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Review Article

Assessment of Molecular Diversity in Groundnut through Simple Sequence Repeats (SSR) Markers - A Review

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Abstract: Groundnut (*Arachis hypogaea* L.), an important oilseed crop is a rich source of oil and protein. Molecular marker technologies are the effective tools and they are used for the assessment of genetic variability because they are not influenced by the environment. Among the molecular markers, Simple Sequence Repeat (SSR) has proved to be the most powerful tool for variety identification in groundnut and has much potential in genetic and breeding studies. Through morphological variability was reported to be very high in groundnut, but the polymorphism revealed at DNA level using different SSR primers was found to be low initially. With the use of more number of primer combinations, substantial polymorphism was detected among different cultivars of cultivated groundnut species. SSR or Microsatellite-based markers therefore represent a useful tool for dissecting genetic variations in cultivated crops, especially groundnut. The SSR markers could discern variations and differentiate between the closely related groundnuts genotypes, makes this technology a powerful tool for genomic characterisation of groundnut.
Keywords: Simple Sequence repeat (SSR) markers, Polymorphism, Molecular diversity, Groundnut

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed crops in the world. Currently, China, India and Nigeria account for largest groundnut production in the world, (FAO, 2014). In India, 70 per cent of the groundnut area and 75 per cent of the production are concentrated in the four states of Gujarat, Tamil Nadu, Andra Pradesh and Karnataka. It is difficult to classify the accessions solely based on their morphological characters. The development of reliable methods is necessary to allow for the assessment of genetic variability in germplasm collections or pedigree reconstruction. In various methodologies, DNA based technologies are the most reliable tools allowing for the assessment of genetic variability because they are not influenced by the environment SSR markers have great potential in genetic and breeding studies.

In any plant breeding programme, assessment of parental divergence is an important and foremost objective. The threat to genetic erosion has led to a significant interest in the assessment of genetic diversity in germplasm collections (Manifesto *et al.*, 2001). It helps in identifying the desirable parents for hybridization programme. Molecular markers are useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar identification early in plant development. The molecular markers based on differences in DNA sequences between individuals generally detect more polymorphisms than morphological and protein based markers (Mignouna *et al.*, 1998; Tanksley *et al.*, 1989). Simple Sequence Repeats (SSR) is used as a primer to amplify regions between the microsatellites. This marker reveals a much larger number of fragments per primer than RAPD analysis (Bajpai *et al.*, 2008).

The molecular tools such as DNA markers are becoming increasingly important as effective tools in crop breeding programmes but their application in genetic enhancement of groundnut is lagging behind due to limited knowledge of the genome. Wide variation for morphological and physiological characteristics in both wild and cultivated groundnut (Halward *et al.*, 1993), but low DNA polymorphism has been observed in cultivated species (Kochert *et al.*, 1991 and Halward *et al.*, 1993) though a large variation for phenotypic characteristics exists. The observed polymorphism may be useful for developing molecular markers for screening various traits in groundnut improvement programmes.

Knowledge of the groundnut genome has so far been limited and only in the recent years have

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molecular techniques been used. Earlier studies on the genus *Arachis* using molecular marker technique such as restricted fragment length polymorphism (RFLP) analysis and random amplification of polymorphic DNA (RAPD) analysis have revealed very little demonstrable polymorphism in the cultivated groundnut. This has lead to the hasty generalization that the groundnut lacks genetic variation at molecular level (Basu and Nigam, 2004).

Molecular markers, in general, and microsatellites or simple sequence repeats (SSRs) in particular have proven very useful for crop improvement (Gupta and Varsheny, 2000). In groundnut, the use of molecular markers for breeding applications, however, has been limited by the low level of the genetic variation in this species. Nevertheless, in recent years, efforts have been made to develop the SSR markers in groundnut (Ferguson *et al*., 2004).

REVIEW OF LITERATURE

The review of literature on molecular diversity using simple sequence repeats (SSRs) or microsatellites in groundnut is presented hereunder.

Hopkins *et al*. (1999) have found only six simple sequence repeat (SSR) markers that detected polymorphisms amongst cultivated groundnut. Dwivedi and Verma (2002) studied molecular diversity in 37 groundnut genotypes with SSR primers and reported that differences in DNA profiles were observed in 537 of the 666 pair-wise comparisons analyzed among 37 drought tolerant genotypes. The dissimilarity ranged from 0.11 to 0.78. ICGV 94100 with ICGV 94106, ICGV 97068, ICGV 99247 and Chico; and ICGV 97068 with CSMG 84-1, ICGV 99231, ICGV 99247 and ICGV 99236 showed large differences at molecular level in their DNA profiles. They also reported that the populations derived from genetically diverse parents are expected to release transgressive segregants with high harvest index and such progenies should perform better under drought environments.

