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Conservation of microsatellite regions across legume genera increases marker repertoire in pigeonpea

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Abstract

Microsatellite markers from chickpea, common bean, fieldpea and lentil were studied for their transferability and ability to reveal polymorphism in pigeonpea with an objective to use them in linkage map construction and tagging of agronomically important traits. Out of total one hundred and sixty three genic and genomic markers from four legume genera screened on six pigeonpea genotypes, 58 were found to be transferable in pigeonpea. Maximum transferability (47%) was shown by markers from common bean, followed by lentil, fieldpea and chickpea. The average polymorphism information content value with genic and genomic markers was found to be 0.60 to 0.50 respectively. These transferable markers will add to the pool of available markers for genotyping and mapping of important traits in *Cajanus*. This study also demonstrated that genic markers are not only transferable across genera but also are at par with genomic markers in detecting polymorphism.

Keywords: *Cajanus cajan*; genic SSRs; genomic SSRs; legumes; polymorphism; transferability.
Abbreviations: EST_Expressed Sequence Tags; PIC_Polymorphism Information Content; SSR_Simple Sequence Repeat; UPGMA_Un-weighted Pair Group Method with Arithmetic Mean.

Introduction

Pigeonpea (*Cajanus cajan* L. Millspaugh) is one of the major grain legume crops of the tropics and subtropics and belongs to the *Cajaninae* sub-tribe of the economically important leguminous tribe *Phaseoleae* that contains soybean (*Glycine max* L.), common bean (*Phaseolus vulgaris* L.) and mungbean (*Vigna radiata* L.). Out of total global production of 4.41 million tons, India alone contributes 2.9 million tons from 4.42 million ha area (FAOSTAT 2011). Inspite of its economic importance, the average productivity is low (740kg/ha) due to various biotic and abiotic production constraints. Conventional breeding efforts could not make much progress in improving productivity of pigeonpea mainly due to its narrow genetic base, high sensitivity to environmental factors and high genotype X environment (GE) interactions (Kumar and Ali, 2006). Moreover, limited research on pre-breeding efforts coupled with repeated use of only few parents in the current breeding programs have further narrowed down the genetic diversity of its cultivated gene pool (Singh et al., 2006). Presently, only a few cultivars are extensively grown in India, which accounts for more than 73% of the global production. This has led to erosion of a large number of land races, a major source for genes for stress tolerance in breeding programs. Recently, with the publication of draft genome sequence (Singh et al., 2011; Varshney et al., 2011), stage has been set now to enrich genomic resources to aid molecular breeding in pigeonpea.

Although, these genomic resources are expected to make available breeder-friendly tools like molecular markers, so far, studies on molecular diversity in pigeonpea have been limited by the paucity of PCR based polymorphic markers. Although the dominant markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) were occasionally employed in pigeonpea, but they tend to present reproducibility problems and were inadequate in assessing diversity within the species (Nadimpalli et al., 1992; Ratnaparkhe et al., 1995; Panguluri et al., 2006). Additionally, pigeonpea is an often cross-pollinated crop (upto 40% out-crossing) and maintenance of the genetic purity of a released cultivar possesses a serious problem (Ratnaparkhe et al., 1995; Souframanien et al., 2003). Therefore use of molecular markers, particularly the neutral markers to unravel genetic relationships between and among cultivars becomes very important. Microsatellite markers are highly polymorphic, locus specific, easily transferable, cost effective and distributed throughout the genome. Significant efforts have been made to develop microsatellite markers in many crop species during the last decade. They are now widely used for investigating genetic diversity among cultivars and genetic resources, for developing genetic maps suitable for Quantitative Trait Loci (QTL) detection studies and marker assisted selection programmes. Together, these characteristics make microsatellite loci one of the best markers for genetic mapping. Microsatellite markers have been developed from plant genomes from both coding and non-coding sequences containing simple repeats (Brown et al., 1996; Blair et al., 2003; Buhariwala et al., 2005). Genomic microsatellites are developed from non-coding regions of genome *viz.* introns or intergenic spacers. DNA libraries and/or enrichment procedure are often used to increase the prevalence of simple sequence repeats (SSR). SSRs are also found in gene coding regions (exons) of higher eukaryotes and recently many markers have been developed from expressed sequence tags

