

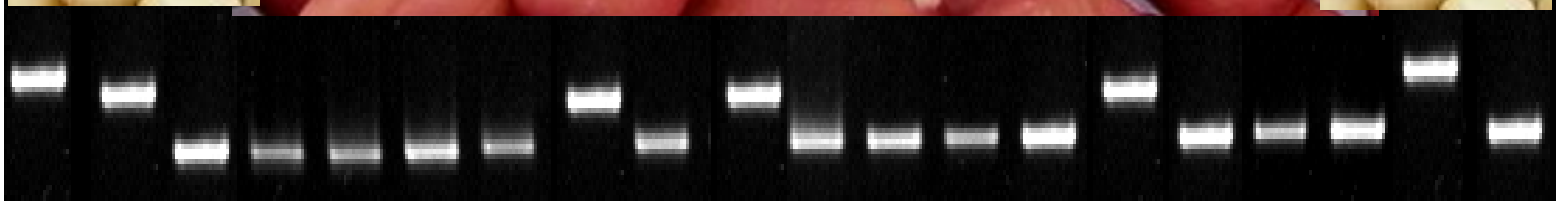
FINAL REPORT

PARB Project # 16

"Genetic Improvement of Groundnut for Herbicide and Disease Resistance"

Host Organization:

Agricultural Biotechnology Research Institute, AARI, Faisalabad



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CHAPTER 1:

INTRODUCTION

Groundnut which is also known as peanut, earthnut, monkeynut, manilanut and ground bean belongs to genus *Arachis*, subtribe *Stylosanthinae*, tribe *Aeschynomeneae* sub family *Fabaceae* and family *Leguminosae*. The genus *Arachis* has 80 species (Valls and Simpson, 1994). All are wild and diploid (2n=2x=20 chromosomes) but solely cultivated groundnut specie is an allotetraploid (2n=4x=40) having genomic formula AABB with monophyletic origin after crossing between two diploid species AA and BB (Kochert *et al*., 1996; Seijo *et al*., 2004) followed by successive selection resulted in a highly conserved genome (Young *et al*., 1996), however in segregation its behave like diploid (Stalker *et al*., 1991). *Arachis hypogaea* L. which is an annual herb that forms underground fruits. There are two subspecies of *Arachis hypogaea*, distinguished primarily on branching pattern and distribution of vegetative and reproductive axes. Subspecies *hypogaea* has two varieties (*hypogaea* and *hirsuta*), whereas subspecies *fastigiata* has four (*fastigiata*, *vulgaris*, *peruviana* and *aequatoriana*). The botanical name of groundnut is derived from the Greek word *arachis* meaning 'legume' and *hypogaea* meaning 'below ground' referring to the formation of pods in the soil (Pattee and Stalker, 1995). *Arachis hypogaea* grown in diverse regions of South America for more than 5000 years and spread worldwide by the time of the European discovery of the New World, or even before that, following pre-Columbian navigation routes in the Pacific Ocean (Krapovickas, 1998). In 2012, groundnut cultivated in more than 100 countries, covering an area of 20.61 million ha with current annual production about 35.13 million metric tons round the world (Anon., 2013). In Pakistan groundnut is grown as cash crop mainly in rain fed conditions. The crop is cultivated on 82.9 thousand ha with annual production 67.8 thousand tones (Anon., 2011). In Pakistan, peanut cultivation on commercial basis started on a meager area of 400 ha in Rawalpindi Division during 1949-50 (Anon, 1982) and then extended toother parts of the country. It is cultivated in pre-monsoon (March-April), monsoon (June-July) or post monsoon season within maximum temperature range of 31 to 35°C and minimum of 18 to 23°C (Sulaiman and Agashe, 1965; Vankataraman and Kazi, 1979). Punjab contributes 89.52 of the area and 76.57% of the production followed by Sindh (13.57 and 19.24%) and NWFP (1.5 and 1.31%), respectively (Anon, 2006). United State stand at the top in per hectare groundnut production (3.45 t/h) followed by Egypt (3.17 t/h), China (2.65 t/h), Argentina (2.51 t/h) and Brazil (2.17 t/h). Pakistan had groundnut yield less than one ton per hectare. The major yield limiting factors are non-availability of high yielding, biotic and abiotic stresses resistant adopted varieties. Diverse gene pool is a prerequisite for development of high yielding varieties. The phenotypic observations about genetic diversity are often misleading. Molecular markers, in general and microsatellites or Simple Sequence Repeats (SSRs) in particular have proven very useful for genetic diversity assessment, germplasm characterization and crop improvement in many species like *Gossypium* (Iqbal *et al*., 1997; Lascape *et al*., 2007; Ullah *et al*., 2012), *Triticum* (Dograr *et al*., 2000; Fufa *et al*., 2005; Maccaferri *et al*., 2007; Mohammadi *et al*., 2009), *Oryza* (Ren *et al*., 2003; Jayamani *et al*., 2007; Kanawapee et at., 2011) *Sacchrum* (Burner *et al*., 1997; Aitken *et al*., 2005; Chen *et al*., 2009; Nawaz *et al*., 2010; Hameed *et al*., 2012) and *Arachis* (Kochert *et al*., 1991; Halward *et al*., 1991, 1992; Paik-Ro *et al*., 1992; Hopkins *et al*., 1999; Jiang *et al*., 2007; Gautami *et al*., 2009). Several studies have identified little genetic diversity among the wild and cultivated groundnut species detected through Randomly Amplified Polymorphic DNAs (RAPD), Amplified Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism

(AFLP) (Hilu and Stalker 1995; Kochert *et al*., 1996; Subramanian *et al*., 2000; Dwivedi *et al*., 2001; He and Prakash, 2001; Herselman, 2003; Bravo *et al*., 2006) and SSR markers (Hopkins *et al*., 1999; Krishna *et al*., 2004; Moretzsohn *et al*., 2004; He *et al*., 2005). The use of molecular markers for breeding applications in groundnut, are limited in the past because of low level of genetic variation may be due to genetic barrier on gene flow from wild diploid to cultivated tetraploid species, self-pollinating habit of cultivated species, intensive selection after hybridization between genetically similar parents for variety development programs, use of inappropriate numbers and unsuitable molecular marker system. Development of SSR markers traditionally requires cloning and sequencing and hence is more cost and labour intensive, compared to PCR arbitrary priming techniques, e.g., RAPDs and AFLP. However, once the SSR markers are developed, their applications in breeding activities particularly using high throughput approaches become very cost effective. By using SSR markers, good progress has been made in developing the genetic maps and diversity studies in AA and BB genome groundnut species. To overcome the low level of polymorphism, one of simple solutions is to develop a critical number of SSR markers in groundnut so that a repertoire of a reasonable number of polymorphic SSR markers for cultivated groundnut germplasm may be available. Only few hundred SSR markers were available until 2005. In recent years, significant efforts have been made to develop the SSR markers in groundnut and more than 9000 SSR markers (Zhao *et al*., 2012) were developed in genus *Arachis* and they showed higher level of polymorphism over other DNA markers, detected in the cultivated peanut (Palmieri, 2002; Ferguson *et al*., 2004; Krishna *et al*., 2004; Moretzsohn *et al*., 2004; He *et al*., 2005; Barkley *et al*., 2007; Gimenes *et al*., 2007; Wang*et al*., 2007; Naito *et al*., 2008; Pandey *et al*., 2011; Zhao *et al*., 2012). Recently, the efficiency of polymorphic marker development was improved remarkably by using *in silico* polymorphism analysis (Shirasawa *et al*., 2012) in comparison with the previous method, in which primers were simply designed based on the flanking sequences of SSR motifs analysis.

A number of biotic and abiotic stresses outbreak in the field of groundnut crop. Among the biotic stresses, there are number of potential pathogens like fungi, bacteria, viruses and mycoplasmas, which collectively results in huge losses to groundnut production. Among fungal diseases Early Leaf Spot (ELS) caused by *Cercospora arachidicola* Hori and late leaf spot (LLS) caused by *Cercosporidum personatum* (Berk.&Curt). Deighton are among major diseases of peanut worldwide (Smith and Littrel, 1980; Subrahmanyam *et al*., 1995; ICRISAT, 1992; Waliyar *et al*., 1993; Ogwulumba *et al*., 2008) and can affect all the aerial parts. ELS are recognized by light brown spot on the leaves. Frequently a bright hollow surrounds each spot while the spots of late leaf spots are black circular and without hollow. Most of early leaf spot spores are formed on the upper leaf surface giving it a slightly raised surface, while lower leaf surface is usually smooth (Naab *et al*., 2005; Ouzounov, 1988; Subrahmanyam *et al*., 1982). Because of too much spotting of leaves there is defoliation and deteriorating of plant resulting in fewer and smaller sized pods and less yield. Worldwide 50% yield losses due to disease have been reported (Smith, 1984; McDonald *et al*., 1985). In a study of ethylene production and leaflet abscission of peanut genotypes infected with *Cercospora arachidicola* Hori, 96% and 71.6% defoliation was occurred in control and surfactant treated plants, respectively (Ketring and Melouk, 1982). Cercospora leaf spot are generally present in every field and its losses can be minimizing by developing disease resistant genotypes. Recent studies (Khedikar *et al*., 2010; Mace *et al*., 2006; Mondal and Badigannavar, 2009) indicated that molecular markers are available for the identification of Cercospora leaf spot resistant genotypes. The Cercospora leaf spot resistant groundnut breeding program may be accelerated with the help of molecular breeding.