Dwivedi *et al*. (2003) reported assessment of molecular diversity should facilitate the identification of agronomically valuable and diverse germplasm for use in linkage mapping and genetic enhancement of specific traits in groundnut. Guohao He *et al*. (2003) reported that the GA/CT repeat was the most frequently dispersed microsatellite in peanut. The primer pairs were designed for fifty -six different microsatellites. 19 of which showed a polymorphism among the genotypes studied. The average number of alleles per locus was 4.25, and up to 14 alleles were found at one locus. They suggested that microsatellite DNA markers produce a higher level of DNA polymorphism than other DNA markers in cultivated peanut.

Girish Kumar Krishna *et al*. (2004) studied in 48 cultivated Valencia peanut genotypes and reported that considerable genetic variations were discovered among the genotypes with f-SSR primers. The f-SSR based clustering could identify the putative pedigree types of the Valencia types of diverse origins and the f-SSR in general is sufficient to obtain estimates of genetic divergence for the material in their study and the results are being utilized in the breeding programme for parental selection and linkage map construction.

Marcio de Carvalho Moretzshon *et al*. (2004) reported that a total of 67 new microsatellite markers were developed for *Arachis*. Only three of these markers, however, were polymorphic in cultivated peanut. Their results showed that the Brazilian peanut germplasm collection has considerable levels of genetic diversity detected by SSR markers. Microsatellite marker transferability was up to 76% for species of the section *Arachis*. A new marker (Ah-041) presented 100% transferability and could be used to classify the peanut accessions in AA and non-AA genome carriers.

He Liangqiong *et al*. (2005) screened 40 SSRs and found 16 amplified bands and of them (PM 36, PM 50 and PM 305) were able to produce bands that specific to *A. correntina* and the specific bands could be detected in several progenies. Their results proved molecular evidence for gene introgression from wild species to cultivated groundnut detected by SSR primers. Jayasree *et al*. (2005) analysed 1312 already developed sequences of which 448 contained microsatellite motifs. At least 39% of the sequences analyzed had significant similarities with sequences from the four databases searched, of which nearly half (47.0%) had significant similarity with *Lotus japonicus* sequences.

A linkage map based on SSR markers was constructed by Moretzsohn *et al*. (2005) using an F_2 population obtained from a cross between two diploid species with AA genome (*A. duranensis* and *A stenosperma*). A total of 271 new microsatellite markers were developed from SSR-enriched genomic libraries, expressed sequence tags (ESTs), and by mining sequences available in the public domain. The new markers and another 162 published for peanut were screened against both progenitors. The 80 co-dominant markers giving expected 1:2:1 segregation ratio was initially used to establish the linkage groups, whereas distorted and dominant markers were subsequently included in the map. The resulting linkage map consisted of 11 linkage groups covering 1,230.89cM of genome, with an average distance of 7.24 cM between markers.

Andrea Akemi Hoshino *et al*. (2006) reported that fifteen microsatellite primer pairs were tested in 76

accessions of 34 species from the nine *Arachis* sections. The data indicated that heterologous primers were very useful in *Arachis* since they had high transferability among the species (91.0%) and allowed the amplification of very polymorphic putative loci, which allowed both the characterization of most accessions and showed highly variability, even when represented by few accessions.

Juliana Pereira Bravo *et al*. (2006) studied to evaluate the transferability of microsatellite primers and the assay of genetic variability between and within the germplasm of some species of the *Arachis* section. Fourteen microsatellite loci developed for three different species of *Arachis* were analyzed and 11 (78.0%) were found to be polymorphic. All loci had transferability to all the species analyzed. The polymorphic loci were very informative, with expected heterozygosity per locus ranging from 0.70 to 0.94 and the germplasm showed wide genetic variation with SSR primers.

