Highly informative genic and genomic SSR markers to facilitate molecular breeding in cultivated groundnut (Arachis hypogaea)

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Highly informative genic and genomic SSR markers to facilitate molecular breeding in cultivated groundnut (*Arachis hypogaea L.*)

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Abstract

With an objective of identification of highly informative set of SSR markers in cultivated groundnut (*Arachis hypogaea* L), a total of 4,485 markers were used for screening using a set of 20 parental genotypes of 15 mapping populations. Though 3,582 (79.9%) markers provided scorable amplification, only 1,351 (37.3%) markers could show polymorphism. The polymorphism information content (PIC) value ranged from 0.10 (GM742) to 0.89 (S009) with an average of 0.31. Similarly number of alleles ranged from 2 to 14 with an average of 3.2 alleles. In general, the SSR markers based on di-nucleotide repeats displayed higher PIC value and number of alleles. Based on these polymorphism features, 199 markers with >0.50 PIC values have been identified. Polymorphism features of these markers along with the primer sequences, for the first time, for a total of 946 SSR markers have been provided. It is anticipated that the identified set of highly informative markers, instead of starting from the random set of SSR markers, should be very useful to initiate molecular genetics and breeding studies in cultivated groundnut.

Key words: Groundnut, Molecular breeding, SSR markers, PIC value, Genetic diversity

Introduction

Groundnut (*Arachis hypogaea* L.), the 3rd most important oilseed crop in the World, is grown extensively throughout the semi-arid tropics (SAT) of Asia, Africa and Latin America with its global production of 35.52 million tons from 23.5 million ha area (FAOSTAT 2009). It is a self-pollinating crop with ten basic chromosomes and allotetraploid genome ($2n = 4x = 40$, AABB) (Stebbins 1957, Stalker and Dalmacio 1986). The origin of cultivated groundnut was probably through a few or even a single hybridization event between two diploid wild species, *A. duranensis* (A-genome) and *A. ipaënsis* (B-genome), followed by a spontaneous chromosome duplication (Halward et al. 1991). The resulting tetraploid plant (AABB genome) was then reproductively isolated from its wild diploid relatives (AA and BB genome). This extreme bottle-neck, coupled with reproductive isolation lead to a limited genetic diversity within the groundnut primary gene pool.

For crop improvement, genetic enhancement of cultivated groundnut to increase the yield and resistance / tolerance to biotic and abiotic stresses have been the most important goals. Although efforts made through conventional breeding has had some measure of success, expected progress could not be achieved in handling complex traits such as tolerance to drought, either due to lack of reliable, precise and cost effective high-throughput phenotyping or due to fertility barriers that hampers the harnessing the genetic variation present in secondary and tertiary gene pool (and even sometimes from primary gene pool also). Recent advances in the area of crop genomics have offered molecular tools to assist breeding (Varshney et al. 2005a). Introgression of desired chromosomal segment in the progeny through precise monitoring using trait-linked marker, the process called marker-assisted selection (MAS) has been successfully applied in several cereal

and some legume crops resulting in development of improved varieties / germplasm (Varshney et al. 2006). Availability of molecular markers and genetic linkage maps are, however, the pre-requisites for undertaking molecular breeding activities particularly identifying and localizing important genes controlling qualitatively and quantitatively inherited traits (Varshney et al. 2006). Such tools would then simply speed up the process of introgression of agronomically desired traits such as yield, quality, biotic and abiotic stress resistance into preferred varieties, especially for complex traits such as drought.

Molecular marker analysis on groundnut germplasm by using a variety of molecular markers such as microsatellites or simple sequence repeats (SSRs), randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphism (AFLPs) in general have shown very low variation in cultivated gene pool because of the evolutionary genetic bottleneck in the form of polyploidy and self pollination (Kochert et al. 1996, Subramaninan et al. 2000, Herselman 2003). On the other hand, wild diploid *Arachis* species showed relatively higher variation (Hilu and Stalker 1995, Moretzsohn et al. 2004, Bravo et al. 2006), providing a rich source of genetic variation for genetic and genomic studies (Stalker and Simpson 1995, Rao et al. 2003, Dwivedi et al. 2007). Among different marker systems analyzed in the groundnut, like other plant species, SSR markers have been found more informative and useful for genetic analysis and breeding applications (Gupta and Varshney 2000).

In the case of groundnut, several hundreds SSR markers have been developed and characterized during last five years all over the World (Hopkins et al. 1999, He et al. 2003, Palmieri et al. 2002, 2005, Ferguson et al. 2004, Moretzsohn et al. 2004, 2005, Nelson et al. 2006, Mace et al. 2007, Proite et al. 2007, Gimenes et al. 2007, Wang et al.

2007, Cuc et al. 2008, Gautami et al. 2009, unpublished markers from University of California-Davis, USA and University of Georgia, USA). However, the development of even low- to moderate- density genetic maps using populations derived from cultivated germplasm has been hindered by the requirement of screening very large numbers of SSR markers to find a sufficient number of polymorphic markers (Varshney et al. 2009a, Khedikar et al. 2010, Ravi et al. 2011, Sarvamangala et al. 2011). The availability of the polymorphism information content (PIC) values and number of alleles detected by a large set of SSR markers would help groundnut community to select the most informative markers to screen the germplasm thus economizing time and cost in the development of the genetic and QTL maps. Here, we have screened, a large number (4485) of SSR markers available in public domain as well as accessed through collaborators across the World on 20 parental genotypes of 15 mapping populations being developed for mapping different traits. An analysis of the marker polymorphism data allowed the identification of a highly informative SSR marker set.