Another major yield limiting factor in groundnut is weeds. A number of broad and narrow leafs weeds like Dela (*Cyprus rotundus*), Barron (*Sorghum helepens*), Khabbal (*Cynodon dactylon*), Lelhi (*Convolvulus arvensis*), Swaank (*Echinochola colonum*), Taandla (*Digera muricata*), Chandri (*Brachairia reptan*), Pohli (*Carthamus oxycantha*), Kulfa (*Portulaca oriacae*) and Madhaana ghaas (*Dactyloctenium aegypticum*) may cause 30-70% yield losses. The application of pre-emergence weedicides is difficult because of non-availability of proper moisture level at the time of sowing. No post emergence weedicide is available for the control of weeds. Genetic engineering offers an alternate technique to the weed control method in crops. The process of weed control in groundnut may be modified through the manipulation of a single enzyme within the groundnut plants to confer tolerance to the herbicide glyphosate. Glyphosate is a systemic, non-selective total herbicide cause inhibition of enzyme 5-enolypyruzylshikimate-3-phoslate synthase (EPSPS). EPSPS is a nuclear encoded chloroplast localized enzyme crucial for the manufacturing of aromatic amino acids via shikimate path way. As this biosynthetic process is unique to plants, microorganisms and fungi therefore glyphosate is relatively non-toxic humans and animals. Glyphosate binds tightly to soil and therefore shows no pre emergence or residual soil activities. It is also short lived in soil and readily degraded by microorganism to produce phosphoric acid, ammonia and carbon dioxide. Use of glyphosate resistant groundnut genotypes in combination with a glyphosate based weed management strategy has the potential to provide efficient and effective weed control in groundnut.

Keeping in view all these problems a comprehensive project was proposed in which three institutes (Agricultural Biotechnology Research Institute, AARI, Faisalabad, Barani Agricultural Research Institute, Chakwal and National Institute of Genomics and Advanced Biotechnology NARC, Islamabad) work in collaboration for finding the solution of these problems. The fields screening, crossing, phenotyping and new variety development through conventional breeding technique was assign to Barani Agricultural Research Institute (BARI), Chakwal. The BARI Chakwal was also assign duty for finding short strategy to control, i.e., chemical control of late leaf spot disease and testing of transgenic glyphosate resistant groundnut genotypes. The mandate of Agricultural Biotechnology Research Institute, AARI, Faisalabad was the estimation of genetic diversity in 70 groundnut genotypes and molecular identification of genotypes having genes for leaf spot resistant. The task for the development of transgenic herbicide resistant genotypes having EPSPS gene was assign to National Institute of Genomics and Advanced Biotechnology NARC, Islamabad.

CHAPTER 2:

REVIEW OF LITERATURE

Gibbons (1966) evaluated the germplasm for *Cercospora arachidicola* and *C. personatum* and found that early maturing sequentially branched peanut cultivars were generally more susceptible to leaf spot than were later maturing alternatively branched cultivars that exhibit various degrees of resistance.

Porter (1970) found that Benlate 50 WP is very effective for controlling cercospora leaf spot and reduce the yield losses due to this disease.

Nei (1972) formulated the measure of genetic Distance (D) based on the identity of genes between population. He defined as D = -logI, where I is the normalized identity of genes between two populations. This genetic distance measures the accumulated allele differences per locus. If the rate of gene substitution per year is constant, it is linearly related to the divergence time between populations under sexual isolation. It is also linearly related to geographical distance or area in some migration models. Since D is a measure of the accumulated number of codon differences per locus. It can also be estimated from data on amino acid sequences in proteins even for a distantly related species. Thus, if enough data are available, genetic distance between any pair of organisms can be measured in terms of D. This measure is applicable to any kind of organism without regard to ploidy or mating scheme.

Mazzani *et al.* (1972) observed that leaf spot counts were higher on cultivars with large, light green leaves. The leaves of alternatively branched cultivars tended to be smaller and have more palisade tissue than those of sequentially branched cultivars.

Gregory *et al.* (1973) used characteristics such as morphological comparisons, geographic distributions, cross compatibility relationships and hybrid fertility to divide the genus *Arachis* into seven sections and 70 species. Fatty acid profiles and the conservation of oleic/linoleic ration done on the sections Extranervosae and Trisminalae led to the conclusions that Extranervosae may be the oldest species in the genus and that the oleic/linoleic ration assist in conditioning endurance in arid environments. *Arachis hypogaea* belongs to one of the seven sections in the genus *Arachis,* namely *Arachis*. The *Arachis* section consists of *A. monticola* as the only wild tetraploid species with 2n = 4x = 40 chromosomes, whereas the rest are diploid species with 2n = 2x = 20 chromosomes.

Clark*et al.* (1974) reported that Benomyl fungicides most abundantly used for the control of cercospora leaf spot in USA. They found that benomyl was acceptable in the USA in the 70's until when resistance to the chemical was detected

Littrell (1974) Isolated the groundnut pathogen *Cercospora arachidicola* (*Mycosphaerella arachidis*) that were tolerant of 5 mu g/ml benomyl *in vitro* collected from 6 locations in 4 counties of Georgia. Sensitive isolates did not grow on PDA containing 0.5 mu g/ml while tolerant ones grew with 160 mu g/ml benomyl. About 1/3 of the lesions collected from experimental plots that had received 6 applications of Benlate yielded tolerant isolates. However, no reduction in groundnut yields occurred in comparison with other fungicide treatments. Cross tolerance was also found to thiophanate-methyl (topsin M) and 2-(methoxycarbamoyl)-benzimidazole (BAS 3560).

Backman *et al.* (1977) found that peanut leaf spot may be caused by either of two fungi that occur wherever peanuts are grown in United State. Most common of the two in the Southeastern United States is *Cercospora arachidicola* Hori, which causes early leafs pot. Symptoms of this fungus are brown to dark brown circular spots usually surrounded by a yellow

halo. Spore production typically occurs on the upper surface. Late leaf spot caused by *Cercosporidium personatum* (Berk. 8cCurt.) Deight., occurs later in the season and is usually darker on the lower surface of the leaflet than early leaf spot. The lesion typically has a pimply lower surface with a less distinct halo than early leaf spot. The disease cycle of *Cercospora* leaf spot in the field is not completely understood, but typically follows this pattern: (1) spores are produced during periods of moisture (dew) on mature lesions (2) the spores are released when the lesion dries and are windborne to young peanut leaflets (3) during the next period of moisture the spores are activated, requiring 14-16 hours at 72°F to germinate and complete the infection process (if this time period is interrupted by a period of dryness the spores are killed (4) 10-14 days after infection their first symptoms of infection are visible and (5) the mature sporulating lesion develops 16-20 days after infection. Late leaf spot seems to follow the same sequence, but is somewhat slower in developing. The disease cycle can greatly influence short-term results with field-applied fungicides. For example, if a 100 percent effective contact fungicide existed and it was applied after infection had occurred, a period of 10-12 days would elapse before any disease reduction could be visible. This time lapse between treatment and response occurs because contact fungicides are effective only on spores or germination tubes on the leaf surface. They have no activity on established infections beneath the leaf cuticle. The establishment of control, therefore, must be developed on a preventative basis. The most desirable fungicides are those that possess both contact and systemic activity. Systemic fungicides not only kill fungal spores on the leaf surface, but can eradicate already established infections within the leaf. Unfortunately, none of these fungicides is presently available to peanut farmers for leaf spot control. Understanding the disease cycle can allow flexibility in establishing spray intervals. During dry periods (when moisture periods do not exceed 12-14 hours), spray intervals can be extended. Conversely, intervals should be shortened during periods of extended moisture that frequently occur with rain or ground fog. From 1971 through 1975 the primary fungicides recommended for leafspot disease control were: Benlate 50WP, 6 ounces per acre (through 1973); Bravo 75WP or 6F, 11/2 pounds per acre or 11/ pints per acre; Kocide 404S F, 2 quarts per acre and Duter 47WP, 6 ounces per acre. Peanuts were grown in a Dothan sandy loam soil in a 1 year rotation with corn (*Zea mays* L.). Plot size was either 150 x 24 feet (1971-72) or 50 x 24 feet. Fungicides were applied every 14 days beginning 40-50 days after planting and ending 14-20 days before harvest. All fungicides were applied by a conventional ground sprayer calibrated to deliver 15 gallons per acre at 80 pounds per square inch. Peanuts were harvested three times between 140 and 160 days after planting. Leafspot incidence (*Cercospora* + *Cercosporidium*)was determined 14 days before harvest by removing 10 non-bearing vertical runners at random from each plot and measuring infection using the following criteria: (i) Total leaflets = number of leaf nodes x 4 (ii) Percent defoliated = number of leaflets lost - total leaflets x 100 (iii) Total leaflets infected = number of leaflets lost + number of leaflets infected and (iv) Percent infection = leaflets infected - total leaflets x 100. This method assumes that defoliation occurred because of previous leaf spot infection. The high figure for disease in Benlate treated plots in 1974 reflects the occurrence of a Benlate resistant race of *Cercospora* that caused severe infection and defoliation. Data on the newly recommended fungicides Difolatan 4F (3 pints per acre) and Duter plus sulfur (6 ounces + 11/pounds per acre). The differences in yield indicate of disease control, for some fungicides yields lower than expected are obtained.

Smith and Littrell (1980) found rapid move towards spray application following the introduction of the highly effective fungicides benomyl, chlorothalonil and fentin hydroxide in the early 1970s.

