Field Crops Research 123 (2011) 53–61

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Genetic mapping and quantitative trait locus analysis of resistance to sterility mosaic disease in pigeonpea [*Cajanus cajan* (L.) Millsp.]

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ARTICLE INFO

Article history:
Received 27 February 2011
Received in revised form 8 April 2011
Accepted 9 April 2011

Keywords:
Pigeonpea
Molecular markers
Simple sequence repeats (SSRs)
Linkage map
QTL

ABSTRACT

Sterility mosaic disease (SMD), considered as the "green plague of pigeonpea" and caused by pigeonpea sterility mosaic virus (PPSMV) is one of the major biotic factors, which leads to heavy yield losses and hence poses a big challenge for pigeonpea production in the Indian subcontinent. Variability in the sterility mosaic pathogen revealed the occurrence of five different isolates in India. Among them, three distinct SMD isolates have been characterized, *viz.,* Patancheru, Bangalore and Coimbatore. Molecular tools offer a viable option to tackle these biotic stresses via identification of the genomic regions associated with the trait such as SMD resistance. With an aim of identifying the gene(s)/QTLs linked with SMD resistance, two F_2 populations, i.e. ICP 8863 × ICPL 20097 (segregating for Patancheru SMD isolate) and TTB 7 × ICP 7035 (segregating for both Patancheru and Bangalore SMD isolates) were developed and $F_{2:3}$ families were phenotyped for resistance to respective isolate(s) of SMD. After screening over 3000 SSR markers on parental genotypes of each mapping population, intra-specific genetic maps comprising of 11 linkage groups and 120 and 78 SSR loci were developed for ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035 populations, respectively. Composite interval mapping (CIM) based QTL analysis by using genetic mapping and phenotyping data provided four QTLs for Patancheru SMD isolate and two QTLs for Bangalore SMD isolate. Identification of different QTLs for resistance to Patancheru and Bangalore SMD isolates is an indication of involvement of different genes conferring the resistance to these two SMD isolates. One QTL namely qSMD4 identified within an interval of 2.8 cM on LG 7 explaining 24.72% of phenotypic variance, once it is validated in other genetic background, seems to be a promising QTL for use in marker assisted selection. In summary, this is the first study on development of intra-specific genetic maps and identification of QTLs for SMD resistance in pigeonpea.

1. Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important grain legume crop of rainfed agriculture in the semi-arid tropics (SATs). It is the only cultivated food crop of the *Cajaninae* sub-tribe with a diploid genome with 11 pairs of chromosomes ($2n = 2x = 22$) and a genome size estimated to be 858 Mbp (Greilhuber and Obermayer, 1998). The genus *Cajanus* comprises 32 species found mainly in India and Australia. Pigeonpea is cultivated in more than 25 tropical and sub-tropical countries, either as the sole crop or a mixed crop with sorghum, pearl millet, maize, or with short duration legumes, e.g., groundnut. It plays an important role in food security, balanced diet and alleviation of poverty because of its diverse usages as a food, fodder and fuel (Rao et al., 2002). India is the largest producer of pigeonpea (2.30 mt) followed by Myanmar (0.54 mt) and Malawi (0.16 mt) (FAOSTAT 2007; http://faostat.fao.org). Although, India leads the world both in area and production of pigeonpea, its productivity is lower than the world average which may be attributed to various abiotic (e.g. drought, salinity and water-logging) and biotic (e.g. diseases like *Fusarium* wilt, sterility mosaic and insects like pod borers) factors.

Among the diseases, sterility mosaic disease (SMD) is considered to be the most important disease of pigeonpea in India and at times

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doi:10.1016/j.fcr.2011.04.011

can cause yield loss upto 95% (Reddy and Nene, 1981; Kannaiyan et al., 1984). The disease is caused by pigeonpea sterility mosaic virus (PPSMV) (Kumar et al., 2003) and transmitted by eriophyid mite (*Aceria cajani* Channabasavanna). The disease is characterized by the symptoms like bushy and pale green appearance of plants followed by reduction in size, increase in number of secondary and mosaic mottling of leaves and finally partial or complete cessation of reproductive structures. Some parts of the plant may show disease symptoms and other parts may remain unaffected (Kumar et al., 2003). SMD infection depends on the availability of mite populations. Mite populations are usually positively correlated with rainfall, relative humidity and lower temperature (Singh et al., 1999). Control of the disease by chemical method though effective but economically not feasible and non eco-friendly (Nene et al., 1989). Breeding for resistant varieties is considered to be one of the most effective and economic methods of reducing crop losses and has received top priority. In case of SMD, the task of developing resistant varieties is complicated in view of the genetic plasticity of the pathogen. A total of five different SMD isolates have been reported and of these three SMD isolates namely Patancheru, Bangalore and Coimbatore have been characterized (Reddy et al., 1993). This dynamic nature of the SMD pathogen has warranted the use of strain specific sources of resistance in crop improvement. So, there is a need for identifying strain specific sources of resistance and its inheritance pattern for better understanding of the disease.

With the advent of genomic tools such as molecular markers, genetic maps, etc., conventional plant breeding has been facilitated greatly leading to the development of improved genotypes/varieties with enhanced resistance/tolerance to biotic/abiotic stresses in several crop species (Varshney et al., 2005, 2006). Identification of environmental insensitive molecular markers associated with SMD resistance would allow rapid screening of cultivars and segregating generations at seedling stage and would subsequently reduce the need for maintaining virulent isolates of the pathogen.