(EST) sequences or gene sequences (Eujayl et al., 2004) and are referred as genic markers. Sequence data obtained from several crop plants indicate sufficient homology existing between genomes in the region flanking the SSR loci. This allows primer pair designed on the basis of the sequence obtained from one crop to detect SSRs in related crop species. Such homology in the flanking region of SSRs loci has extended the utility of these markers to related species or genera where very little information on SSR is available. This phenomenon is sometimes described as transferability of microsatellite primers across species/genera. Several reports have established transferability of SSR markers from one genera/species to other (Pandian et al., 2000; Choumane et al., 2004; Eujayl et al., 2004; Datta et al., 2010a, b, 2012). Pandian et al. (2004) observed high level of sequence conservation of microsatellite markers across vetch, lentil, chickpea and fieldpea. Datta et al. (2010a, b, 2012) analyzed the transferability of microsatellite markers across different legume taxa and reported marker transferability from 36% - 95%. An effort to develop linkage map and studying germplasm diversity of pigeonpea has been slowed due to availability of only few polymorphic markers. As compared to large number of markers in common bean (Yu et al., 2000; Blair et al., 2003; L'taief et al., 2008), soybean (Cregan et al., 2004; Hisano et al., 2007), chickpea (Buhariwalla et al., 2005; Sethy et al., 2006 ; Qadir et al., 2007; Choudhary et al., 2009) and lentil (Hamwieh et al., 2005), only 141 microsatellite markers were reported till recently in pigeonpea (Burns et al., 2001; Odeny et al., 2007, 2009; Saxena et al., 2010) and these are derived from genomic sequences. Dutta et al. (2011) have reported 71 genic SSR markers by deep transcriptome sequencing. Recently, Bohra et al. (2011) have identified 842 polymorphic SSR markers from end sequences of BAC clones and using these markers, a SSR-based genetic map comprising of 239 loci was developed in pigeonpea. Although, this is a significant progress, there is still an urgent need to increase the number of polymorphic microsatellite markers in pigeonpea for diversity analysis and mapping of important traits. Moreover, transferability of markers from closely related legume species also allows studying genome synteny and considerably saves cost in marker development. Linkage maps are now available for a variety of leguminous crops including chickpea (Winter et al., 1999, 2000; Nayak et al., 2010), lentil (Hamwieh et al., 2009) and common bean (Blair et al., 2003). But till very recently, there was no genetic map available for pigeonpea and the two published maps do not cover the whole genome (Yang et al., 2011; Bohra et al., 2012). A saturated genetic map of pigeonpea based on microsatellite markers would be tremendously useful for marker assisted selection projects as well as further genetic studies. To this end, we have tried to develop a set of transferable microsatellite markers from other legume genera/species for mapping of different traits in pigeonpea along with other available microsatellite markers.

Results

In order to assess the transferability and polymorphism detection ability of microsatellite markers from four different legume genera in pigeonpea, genomic DNA from six pigeonpea cultivars were amplified with 163 markers, of which most were genomic microsatellite markers. Majority of markers were from chickpea (33%) and fieldpea (28%); whereas rest were from common bean (21%) and lentil (18%). Markers from common bean produced maximum amplification (47%), whereas, minimum amplification (26%) was recorded with chickpea markers (Table 1). Maximum numbers of genomic markers were from chickpea (44.2%) whereas maximum number of genic markers belongs to field pea (54%). These makers amplified a total of 92 alleles of which 57 alleles were from genomic markers and 35 were from genic markers in all the six genotypes studied. Maximum number of alleles (4) was amplified by PSBLOX13.1, PEAACPLHPPS and PSP4OSG whereas majority of markers amplified only one allele. Alleles that did not produce any amplicon in pigeonpea genotypes were considered as null alleles.