Materials & Methods

Plant material: A total of 20 genotypes representing parents of 15 mapping populations segregating for resistance / tolerance to biotic and abiotic stresses were used to screen with SSR markers (Table 1, 2). This set includes drought tolerant genotypes (ICGS 44, ICGS 76, CSMG 84-1 and ICGV 86031), drought sensitive genotypes (TAG 24 and Chico), resistant genotypes for different foliar disease (GPBD 4, ICG 11337, ICGV 86590, R 9227, ICG (FDRS) 10 and TxAG-6) and susceptible to foliar diseases (TAG 24, JL 24, GPBD 5, TG 19, TG 26 and TMV-2). In addition, two AA- genome (diploid)

species genotypes (K7988 and V10309) and a synthetic allotetraploid genotype (TxAG-6) developed from the cross *A. batizocoi* and (*A. cardenasii* × *A. diogoi*) was also included in the set.

DNA isolation: Total genomic DNA was isolated from unopened leaves harvested from 10-15 days old seedlings according to modified CTAB-based method as given in Cuc et al. 2008. The DNA quality and quantity were checked on 0.8% agarose gels and DNA concentration was normalized to ~5 ng/µl for PCR.

Polymerase chain reaction (PCR) with SSR markes: A total of 4485 SSR markers, as given in Table 3 were used for screening the genotypes.

PCR reactions for all the markers were performed in 5 µl volume following a touchdown PCR profile in an ABI thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR reaction was comprised of ~5 ng of genomic DNA, 2 picomoles of each primer, 2 mM of each dNTP, 2mM $MgCl_2$, 1X amplification buffer and 0.1 U of Taq DNA polymerase (Qiagen, Hilden, Germany). The touchdown PCR amplification profile had initial denaturation step for 3 min at 94°C followed by first 5 cycles of 94°C for 20 sec, 65°C for 20 sec and 72°C for 30 sec, with 1°C decrease in temperature each cycle, followed by 35 cycles of 94°C for 20 sec with constant annealing temperature (59°C) for 20 sec and 72°C for 30 sec, followed by a final extension for 20 min at 72°C. The amplified products were tested on 1.2% agarose gels to check the amplification.

SSR fragment analysis: After confirmation for amplification, PCR products were diluted to varied folds (60-100) and used for multiplexing based on different fluorescent labels and amplicon length. Markers that had different labels and allele size ranges were

considered together along with markers with the same label separated by more than 50 bp. Formamide (1µl) was added to each well containing PCR product (1µl) along with GeneScan 500 standard (Applied Biosystems) internal lane standard labelled with either ROX or LIZ. GeneScan Filter Set D and the ROX 500/LIZ 500 internal lane were used for analysis of amplicons labelled with different fluorescent dyes such as FAM, VIC, NED, PET, HEX and TAMARA. Allele sizing and scoring based on capillary electrophoresis (ABI 3700 Genetic Analyzer-Applied Biosystems) data was carried out using GENESCAN 3.1 software (Applied Biosystems). PCR products for a few markers were also analzyed on 6% non-denaturingpolyacrylamide gels (PAGE) (29:1 acrylamide/bisacrylamide) and visualized by silver staining as given in Varshney et al. (2009).

Data analysis: Major allele frequency, gene diversity and PIC values for all loci were computed using allelic data with PowerMarker V3.25 (Liu and Muse 2005). For assessing the genetic relationships among the genotypes, allelic data were converted into binary form i.e., 0 and 1. The similarity matrix was computed using Jaccard's coefficient utilizing the unweighted pair group method with arithmetic averages (UPGMA) method and further a Neighbor Joining (NJ) dendrogram was constructed using the software NTSYSpc version 2.02 (Rohlf 2000).

Results

Marker analysis

A set of 20 groundnut genotypes representing parents of 15 mapping populations were screened with 4,485 SSR markers to identify a set of highly informative SSR markers to use in genetic analysis and breeding applications in groundnut (Table 1, 2, 3). All these SSR markers were initially optimized on 2 genotypes (ICGV 86031 and TAG 24) for PCR components and PCR profiles. Out of 4485 primers pairs screened, 3582 (79.9 %) primer pairs provided scorable amplification with a touchdown PCR profile. Subsequently these 3582 primer pairs only used for screening on 5-16 genotypes out of a total 20 genotypes. Although a total of 1351 (37.7 %) markers showed polymorphism, high-quality scoring data for at least 11 genotypes were available for only 1020 SSR markers. Primer sequence information along with the polymorphism features of 946 new SSR markers have been provided in ESM 1.

Polymorphism features

All identified polymorphic markers (1020) detected a total of 3214 alleles with an average of 3.2 alleles per marker. The number of alleles per marker ranged from 2 for 463 markers to 14 for 2 markers namely GNB18 and GNB515 per marker**.** Similarly, the PIC values for polymorphic markers ranged from 0.10 (GM742) to 0.89 (S009) with an average of 0.31 per marker. In total, only 15.67% markers had PIC value more than 0.50.

In terms of marker polymorphisms per mapping population, a higher level of polymorphism was detected in AA-genome mapping population namely K7988 × V10309 (60.5%) followed by followed by TMV 2 × TxAG-6 (42%) (Table 4). The remaining populations showed comparatively very low polymorphism ranging from 2.4% (GPBD 5 × GPBD 4) to 11.1 % (Chico × CSMG 84-1) and average being 7.09 % per population.