Among various type of marker systems available, microsatellite or simple sequence repeat (SSR) markers have proven to be more reliable, hypervariable and reproducible as compared to RAPD (random amplified polymorphic DNA) markers and less cumbersome and time consuming than the RFLP (restriction fragment length polymorphism) or AFLP (amplified fragment length polymorphism) markers. In case of pigeonpea, a very limited number of genomic resources including few SSR markers were available until recently (Varshney et al., 2009, 2010). For instance, 156 SSR markers (Burns et al., 2001; Odeny et al., 2007, 2009; Saxena et al., 2010a) derived from genomic DNA library were available which were not enough for developing the genetic map especially considering a very low diversity in the elite germplasm collection of pigeonpea. Recently a large set of >3000 bacterial artificial chromosome (BAC)-end sequences (BESs) and expressed sequence tag (EST)-derived SSR markers have been developed (Bohra et al., 2011; Raju et al., 2010). Availability of these markers should facilitate genetic mapping and molecular breeding in pigeonpea.

Realizing the importance of mapping of SMD resistance in pigeonpea, this study was undertaken with following objectives: (i) development and phenotyping of two mapping populations for SMD resistance, (ii) construction of intra-specific genetic maps, and (iii) identification of gene(s)/QTLs associated with SMD resistance.

2. Materials and methods

2.1. Development of segregating populations

For development of suitable segregating populations for mapping SMD resistance, four molecularly and morphologically diverse parents for SMD, based on the previous reports (Rangaswamy et al., 2005; Ganapathy et al., 2010; Saxena et al., 2010b) were selected. Among these four genotypes, ICP 8863 and TTB 7 are indeterminate, mid-late and susceptible to SMD while ICPL 20097 and ICP 7035 are indeterminate, mid-late but resistant to SMD.

The individual flowers of the selected female parents ICP 8863 and TTB 7 were hand emasculated and pollinated with the pollen dust from the male parents ICPL 20097 and ICP 7035 respectively, to get sufficient F_1 seeds (*Kharif* 2006). F_1 plants along with their parents were grown during summer 2007 and were selfed by covering nylon net to prevent out crossing from honey bees and other insect pollinators. Selfed seeds from the F_1 plants were collected and used for raising F_2 generation during *Kharif* 2007. All the F_2 plants were covered with nylon net to prevent insect pollination. Seeds obtained from F_2 plants were collected and forwarded to $F_{2:3}$ generation for phenotyping against SMD.

2.2. Resistance screening for SMD

The SMD infection leads to partial or complete cessation of reproductive structures. Screening for SMD is destructive in nature and practicing it in F_2 may result in non availability of further seeds from susceptible plants, hence to avoid this, screening was performed in $F_{2:3}$ families instead of F_2. The mapping population ICP 8863 × ICPL 20097 was phenotyped for resistance to Patancheru SMD isolate at ICRISAT, Patancheru, while the other mapping population (TTB 7 × ICP 7035) was phenotyped during *Kharif* 2008 for resistance to Patancheru SMD isolate at ICRISAT, Patancheru and for Bangalore SMD isolates at UAS-Bangalore following "Leaf Stapling Technique" (Nene and Reddy, 1976). All $F_{2:3}$ individuals with 15 plants per family alongwith their parents, F_1s and susceptible check (ICP 8863) were raised in poly bags in randomized complete block design (RCBD) with two replications in case of TTB 7 × ICP 7035 population and three replications in ICP 8863 × ICPL 20097 population. SMD infected leaves were stapled to leaves of test plants, as mentioned above, at 12 to 15 days (2–3 leaf stage). As the stapled leaflets from the infected plants get dried, mites from the infected leaves migrate to healthy leaves and inoculate the virus. The susceptible control ICP 8863 was included in both sets, at frequent intervals, to monitor the extent of disease spread. At both locations, plants were scored for incidence of SMD at 15 days interval up to 75 days by counting the healthy plants (no mosaic symptoms) and diseased plants (with mosaic symptoms) as per the criterion followed in All India Co-ordinated Research Project (AICRP) on pigeonpea. The plants were grouped as resistant (0–10% of plants infected); moderately resistant (10.1–30% of plants infected) and susceptible (30.1–100% of plants infected) (Singh et al., 2003).

The analysis of variance (ANOVA) for scoring of SMD at two different locations was performed to test the significance of differences between genotypes. The adjusted mean values of quantitative trait were used to estimate coefficients of skewness and kurtosis using 'STATISTICA' version 9 (Statsoft, Inc., Tulsa, Oklahoma, USA).

2.3. DNA extraction and polymerase chain reaction (PCR)

Genomic DNA was extracted from the young leaf tissues of the mapping parents (ICP 8863, ICPL 20097, TTB 7 and ICP 7035) and F_2 using standardized high throughput mini-DNA extraction method as mentioned in Cuc et al. (2008).

PCRs for all SSR markers were performed in 5 μl reaction volume in an ABI 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA), in 384-well PCR plates (Applied Biosystems, Foster city, CA, USA), consisting of 5 ng/μl DNA template, 2 pmol of primer, 15 mM $MgCl_2$, 2 mM dNTPs, 0.3 U of Taq DNA polymerase (Jonaki, Hyderabad, India) and 1X PCR buffer (Jonaki, Hyderabad, India). A touch

Table 1
Phenotypic variation of the SMD in $F_{2:3}$ families of ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035.