Transferability of legume microsatellite markers in pigeonpea

Microsatellite markers from common bean (34) exhibited highest transferability of 47%, whereas 30 markers from lentil showed 40% transferability (Fig.2, Table 1). The extent of genomic similarity between two species determines the extent of transferability and use of molecular markers from one species to other related species (Gupta et al., 2013).The high level of transferability of common bean markers in pigeonpea might be ascribed to the fact that common bean and pigeonpea belong to the same tribe *Phaseoleae*. Transferability of 45 fieldpea markers was also found to be low where only 35.5% transferability was recorded. Of the 54 markers from chickpea, only 25.9% were found to be transferable in pigeonpea. The low level of transferability of chickpea markers in *Cajanus* might be attributed to the fact that they belong to two different tribes, chickpea belongs to *Cicerae* and pigeonpea falls in *Phaseoleae* (Choi et al., 2004).

Transferability of genic microsatellite markers

Majority of genic markers used in the present study were from *Pisum* (27) and rest were from *Phaseolus* (19) and *Cicer* (4). Of the total 50 genic markers, only 19 markers produced good amplification. Out of 19 markers, BMd 47 and BMd 52 showed the maximum (100%) whereas BMd 53 and PEAATPSYND showed the minimum transferability (17%). In the six genotypes studied, Asha produced amplification with maximum (84.2%) markers, whereas, 36.8% markers amplified in 67 B (Fig. 3). The genic markers from *Cicer* did not amplify any allele in pigeonpea, however 47.3% transferability was observed with 19 genic markers from *Phaseolus.* Out of 27 genic markers from *Pisum*, only 10 (37%) amplified specific alleles in pigeonpea. Marker PEACPLHPPS was found to be most informative by amplifying highest number (4) of alleles. The overall transferability of the genic markers was found to be 38%.

Transferability of genomic microsatellite markers

A total of 113 genomic markers were used in the present study, out of which 50 (44.24%) were from *Cicer*, 15 (13.27%) from *Phaseolus*, 18 (15.9%) from *Pisum* and 30 (26.54%) from *Lens*. Total 39 markers amplified alleles in pigeonpea and showing a transferability frequency of 34.5%. Maximum transferability (100%) was observed with TR1, TA76 S, AGLC 34, AGLC 52, TR 26, NPS 7, NPS 13, SSR 13, SSR 59-2, SSR 99 and minimum (16%) with TA59, BMd 40, BMd 42. Of all the six genotypes studied, highest marker transferability (89%) was found in Asha, whereas, lowest transferability (43.5%) was seen in 67B. Average transferability with all the six genotypes was 62.5%. (Fig.3) Among genomic markers from four legume genera, maximum (46%) transferability was observed with markers

Table 1. Amplification results of genic and genomic markers from different legumes in pigeonpea.

	Chickpea		Common bean		Lentil		Field pea	
	Genic	Genomic	Genic	Genomic	Genic	Genomic	Genic	Genomic
Marker tested	4	50	19	15	-	30	27	18
No amplification	4	36	10	8	-	18	17	12
Monomorphic	-	7	2	0	-	3	-	-
Polymorphic	-	7	7	7	-	9	10	6