Yaqoob *et al.* (1989) concluded that yield of peanut in Pakistan may be enhanced from 33 to 119% with appropriate improvements in the disease management.

Weising *et al.* (1989) found that simple quadruplet repeats $(GATA)_n$ and $(GACA)_n$ are constituents of repetitive DNA in most animal genomes investigated to date. Since restriction fragments harbouring these sequences show considerable polymorphism between individuals, GATA/GACA repeats have been successfully used for DNA fingerprinting of e.g. human DNA. They demonstrated the presence of tandemly arranged $(GATA)_n$ and $(GACA)_n$ repeats in the genome of chickpea (*Cicer arietinum*) and barley (*Hordeum vulgare*), a dicot and a monocot crop plant, respectively. DNA was isolated according to from leaves of individual plants derived from different accessions (collection of ICARDA, Syria), digested with Alu I or Taq I and electrophoretically separated on 1.2% agarose gels. Gels were denatured, neutralized, dried and hybridized to 32P-labeled synthetic $(GATA)_4$ or $(GACA)_4$ oligonucleotide probes. Both GATA and GACA repeats are present in the genomes of chickpea and barley. Whereas band heterogeneity is limited in case of barley/GATA and chickpea/GACA, hybridization of chickpea DNA to the $(GATA)_4$ probe and of barley DNA to the $(GACA)_4$ probe resulted in considerable band diversity. These results confirm and extend recent data obtained by hybridizing human and M13 minisatellite probes to several plant DNAs. Furthermore, they demonstrate the ubiquitous presence and polymorphism of simple repetitive DNA motives in the Eukaryotic kingdoms. The applicability of these probes for variety identification in crop plants is presently under investigation.

Wynne and Halward (1989) found significant amount of morphological variation, but not much genetics polymorphism with molecular markers among *A. hypogaea* cultivars, as well as exotic lines.

Condit and Hubbell (1991) found that tandem DNA repeats of 2 bp are potentially important tools for population genetic studies because of their abundance and length variation. As part of their research into the ecology of tropical forest plants, they began a study of dinucleotide repeat regions in several genera of tropical trees. Genomic libraries in bacteriophage lambda were screened with the oligonucleotide probes poly (GT) and poly (AG). Both types of repeat regions were abundant in the genomes of all six plant species examined. Using the size of inserts in the phage libraries and number of phage screened, they estimated that there were 5 x 10(3) to 3 x 10(5) poly (AC) sites per genome, with slightly more AG than AC sites. When libraries were made from smaller fragments of genomic DNA, abundance estimates were higher, suggesting that two-base repeat sites were clustered in the genome. Poly (AC) sites were 16-22 bp in length and four of the five sequenced were adjacent to either poly (AG) or poly (AT) sites. Other repeat regions appeared in DNA flanking the AC sites. This further demonstrated that two-base repeats and other repetitive DNA were clustered in the genome. Two-base repeats are abundant in plant genomes and could provide a large number of polymorphic markers for studies of plant population genetics

Kochert *et al.* (1991) used RFLP markers to study the relatedness among different *Arachis* sp. and introgression from wild crosses. RFLPs were used to understand the relatedness of different peanut species and the techniques showed that *A. ipaenis*, *A. duranesis* and *A. spegazzinii* are the most closely related and that wild *Arachis* sp. were rather closely related to the diploid progenitor species of the allotetraploid cultivated peanut.

Akkaya *et al.* (1992) studied to ascertain the presence and degree of simple sequence repeat (SSR) DNA length polymorphism in the soybean. A search of GenBank revealed no (CA)n or (GT)n SSRs with n greater than 8 in soybean. In contrast, 5 (AT)n and 1 (ATT)n SSRs with n ranging from 14 to 27 were detected. Polymerase chain reaction (PCR) primers to regions flanking the six SSR loci were used in PCR amplification of DNA from 43 homozygous soybean genotypes.

At three loci, amplification produced one PCR product per genotype and revealed 6, 7 and 8 product length variants (alleles) at the three loci, respectively. F_1 hybrids between parents carrying different alleles produced two PCR products identical to the two parents. Codominant segregation of alleles among F2 progeny was demonstrated at each locus. A soybean DNA library was screened for the presence of (CA/GT)n SSRs. Sequencing of positive clones revealed that the longest such SSR was (CA)9. Thus, (CA)n SSRs with n of 15 or more are apparently much less common in soybean than in the human genome. In contrast to humans, $(CA)_n$ SSRs will probably not provide an abundant source of genetic markers in soybean. However, the apparent abundance of long $(AT)_n$ sequences should allow this SSR to serve as a source of highly polymorphic genetic markers in soybean.

Halward *et al.* (1993) concluded that abundant germplasm resource available to peanut breeders, they have for long been depended on the crossing of elite breeding lines for the development of imported cultivars. This practice led to the erosion of the germplasm base of domesticated peanut.

Morgante and Olivieri (1993) assessed the feasibility of using microsatellites as markers in plant genetics. A survey of published DNA sequence data for presence, abundance and ubiquity in higher plants of all types of dinucleotide and trinucleotide repeats with a minimum number of 10 and 7 units, respectively, was conducted. This search revealed that such microsatellites are frequent and widely distributed. They were uncovered in 34 species, with a frequency of one every 50 kb. AT repeats was by far the most frequently observed class of dinucleotide microsatellites, whereas AC/TG repeats, which are common in animals, were observed only once. TAT repeats prevailed among trinucleotides. Polymerase chain reaction amplification of (AT) and (TAT), microsatellites in soybean (*Glycine max* (L.) Merr. revealed that they are highly polymorphic, as a consequence of length variation, somatically stable and inherited in a co-dominant Mendelian manner. The abundance and amount of information derived from such markers, together with the ease by which they can be identified, make them ideal markers for plant genetic linkage and physical mapping, population studies and varietal identification.

Thomas and Scott (1993) investigated microsatellite repeat sequences as Sequenced Tagged Site (STS) DNA markers to determine the potential for genetic analysis of the grapevine genome. The PCR generated markers detect co-dominant alleles at a single locus or site in the genome. The marker type is very informative detecting high heterozygosity (69-88%) within individual grapevine cultivars and high genetic variation between cultivars, making it a useful marker type for plant genome mapping and genome typing. For five loci a screening of 26 *V. vinifera* cultivars found 13, 12, 8, 5 and 4 different length alleles respectively with some alleles more common than others. The genomic DNA sequences surrounding microsatellite sequences were conserved within the genus permitting STS primers to amplify STSs from other Vitis species. These Vitis species were found to have some unique alleles not present in *V. vinifera*.

Wu and Tanksley (1993) found that dinucleotide microsatellites have been characterized and used as genetic markers in rice. Screening of a rice genomic library with poly (dG-dA) (dC-dT) and poly (dG-dT) (dC-dA) probes indicated that $(GA)_n$ repeats occurred, on average, once every 225 kb and $(GT)_n$ repeats once every 480 kb. DNA sequencing of ten randomly selected microsatellites indicated that the numbers of repeats ranged from 12 to 34 and that the patterns of microsatellites in rice were similar to those of humans and other mammals. Primers to these microsatellite loci as well as to four published microsatellite-containing sequences have been designed and degrees of polymorphism have been examined with 20 rice accessions. Multiple alleles, ranging from 5 to 11, have been observed at all the microsatellite loci in 20 rice accessions.

Alleles specific to two cultivated subspecies, indica and japonica, were found in some microsatellite loci. Heterozygosity values of all the microsatellite markers were significantly higher than those of RFLP markers, based upon a parallel comparison. Ten microsatellite loci have been genetically mapped to four rice chromosomes. The genomic distribution of microsatellites appears to be random in rice.

Zhao and Kochert (1993) observed that DNA microsatellites are ubiquitously present in eukaryotic genomes and represent a vast source of highly informative markers. They found in this article a $(GGC)_n$ microsatellite which is widely distributed in eukaryotic genomes. Using Polymerase Chain Reaction (PCR) techniques and DNA sequencing, they demonstrated for the first time in plant species that a $(GGC)_n$ microsatellite locus is moderately polymorphic. Six alleles are present at this locus in rice and length polymorphisms are caused by variation in the number of tandem GGC repeats. By scoring a backcross mapping population, they were able to demonstrate that this locus is stably inherited and does not link to any known RFLP markers on the rice RFLP map. They suggested that DNA microsatellites should be useful in plants for construction of genetic linkage maps, extension of the existing genetic linkage maps, linkage analysis of disease and pest resistance genes and the study of population genetics.

Terauchi and Konuma (1994) characterized six microsatellite loci in *Dioscorea tokoro*, a wild yam species in East Asia. All six loci were polymorphic in a sample of 23 individuals from natural populations in Japan. The microsatellite loci displayed many alleles (6.2 alleles per locus on average) and the observed heterozygosity (H_o = 0.54) as well as expected heterozygosity (H_e = 0.68) were high. The heterozygosities were far more than that previously detected by allozyme analysis of *D. tokoro* (H_o = 0.23, H_e = 0.28). Five microsatellite loci were sufficient to provide a paternity exclusion rate (Q) of Q = 0.98, which enables monitoring of the pollen-mediated gene flow between plants in a population. Microsatellite loci were abundant and highly polymorphic in *D. tokoro* and other plants and were therefore ideal markers for plant population genetic studies.