Isolate/Mapping population	Sample size	Mean	Minimum	Maximum	Standard deviation	Standard ERROR	Skewness	Kurtosis
Patancheru SMD isolate								
ICP 8863 × ICPL 20097	190	55.08	0	100	29.04	2.10	−0.38	−0.97
TTB 7 × ICP 7035	130	59.56	0	100	32.28	2.83	−0.57	−0.85
Bangalore SMD isolate								
TTB 7 × ICP 7035	130	78.35	6.3	100	21.60	1.89	−1.25	1.28

down PCR amplification profile with 3 min of initial denaturation cycle, followed by first five cycles of 94 °C for 20 s, 60 °C for 20 s and 72 °C for 30 s, with 1 °C decrease in annealing temperature per cycle, then 30 cycles of 94 °C for 20 s with constant annealing temperature (56 °C) and 72 °C for 30 s followed by a final extension for 20 min at 72 °C.

The amplified products were checked for amplification on 1.2% agarose gel. Amplified products were size fractioned using capillary electrophoresis on an ABI 3730 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA). Allele sizing of the electrophoretic data thus obtained was done using software GeneMapper version 4 (Applied Biosystems, Foster City, CA, USA).

2.4. Genetic mapping

Segregation data were assembled for all polymorphic markers on 190 F_2 individuals of ICP 8863 × ICPL 20097 and 130 F_2 individuals of TTB 7 × ICP 7035 populations. The linkage analysis for both populations was performed with the help of JoinMap 3.0 software (Van Ooijen and Voorrips, 2001). Linkage groups (LGs) were established at LOD ≥ 3 with other parameters like recombination threshold of 0.40, ripple value of 1 and jump threshold of 5. Deviation from Mendelian segregation ratio (1:2:1) was estimated with the help of *locus genotype frequency* function. Map distances were calculated using Kosambi mapping function (Kosambi, 1944).

2.5. QTL analysis

Genotyping data and phenotyping data obtained for SMD were analyzed for mapping of QTLs by using the method composite interval mapping (CIM), proposed by Zeng (1994) in the WinQTL Cartographer, version 2.5 (Wang et al., 2007). CIM analysis was performed using the Standard Model 6, scanning the genetic map and estimating the likelihood of a QTL and its corresponding effects at every 1 cM (walk speed), while using significant marker cofactors to adjust the phenotypic effects associated with other positions in the genetic map. The number of marker cofactors for the background control was set by forward–backward stepwise regression. A window size of 10 cM was used, and therefore cofactors within 10 cM on either side of the QTL test site were not included in the QTL model. Thresholds were determined by permutation tests (Churchill and Doerge, 1994; Doerge and Churchill, 1996), using 1000 permutations and a significance level of 0.05. The significant QTLs were plotted in graphics. Graphical presentation of the LGs and the QTLs was obtained by using MapChart, version 2.2 (Voorrips, 2006).

3. Results

3.1. Development and phenotyping of populations for SMD resistance

By using four diverse genotypes, two mapping populations namely ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035 comprising of 190 and 130 F_2 individuals, respectively, were developed. The SMD phenotyping which was destructive was avoided in F_2, so that seeds can be harvested from these plants to obtain $F_{2:3}$ families. Susceptible parents ICP 8863 and TTB 7 exhibited 100% disease incidence for Patancheru SMD isolate and both Bangalore and Patancheru SMD isolates respectively, with severe mosaic symptoms. While resistant parents ICPL 20097 and ICP 7035 showed complete resistance to Patancheru SMD isolate but 6.6% disease incidence was observed for Bangalore SMD isolate (in case of ICP 7035). The mean SMD percentage disease reactions of $F_{2:3}$ progenies against Bangalore and Patancheru SMD isolates were subjected to ANOVA. The 'F' calculated value was significant at 1% level of significance, suggesting that the genotypes under consideration showed considerable variation for the SMD reactions. Descriptive statistics of SMD incidence in $F_{2:3}$ populations of ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035 has been presented in Table 1. The SMD incidence for Patancheru SMD isolate in ICP 8863 × ICPL 20097 ranged from 0 to 100% with an average incidence of 55.08%. The coefficients of skewness and kurtosis were −0.38 and −0.97 respectively. Similarly, in TTB 7 × ICP 7035, SMD incidence ranged from 0 to 100% (with a mean of 59.56%) and 6.3 to 100% (with a mean of 78.35%) for Patancheru and Bangalore SMD isolates respectively. For Patancheru SMD isolate, coefficient of skewness was −0.57 and kurtosis was −0.85 while in case of Bangalore SMD isolate, coefficient of skewness and kurtosis were found to be −1.25 and 1.28 respectively.

The variation existed in the $F_{2:3}$ families of the crosses ICP 8863 × ICPL 20097 (Fig. 1) and TTB 7 × ICP 7035 (Fig. 2) for SMD incidence showed near normal curves for both the SMD isolates indicating presence of a number of genes governing resistance against SMD. In case of Bangalore isolate, skewness towards susceptibility for SMD was observed due to more virulent nature of this isolate. In order to take care of distribution abnormalities, arc-sine transformed means for SMD were utilized for QTL identification.

3.2. Marker polymorphism assessment

With an objective to develop genetic maps based on two segregating populations, >3,000 SSR markers were tested on the parental genotypes. In case of ICP 8863 × ICPL 20097, screening of 3,072 BES-SSR markers provided 2,298 (74.80%) amplifiable

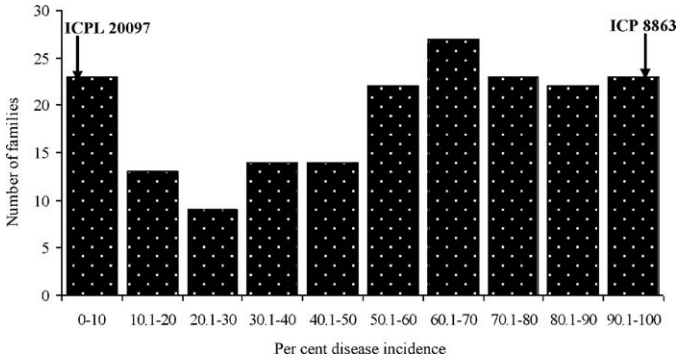

Fig. 1. Frequency distribution of per cent disease incidence for Patancheru SMD isolate in 190 $F_{2:3}$ families derived from a cross ICP 8863 × ICPL 20097. The mean scores for resistant (ICPL 20097) and susceptible (ICP 8863) parents are indicated by arrows.