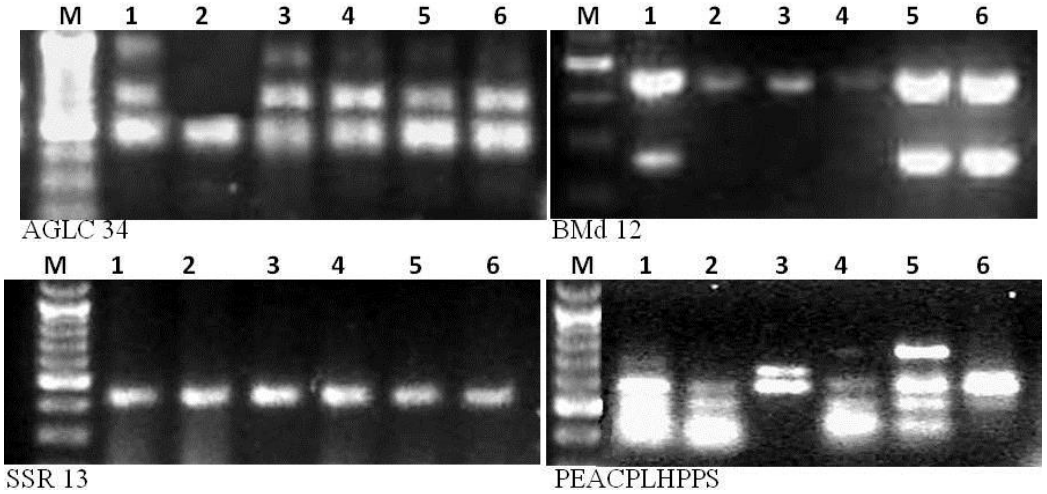


Fig 1. Amplification profile of different cross genera SSR markers in Pigeonpea genotypes. PCR amplification of markers AGLC 34, BMd 12, SSR 13 and PEACPLHPPS in pigeonpea genotypes. The PCR amplification products were separated on 3% Agarose gel and visualized under UV light in the presence of ethidium bromide. Lanes (left to right): 100 base pair DNA ladder, pigeonpea genotype Asha, UPAS 120, Bahar, 67-B, Type 7 and Dholi dwarf.

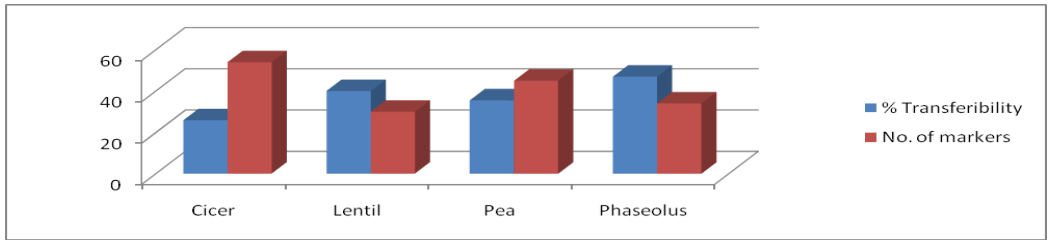


Fig 2. A summary of the average transferability obtained with cross genera legume markers in *Cajanus.*

derived from *Phaseolus* which was closely followed by markers from *Lens* (40%), whereas *Pisum* and *Cicer* markers showed transferability of 33% and 28% respectively.

Polymorphism with genic markers

Nineteen genic markers, which were transferable in pigeonpea, were further analyzed for their ability to reveal polymorphism. Amplification results based on presence/absence and size variation among alleles showed that 17 (89.5%) markers were polymorphic, whereas, only 2 markers produced monomorphic bands. These 19 markers amplified a total of 35 easily scorable alleles with an average of 1.84 alleles per locus, ranging from 100 to 450 base pairs. Maximum numbers of alleles (4) were amplified by marker PEACPLHPPS. Three alleles each were amplified by markers PSARGDECA and BMd 35. Two alleles each were amplified by BMd 27, BMd 28, BMd 47, BMd 48, BMd 55, PSGAPA1, PSY14273, PSU58830, PEAATPSYD, PEAOM14A and PSU51918. Nineteen markers amplified a total of 107 alleles in all the six genotype tested with an average being 17.8 alleles per genotype. Dholi dwarf amplified a maximum of 31 alleles whereas only 10 alleles were amplified in 67 B. Maximum PIC value was obtained with BMd 53 (0.97) and minimum (0.06) was observed with BMd 28 with the average PIC with all the genic markers being 0.60. With microsatellites derived from common bean, the similarity coefficient between cultivars ranged from 0.55 to 0.91 (Avg. 0.83) while markers from *Pisum* showed similarity coefficient value ranges from 0.13 to 1.0 (Avg. 0.56) indicating average genetic diversity of 16.9%.

Polymorphism with genomic markers

Of the 39 transferable genomic markers, 29 (74.3%) were polymorphic and 10 were monomorphic. Amplicon size ranging from 150 to 900 base pairs was obtained by the cross species amplifications. Maximum number (4) of alleles were amplified by markers PSBLOX13.1 and PSP4OSG while majority of markers amplified only one allele. Thirty nine markers amplified a total of 182 alleles in all the six genotype tested with an average of 30.3 alleles per genotype. Genotype Asha amplified a maximum of 35 alleles, whereas, only 17 alleles were amplified in the genotype 67 B. Maximum PIC value (0.99) was obtained with marker NPS 5 and minimum (0.31) with the markers AGLC 16, NPS 2, NPS 35, SSR 107, SSR130, SSR 212-1, SSR 233. The average PIC with all the genomic markers was 0.50. Genetic similarity matrix based on Jaccard's similarity coefficient with genomic markers ranged from 0.48 to 0.87 with the average being 0.77. Eight markers from *Phaseolus* showed similarity coefficient values ranging from 0.22 to 1.00 with an average 0.65. Microsatellite markers from *Lens* revealed similarity coefficient values ranged from 0.50 to 0.96 with an average of 0.82.