Liu *et al.* (1995) established TaqI genomic library of seashore paspalum (*Paspalum vaginatum* Swartz) and screened for the presence of $(GA)_n$and $(CA)_n$ simple sequence repeats (SSRs). A total of 54 clones with a positive signal were detected among 13,000 clones screened. Forty seven clones having repeats of $n \geq 3$ were identified, of which 85% were perfect, 13% were imperfect and 2% were compound repeat sequences. Five of ten primer pairs synthesized to amplify selected loci resulted in a product in the expected size range and were subsequently used to examine SSR polymorphisms among 46 ecotypes of *P. vaginatum*. The number of alleles resolved on agarose or polyacrylamide gels were similar and ranged from 6 to 16 with an average of 14 per locus. Phenetic analysis of SSR polymorphisms revealed genetic relationships among the *P. vaginatum* ecotypes that were in general agreement with relationships determined previously by RAPD analysis of the same plant materials. Further screening of the genomic library did not identify $(AT)_n$, trimeric or tetrameric repeats. Hybridization of an $(ATT)_8$ oligonucleotide probe to genomic DNA isolated from *I. batatas*, *E. coli*, *Citrullis lanatus* and *P. vaginatum* suggested that the *P. vaginatum* genome contained significantly fewer ATT repeats than either the *I. batatas* or *C. lanatus* genome.

Rongwen *et al.* (1995) used microsatellite or simple sequence repeat (SSR) DNA markers to develop unique DNA profiles of soybean genotypes. Microsatellites are DNA sequences such as $(AT)_n/(TA)_n$ and $(ATT)_n/(TAA)_n$ that are composed of tandemly repeated 2-5 base pair DNA core sequences. The DNA sequences flanking microsatellites are generally conserved allowing the selection of polymerase chain reaction (PCR) primers that will amplify the intervening SSR. Variation in the number of tandem repeats, "n", results in PCR product length differences. The

SSR alleles present at three $(AT)_n/(TA)_n$ and four $(ATT)_n/(TAA)_n$ loci were determined in each of 96 diverse soybean genotypes. Between 11 and 26 alleles were found at each of the seven loci. Only two genotypes had identical SSR allelic profiles and these had very similar pedigrees. The gene diversity for the seven markers averaged 0.87 for all 96 genotypes and 0.74 for a subset of 26 North American cultivars. These are much higher than soybean gene diversity values obtained using RFLP markers and are similar to the average values obtained for human microsatellite markers. SSR markers provide an excellent complement to the conventional markers that are currently used to characterize soybean genotypes.

Bhagwat *et al.* (1997) studied variation in RAPD profiles between groundnut cultivar Spanish, improved and its mutants originated by X-ray irradiation. Twelve RAPD primers produced 1182 fragments of which 65 fragments were polymorphic (5.5%) thus giving on average 1.51 polymorphic bands per primer. Primer OPJ 06 yielded high polymorphism among the mutants.

Goldstein and Pollock (1997) reviewed mutation processes and methods of phylogenetic inference though microsatellites. Microsatellite due to their exceptional variability and relative ease of scoring are now generally considered the most powerful genetic marker. It is typical to observe loci with more than 10 alleles and heterozygosities above 0.60, even in relatively small samples, while certain loci can be considerably more variable. In addition to being highly variable, microsatellites are also densely distributed throughout eukaryotic genomes, making them the preferred marker for very high resolution genetic mapping. Microsatellites are rapidly replacing RFLPs and RAPDs in most applications in population biology, from identifying relatives to Inferring demographic parameters. Part of the appeal of microsatellites over RFLPs and RAPDs is that the genetic basis of microsatellite variability is readily apparent. Unique primers amplify a genomic region including a well-defined repeat structure that is responsible for the observed variation. One perceived difficulty with microsatellites is the long lead time in identifying and characterizing microsatellites in new taxonomic groups. This problem is partially alleviated, however, by the continuing popularity of microsatellites in genetic mapping. Microsatellite maps are now available in nearly all organisms of genetic and/or economic interest including humans, mice, fruit flies, cows, sheep, chickens, pigs, tomatoes, soybeans and rice. In addition, large databases of microsatellites isolated for population work are accumulating. One maintained at the Smithsonian laboratory of molecular systematics includes 25 species and is certainly an underestimate of those available. One practical long-term difficulty with microsatellite markers is the requirement of determining fragment lengths, which would seem to complicate automation. Ultimately, the future may belong to markers amenable to yes/no tests which can be set up on dense chips (e.g., single nucleotide polymorphisms). In contrast with their importance in intraspecific studies, microsatellites have yet to make any real contribution to phylogeny reconstruction. This failure has come as a surprise to those who suspected that the huge number of microsatellites available, coupled with their very rapid rate evolution would make them particularly useful in working out the relationships among very closely related species. Although, it is not yet entirely clear why microsatellites have not been more successful in reconstructing phylogenies, part of the difficulty certainly stems from restrictions to divergence imposed by range constraints, irregularities and asymmetries in the mutation process and the degradation of microsatellites over time. A number of recent studies have developed theoretical methods to both estimate the relevant molecular details and to correct for them statistically, but they have yet to be tested.

Guilford *et al.* (1997) screened an apple genomic library with $(GA)_{15}$ and $(GT)_{15}$ probes and demonstrated that these repeats are abundant, occurring about every 120 and 190 kb, respectively. Microsatellites isolated from a small insert library enriched for (GA) repeats contained numbers of repeats ranging from 7 to 39. Primers to these microsatellite loci were able to direct the amplification of the repeats in 21 different cultivars. The majority of markers was highly polymorphic, diploid and showed simple Mendelian inheritance, although about 25% of markers generated complex banding patterns consistent with the amplification of more than one locus. As few as three microsatellite markers were sufficient to differentiate between all 21 apple cultivars.

He and Prakash (1997) used DNA amplification fingerprinting (DAF) and amplified fragment length polymorphism (AFLP) approaches for testing the potential to detect genetic variation in peanut. The AFLP approach was more efficient as 43% of the primer combinations detected polymorphic DNA markers in contrast to 3% with the DAF approach. However, the number of polymorphic bands identified using primers selected in both approaches was comparable. In the DAF study, when 559 primers of varying types were screened, 17 (mostly 10-primer types) detected polymorphism producing an average of 3.7 polymorphic bands per primer with a total of 63 polymorphic markers. In the AFLP study, when 64 primer combinations (three selective nucleotides) corresponding to restriction enzymes Eco RI and MseI were screened, 28 detected polymorphism. On an average, 6.7% of bands obtained from these 28 primer pairs were polymorphic resulting in a total of 111 AFLP markers. Our results demonstrate that both AFLP and DAF approaches can be employed to generate DNA markers in peanut and thus have potential in the marker-assisted genetic improvement and germplasm evaluation of this economically important crop.

Innan *et al.* (1997) studied variation in repeat number at 20 microsatellite loci of *Arabidopsis thaliana* in a worldwide sample of 42 ecotypes to investigate the pattern and level of polymorphism in repetitive sequences in natural plant populations. There is a substantial amount of variation at microsatellite loci despite the selfing nature of this plant species. The average gene diversity was 0.794 and the average number of alleles per locus was 10.6. The distribution of alleles was centered around the mean of repeat number at most loci, but could not be regarded as normal. There was a significantly positive correlation between the number of repeats and the amount of variation. For most loci, the observed number of alleles was between the expected values of the infinite allele and stepwise mutation models. The two models were rejected by the sign test. Linkage disequilibrium was detected in 12.1% of the pairwise comparisons between loci. In phylogenetic tree, there was no association between ecotype and geographic origin.

Olufowote *et al.* (1997) studied to determine an efficient way of detecting within cultivar variation in rice varieties obtained from national and international germplasm collections. Seventy one rice cultivars were evaluated for within cultivar variation using a combination of phenotypic, RFLP and microsatellite or Simple Sequence Length Polymorphism (SSLP). Variation between individuals within an accession and between duplicate accessions within a cultivar was detected even in cultivars that had been purified by phenotypic evaluation. Landrace cultivars were more heterogeneous and displayed a larger number of both RFLP and SSLP alleles than did modern cultivars. Microsatellite markers detected a greater number of alleles and were able to discriminate between even closely related individuals more efficiently than RFLPs. Some microsatellite markers were more informative than others for assessing genetic diversity. Single markers revealed 5.6-61.1% of the total variation detected by the 10 SSLP markers. Some marker combinations were complementary, providing more information than others. Several combinations of 4 SSLP

markers detected as much as 94% of the total within cultivar variation detected by the 10 SSLP markers. These results suggest that the use of four well-chosen microsatellites would be an efficient method for evaluating the heterogeneity of rice accessions.

Stalker (1997) reported that breeders have been using different techniques such as pedigree, modified pedigree, mass selection, mutation breeding and back crossing to develop different cultivars of *A. hypogaea*. These methods limited genetic variation among genotypes in commercial peanut production over the years although diversity is believed to have increased in recent year.

Hopkins *et al.* (1999) identified SSR markers in cultivated peanut and tested them to discriminate among 22 groundnut accessions. Peanut total genomic DNA libraries were constructed and screened with '2~-labelled dinucleotide repeats, GT and CT. DNA sequences obtained from the SSR containing clones designed on the basis of DNA sequences flanking the repeat motif were tested in Polymerase Chain Reaction (PCR) assays using 22 peanut DNAs, in both cultivated and wild peanut species. Six SSR markers developed showed polymorphism among cultivated peanuts. From 2 to 14 DNA fragments were amplified per SSR markers and as a group, the six markers amplified up to 10 putative SSR loci.