The results of Marcos A Gimenes *et al*. (2007) showed that microsatellite primer pairs from *A. hypogaea* have multiple uses. A higher level of variation among *A. hypogaea* accessions can be detected using microsatellite markers. The microsatellite primers of *A. hypogaea* showed a very high rate of transferability to other species of the genus. These primer pairs provide important tools to evaluate the genetic variability and to asses the mating system in *Arachis* species. Chuan Tang Wang *et al*. (2007) studied 123 newly designed primer pairs tested in 12 peanut varieties/lines and found only 44 (35.8%) produced polymorphic bands. Nalini Mallikarjuna *et al*. (2007) conducted experiment with SSR primers to know the molecular genetic relationship among *Arachis diogoi* and *A. chiquitana* accessions and seventeen SSR primers showed polymorphism between *Arachis diogoi* and *A. chiquitana* accessions.

Luu M Cuc *et al.* (2008) constructed a microsatellite-enriched library from the genotype TMV 2. Sequencing of 720 putative SSR-positive clones from a total of 3,072 provided 490 SSRs. 71.2% of these SSRs were perfect type, 13.1% were imperfect and 15.7% were compound. Among these SSRs, the GT/CA repeat motifs were the most common (37.6%) followed by GA/CT repeat motifs (25.9%). The primer pairs could be designed for a total of 170 SSRs and were optimized initially on two genotypes. 104 (61.2%) primer pairs yielded scorable amplicon and 46 (44.2%) primers showed polymorphism among 32 cultivated groundnut genotypes. The polymorphic SSR markers detected 2 to 5 alleles with an average of 2.44 per locus. The polymorphic information content (PIC) value for these markers varied from 0.12 to 0.75 with an average of 0.46. Varsheny *et al*. (2009) constructed the first genetic map for cultivated groundnut and demonstrated its utility for molecular mapping of QTLs controlling drought tolerance related traits *i.e* transpiration, transpiration efficiency, specific leaf area and SPAD chlorophyll meter reading (SCMR) as well as established relationships with diploid AA genome of groundnut. They developed a linkage map for tetraploid cultivated groundnut, a total of 1145 microsatellite or simple sequence repeat (SSR) markers were screened on two genotypes TAG-24 and ICGV- 86031 that are parents of a recombinant inbred line mapping population. They reported that 144 (12.6%) polymorphic markers were identified and these amplified a total of 150 loci. A total of 135 SSR loci could be mapped.

Genetic diversity studies in cultivated groundnut using SSR markers were reported by various authors. Twenty three SSRs were screened across 22 groundnut genotypes with differing levels of resistance to rust and LLS by Mace *et al.* (2006) and they reported that twelve of the 23 SSRs (52 per cent) showed a high level of polymorphism with PIC values ≥0.5. Molecular diversity and association of simple sequence repeat (SSR) markers with rust and late leaf spot (LLS) resistance were detected in a set of 20 cultivated groundnut genotypes differing in resistance against both diseases and were reported by Mondal and Badigannavar (2009). In their report, out of 136 bands amplified from 26 primers, 104 were found polymorphic (76.5 per cent). Thirty four SSR markers were used to assess the genetic variation of four sets of twenty-four accessions each from the four botanical varieties of the cultivated peanut were as reported by Tang *et al*. (2007). In their report, among the tested accessions, ten to sixteen pairs of SSR primers showed polymorphisms and the dendrograms based on genetic distances were constructed for the four botanical varieties, which revealed the existence of different clusters. Finally, they concluded that there was abundant intra-variety SSR polymorphism, and with more and more SSR markers being developed, the intrinsic genetic diversity would be detected and the development of genetic map and marker-assisted selection for cultivated peanut would be feasible.

CONCLUSION

The assessment of parental divergence is an important and foremost objective for development of wide divergent material for specific traits. Molecular markers are useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar identification early in plant development. It helps in identifying the desirable parents for hybridization programme to develop new plant types. The molecular markers based on differences in DNA sequences between individuals

generally detect more polymorphisms than morphological and protein based markers (Mignouna *et al.,* 1998; Tanksley *et al.,* 1989). Simple Sequence Repeats (SSR) is used as a primer to amplify regions between the microsatellites. This marker reveals a much larger number of fragments per primer than RAPD analysis (Bajpai *et al.,* 2008). The SSR-based markers were found to be quite discriminatory in discerning variations between and among groundnut lines even where the level of variation was low. Microsatellite-based markers therefore represent a useful tool for dissecting genetic variations in cultivated crops, especially groundnut. The information through SSR markers clearly indicates that it has a lot of scope as a tool to distinguish genotypes in groundnut as sufficient polymorphism was revealed among groundnut genotypes. The observed polymorphism may be useful for developing molecular markers for screening various traits in groundnut improvement programmes.

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