Comparison between genic and genomic markers

The Mantel matrix correspondence test (Mantel et al., 1967) was used to compare the similarity matrices and the correlation coefficient between genic and genomic SSRs was found to be 0.87. The test indicated that clusters produced based on genic and genomic microsatellite markers were conserved since the minimum required matrix correlation value was 0.80. The finding of this study showed that genic SSRs are equally good for polymorphism studies along with genomic SSRs. Hanai et al. (2007) observed similar results while comparing genic and genomic SSRs in common bean.

Genetic relationship and diversity

Polymorphism data from the transferable SSRs were used to test their potential in genetic studies by ascertaining the genetic diversity/interrelationship among the genotypes (Fig. 4). Unweighted Paired Group Method of Arithmetic Averages (UPGMA) dendrogram based on all the transferable markers clearly divided the six genotypes into two clusters. Two genotypes Asha and Dholi Dwarf grouped together in first cluster. The second cluster further subdivided into two sub clusters, where, genotypes UPAS 120 and Bahar grouped together in first sub cluster and 67 B and Type 7 in the second. Overall topology including clades within major clusters did not change except minor variation in dendrogram based on the genic and genomic markers separately as well as together.

Discussion

In this study, we tested the ability of genic and genomic SSRs from different legume genera to amplify in pigeonpea and reveal the polymorphism. The genic markers from *Phaseolus* showed maximum transferability. The possible reason behind this may be that *Cajanus* and *Phaseolus* belong to the same tribe, *Phaseoleae*, and share higher genome synteny. In our study, overall transferability of the genic microsatellite markers was greater (38%) than the genomic microsatellite markers (34.5%) which may be due to the fact that there is greater sequence conservation in transcribed region of the genome. Transferability of genic microsatellite markers to related species or genera has been demonstrated in several studies. In *Medicago truncatula* genic SSRs, Eujayl et al. (2004) observed that 89% of the genic SSRs were transferable. Similarly, Castillo et al. (2008) observed 100% transferability of barley genic microsatellite markers in *Hordeum chilense*, and 25.6% genic markers to be polymorphic. In a similar study with barley genic SSRs, Varshney et al. (2005a) could successfully amplify a sub-set of 165 genic-SSR markers of barley to wheat, rye and rice. A higher proportion, i.e., 78.2% of barley markers showed amplification in wheat followed by 75.2% in rye and 42.4% in rice. Furthermore, *in silico* comparison with 185 mapped barley genic-SSR loci against 13,69,182 publicly available cereal ESTs showed significant homology with ESTs of wheat (93.5%), rye (37.3%), rice (57.3%), sorghum (51.9%) and maize (51.9%). In addition, the genic SSRs are good candidates for the development of conserved orthologous markers for genetic analysis and breeding of different species (Varshney et al., 2005b). For example, a set of 12 barley EST-SSR markers was identified that showed significant homology with the ESTs of four monocotyledonous species (wheat, maize, sorghum and rice) and two dicotyledonous species (*Arabidopsis* and *Medicago*) and could potentially be used across these species (Varshney et al., 2005a). We found that the transferability rate of microsatellite markers from all four selected legume genera to pigeonpea was lower (35.6%) than the earlier reports in other legumes (Pandian et al., 2000; Choumane et al., 2004; Gutierrez et al., 2005; Datta et al., 2010a,b, 2012). Peakall et al. (1998) reported 65% cross-species amplification within the genus *Glycine.* Reddy et al. (2009) examined cross-genera transferability of SSR markers from *Trifolium pratense*, *Medicago truncatula*, and *Pisum sativum* and observed successful amplification of 62% *Trifolium* markers followed by *Medicago* (36%) and *Pisum*