Polymorphism trends

Due to unavailability of repeat motif information for 16 SSR markers, the relationship between types of SSRs with number of alleles and PIC value was analyzed for 1004 polymorphic SSR markers. Based on the repeat motifs, all markers were classified into three classes namely Class I (<10 repeat units), Class II (>10 repeat units) and compound SSRs (more than one type of repeats are present). By using these criteria, the Class I contained 323 (32.2%) markers, Class II had 609 (60.6%) markers and the compound SSR class included 72 (7.2%) markers. In Class I type markers, di- (124) and tri- (164) repeat motifs were abundant followed by tetra- (19), penta- (5) and hexa- (8) nucleotide repeats (Table 5). The average PIC values for these repeat motifs varied from 0.28 (hexa-nucleotide SSRs) to 0.43 (di-nucleotide SSRs). As compared to 5 types of repeat motifs in the case of Class I SSR markers, the Class II markers possessed only 3 repeat types i.e., di- (142), tri- (443) and tetra- (29) nucleotide repeats with an average PIC values as 0.31, 0.26, and 0.24, respectively. It is noteworthy that di-nucleotide repeats from both the classes (Class I and Class II), in general, produced large number of alleles (upto 14) while hexa-nucleotide repeats could produce only 2-3 alleles per markers (Table 5, Fig. 1). In summary, a negative correlation was observed between repeat motifs and average number of alleles produced by markers for both classes. Similarly, negative correlation was also observed between repeat motifs and PIC value. Markers with larger repeat motifs tended to have lower PIC values. In contrast, as expected, there was a positive correlation between average number of alleles and PIC values. Considering the PIC values, there were 199 SSR markers that showed high (>0.50) PIC values. This set is recommended as an informative set of SSR markers that can be used as a starting point for undertaking genetic analysis and breeding applications in groundnut. The markers of

this set detect 3-14 alleles with an average of 4.87 per markers. However after excluding the AA- genome (K7988, V10309) and synthetic amphidiploid genotype (TxAG-6), the average PIC value and no of alleles detected by these markers are reduced to 0.56 and 3.84, respectively (Table 6).

Comparison between genomic and genic SSRs

All 1020 polymorphic markers were classified into genomic and genic SSRs based on their origin from genomic vs transcribed portion i.e. ESTs. As a result, 260 markers were found to belong to genomic SSR and 760 to genic SSR classes. In terms of comparison of markers from these two classes, the PIC values of all the polymorphic SSR markers were analyzed in terms of the above mentioned two classes. While higher PIC value (>0.50) was shown for 29.6% genomic and 9.5% genic SSR markers, the remaining 70.1% genomic and 90.5% genic SSR markers had the lower PIC value (<0.50) (Table 7, Fig. 2). This clearly indicates that genomic SSR markers as compared to genic SSR markers display more polymorphism.

Genetic relationships among parental genotypes

Based on the allelic data obtained for all 1020 polymorphic SSR loci on 11 parental genotypes, a similarity matrix was generated (ESM 2). Similarity index of these 1020 marker loci ranged from 0.044 to 0.842. It was found that the two most closely related genotypes were ICGS 44 and ICGS 76 with the highest similarity index (0.842). On the other hand two most distantly related cultivars were T*x*AG-6 and ICG 11337 with lowest similarity index (0.044). Similarity matrix was used to prepare dendrogram using software NTSYSpc which grouped 11 tetraploid parental genotypes into three major clusters Cluster A (‘cl A’), Cluster B (‘cl B’) and Cluster C (‘cl C’) (Fig. 3). While ‘clB’

(ICG 11337) and ‘clC’ (TxAG-6) contained single genotype each, the ‘clA’ contained remaining 9 genotypes. The major cluster, ‘cl A’ is consisting of two sub clusters i.e. ‘cl AI’ (ICGS 44, ICGS 76, CSMG 84-1) and ‘cl AII’ (ICGV 86031, TAG 24, TG 26, GPBD 4, TMV 2, JL 24).

Discussion

In many regions of the World, the genetic yield potential of groundnut is not reached because of biotic and abiotic stresses. Marker-assisted selection offers an important tool to enhance tolerance/resistance to these stresses and has the potential to enable faster and larger gains through genetic improvement. However, until recently the implementation of marker assisted selection was severely hampered by the very limited genomic resources available for groundnut (Varshney et al. 2007). Over the last few years about 5000 SSR markers have been developed for groundnut (Ferguson et al. 2004, Moretzsohn et al. 2004, Nelson et al. 2006, Proite et al. 2007, Wang et al. 2007, Cuc et al. 2008, Liang et al. 2009). However only a few hundred SSR markers have been mapped. This was mainly because of two reasons: (a) limited genetic diversity in the mapping populations, and (b) use of limited number of SSR markers by different research groups. While low level of genetic diversity is an inherent genetic constraint in cultivated groundnut, we reasoned that the identification of a highly informative set of SSR markers would help the community focus marker screening on potentially polymorphic markers instead of using all available SSR markers, most of which have a low potential. Therefore an attempt was made to identify a highly informative set of SSR markers using a starting set of >4400 SSR markers, and 20 genotypes representing parents of 15 mapping populations.

Although 1351 SSR markers showed polymorphism in the genotypes analysed in the study, only 1020 SSR markers that had high quality data for at least 11 out of 20 genotypes were fully analyzed. Out of the 1020 polymorphic markers, the highest polymorphism was obtained in the diploid AA-genome mapping population (60.5%) followed by TMV 2 × TxAG-6 (42%) population. On the other hand a low level of polymorphism was observed in the mapping populations of cultivated genotypes ranging from 2.4% (GPBD 5 × GPBD 4) to 11.1% (Chico × CSMG 84-1) with an average of 5.58%. A high level of polymorphism (46.8% of SSRs and ca. 1 single nucleotide polymorphism/90 bp) has been previously observed earlier in the AA-genome mapping population (K7988 × V10309, Moretzsohn et al. 2005, Bertioli et al. 2009). Similarly in the mapping population involving synthetic amphidiploid (TxAG6) a high polymorphism (66.0 %) rate has been previously observed (Burrow et al. 2001). The genetic base of the cultivated groundnut is very narrow, and the low levels of genetic diversity observed in cultivated material in the present study is in line with that of earlier studies (Varshney et al. 2009a, Khedikar et al. 2010, Ravi et al. 2011, Sarvamangala et al. 2011).