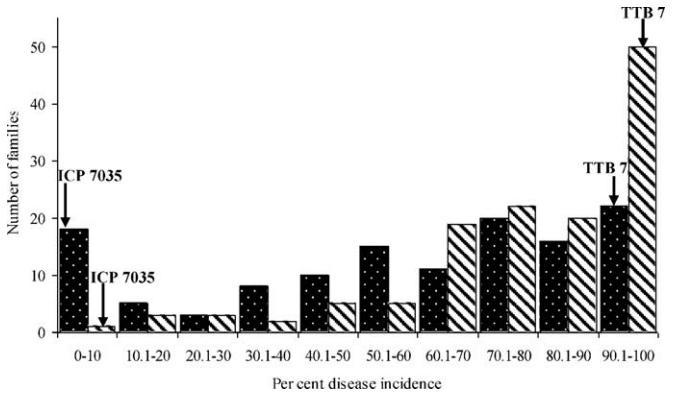

Fig. 2. Frequency distribution of per cent disease incidence for Patancheru (■) and Bangalore (◣) SMD isolates in 130 'TTB 7 × ICP 7035' $F_{2:3}$ families. The mean scores for susceptible (TTB 7) and resistant (ICP 7035) parents are indicated by arrows.

markers and 143 (4.65%) polymorphic markers. On the other hand, a total of 3,320 SSR markers including 3,072 BES derived, 164 genomic DNA libraries derived and 84 expressed sequence tag (EST)-derived markers were checked on parental genotypes of TTB 7 × ICP 7035 population. As a result, 2,107 (63.5%) marker provided amplicons of expected size and 84 (2.5%) markers including 83 BAC-ends/genomic and one EST-SSR markers were found to be polymorphic.

3.3. Construction and comparison of genetic maps for two populations

A total of 143 and 84 polymorphic markers, as identified above, were used to generate segregation data on all F_2 individuals of ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035 populations, respectively.

In case of ICP 8863 × ICPL 20097 population, segregation data assembled for 143 polymorphic markers tested for goodness of fit. While 138 (96.50%) markers showed a goodness of fit, the remaining 5 (3.49%) markers (CcM0974, CcM1001, CcM1820, CcM1821, and CcM2227) showed significant deviation from Mendelian ratio. Thus a total of 120 markers got mapped at a total map length of 534.89 cM. The number of LGs was 11, which is equivalent to the haploid ($n = x = 11$) chromosome number of *Cajanus*. The number of mapped markers was ranged from 2 (LG 1 and LG 11) to 21 (LG 6) with an average of 10 markers per LG. Similarly, length of LGs varied from 7.60 cM (LG 11) to 105.89 cM (LG 2) with an average value of 48.62 cM per LG (Fig. 3).

Similarly in case of TTB 7 × ICP 7035 population, segregation data were obtained for all 130 F_2 lines with all 84 polymorphic markers. While 77 (91.66%) markers showed a goodness of fit, the remaining seven (8.33%) markers namely CcM0085, CcM0374, CcM0443, CcM1493, CcM1813, CcM2228 and CcM2818 showed segregation distortion. Linkage map analysis of 84 markers assigned a total of 78 markers to 11 LGs spanning 466.97 cM with an average marker interval of 5.98 cM (Fig. 3). Six SSR markers, however, remained unlinked. The number of markers mapped per LG ranged from 3 (LG 7) to 12 (LG 1). The length of LGs ranged from 4.32 (LG 7) to 89.51 cM (LG 1).

In both genetic maps, names of different LGs were assigned on the basis of common markers from the reference genetic map derived from an inter-specific F_2 population, i.e. ICP 28 × ICPW 94 (Bohra et al., 2011) and the consensus genetic map (unpublished). A detailed comparison of genetic maps of these two intra-specific mapping populations showed 15 markers to be common and distributed on 8 LGs (Fig. 3). While comparing the intra-specific map for ICP 8863 × ICPL 20097 population with the inter-specific map for ICP 28 × ICPW 94 population, 11 markers were found common. While four of these 11 markers were present on four different LGs, seven markers were present on three LGs namely LG 2 (CcM1101, CcM1198, CcM2097), LG 5 (CcM1139, CcM2281) and LG 6 (CcM0361, CcM2538). As expected, the order of these markers on the three LGs in both maps is in congruence (Fig. 4). Similarly, 13 markers were found common between the maps of TTB 7 × ICP 7035 and ICP 28 × ICPW 94 crosses. Congruence of 7 markers on three LGs has been shown in Fig. 5.

3.4. Identification of QTLs associated with SMD resistance

Phenotyping data (arc-sine transformed means) and genetic mapping data, as mentioned above, were used for identification of QTL(s) for resistance to Patancheru SMD isolates based on two populations (ICP 8863 × ICPL 20097, TTB 7 × ICP 7035) and for Bangalore SMD isolates on one population (TTB 7 × ICP 7035).