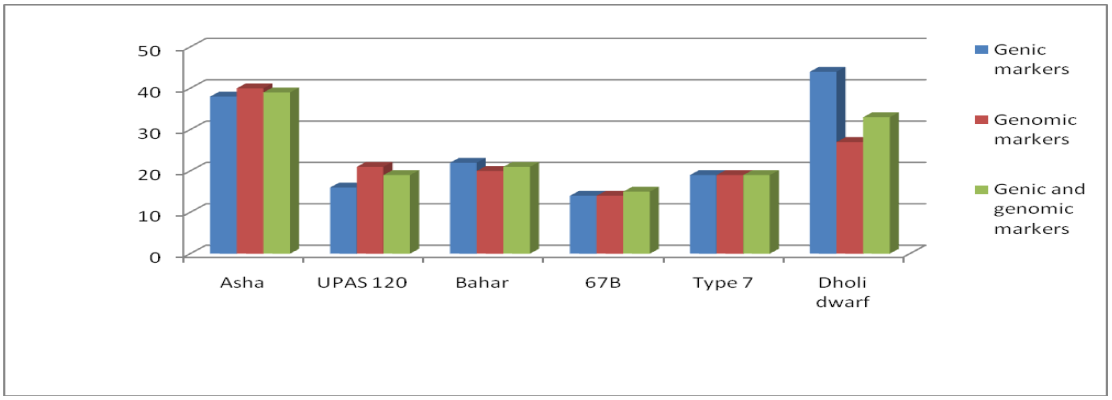
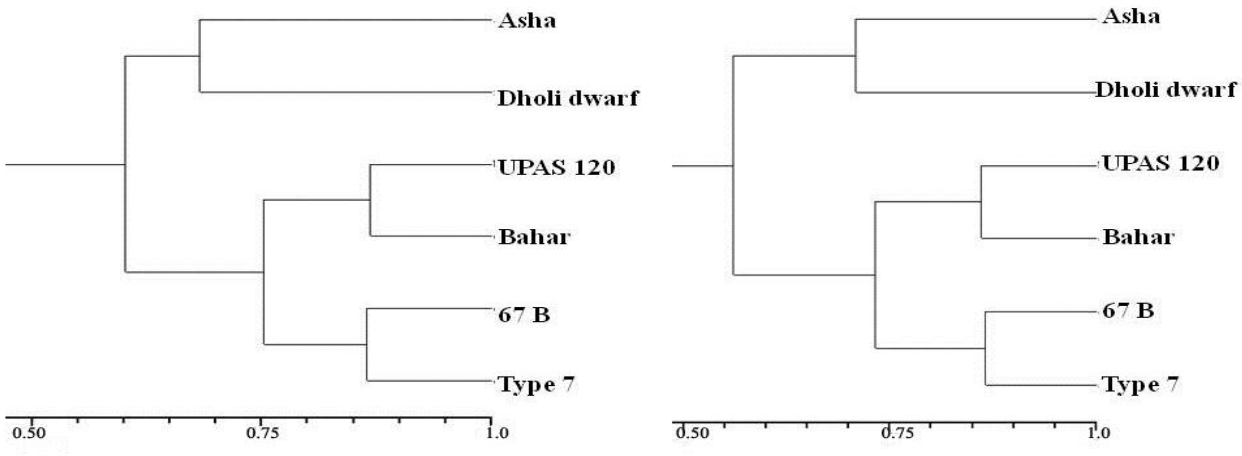


Fig 3. Average transferability of individual genotype with genic and genomic markers.



Similarity coefficient value with genic markers

Similarity coefficient value with genomic markers

Fig 4. Phylogenetic relationship among six pigeonpea genotypes based on amplification events obtained using genic and genomic sequences from cross genera legume species markers Dendrogram showing phylogenetic relationship among 6 *Cajanus cajan* genotypes generated from 58 genic and genomic SSR markers. Scale at the bottom of the dendrogram indicates the level of similarity between the genotypes.