Kubik *et al.* (1999) advocated that simple sequence repeats (SSRs) have proven to be useful genetic markers in a wide variety of plants, but have yet to be widely applied to turfgrasses. They studied of SSRs in perennial ryegrass (*Lolium perenne* L.). A library of perennial ryegrass genomic DNA was screened with (GA) and (GT) probes and SSR-containing clones were isolated and sequenced. On the basis of this screening, they estimated that there are roughly 5800 $(GA)_n$ and $(GT)_n$ SSRs in haploid perennial ryegrass genome. Polymerase chain reaction (PCR) primers were designed to amplify the isolated SSRs and six polymorphic SSRs were identified. Polymorphism in these six SSRs was sufficient to discriminate among 18 individuals representing 11 perennial ryegrass clones and seven other *Lolium* species. Half-sibs could be distinguished with data from as few as three SSRs. The SSR genotype data was also used to infer genetic relationships among the individuals of the sample. The relationships were in broad agreement with those established by previous analyses, suggesting that SSR data will be using for exploring relationships among perennial ryegrass cultivars. In total, this study indicates that SSRs are sufficiently abundant and sufficiently polymorphic to be useful genetic markers in perennial ryegrass.

Prevost and Wilkinson (1999) studied the potential of ISSR PCR for fingerprinting using four primers on 34 potato cultivars. The complex band profiles generated were reproducible among repeat PCRs, DNA extractions, electrophorese and gel scorings. Two primers were each able to distinguish all cultivars. The combined use of any two of the four primers also allowed complete diagnosis. It is concluded that ISSR-PCR provides a quick, reliable and highly informative system for DNA fingerprinting that is amenable for routine applications. Two possible correlates of the ability of primers to distinguish between genotypes were then examined. Marker Index failed to correlate significantly with genotype diagnosis, but a strong and seemingly linear relationship was observed between Resolving Power of a primer and its ability to distinguish genotypes (r^2=0.98). Resolving Power of one or a pair of primers was found to provide a moderately accurate estimate of the number of genotypes identified. Possible implications for future studies on DNA fingerprinting were discussed.

Laprade *et al.* (1999) found that DNA fingerprinting has been successfully applied to plants to develop genetic profiles. They observed that these tools were imported in diverse fields of plant population research, e.g., the study of breeding systems (sexual versus asexual reproduction in clonal plant species, estimating of selfing rates, paternity and maternity analysis, genetic

relatedness between or within species and populations, assessment of gene flow and gene identification.

Tessier *et al.* (1999) developed a cultivar identification tool based on molecular analysis and a statistical approach. From the PIC parameter they defined the Discriminating power (D parameter) which evaluates the efficiency of a primer for the purpose of identification of varieties; i.e. the probability that two randomly chosen individuals have different patterns. D can be used to compare different types of markers even if only the allelic frequencies are known. They used this parameter to develop an algorithm for selecting the optimal combination of primers necessary to identify a set of varieties. The optimal combination of primers determined for a small elite group of varieties applied on a larger set induces a risk of confusion involving one of the elite varieties. They estimated the risk of confusion using the D value of each primer of the combination. They applied this methodology on a set of 224 varieties of *Vitis vinifera* screened with 21 RAPD primers and two microsatellite loci. The discriminating power of the primers did not only depend on the number of patterns it generates but also on the frequencies of the different patterns. A combination of 8 primers (6 RAPD and two microsatellites) was found to be optimum for the discrimination of these 224 varieties. A subset of 38 elite varieties was also investigated. The determined optimal combination of 4 primers (3 RAPD and one microsatellite) applied on the 224 varieties gave 9 risks of confusion involving 1 of the elite varieties. Confusion can happen between varieties with the same origin as well as between varieties of very diverse geographical origins.

Brenneman and Culbreath (2000) found that Bentex T significantly reduced the incidence and severity of the disease compared to the other fungicides. This was followed by Benlate 50 WP. The corresponding yields following treatment with these chemicals were also higher than with the other chemicals and the control.

Subramanian *et al.* (2000) evaluated 70 groundnut genotypes representing variability for several morphological, physiological and other characters for polymorphism employing random amplified polymorphic DNA (RAPD) assay with 48 oligonucleotide primers and found 7 (14.7%) as polymorphic. The seven polymorphic primers produced 408 bands out of these 27 were polymorphic.

Ijaz (2011) studied on various aspects of Cercospora leaf spot (CLS)related to host pathogen interaction, epidemiology, germplasm screening and cultural and chemical control measures. Survey and multi-locational field experiments revealed higher CLS severities under high rainfall, especially in early monsoon zones or years. *Cercospora personatum* (LLS) pathogen is more significant than *C. arachidicola* (ELS) under temperature regimes of 20-24°C and 25-30°C with leaf wetness conditions. Forecasting system based on humid thermal ratios increasing 3.1 for more than 3.5 days may be used to forecast disease initiation. This may be helpful to obtain better yields by adopting plant protection measures in time. This system may be used by extension workers, other public or private sector agencies to forecast spray schedules. Under poor ecologies, genotypes had higher disease severities and defoliation however promising lines and newly approved commercial cultivars evolved under rainfed conditions with good agronomic characteristics were found tolerant. In peanut germplasm reaction studies to CLS at pod development stage, most of the genotypes Virginia, Valencia or Spanish were susceptible to CLS. Genotypes even resistant at flowering stage became highly susceptible to CLS at later stages of plant development. Most of the peanut germplasm available is Virginia type and is late maturing so higher yield losses are expected from CLS severities at pod development stage. There are genotypes available in Virginia, Spanish and Valencia type exotic material possessing resistance to CLS in form of spots per leaf, sporulation or defoliation. There is a need to expand variability

in germplasm either through hybridization of CLS resistant genotypes and genotypes containing good agronomic characteristics or using mutation technologies. Studies on management strategies suggest that yield may be increased significantly under poor ecologies in genotypes with good agronomic characters through fungicide protection from CLS.

Moraes *et al.* (2001) conducted four field trials in Ribeirão Preto and Pindorama, SP, Brazil (1996 and 1997) to evaluate the efficiency of fungicides in the control of late leaf spot (*Cercosporidium personatum*) and scab (*Sphaceloma arachidis*) in peanut (*Arachis hypogaea*), cultivar Tatu. The treatments consisted of: control, not sprayed; conventional control, with four sprayings of chlorothalonyl at 14-day intervals starting at 41 to 43 days after planting monitored control with chlorothalonyl, tebuconazole, difenoconazole and propiconazole, respectively, in the recommended dosages for the peanut crop. Monitoring consisted of doing the first spraying when 5 to 15% of the leaflets were infected by the late leaf spot and the following sprayings, at a minimum of 14-day intervals between applications, after three days, consecutive or not, in which rain precipitations greater than 2.5 mm were registered, in periods of seven days. Late leaf spot severity was assessed at weekly intervals during the growing season with diagrammatic scales of infected leaf area. Scab severity was evaluated at 84-92 days after planting with a specific scale of notes, ranging from 1 to 4 according to the symptoms exhibited in stems and petioles. The results showed that in the monitored control, where the number of sprayings was reduced to one to three, the triazole fungicides were more efficient than chlorothalonyl, resulting in pod yields close to the treatment where four fixed sprayings were made. Tebuconazole promoted the highest reductions in late leaf spot intensity and, difenoconazole showed outstanding efficiency in the control of scab.

Culbreath *et al.* (2002) tested sterol biosynthesis inhibitor and strobilurin fungicides for control of early (*Cercospora arachidicola*) and late (*Cercosporidium personatum*) leaf spot diseases of peanut (*Arachis hypogaea*). They want to find that systemic fungicide benomyl, the protectant fungicide chlorothalonil and late leaf spot of peanut as a model system to compare fungicide application strategies for fungicide resistance management. Field experiments were conducted at Tifton and Plains, GA, in 1995 and 1996 to determine the effects of alternate applications, mixtures and alternating block applications of chlorothalonil and benomyl compared with full-season applications of two rates of chlorothalonil and two rates of benomyl alone on late leaf spot of peanut and on the proportion of the pathogen population resistant to benomyl following the various regimes. Tank mix combinations of half rates of the two fungicides and alternations of the full rates of the two fungicides provided better ($p \leq 0.05$) control of late leaf spot than full season applications of either rate of benomyl alone and were comparable to full rates of chlorothalonil alone. Neither tank mixes nor alternating sprays prevented an increase in the relative frequency of Benomyl resistant isolates compared with other treatments in which benomyl were used. Both mixtures and alternate applications of chlorothalonil and benomyl were effective for management of leaf spot in fields where benomyl alone did not provide season-long leaf spot control.

Palmieri (2002) found that *Arachis pintoi* is an alternative to forage production in the tropics. Its germplasm comprises more than 150 accessions that could be used to improve it. The objective of this study was isolation and characterization of microsatellite loci in *A. pintoi* to be used to molecular evaluation of this germplasm and of *A. repens* (section Caulorrhizae). Seven loci were analyzed using five accessions of *A. repens* and 20 accessions of *A. pintoi*. The high variation found makes clear the high potential of this marker in genetic studies in these species. The developed markers showed total transferability to *A. repens*.

He *et al.* (2003) surveyed 56 SSR markers on 24 cultivated peanut genotypes and found 19 as polymorphic. The average number of alleles per locus was 4.25. The maximum number of

alleles was 14 and found in primer PM50. When 48 genotypes surveyed by using five such highly polymorphic markers they could differentiate 24 cultivated groundnut genotypes.