Number of alleles detected and the PIC value based on the frequencies of different alleles in the germplasm surveyed by a particular marker indicates the quality (discriminatory power) of the marker. Number of alleles ranged from 2-14 (average 3.2) per marker in the present study was high as compared to the earlier genetic diversity studies (He et al. 2003, Krishna et al. 2004, Moretzsohn et al. 2004, Cuc et al. 2008, Gautami et al. 2009, Liang et al. 2009) as they reported 2 to 8 alleles per marker. Comparable results (2-13 alleles) by Song et al. (2010) and higher alleles (2-20) by Varshney et al. (2009b) were also reported. Similarly, the PIC value for polymorphic markers ranged from 0.10 to 0.89 with an average of 0.31 per marker. In total, only 15.67% markers could show PIC value

more than 0.50. The PIC values observed here are in the agreement of earlier genetic diversity studies (Mace et al. 2006, Cuc et al. 2008, Gautami et al. 2009, Liang et al. 2009, Varshney et al. 2009b). Like earlier studies (Varshney et al. 2002, Moretzsohn et al. 2005, Song et al. 2010), the present study also reported di- and tri-nucleotide repeats SSR markers as highly polymorphic markers. While a negative correlation was observed between the PIC value and repeat unit classes, a positive correlation observed between PIC value and number of alleles (Cuc et al. 2008).

In terms of comparison of informativeness of SSR markers based on the origin of DNA sequences, the genomic SSRs showed higher level of polymorphism as compared to genic SSR markers. This is in agreement to general conception that genic SSRs show low level of polymorphism as compared to genomic SSRs as genic SSRs originate from highly conserved portion of the genome (Varshney et al. 2005b). Hence, we suggest that development of genomic SSR markers should be given priority over genic SSRs in crops like groundnut that have a narrow genetic background.

The dendrogram constructed based on allelic data for all 1020 polymorphic markers classified all the genotypes into 3 groups. Majority of the genotypes clustered according to their pedigree and origin. It has also been found that even though the parents of the mapping population were found to be diverse based on the morphological traits, they (ICGS 44 and ICGS 76) clustered together with the highest similarity index (0.842). This has also reflected in polymorphism percentage between two populations developed using 3 parents (ICGS 44, ICGS 76, CSMG 84-1) for drought related traits (ICGS 44× ICGS 76 and ICGS 76 × CSMG 84-1). The population derived from the cross ICGS 76 × CSMG 84-1 showed higher polymorphism (4.9%) as compared to the population developed from

the cross ICGS 44× ICGS 76 (3.4%). Two most distantly related cultivars were TxAG 6 and ICG 11337 with low similarity index (0.044) and grouped separated in two clusters. This is because TxAG 6 is a synthetic amphidiploid derived from the cross *A. batizocoi* × (*A. cardenasii* × *A. diogoi*) and TMV 2 being a cultivated variety.

The most important feature of this study is the identification of a set of 199 SSR markers that have higher PIC values and have the potential to detect more alleles in a set of germplasm accessions, or more polymorphism between a pair of parentals. This set was identified after analyzing a range of genotypes including cultivated, two AA-genome species genotypes and one synthetic amphidiploid. Therefore, the markers of this set should be very useful for genetic analysis in wild *Arachis* species as well as applications in the groundnut molecular breeding. The use of this SSR marker set should economize screening time and would facilitate the cross-references of genetic maps, including the linking of cultivated maps to information-rich diploid maps, and a unified genetic map for the legumes (Bertioli et al. 2009, Foncéka et al. 2009, Leal-Bertioli et al. 2009, unpublished results). Therefore, we recommend that the community should give the identified set of SSR markers priority while framing strategies for studying genetic diversity, linkage mapping, QTL analysis and marker-assisted breeding.

In summary, this study reports the primer sequences for 946 novel SSR markers for the first time, the analysis of 4,485 SSR markers on a set of 20 genotypes and the identification of a most informative set of 199 SSR markers. We hope that the details provided in tables and ESM 1 for all polymorphic SSR markers in addition to the

informative set of SSR markers will benefit international groundnut research and molecular breeding.

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Table 1: Pedigree of parental genotypes used in the study

S. No.	Genotypes	Pedigree	Botanical type	Market type	Origin
1	ICGS 44	Robut 33-1-1-5-B1-B1-B2	*vulgaris*	Spanish	India
2	ICGS 76	TMV 10 × CHICO	*hypogaea*	Virginia	India
3	ICGV 86031	F 334 A-B-14× NC Ac 2214	*vulgaris*	Spanish	India
4	ICGV 86590	X 14-4-B-19-B × PI 259747	*hypogaea*	Virginia	India
5	ICGV 11337	Cs 46	-	-	India
6	CSMG 84-1	Selection from MA 10	*hypogaea*	Virginia	India
7	TAG 24	TG S2 × TGE 1	*hypogaea*	Virginia	India
8	TG 19	TG 17 × TG 1	*hypogaea*	Virginia	India
9	TG 49	TG 28A × TG 26	*vulgaris*	Spanish	India
10	TG 26	BARCG 1 × TG 23	*hypogaea*	Virginia	India
11	GPBD 4	KRG 1 × CS 16 (ICGV 86855)	*vulgaris*	Spanish	India
12	GPBD 5	TG 49 × GPBD 4	*vulgaris*	Spanish	India
13	TMV 2	Mass selection from Gudhiatham bunch	*vulgaris*	Spanish	India
14	TxAG-6	[A. *batizocoi* × (*A. cardenasii* × *A. Diogoi*)]^{4x}	-	-	USA
15	R 9227	ICGS 7 × (NC Ac 2214 × ICGV 86031)	*vulgaris*	Spanish	India
16	JL 24	Selection from EC 94943	*vulgaris*	Spanish	India
17	Chico	Short duration genotype	v*ulgaris*	Spanish	USA
18	ICG (FDRS) 10	Ah 65 × NCAc 17090	*vulgaris*	Spanish	India
19	K7988	*A. duranensis* (AA genome)	*duranensis*	-	Brazil
20	V10309	*A. stenosperma* (AA genome)	*stenosperma*	-	Brazil