(25%) in lentil. Gutierrez et al. (2005) reported 40%, 36.4% and 37.6% transferability of *M. truncatula* microsatellites in fababean, chickpea and fieldpea, respectively. In another study, Choumane et al. (2004) observed 54.4% sequence conservation among *Cicer*, *Lens* and *Pisum*. We have earlier analyzed the transferability of microsatellite markers across different taxa *viz*. *Lens*, *Cajanus*, *Lathyrus*, *Vigna* and *Phaseolus.* The transferability of microsatellite markers as determined through robust amplification on pigeonpea genotypes was 47%, 46%, 36% and 45% for common bean, chickpea, fieldpea and lentil derived markers, respectively. The transferability was 55% with chickpea markers and 65% with lentil markers. Similarily, 28% chickpea markers, 60% pigeonpea markers and 46% lentil specific markers were transferrable to *Phaseolus* species (Datta et al., 2010 a, b, 2012). These results indicate that transferability of markers amplifying the microsatellite loci across related genera/species have vast potential for their utilization genomics enabled improvement of food legumes.The lower rate of marker transferability in few cases, compared well with the results of Pandian et al. (2000) who reported 5% and 18% transferability of microsatellite markers from *Cicer* to *Lens* and *Pisum*. The transferability rate of SSRs depends on many factors which includes number of markers used for SSR amplification, PCR conditions and plant species involved in the study. It has been observed that genomic microsatellite markers exhibits low transferability when used across genera (Peakall et al., 1998). Evolutionary distance and rate of evolution also contributes in amplification of particular locus from one plant species with primer designed from another species (Torres et al., 2008). The conserved primer binding sites in all four legume genera and pigeonpea can be exploited for enhancing the genomic resources for comparative genome analysis within and beyond legumes and will also contributes as additional markers for diversity analysis and mapping of for useful genes in pigeonpea. The microsatellite markers derived from EST are considered less powerful in the discrimination of genotypes than genomic region derived SSR marker. This is mainly due to the conserved nature of the EST sequences. Eujayl et al. (2001) compared genic and genomic SSR markers to investigate genotypic variation of 64 durum wheat lines, land races, and varieties obtaining 255 polymorphic loci among 137 EST SSR markers and 505 among 108 genomic SSR markers with

an average of 4.1 and 5 alleles per locus, respectively. However Scott et al. (2000) found the opposite results in the case of apricot and grape, where the genic microsatellite markers showed higher polymorphism. In our study, EST derived microsatellite markers showed higher polymorphism percentage and PIC value (89.5%, 0.60) as compared to that of genomic markers (74.3%, 0.50). Odeny et al. (2009) tested transferability of 161 genic-SSRs from soybean to pigeonpea and found 24 markers were polymorphic in pigeonpea. Similar results were obtained by Hempel and Peakall (2003); they found 23 primers pairs transferable from *Glycine max* to its wild relative *G. clandestina* and produced 19 polymorphic loci. Recently, Dutta et al. (2011) observed high level polymorphism (PIC 0.63) in pigeonpea with genic SSR markers of ≥ 20 bp. In a similar study, Yue et al. (2004) found that microsatellite derived from EST's showed higher allele number than the ones isolated from genomic libraries. This finding was true in our study also, genic markers were found to amplify higher number of alleles (1.84) per locus than the genomic markers, which amplified 1.46 alleles per locus. Several studies have found that genic microsatellite markers are useful for estimating genetic relationship and at the same time provides provide opportunities to examine functional diversity in relation to adaptive variations. The low level of polymorphism detected with genic SSRs in several other studies may be compensated by their higher cross species/genus transferability. Although cross species/genera amplification was observed with genomic SSRs as well, comparatively higher rate of transferability has been observed in this study especially across genera.

Material and Methods

Plant material and DNA extraction

Total genomic DNA from six pigeonpea genotypes (which have been used as parents in mapping population development at IIPR, Kanpur, India) was isolated from young leaves, using modified CTAB method (Abdelnoor et al., 1995). The extracted DNA was purified with RNase treatment (10 µg/ml) for 1 hour at 37°C followed by treatment with phenol: chloroform: isoamyl alcohol (25:24:1). The pellet was dissolved in appropriate amount of TE (Tris10mM, EDTA 1mM) buffer. DNA from different samples was quantified both by visual quantification, by comparing the DNA band intensity with that of λ DNA of known concentration, as well as through UV spectrophotometer and finally diluted to a concentration of 5 ng/µl.