Ferguson *et al.* (2004) observed that major constraint to the application of biotechnology to the improvement of the allotetraploid peanut, or groundnut (*Arachis hypogaea* L.) has been the paucity of polymorphism among germplasm lines using biochemical (seed proteins, isozymes) and DNA markers (RFLPs and RAPDs). Six Sequence Tagged Microsatellite (STMS) markers were previously available that revealed polymorphism in cultivated peanut. They identified and characterized 110 STMS markers that reveal genetic variation in a diverse array of 24 peanut landraces. The Simple Sequence Repeats (SSRs) were identified with a probe of two 27,648 clone genomic libraries: one constructed using PstI and the other using Sau3AI/BamHI. The most frequent, repeat motifs identified were ATT and GA, which represented 29 and 28%, respectively, of all SSRs identified. These were followed by AT, CTT and GT. Of the amplifiable primers, 81% of ATT and 70.8% of GA repeats were polymorphic in the cultivated peanut test array. The repeat motif AT showed the maximum number of alleles per locus (5.7). Motifs ATT, GT and GA had a mean number of alleles per locus of 4.8, 3.8 and 3.6, respectively. The high mean number of alleles per polymorphic locus, combined with their relative frequency in the genome and amenability to probing, make ATT and GA the most useful and appropriate motifs to target and to generate further SSR markers for peanut.

Moretzsohn *et al.* (2004) developed 67 new microsatellite markers (mainly TTG motif) for *Arachis*. Only three of these markers were polymorphic in cultivated peanut. These three new markers plus five other markers characterized previously were evaluated for number of alleles per locus and gene diversity using 60 accessions of *A. hypogaea*. Genetic relationships among these 60 accessions and a sample of 36 wild accessions representative of section *Arachis* were estimated using allelic variation observed in a selected set of 12 SSR markers. Results showed that the Brazilian peanut germplasm collection has considerable levels of genetic diversity detected by SSR markers. Similarity groups for *A. hypogaea* accessions were established, which is a useful criteria for selecting parental plants for crop improvement. Microsatellite marker transferability was up to 76% for species of the section *Arachis*, but only 45% for species from the other eight *Arachis* sections tested. A new marker (Ah-041) presented 100% transferability and could be used to classify the peanut accessions in AA and non-AA genome carriers.

Kumar (2004) initiated to assess diversity using SSR markers among 29 groundnut accessions belonging to two subspecies *fastigiata* and *vulgaris* andthree botanical varieties *vulgaris, fastigiata* and *hypogaea*,originating from fifteen countries, which include 25 early-maturing and four late-maturing accessions. Initially intra accession variation among ten accessions of E G 3540, ICG 4558, ICG 4890, ICG 9427, ICG 11914, ICG 14814, Gangapuri, JL 24, Chico andTMV2, was assayed using *5* SSR primer pairs. UPGMA clustering of the SSR band profiles revealed significant variation within the accessions. He suggested that small number of individuals or single plant may not be fully representative of that particular accession. So, use of more individuals from each accession and pooling the template DNA from several individuals is an attractive strategy because it is not possible to detect all the constituent bands in the composite profile. A total of 22 alleles were detected by five primer pairs with an average number of 4.4 alleles per primer pair. The number of alleles ranged from 2 (2B10) to 8 (2D12B). To capture this intra-accession diversity in main study, equal amounts of DNA from individual plants were pooled for each accession. Inter-accession diversity analysis of 29 accessions was performed using 20 SSR primer pairs, which detected a total of 57 alleles with an average of 2.85 alleles per primer

pair. The number of alleles ranged from 2-5. The PIC values, ranged from 0.53 (17F6) to 0.93 (15C12), with an average of 0.78.

Krishna *et al.* (2004) recorded morphological, physiological and agronomic traits, whereas few molecular variations have been recorded for this crop. The identification and understanding of molecular genetics diversity in cultivated peanut types will help in effective genetics conservation along with efficient breeding programs in this crop. The New Mexico breeding program has embarked upon a program of improvement of Valencia type peanut (belonging to the sub species *fastigiata*), because efforts to improve the yield potential are lacking due to lack of identified divergent exotic types. For the first times, this study has shown molecular diversity using microsatellite markers in the cultivated Valencia peanut (sub spp. *fastigiata*) from around the globe. In this investigation, 48 cultivated Valencia peanut genotypes have been selected and analyzed using 18 fluorescently labeled SSR (f-SSR) primers pairs. These primers pairs amplified 120 polymorphic loci among the genotypes screened and amplified from 3 to 19 alleles with an average of 6.9 alleles per primers pair. The f-SSR markers data was further analyzed using cluster algorithms and principal component analysis. The results indicated that considerable genetics variations were discovered among the analyzed genotypes. The f-SSR based clustering could identify the putative pedigree types of the present Valencia types of diverse origins and the f-SSR in general is sufficient to obtain estimates of genetics divergence for the material in study. The results are being utilized in our breeding program for parental selection and linkage map construction.

Pinto *et al.* (2004) reported that sugarcane microsatellites or Simple Sequence Repeats (SSR) developing is economical and practical way by mining EST databases. A survey in the SUCEST (sugarcane EST) database revealed a total of 2005 clusters out of 43,141 containing SSRs. Of these, 8.2% were dinucleotide, 30.5% were trinucleotide and 61.3% were tetranucleotide repeats. Except for dinucleotides, the CG-rich motif types were the most common. Differences in abundance of trinucleotide motif types were observed between EST-SSRs and those isolated from sugarcane genomic libraries. Among the different cDNA libraries used for EST sequencing, SSRs were more frequent in the ones derived from leaf roll (LR). Twenty-three out of 30 tested SSRs produced scorable polymorphisms in 18 sugarcane commercial clones. These EST-SSRs showed a moderate level of polymorphism with some SSRs producing unique fingerprints. The number of alleles observed among the 18 clones evaluated varied from 2 to 15, with an average of 6.04 alleles/locus. The polymorphism information content (PIC) values ranged from 0.28 to 0.90 with a mean of 0.66. The EST-SSRs screened over both parents (SP 80-180, SP 80-4966) and 6 F_1 individuals produced 52 segregating markers that could potentially be used for sugarcane mapping. The EST-SSRs were found in clusters that had significant homology to proteins involved in important metabolic pathways such as sugar biosynthesis, proving that EST-SSRs are a valuable tool for the construction of a functional sugarcane map.

He *et al.* (2005) identified DNA markers associated with botanical varieties that are useful in plant genotyping, germplasm management and evolutionary studies. They have developed 130 Simple Sequence Repeat (SSR) markers in peanut, 38 of which were used in this study because of their ability in detecting genetic polymorphism among 24 peanut accessions. Eight SSR markers were found useful to classify botanical varieties. Among them, six SSR markers were specific to botanical varieties *fastigiata* and *vulgaris*, one to botanical varieties *hypogaea* and *hirsuta* and one to botanical varieties *peruviana* and *aequatoriana*. Also, three of them derived from peanut expressed sequence tags (ESTs) were associated with putative genes. As botanical varieties have different morphological traits and belong to different subspecies in *A. hypogaea*, these markers

might be associated with genes involved in the expression of morphological traits. The results also suggested that SSRs (also called microsatellites) might play a role in shaping evolution of cultivated peanut. Multiplex PCR of botanical variety-specific markers could be applied to facilitate efficient genotyping of the peanut lines.

Hagan *et al.* (2006) estimated losses due to cercospora leaf spot in groundnut in the USA and found that this disease caused up to 50% of yield losses.

Mace *et al.* (2006) studied rust (*Puccinia arachidis*) and late leaf spot (LLS, *Phaseoisariopsis personata*) the major diseases causing significant yield loss in groundnut. The development of varieties with high levels of resistance has been constrained by adaptation of disease isolates to resistance sources and incomplete resistance in resistant sources. The current study was conducted to identify diverse disease resistant germplasm for the development of mapping populations and for their introduction into breeding programs. Twenty three SSRs were screened across 22 groundnut genotypes with differing levels of resistance to rust and LLS. Overall, 135 alleles across 23 loci were observed in the 22 genotypes screened. Twelve of the 23 SSRs (52%) showed a high level of polymorphism, with PIC values ≥0.5. This is the first report detecting such high levels of genetic polymorphism in cultivated groundnut. Multi-dimensional scaling and cluster analyses revealed three well-separated groups of genotypes. Locus by locus ANOVA and Kruskal-Wallis one-way ANOVA identified candidate SSR loci that may be valuable for mapping rust and LLS resistance. The molecular diversity analysis presented here provides valuable information for groundnut breeders designing strategies for incorporating and pyramiding rust and late leaf spot resistances and for molecular biologists wishing to create recombinant inbred line populations to map these traits.

Barkley *et al.* (2007) survey 141 peanut mini core collection with 31 genomic SSR markers with a M13 tail attached to assess the genetic diversity. A total of 477 alleles were detected with an average of 15.4 alleles per locus. The mean Polymorphic Information Content (PIC) score was 0.687. The cultivated peanut (*Arachis hypogaea* L.) mini core produced a total of 312 alleles with an average of 10.1 alleles per locus. A neighbor-joining tree was constructed to determine the interspecific and intraspecific relationships in this data set. Almost all the peanut accessions in this data set classified into subspecies and botanical varieties such as subsp. *hypogaea* var. *hypogaea*, subsp. *fastigiata* var. *fastigiata* and subsp. *fastigiata* var. vulgaris clustered with other accessions with the same classification, which lends further support to their current taxonomy. Alleles were sequenced from one of the SSR markers used in this study, which demonstrated that the repeat motif is conserved when transferring the marker across species borders.