In case of SMD resistance to Patancheru SMD isolate, composite interval mapping (CIM) in ICP 8863 × ICPL 20097 population identified two QTLs *viz.* qSMD1 and qSMD2 flanked by the markers *CcM1982-CcM1447* and *CcM0588-CcM2781* on LG 9 at LOD scores of 3.1 and 3.07 respectively (Fig. 2). These two QTLs explained 9.2 and 8.3% phenotypic variance (%PV) with a positive additive effects of 0.16 and 0.15 and negative dominance effects of −0.16 and −0.13 respectively (Table 2). In case of TTB 7 × ICP 7035 population, CIM identified two major QTLs namely qSMD3 and qSMD4 on marker intervals *CcM2149-CcM0468* (LG 2) and *CcM1825-CcM1895* (LG 7) at LOD scores of 3.86 and 6.74, respectively (Fig. 2). One QTL (flanked by *CcM2149-CcM0468*) explained 12.32% PV with positive additive effect (0.24) and the second QTL (bracketed in *CcM1825-CcM1895* region) explained 24.72% PV having a positive additive effect of 0.33 (Table 2). However not a single common QTL for Patancheru SMD isolate was observed based on analysis of two mapping populations.

For resistance to Bangalore SMD isolate, CIM in TTB 7 × ICP 7035 mapping population identified two QTLs *viz.* qSMD5 and qSMD6 flanked by the markers *CcM0970-CcM2485* and *CcM0416-CcM2337* with LOD score 3.35 and 2.92, respectively were identified (Fig. 2). The 'qSMD5' (bracketed in *CcM0970-CcM2485* region) was located on LG 1 accounted for 15.93% PV with a positive additive effect of 0.23. Similarly, 'qSMD6' (present in *CcM0416-CcM2337* region) was located on LG 3 explained 10.58% PV with a positive additive effect (0.18).

4. Discussion

Among various biotic stresses, SMD is considered to be the most important disease of pigeonpea causing a yield loss upto 95% (Kannaiyan et al., 1984). Development of cultivars with resistance to SMD is the best strategy to diminish cost of cultivation, soil and environment pollution. Though significant breeding efforts have been made in this direction but limited success has been achieved in developing SMD resistant cultivars. Availability of genomic resources such as molecular markers and genetic linkage maps would greatly facilitate identification and introgression of specific genomic regions associated with trait of interest. Without the availability of a genetic map, it is difficult to utilize molecular markers or to combine molecular and conventional genetic techniques in pigeonpea improvement programs. SSR are the markers of choice because they are ubiquitous throughout the genome, multi-allelic, co-dominant and breeder friendly (Gupta and Varshney, 2000). In pigeonpea, a very limited number of genomic resources were available so far and only a few SSR markers were developed. However, recent efforts at international level have facilitated development of a large number of genomic resources (Varshney et al., 2010). Since, there is no genetic map available in the cultivated pigeonpea