Table 2: Details of the mapping populations available based on genotypes used in the analysis

S. No.	Mapping populations	Source	Segregating traits
Abiotic tolerance			
1	ICGS 44 × ICGS 76	ICRISAT, India	Drought tolerance related traits viz., transpiration, transpiration efficiency, specific
2	ICGS 76 × CSMG 84-1	ICRISAT, India	leaf area and SPAD chlorophyll meter reading (SCMR)
3	TAG 24 × ICGV 86031	ICRISAT, India	
4	Chico × CSMG 84-1	ICRISAT, India	
5	K7988 × V10309	EMBRAPA, Brazil	Linkage mapping (diploid, AA genome)
Biotic resistance			
6	TMV 2 × TxAG-6	ICRISAT, India	Late leaf spot (LLS) resistance, root-knot nematode
7	ICG 11337 × JL 24	ICRISAT, India	Late leaf spot (LLS) resistance
8	JL 24 × ICG(FDRS) 10	ICRISAT, India	Late leaf spot (LLS) resistance
9	TAG 24 × GPBD 4	UAS-Dharwad, India	Rust and late leaf spot (LLS) resistance
10	TG 26 × GPBD 4	UAS-Dharwad, India	
11	GPBD 5 × GPBD 4	UAS-Dharwad, India	
12	TG 19 × GPBD 4	UAS-Dharwad, India	*Aspergillus* crown rot, rust and late leaf spot (LLS) resistance
13	TG 49 × GPBD 4	UAS-Dharwad, India	
14	TAG 24 × R 9227	UAS-Dharwad, India	*Sclerotium* rot resistance
15	JL 24 × ICGV 86590	DGR, Junagadh, India	Rust and *Sclerotium* rot resistance

ICRISAT: International Crops Research Institute for the Semi Arid Tropics, Hyderabad, India; UASD: University of Agricultural Sciences-Dharwad, India, DGR: Directorate of Groundnut Research, Junagadh, India

Table 3: Source of markers used for polymorphism survey among parental genotypes of 15 mapping populations

S. No.	Series	No. of markers	Source of markers
1	Ah, Lec	26	Hopkins et al. 1999
2	pPGPseq, pPGSseq	226	Ferguson et al. 2001
3	Ap	18	Palmeri et al. 2002, 2005
4	PM	59	He et al. 2003
5	AC, Ah, gi, RN, TC, Seq	338	Moretzsohn et al. 2004, 2005
6	LG, Lup	103	Nelson et al. 2006
7	Lup, Dal, Stylo, Ades, Amor, Chaet	51	Mace et al. 2007
8	RN, RM	53	Proite et al. 2007
9	Ah	14	Gimenes et al. 2007
10	S	123	Wang et al. 2007
11	IPAHM	104	Cuc et al. 2008
12	GA	97	Nagy et al. 2009
13	ICGM	23	Gautami et al. 2010
14	GM	2098	S. J. Knapp, UG, Georgia, USA (Unpublished)
15	GNB	1152	Doug R Cook, UC, Davis, USA (Unpublished)
Total markers		**4485**	

Table 4: Comparative marker polymorphism in different parental combinations

Mapping population	No. of markers tested	No. of markers amplified (%)	No. of polymorphic markers	% polymorphism
ICGS 44 × ICGS 76	4245	2637 (62.1)	90	3.4
ICGS 76 × CSMG 84-1	4245	2582 (60.8)	129	4.9
TAG 24 × ICGV 86031	4485	2620 (58.4)	211	8.1
TAG 24 × GPBD 4	4100	2737 (66.7)	163	5.9
TMV 2 × T*x*AG-6	3222	1571 (48.4)	660	42.0
ICG 11337 × JL 24	3099	1227 (39.6)	82	6.7
TG 26 × GPBD 4	4100	2202 (53.7)	142	6.4
TG 19 × GPBD 4	1152	715 (62.1)	26	3.6
TG 49 × GPBD 4	1152	685 (59.5)	27	3.9
GPBD 5 × GPBD 4	1152	673 (58.4)	16	2.4
TAG 24 × R 9227	1152	546 (47.4)	16	2.9
JL 24 × ICGV 86590	1152	748 (64.9)	35	4.7
JL24 × ICG (FDRS) 10	2070	1305 (63.0)	112	8.6
Chico × CSMG 84-1	2070	1330 (64.2)	148	11.1
K7988 × V10309	1947	660 (33.9)	399	60.5

Table 5: Distribution of polymorphic markers into different repeat classes

SSR type	Repeat classes	Polymorphic markers	PIC value range (mean)	Number of alleles (mean)
Compound		72 (7.2 %)	0.12-0.80 (0.39)	2-9 (3.5)
Class I	NN	124 (38.4%)	0.11-0.86 (0.43)	2-14 (3.9)
	NNN	164 (50.8%)	0.11-0.76 (0.36)	2-6 (2.9)
	NNNN	19 (5.9%)	0.11-0.49 (0.32)	2-4 (3.0)
	NNNNN	8 (2.5%)	0.11-0.54 (0.31)	2-4 (2.8)
	NNNNNN	8 (2.5%)	0.12-0.43 (0.28)	2-3 (2.5)
	Total	323 (32.2)	0.11-0.86 (0.36)	2-14 (3.3)
Class II	NN	142 (23.3%)	0.11-0.89 (0.31)	2-11 (3.0)
	NNN	443 (72.7%)	0.11-0.87 (0.26)	2-14 (2.6)
	NNNN	24(3.9)	0.12-0.61 (0.24)	2-5 (2.6)
	Total	609 (60.6%)	0.11-0.89 (0.27)	2-14 (2.7)
Grand total		1004	0.11-0.89 (0.31)	2-14 (3.2)