Gimenes *et al.* (2007) cloned 68 sequenced and found 38 (55.9%) of them containing microsatellites were isolated and characterized using 16 accession of 16 *A. hypogaea*. The level of variation found in *A. hypogaea* using microsatellites was higher than with other markers. Cross-transferability of the markers was also high. Sequencing of the fragments amplified using the primer pair Ah11 from 17 wild *Arachis* species showed that almost all wild species had similar repeated sequence to the one observed in *A. hypogaea*. Sequence data suggested that there is no correlation between taxonomic relationship of a wild species to *A. hypogaea* and the number of repeats found in its microsatellite loci. A higher level of variation among *A. hypogaea* accessions can be detected using microsatellite markers in comparison to other markers, such as RFLP, RAPD and AFLP. 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 assess the mating system in *Arachis* species.

Kamburona (2007) utilized both the AFLP and SSR markers for evaluating the degree of polymorphism among 18 RSA peanut lines and an OC-1 transformed line. In AFLPs studied he detected 246 fragments of which 148 were polymorphic and 88 were monomorphic. The 60% of fragment analyzed were polymorphic and only 40% were monomorphic. The polymorphic information content (PIC) and marker index (MI) for the primer pairs, giving information on how polymorphic they are in relation to one another, were also calculated. Primers E-ACT/M-CCG was the most informative (i.e., most polymorphic with a POL of 65.3%) and had a PIC of 0.45 and a marker index of 29.59. He used six SSR primers pairs and found that fragment size varied between 60-520 bp. Primer pair Lee-1 resulted in the highest number of amplified loci (four) across the 19 peanut lines, while Ah4-4 only amplified one locus across the samples.

Jiang *et al.* (2007) assessed genetic relationships of 31 peanut genotypes with various levels of resistance to Bacterial Wilt (BW) based on SSR and AFLP analysis. Twenty nine of 78 SSR primers and 32 of 126 AFLP primer combinations employed were polymorphic amongst the peanut genotypes tested. The SSR primers amplified 91 polymorphic loci in total with an average of 3.14 alleles per primer and the AFLP primers amplified 72 polymorphic loci in total with an average of 2.25 alleles per primer. Four SSR primers (14H06, 7G02, 3A8, 16C6) and one AFLP primer (P1M62) were found to be most efficient in detecting diversity. The genetic distance between pairs of genotypes ranged from 0.12-0.94 with an average of 0.53 in the SSR data and from 0.06-0.57 with an average of 0.25 in the AFLP data. The SSR based estimates of the genetic distance were generally larger than that based on the AFLP data. The genotypes belonging to subsp. *fastigiata* possessed wider diversity than that of subsp. *hypogaea*. The clustering of genotypes based on the SSR and AFLP data were similar but the SSR clustering was more consistent with morphological classification of *A. hypogaea*. Optimum diverse genotypes of both subsp. *hypogaea* and subsp. *fastigiata* can be recommended based on this analysis for developing mapping populations and breeding for high yielding and resistant cultivars.

Wang*et al.* (2007) surveyed 34 SSR markers to assess the genetic variation of four sets of 24 accessions each from the four botanical varieties of the cultivated peanut. Among the tested accessions, 10-16 pairs of SSR primers showed polymorphisms. The maximum differentiation index, which was defined as the degree of genetic differentiation, was as high as 0.992 in the tested accessions. Each accession could be discriminated by a specific set of polymorphic SSR primers and the intra-variety genetic distance was determined among accessions, with an average of 0.59 in var. *fastigiata*, 0.46 in var. *hypogaea*, 0.38 in var. *vulgaris* and 0.17 in var. *hirsuta*. Dendrograms based on genetic distances were constructed for the four botanical varieties, which revealed the existence of different clusters. It was 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.

Cuc *et al.* (2008) found that due to the origin of *Arachis hypogaea* L. through a single and recent polyploidization event, followed by successive selection during breeding efforts, cultivated groundnut has a limited genetic background. In such species, microsatellite or Simple Sequence Repeat (SSR) markers are very informative and useful for breeding applications. The low level of polymorphism in cultivated germplasm, however, warrants a need of larger number of polymorphic microsatellite markers for cultivated groundnut. They constructed microsatellite enriched library from the genotype TMV2. 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. Based on 112 alleles obtained by 46 markers, a phenogram was constructed to understand the relationships among the 32 genotypes. Majority of the genotypes representing subspecies *hypogaea* were grouped together in one cluster, while the genotypes belonging to subspecies *fastigiata* were grouped mainly under two clusters.

Naito *et al.* (2008) analyzed 201 cultivated and 15 wild accessions of *A. hypogaea* with 13 SSR markers. These 13 primers pairs amplified 108 polymorphic alleles in *A. hypogaea*. The detected alleles were 3-15 at each of the 13 markers with an average of 8.3 per marker. The phenogram based on the SSR genotypes revealed that *A. hypogaea* and *A. monticola* made a separate group from diploid species. They were classified into 150 genotypes. *A. hypogaea* and *A. monticola* were divided further into two groups. The first group consisted mainly of spp. *fastigiata* accessions, the second groups consisted mainly of sp. *hypogaea* accessions and tetraploid wild peanut *A. monticola*.

Gomez *et al.* (2008) differentiated between self and hybrids in peanut F_1 and F_2 populations using SSR markers. Banding patterns of the parents showed that 70% of the putative hybrids possessed marker allele from the male parent. The H-PAGE gels gave better band separation and differentiation of selfed progenies than agarose gels and were compatible with the common horizontal agarose gel units. This method provides a quick assay to distinguish hybrids from inadvertent selfs and should result in greater efficiency and more effective use of resources in peanut breeding programs.

Gautami *et al.* (2009) constructed SSR enriched library from the genotype ICGV 86031. Out of 29 SSR isolated, primer pairs were designed for 23 SSR loci of which 14(61%) primers pairs yielded scorable amplicon. Eight (57%) primer pairs showed polymorphism among 23 groundnut genotypes that are parents of different groundnut mapping population at ICRISAT, India and EMBRAPA, Brazil. The PIC for polymorphic SSR markers ranged from 0.13 to 0.36 with an average of 0.25. Newly isolated SSR loci showed good interspecific transferability rate ranging from 13-100% across seven legumes and 43-100% within the seven legumes. The present set of newly developed SSR markers enriches the existing groundnut SSR repertoire and the transferrable SSR markers will be useful for comparative genome analysis in related legumes.

Varshney *et al.* (2009) assessed genetic variation among 189 groundnut accessions with 25 simple sequence repeat loci. A total number of 265 alleles were detected with the range of 3 (Ah1TC6G09) to 20 (Ah1TC11H06) with an average of 10.6 alleles per locus. The polymorphism information content value at these loci varied from 0.38 (Ah1TC6G09) to 0.88 (Ah1TC11H06) with an average of 0.70. A total of 59 unique alleles and 127 rare alleles were detected at almost all the loci assayed. Cluster analysis grouped 189 accessions into four clusters. In general, genotypes of South America and South Asia showed high level of diversity. Extraordinary level of natural genetic variation reported here provides opportunities to the groundnut community to make better decisions and define suitable strategies for harnessing the genetic variation in groundnut breeding.

Liang *et al.* (2009) investigated 24,238 ESTs for the identification and development of SSR markers. In total, 881 SSRs were identified from 780 SSR-containing unique ESTs. On an average, one SSR was found per 7.3 kb of EST sequence. Tri-nucleotide motifs (63.9%) being the most

abundant followed by di-(32.7%), tetra-(1.7%), hexa-(1.0%) and penta-nucleotide (0.7%) repeat types. The top six motifs included AG/TC (27.7%), AAG/TTC (17.4%), AAT/TTA (11.9%), ACC/TGG (7.72%), ACT/TGA (7.26%) and AT/ TA (6.3%). Based on the 780 SSR containing ESTs, a total of 290 primer pairs were successfully designed and used for validation of the amplification and assessment of the polymorphism among 22 genotypes of cultivated peanuts and 16 accessions of wild species. The results showed that 251 primer pairs yielded amplification products, of which 26 and 221 primer pairs exhibited polymorphism among the cultivated and wild species examined, respectively. Two to four alleles were found in cultivated peanuts, while 3-8 alleles presented in wild species. Sequence analysis of selected amplified alleles revealed that allelic diversity could be attributed mainly to differences in repeat type and length in the microsatellite regions. In addition, a few single base mutations were observed in the microsatellite flanking regions.

Duarte Filho *et al.* (2010) analyzed the genetic similarity in commercial cultivars of sugarcane from the breeding program cultivars RB (Republic of Brazil), using SSR markers and coefficient of parentage. Eighteen microsatellite markers were used to estimate genetic similarity in 30 genotypes and coefficient of parentage was estimated in 28 accessions. Eighteen primer pairs produced an average of 3.2 alleles, the level of polymorphism (PIC value) ranged from 0.34 to 0.78 in SMC248CG and SCC2 primers, respectively. The parentage coefficient was high among cultivars, with a mean of 0.14, suggesting high relationship among the cultivars. The results here suggest that to analyzed accessions, there is a high genetic similarity which could reduce the genetic gain in breeding. However, crosses among genotypes of sugarcane produce a high variability in the progenies, suggesting a combination between the genomes of species that originated the current cultivars.