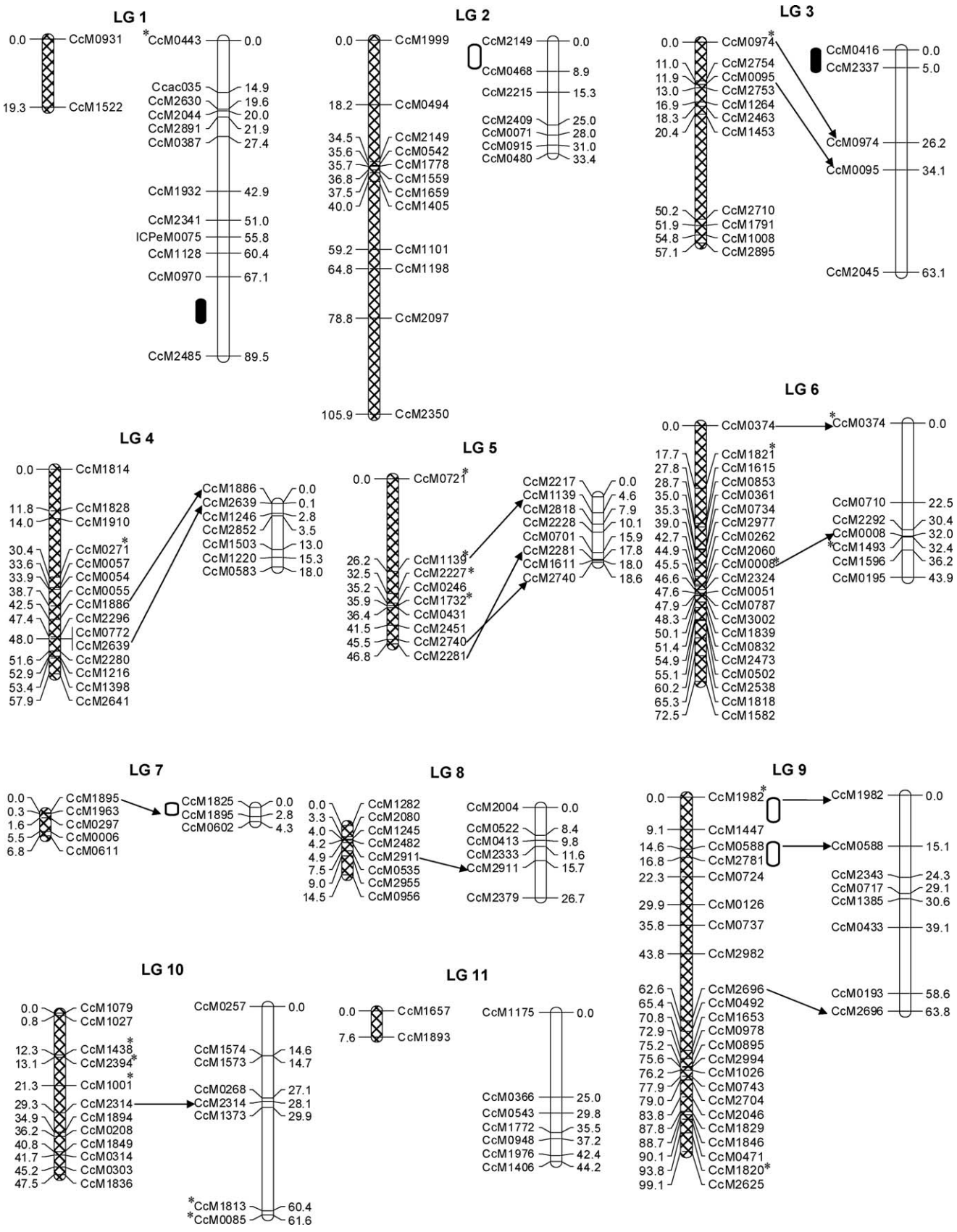

Fig. 3. Genetic linkage maps derived from intra-specific F_2 populations *viz.* ICP 8863 × ICPL 20097 (LGs are indicated by ▨) and TTB 7 × ICP 7035 (LGs are indicated by ▭). Maps were constructed using JoinMap 3.0. Markers are on right (ICP 8863 × ICPL 20097) and left (TTB 7 × ICP 7035) side of bar while distances are shown on left (ICP 8863 × ICPL 20097) and right (TTB 7 × ICP 7035) side of bar. The positions of QTLs associated with SMD resistance are indicated as white (for Patancheru SMD isolate) and black (for Bangalore SMD isolate) bars along different LGs. Common markers between two linkage maps are shown by arrows and distorted markers are indicated by asterisk (*).

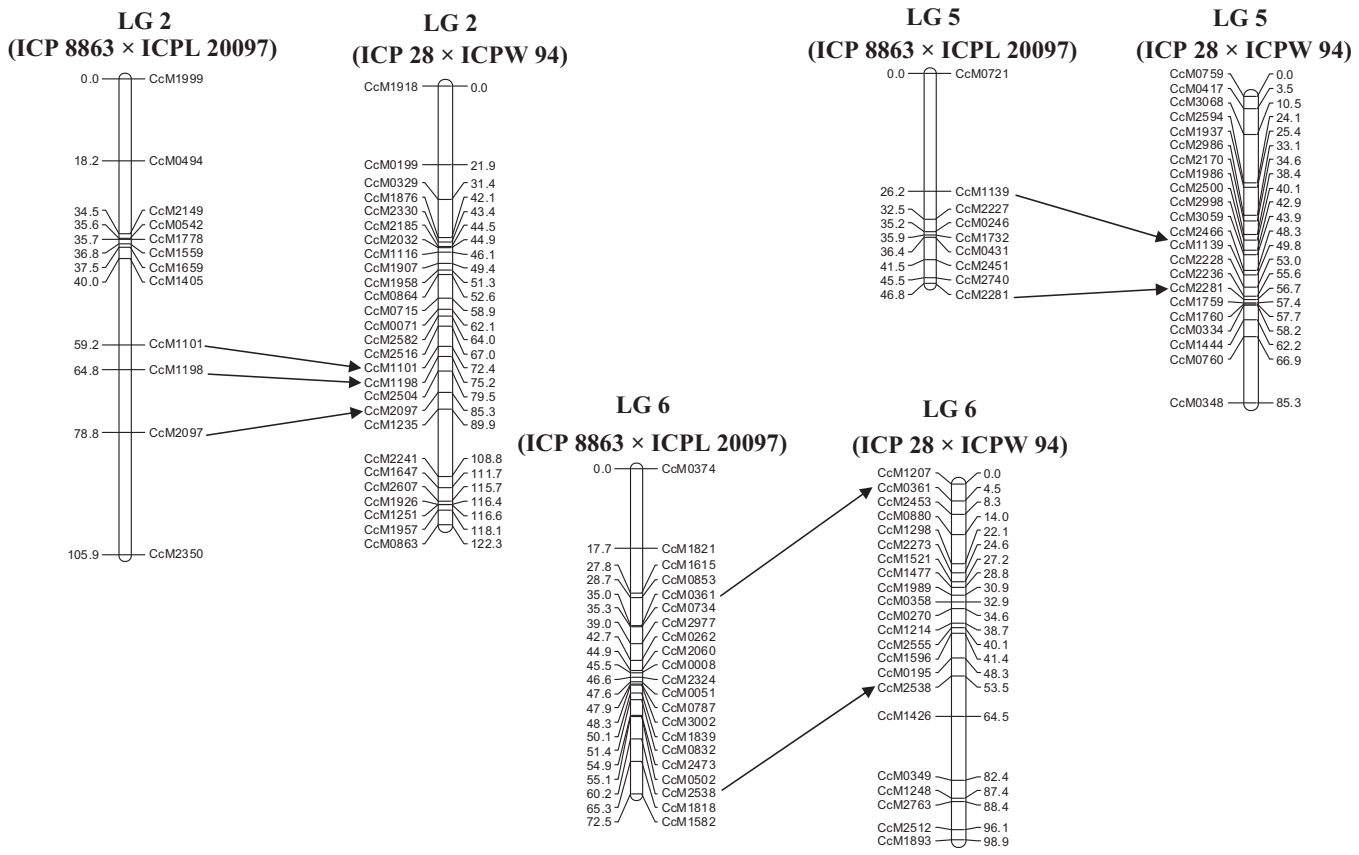

Fig. 4. Marker based correspondence between LGs of intra-specific (ICP 8863 × ICPL 20097) and inter-specific (ICP 28 × ICPW 94) genetic maps. Common markers are taken into consideration to show a good agreement of marker orders with reference genetic map (ICP 28 × ICPW 94). Arrows are indicating positions of common markers in two different LGs.