Table 6: Details of highly polymorphic markers identified in the present study

S. No.	Markers	Total genotypes		Cultivated genotypes	
		Allele no.	PIC values	Allele no.	PIC values
1	pPGPSeq04D02	3	0.56	3	0.50
2	pPGPSeq04G01	3	0.56	3	0.56
3	pPGPSeq15F12	4	0.67	4	0.67
4	TC11F12	3	0.56	2	0.36
5	TC11H06	4	0.67	5	0.64
6	TC2B09	4	0.67	3	0.56
7	TC3B05	3	0.56	4	0.61
8	TC3G05	3	0.56	3	0.56
9	TC4F12	4	0.67	4	0.67
10	TC7A02	4	0.67	4	0.67
11	TC7E04	4	0.67	5	0.64
12	gi-427	3	0.56	3	0.56
13	IPAHM177	3	0.56	3	0.56
14	IPAHM229	3	0.56	6	0.77
15	IPAHM395	4	0.67	4	0.67
16	IPAHM509	4	0.67	4	0.67
17	IPAHM689	5	0.77	5	0.77
18	IPAHM93	3	0.56	3	0.56
19	PM183	3	0.56	3	0.50
20	PM238	3	0.56	3	0.50
21	PM3	4	0.67	4	0.67
22	PM35	4	0.67	3	0.50
23	PM434	4	0.67	4	0.67
24	S001	8	0.81	7	0.80
25	S003	5	0.62	3	0.49
26	S009	11	0.89	3	0.56
27	S011	5	0.58	3	0.41
28	S016	4	0.55	5	0.72
29	S019	9	0.85	4	0.61
30	S021	5	0.62	4	0.61
31	S022	3	0.50	8	0.84
32	S023	6	0.75	4	0.58
33	S024	5	0.68	3	0.47
34	S026	4	0.56	5	0.70
35	S038	8	0.76	3	0.55
36	S040	6	0.75	2	0.36
37	S046	4	0.62	4	0.58

38	S048	3	0.58	7	0.76
39	S049	8	0.80	6	0.75
40	S052	6	0.72	3	0.56
41	S057	6	0.76	3	0.55
42	S059	4	0.55	7	0.80
43	S068	8	0.80	6	0.77
44	S070	5	0.69	6	0.77
45	S072	3	0.55	3	0.50
46	S073	4	0.64	7	0.80
47	S076	5	0.64	4	0.65
48	S080	5	0.69	3	0.55
49	S083	6	0.78	4	0.61
50	S084	4	0.67	4	0.61
51	S086	5	0.68	4	0.65
52	S093	5	0.71	6	0.77
53	S096	4	0.53	4	0.69
54	S101	3	0.54	4	0.61
55	S108	4	0.62	9	0.87
56	S113	5	0.69	3	0.59
57	S118	5	0.64	3	0.47
58	GM1043	4	0.60	3	0.49
59	GM1073	4	0.56	3	0.57
60	GM1076	4	0.50	3	0.44
61	GM1089	4	0.53	2	0.37
62	GM1097	4	0.52	3	0.50
63	GM1098	5	0.59	3	0.44
64	GM1202	4	0.54	3	0.50
65	GM1256	3	0.54	3	0.57
66	GM1357	5	0.60	2	0.37
67	GM1369	4	0.54	2	0.35
68	GM1411	3	0.56	3	0.50
69	GM1469	4	0.50	2	0.35
70	GM1477	5	0.61	2	0.35
71	GM1483	5	0.60	2	0.37
72	GM1489	4	0.62	4	0.57
73	GM1501	4	0.54	2	0.37
74	GM1502	7	0.74	4	0.48
75	GM1515	4	0.54	3	0.50
76	GM1533	5	0.67	4	0.69
77	GM1538	3	0.51	2	0.29
78	GM1555	4	0.58	3	0.53
79	GM1562	3	0.56	3	0.50

80	GM1565	4	0.50	2	0.35
81	GM1575	3	0.52	3	0.50
82	GM1577	5	0.69	6	0.75
83	GM1664	3	0.59	4	0.48
84	GM1745	3	0.55	3	0.34
85	GM1760	5	0.58	4	0.57
86	GM1773	3	0.54	4	0.66
87	GM1834	5	0.61	2	0.37
88	GM1839	3	0.50	2	0.29
89	GM1842	4	0.56	3	0.50
90	GM1845	3	0.59	2	0.18
91	GM1863	5	0.72	6	0.79
92	GM1864	5	0.73	6	0.76
93	GM1869	3	0.50	2	0.35
94	GM1879	3	0.55	4	0.66
95	GM1907	3	0.50	4	0.57
96	GM1911	5	0.69	5	0.68
97	GM1937	4	0.60	4	0.61
98	GM1949	3	0.53	3	0.59
99	GM1954	4	0.57	4	0.57
100	GM1958	4	0.52	3	0.49
101	GM1959	6	0.65	3	0.49
102	GM1960	4	0.50	2	0.35
103	GM1977	4	0.53	2	0.35
104	GM1986	6	0.78	4	0.66
105	GM1991	6	0.71	4	0.57
106	GM1992	4	0.53	3	0.34
107	GM1996	6	0.73	5	0.68
108	GM2009	7	0.77	4	0.57
109	GM2024	4	0.57	3	0.53
110	GM2053	4	0.65	4	0.64
111	GM2084	5	0.64	3	0.53
112	GM2103	5	0.62	3	0.49
113	GM2165	4	0.58	3	0.59
114	GM2206	5	0.50	3	0.44
115	GM2215	4	0.56	3	0.57
116	GM2348	4	0.70	3	0.34
117	GM2407	4	0.53	2	0.37
118	GM2444	5	0.60	3	0.50
119	GM2478	6	0.51	2	0.18
120	GM2482	3	0.52	3	0.44
121	GM2504	7	0.74	5	0.70