Microsatellite markers and PCR amplification

A total of 163 SSR primer sequences representing 54 from chickpea (*Cicer arietinum*) (Winter et al., 1999; Buhariwalla et al., 2005; Qadir et al., 2007), 34 from common bean (*Phaseolus vulgaris*) (Blair et al., 2003), 30 from lentil (*Lens culinaris* ssp. *culinaris*) (Hamwieh et al., 2005) and 45 from pea (*Pisum sativum*) (Burstin et al., 2001) were used for PCR amplification to study transferability as well as their utility in parental polymorphism within six genotypes, which are used as mapping parents (Supplementary Table 1 and Supplementary Table 2). The length of these primers varied from 18 to 29 nucleotides. These primers were custom synthesized from Operon Technologies, Alameda, USA. PCR amplification was performed in a 5 µl reaction volume containing 1X PCR buffer (10 mM Tris HCl pH 9.0, 1.5 µM $MgCl_2$, 50 mM KCl, 0.01% gelatin), 0.2 mM each of dNTP (Bangalore Genei), 0.1 U of *Taq* DNA polymerase (Bangalore Genei, Bengaluru) and 5 pmol each of forward and reverse primers. PCR tubes contained 5 ng of genomic DNA as template. An initial denaturation was given for 3 min. at 95°C. Subsequently, five touch-down PCR (Don et al., 1991) cycles comprising of 94°C for 20 s, 60°-55°C (depending on the marker as given in supplementary Table 1) for 20 s, and 72°C for 30 s were performed. These cycles were followed by 40 cycles of 94°C for 20 s with constant annealing temperature (depending on marker) for 20 s, and 72°C for 20 s, and a final extension was carried out at 72°C for 20 min. All PCR amplicons were resolved by electrophoresis on 3% agarose gel and visualised with ethidium bromide under UV illumination to identify the informative SSR loci across all the six genotypes. GeneRuler 100 bp DNA ladder (MBI Fermentas, La Jolla, CA, USA) was used to estimate the allele size (Fig.1). The gels were run for 4 hours at 45 volts and SSR fingerprint profiles were recorded with BioRad Gel Doc XR version 2.0. The SSR bands were scored as present (1) or absent (0) for each primer genotype combination and a binary raw matrix was generated.

Molecular data analysis

The amplification data generated by transferable markers were analyzed using SIMQUAL route to generate Jaccard's similarity coefficient (Jaccard 1908) using NTSYS-PC, software version 2.1 (Rohlf 1998). These similarity coefficients were used to construct a dendrogram depicting genetic relationships among the cultivars by employing the (UPGMA) algorithm and Sequential, Agglomerative, Hierarchical, Non-overlapping (SAHN) clustering. The Polymorphism Information Content (PIC) (Anderson et al., 1993) was calculated for each marker using the following equation:

$$(PIC_i) = 1 - \sum_{j=1}^{n} P_{ij^2}$$

Where, P_{ij} is the frequency of the j^{th} allele for the i^{th} marker, and summed over 'n' alleles. In order to estimate the congruence among dendrograms, product moment correlation (r) was computed and compared using Mantel statistics (t) in MXCOMP program.

Conclusions

Our study suggests that microsatellites can be utilized across species or sometimes even across unrelated genera. This is not surprising, because the coding sequences for similar functions should remain conserved over a wide range of species, so that the microsatellites and their flanking sequences, carried by these coding sequences, should also remain conserved. The transferability results suggest that a proportion of microsatellites can certainly be utilized for comparative genome mapping in legumes. The transferable genic SSR markers can be further tested for studying genome structure and evolution. The present study clearly indicates potential for the transferability of SSR markers from cross-genera legumes, thus circumventing laborious cloning and screening procedures involved in characterizing SSR loci for

pigeonpea. These markers would be useful for improving availability of markers of breeder's choice for genetic diversity analysis and ultimately toward development of a widely distributed and well-saturated linkage map of pigeonpea. The present study has also provided insights into the genetic base of few selected important pigeonpea cultivars in India and will enable breeders to involve new germplasm in hybridization programs.

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