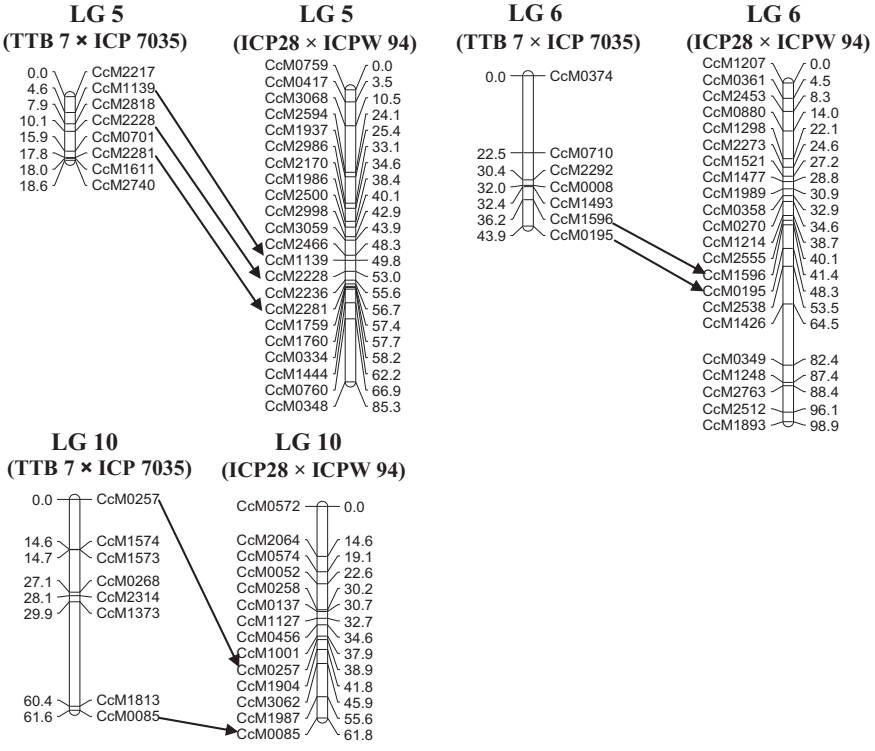

Fig. 5. Marker based correspondences between LGs of intra-specific (TTB 7 × ICP 7035) and inter-specific (ICP 28 × ICPW 94) genetic maps. A total of seven markers distributed among three different LGs exhibited a good agreement of marker orders between two genetic maps. The positions of common markers between two different LGs are shown by arrows.

Table 2
Composite interval mapping (CIM) analysis of QTLs associated with resistance to SMD in $F_{2:3}$ families.

QTLs	Linkage group (LG)	Position (cM)	Marker interval	LOD score	[a]R^2 (%)	[b]Additive effect	Dominance effect
Patancheru SMD isolate							
ICP 8863 × ICPL 20097							
qSMD1	LG 9	4.0	CcM1982-CcM1447	3.1	9.2	0.16	−0.16
qSMD2	LG 9	14.6	CcM0588-CcM2781	3.07	8.3	0.15	−0.13
TTB 7 × ICP 7035							
qSMD3	LG 2	0.01	CcM2149-CcM0468	3.86	12.32	0.24	−0.02
qSMD4	LG 7	2.01	CcM1825-CcM1895	6.74	24.72	0.33	−0.10
Bangalore SMD isolate							
TTB 7 × ICP 7035							
qSMD5	LG 1	83.08	CcM0970-CcM2485	3.35	15.93	0.23	−0.02
qSMD6	LG 3	0.01	CcM0416-CcM2337	2.92	10.58	0.18	−0.10

[a] R^2 = percent phenotypic variation (PV).
[b] Positive additive effect indicates that favorable alleles has come from susceptible parents, i.e. ICP 8863 and TTB 7.

so far; the present investigation emphasizes on development and phenotyping of mapping populations, construction of SSR based intra-specific genetic linkage maps and subsequent identification of QTLs contributing to SMD resistance.

4.1. Mapping populations and SMD phenotyping

The two mapping populations *viz.* ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035 consisting of 190 and 130 $F_{2:3}$ families respectively, exhibited significant variation in resistance to SMD. Genetics of SMD has been studied earlier, depending on the resistance source, SMD isolate and scoring method, resistance to SMD in pigeonpea appears to be complex (Saxena, 2008). In the present study, the patterns of frequency distribution of SMD were continuous indicating involvement of two or more segregating genes with majority of them were having increasing effects. However, large number of plants could be classified into categories of moderately resistant and susceptible class. As expected only a few plants were resistant against Bangalore SMD isolate due to its high virulent nature as compared to mild virulence of Patancheru SMD isolate (Kulkarni et al., 2003).

4.2. Marker polymorphism and intra-specific genetic maps

Even after using large number of SSR markers, a small number of markers showed polymorphism between the parental genotypes. Identification of 4.65% polymorphic markers in ICP 8863 × ICPL 20097 and 2.50% polymorphic markers in case of TTB 7 × ICP 7035 cross once again confirmed the narrow genetic base existing in cultivated pigeonpea genepool (Odeny et al., 2007; Saxena et al., 2010a). Furthermore, in comparison to the genomic SSRs, EST-SSRs were found less polymorphic (1.2%) which is due to greater DNA sequence conservation in transcribed regions (Varshney et al., 2005). Low level of polymorphism in parental genotypes of the mapping populations posed a challenge in developing a good genetic map. Hence, while developing mapping populations for the traits of interest, screening of different genotypes or germplasm using molecular markers and the combination of genotypes exhibiting higher polymorphism could be a better approach (Mace et al., 2006; Saxena et al., 2010b).