122	GM2522	4	0.52	3	0.50
123	GM2528	6	0.54	3	0.34
124	GM2531	6	0.51	3	0.34
125	GM2589	5	0.52	4	0.48
126	GM2602	6	0.70	3	0.44
127	GM2603	4	0.58	4	0.57
128	GM2605	7	0.78	4	0.64
129	GM2606	5	0.50	2	0.29
130	GM2623	4	0.54	2	0.37
131	GM2637	6	0.75	5	0.68
132	GM2638	5	0.68	6	0.71
133	GM2671	4	0.57	2	0.35
134	GM2730	4	0.62	4	0.69
135	GM2746	4	0.50	4	0.57
136	GM744	8	0.85	7	0.79
137	GM761	3	0.50	2	0.29
138	GM822	5	0.72	6	0.79
139	GM840	9	0.86	6	0.79
140	GM995	5	0.65	4	0.61
141	GNB 1069	5	0.63	3	0.54
142	GNB 1072	9	0.64	9	0.70
143	GNB 1148	5	0.53	2	0.38
144	GNB 1056	5	0.62	3	0.49
145	GNB 1112	4	0.53	3	0.50
146	GNB 1114	7	0.79	6	0.76
147	GNB 1055	5	0.68	3	0.50
148	GNB 18	14	0.86	8	0.79
149	GNB 38	4	0.56	3	0.55
150	GNB 608	3	0.53	3	0.52
151	GNB 58	8	0.63	7	0.68
152	GNB 73	4	0.61	3	0.57
153	GNB 428	5	0.71	5	0.70
154	GNB 98	6	0.68	5	0.65
155	GNB 643	3	0.50	3	0.53
156	GNB 100	5	0.64	4	0.57
157	GNB 461	4	0.53	2	0.37
158	GNB 107	5	0.69	3	0.59
159	GNB 464	6	0.79	6	0.79
160	GNB 126	5	0.56	3	0.50
161	GNB 667	9	0.68	5	0.58
162	GNB 136	7	0.70	6	0.70
163	GNB 467	5	0.70	3	0.49

164	GNB 679	5	0.70	5	0.69
165	GNB 682	10	0.76	8	0.76
166	GNB 145	6	0.73	4	0.64
167	GNB 155	6	0.69	4	0.57
168	GNB 159	4	0.62	4	0.66
169	GNB 167	5	0.67	4	0.61
170	GNB 178	5	0.68	5	0.73
171	GNB 181	5	0.67	4	0.64
172	GNB 712	5	0.72	3	0.57
173	GNB 716	4	0.60	3	0.54
174	GNB 981	4	0.55	3	0.49
175	GNB 733	5	0.66	4	0.61
176	GNB 991	5	0.52	4	0.57
177	GNB 515	14	0.87	10	0.84
178	GNB 1001	5	0.72	4	0.64
179	GNB 569	6	0.68	4	0.66
180	GNB 775	4	0.58	3	0.54
181	GNB 782	4	0.53	3	0.50
182	GNB 262	5	0.66	3	0.57
183	GNB 1026	4	0.51	2	0.18
184	GNB 1027	6	0.71	6	0.77
185	GNB 284	4	0.58	3	0.57
186	GNB 303	7	0.75	4	0.69
187	GNB 317	5	0.68	5	0.68
188	GNB 344	4	0.66	4	0.57
189	GNB 357	5	0.72	5	0.70
190	GNB 840	8	0.82	5	0.70
191	GNB 842	9	0.75	5	0.61
192	GNB 850	3	0.51	3	0.50
193	GNB 378	6	0.62	5	0.62
194	GNB 853	5	0.64	3	0.49
195	GNB 387	5	0.54	4	0.57
196	GNB 392	4	0.51	2	0.35
197	GNB 397	4	0.55	3	0.45
198	GNB 555	7	0.74	5	0.73
199	GNB 417	5	0.68	4	0.64
	Average	4.86	0.63	3.84	0.56

Table 7: Variation in PIC value between genomic and genic SSRs

S. No.	PIC range	Genomic SSRs		Genic SSRs		Total markers	
		Number	(%)	Number	(%)	Number	(%)
1	0.10-0.20	54	20	284	37.4	339	32.8
2	0.21-0.30	59	21.8	196	25.8	255	24.7
3	0.31-0.40	35	12.9	146	19.2	181	17.6
4	0.41-0.50	32	11.8	62	8.2	94	9.11
5	0.51-0.60	24	8.9	44	5.8	68	6.6
6	0.61-0.70	39	14.4	15	1.9	54	5.2
7	0.71-0.80	21	7.8	11	1.4	32	3.1
8	0.81-0.90	6	2.2	2	0.3	8	0.77
Total markers		260		760		1020	

Figure 1: Relationships of average number of alleles detected and PIC values of SSR markers with their respective classes and repeat types

