible genotypes reported in this study will be useful to generate mapping populations for FW mapping and work in progress at the centre.

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