Two intra-specific genetic maps thus generated, consisted of 120 and 78 SSR marker loci covering all 11 LGs and spanned distances of 534.89 cM and 466.97 cM, respectively. The results were corroborative to that of Bohra et al. (2011) in terms of number of observed LGs, however, inter-marker distances were higher than found in case of inter-specific map for ICP 28 (*C. cajan*) × ICPW 94 (*C. scarabaeoides*) cross (one marker per 3.8 cM) as relatively large number of markers (239) were integrated into the inter-specific genetic map. Inter-specific genetic maps are constructed from wide crosses, i.e. between different species of same genus, to provide an opportunity for more DNA polymorphism ultimately leading to generation of high density genetic maps. On the other hand, intra-specific genetic maps are derived from narrow crosses (hybridization within species) making it more useful for direct application in cultivated gene pool. The intra-specific genetic maps developed in this study were compared in detail with each other and with the SSR-based reference inter-specific genetic map (ICP 28 × ICPW 94). In general, a good congruence was observed in marker orders not only in narrow but also in broad base genetic map. Occurrence of common markers across different mapping populations provides an opportunity for construction of consensus or composite linkage maps which would facilitate placement of more markers in a single genetic map for better genome coverage especially in case of cultivated crosses which mostly suffer from the problem of low polymorphism.

4.3. QTLs associated with SMD resistance

The present investigation is a pioneering attempt to identify QTLs associated with SMD resistance and it was carried out by using genotypic and phenotypic segregation data based on F_2 population and $F_{2:3}$ progenies respectively. In the present study, no common QTL was observed between two populations for the Patancheru and Bangalore SMD isolates indicating distinctiveness of these two isolates. Non-occurrence of common QTLs for Patancheru SMD isolate in two mapping populations suggests existence of different genomic regions associated with resistance to Patancheru SMD isolate. Identification of QTLs for SMD trait in pigeonpea is the first of its kind. As a result, no comparison could be made on QTLs identified in this study with other studies in pigeonpea, but was compared with other diseases in other crops. All the QTLs associated with SMD resistance were mapped near chromosome ends indicating that the linkage maps obtained need further saturation with additional markers like DArTs and SNPs which are amenable to high throughput genotyping.

All the six QTLs identified for Bangalore (detected from TTB 7 × ICP 7035) and Patancheru SMD isolates (detected from both ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035) inherited the "resistant" allele from the susceptible parents ICP 8863 and TTB 7, indicating the resistant alleles are present in the susceptible parent. This is not uncommon and has been reported in many plant species (Young et al., 1993; Lefebvre and Palloix, 1996; Pilet et al., 1998). Similarly, for early blight resistance in tomato, a QTL was detected on chromosome 3 inheriting "resistant" allele from the susceptible parent (Zhang et al., 2003). On the contrary, Phan et al. (2007) detected two regions significantly associated with anthracnose resistance on LG 4 and LG 17 at LOD of >3. These QTLs explain over 31 and 26% of the phenotypic variance respectively, and were inherited from the resistant parent P 27174.

It is however, important to mention here that though a total of six QTLs were identified for Bangalore and Patancheru SMD isolates from two populations. One QTL for Patancheru SMD isolate on LG 7 (TTB 7 × ICP 7035) explained a high phenotypic variation (24.72%) with LOD value of 6.74; similarly for Bangalore SMD isolate QTL on LG 1 (TTB 7 × ICP 7035) explained 15.93% PV with LOD value of 3.35. These QTLs having large phenotypic effects may be useful for marker assisted breeding in pigeonpea while deployment of small effect QTLs in breeding programme would require strategies based on complex crosses such as multi-parent advanced generation intercross (MAGIC) and marker assisted recurrent selection (MARS) to accumulate all the favorable but small effect QTLs into superior genotypes. Based on QTL mapping studies in other species, it can be generalized that sufficient amount of phenotypic variability for the given trait in mapping populations and high/reasonable marker density are pre-requisites to identify the major QTLs explaining higher phenotypic variation. For breeding purposes, QTL with large additive effect which are stable across environments without any epistatic interactions, are most desirable. Unfortunately, due to limitation of seed quantity, confirmation of the QTLs was not possible across different seasons for each isolate.

5. Conclusions

The first reference genetic linkage map in pigeonpea was developed using an inter-specific mapping population and the present study represents the first attempt towards development of linkage maps using SSR markers for cultivated pigeonpea. Low level of polymorphism, observed in the present study like in earlier studies, necessitates large scale development of markers such as DArTs and SNPs, so that genetic maps with reasonable marker density can be developed for cultivated pigeonpea in future. The present study also demonstrates the application of genetic map for identification of QTLs responsible for SMD resistance. In summary, the developed genetic map should be useful for the pigeonpea community and would be useful in integrating future genetic maps to synthesize a consensus map derived from multiple populations with better genome coverage, and to transfer the sequence information from model legume species like *Lotus* and *Medicago* for enhancing the knowledge of comparative genome evolution of legumes as well as pigeonpea improvement.

Acknowledgements

Authors are thankful to the Indo-US Agricultural Knowledge Initiative of Indian Council of Agricultural Research (ICAR) and Department of Biotechnology (DBT) of the Government of India and CGIAR Generation Challenge Programme for financial support to undertake this study. Thanks are also due to Dr Abhishek Rathore, ICRISAT; Dr T. Nepolean, Indian Agricultural Research Institute (IARI), New Delhi; Dr K. N. Ganapathy, Directorate of Sorghum Research (DSR), Hyderabad and Dr Y. P. Khedikar, University of Saskatchewan, Canada, for their valuable suggestions.

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