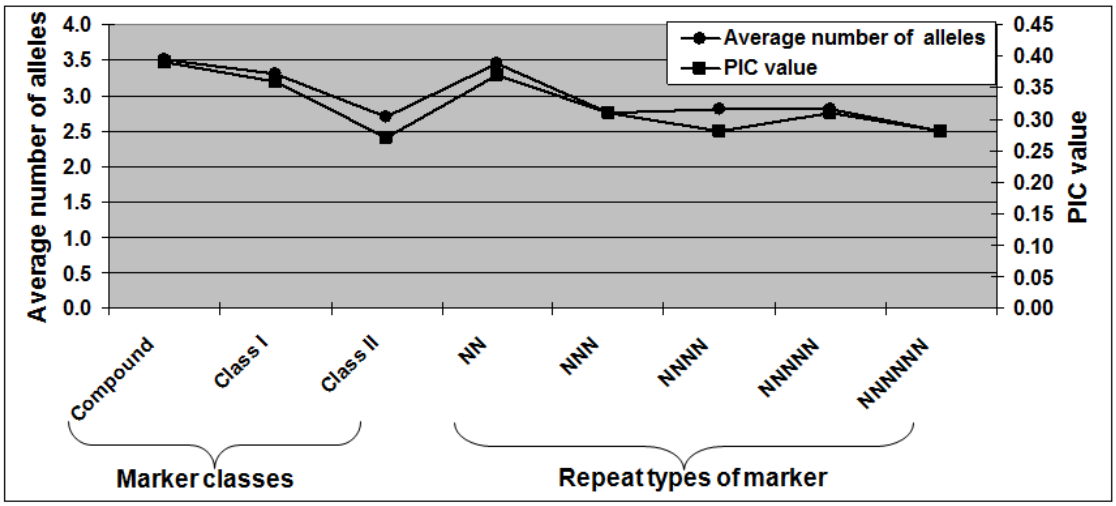


Figure 2: Classification of polymorphic genomic and genic SSR markers into different classes of PIC values

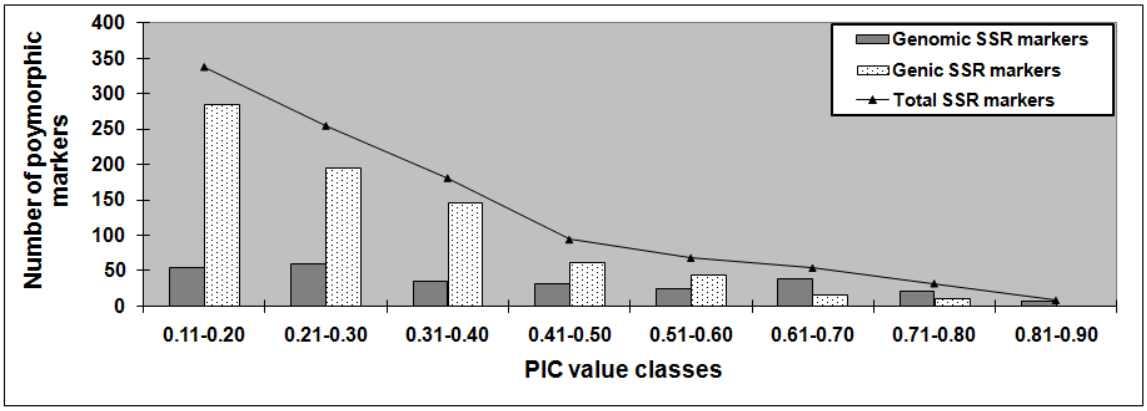
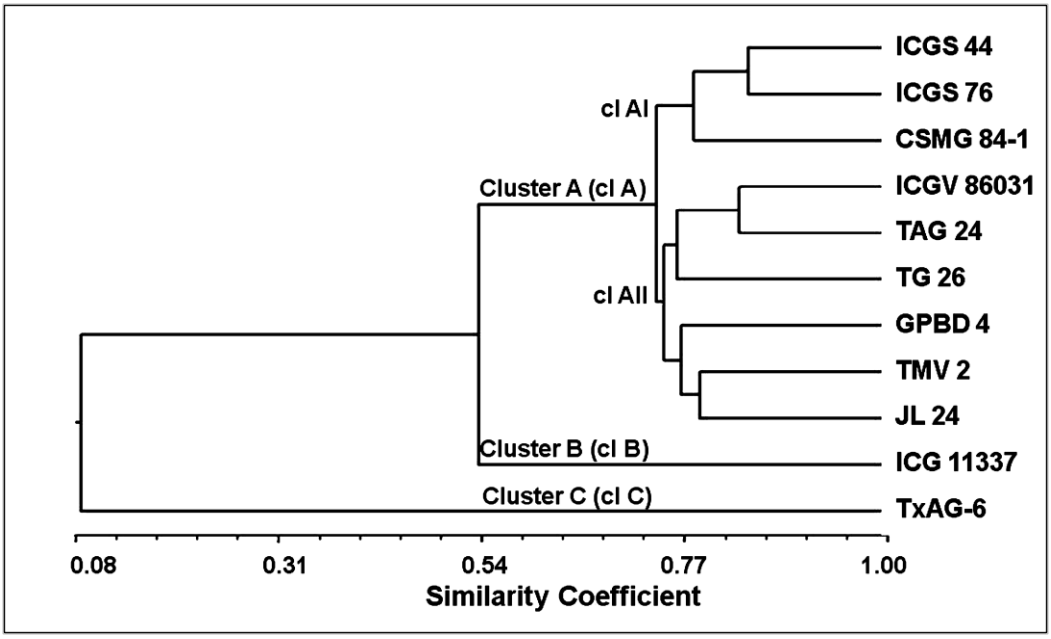


Figure 3: Dendrogram showing genetic relationship among parental genotypes of different mapping populations



Legends for ESM:

ESM 1: Details of new polymorphic genomic and genic SSR markers in groundnut

This ESM provides details on repeat motifs, product size, forward sequence, reverse sequence, number of alleles and polymorphic information content (PIC).

ESM 2: Genetic similarity among 11 groundnut genotypes based on 1020 polymorphic markers

This ESM provides details on genetic similarity distance among 11 groundnut genotypes.