Khedikar *et al.* (2010) studied 268 recombinant inbred lines of a mapping population TAG 24 × GPBD 4 segregating for LLS and rust were used to undertake QTL analysis. Phenotyping of the population was carried out under artificial disease epiphytotics. Positive correlations between different stages, high to very high heritability and independent nature of inheritance between both the diseases were observed. Parental genotypes were screened with 1,089 Simple Sequence Repeat (SSR) markers, of which 67 (6.15%) were found polymorphic. Segregation data obtained for these markers facilitated development of partial linkage map (14 linkage groups) with 56 SSR loci. Composite interval mapping (CIM) undertaken on genotyping and phenotyping data yielded 11 QTLs for LLS (explaining 1.70-6.50% phenotypic variation) in three environments and 12 QTLs for rust (explaining 1.70-55.20% phenotypic variation). Interestingly a major QTL associated with rust (QTLrust01), contributing 6.90-55.20% variation, was identified by both CIM and single marker analysis (SMA). A candidate SSR marker (IPAHM 103) linked with this QTL was validated using a wide range of resistant/susceptible. A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.)

Molla *et al.* (2010) studied a set of three SSR markers namely, PM36, PM50 and PM238 for identification of ten cultivated groundnut varieties (Dhaka-1, Bashanti, Tridana, Zhinga badam, BARI badam 5,6,7; BINA Cheenabadam 1,2,3) available in Bangladesh. All the cultivars were successfully discriminated by these three SSR primers. The primer PM50 alone was able to distinguish four varieties (Dhaka-1, Bashanti, Tridana and Zhinga Badam). Six variety- specific alleles were identified, these are, PM36/227, PM50/110, PM50/116, PM50/118, PM50/137, PM238/200. The three primers produced a total of 13 alleles with size ranging from 109bp to 241bp. The Polymorphism Information Content (PIC) value for the primer PM36, PM50 and

PM238 was found 0.81, 0.76 and 0.82 respectively. This approach will be useful for developing a set of limited number of SSR loci for the identification of commercially important groundnut varieties for purpose of obtaining Plant Variety Protection (PVP) in Bangladesh.

Mondal and Badigannavar (2010) studied molecular diversity and association of simple sequence repeat (SSR) markers with rust and late leaf spot (LLS). The LLS resistance was detected in a set of 20 cultivated groundnut genotypes varying in resistance against rust and LLS. Out of 136 bands amplified from 26 primers 104 were found polymorphic (76.5%) cluster analysis (UPGMA) revealed two main clusters separated at 52% Jaccard's similarity coefficient according to disease reaction to rust and LLS. Based on the Kruskal-Wallis one way ANOVA and simple regression analysis three and four SSR alleles were found associated with rust and LLS resistance, respectively.

Shoba *et al.* (2010) used seventeen SSR markers for analyzing 11 groundnut genotypes and found six primer pairs (24.0%) as polymorphic. The genotype TMV 2 was susceptible to rust and late leaf spot diseases and it was separately clustered in the dendrogram and among the eleven foliar disease resistant genotypes, the genotypes viz., COG 0423, COG 0436 and COG 0432 were distantly clustered from TMV 2. Hence, by using the genotypes viz., TMV 2, COG 0423, COG 0436 and COG 0432, three combinations viz., TMV 2 x COG 0423, TMV2 x COG 0436 and TMV 2 x C0G 0432 could be made for further studies for the development of late leaf spot resistant genotypes.

Song *et al.* (2010) used wild type peanuts to increase the genetic diversity and gene resources of the peanut cultivars. They found Marker Assisted Selection (MAS) could shorten the process of inter-specific hybridization and provide a possible way to remove the undesirable traits. The limited number of molecular markers available in peanut retarded its MAS process. They cloned the gene of agronomic interest for the development of molecular markers. They found 610 ESTs that contained one or more SSRs from 12,000 peanut ESTs. The most abundant SSRs in peanut were trinucleotides (66.3%) SSRs followed by dinucleotide (28.8%) SSRs. AG/TC repeats were 10.7% followed by CT/GA (9.0%), CTT/GAA (7.4%) and AAG/TTC (7.3%) repeats respectively. Ninety-four SSR containing ESTs were randomly selected for primer design and synthesis, of which 33 pairs could generate good amplification and were used for polymorphism assessment. Results showed that polymorphism was very low in cultivars, while high level of polymorphism was revealed in wild type peanuts.

Han *et al.* (2011) used 238 Simple Sequence Repeat (SSR) markers for prominent gene identification and 36 Korean varieties discrimination. Twelve SSR primers were found that can differentiate between these 36 varieties. These 12 primer pairs amplified 27 alleles with an average of 2.3 alleles per primer.

Macedo *et al.* (2012) constructed a plasmid genomic library enriched for TC/AG repeats and 1401 clones sequenced. From the sequences obtained 146 primer pairs flanking mostly TC microsatellites were developed. The average number of repeat motifs amplified was 23. These 146 markers were characterized on 22 genotypes of cultivated peanut. In total 78 of, the markers were polymorphic within cultivated germplasm. Most of those 78 markers were highly informative with an average of 5.4 alleles per locus being amplified. Average gene diversity index (GD) was 0.6 and 66 markers showed a GD of more than 0.5. Genetic relationship analysis was performed and corroborated the current taxonomical classification of *A. hypogaea* subspecies and varieties.

Mei *et al.* (2012) assessed amplification efficiency and its application potential for the research on peanut genetic diversity by using cultivars- derived EST-SSR primers in 89 *Arachis aeecssions*. From randomly selected 235 EST-SSR primer pairs, they detected 223 amplification

bands in the 89 accessions with the amplification efficiency of 94.89%. Among 53 primer pairs exhibited 206 polymorphic bands, which showed 1-12 bands per primer pair with an average of 3.89. The polymorphism index ranged from 0.044 to 4.040 with an average of 1.173. The average similarity coefficient was 0.685 among 89 peanut accessions, ranging from 0.442 to 0.976. The accessions from the same section were mainly clustered together, peanut cultivars were grouped into the section of *Arachis* and *A. duranensis* had a close relation to cultivated peanut, which is coincident with botanical classification. According to the statistical analysis from the results of oil content and SSR data in wild peanuts, some interesting bands such as POCR437-180/170 were found to be potential for candidate markers related to oil content. BLAST analysis indicated that the alignment sequence of primer POCR437 corresponded to the coding genes of malonyl-CoA-ACP transacylase and acyl-carrier-protein, both of which are involved in fatty acid synthesis.

Macedo *et al.* (2012) studied plasmid genomic library enriched for TC/AG repeats. 146 sequences primer pairs flanking mostly TC microsatellites were developed. The average number of repeat motifs amplified was 23. These 146 markers were surveyed on 22 cultivated peanut genotypes. Seventy eight markers were polymorphic with an average of 5.4 alleles per locus being amplified. The average Gene Diversity (GD) index was 0.6, whereas 66 markers showed a GD of more than 0.5. Genetic relationship analysis was performed and corroborated the current taxonomical classification of *A. hypogaea* subspecies and varieties.

Mondal *et al.* (2012) studied 164 recombinant inbred lines derived from resistant (VG 9514) and susceptible (TAG 24) cultivated groundnut parents and screened for rust resistance in five environments. Subsequent genotyping of these lines with 109 SSR markers generated a genetic linkage map with 24 linkage groups. The total length of the linkage map was 882.9 cM with an average of 9.0 cM between neighboring markers. The markers pPGPseq4A05 and gi56931710 flanked the rust resistance gene at map distances of 4.7 and 4.3 cM, respectively, in linkage group 2. The significant association of these two markers with the rust reaction was also confirmed by discriminant analysis. The informative SSR markers classified rust-resistant and susceptible groups with 99.97% correctness. The SSR markers pPGPseq4A05 and gi56931710 were able to identify all the susceptible genotypes from a set of 20 cultivated genotypes differing in rust reaction. Tagging of the rust resistance locus with linked SSR markers will be useful in selecting the rust resistant genotypes from segregating populations and in introgressing the rust resistance genes from diploid wild species.

Lukanda *et al.* (2012) analyzed plant and disease development cycles of leaf spot diseases in *A. hypogaea* and observed significant differences among the groundnut varieties evaluated for resistance to the leaf spot disease. The results show that plant development cycle can be divided into three developmental stages. A first stage characterized by a low production of leaves, a second stage with a significant leaf development and finally a third stage with a reduction of leaves. Interestingly, the leaf spot disease cycle was also divided in three stages. The disease stage characterized by the highest level of symptom expression was not associated with the plant phase with the highest emerged leaves. Disease symptoms reached the highest pick only after the phase of intense leaf development. The molecular analysis revealed that all the groundnut varieties analyzed were genetically closely related even though they showed different reactions to the leaf spot disease.

Pandey *et al.* (2012) used 4,485 markers for the identification of highly informative set of SSR in cultivated groundnut (*Arachis hypogaea* L.), using a set of 20 parental genotypes of 15 mapping populations. A total of 3,582 (79.9%) markers produced scorable amplification whereas only 1,351 (37.3%) markers showed polymorphism. The 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 per marker. In general, the SSR markers based on di-nucleotide repeats displayed higher PIC value and number of alleles. They suggested initiating with an identified set of highly informative markers, instead of from the random set of SSR markers for diversity and molecular breeding study.

Pandey *et al.* (2012a) studied blockage of gene flow from diploid wild relatives to the tetraploid. cultivated peanut, recent polyploidization combined with self-pollination. They concluded that narrow genetic base of the primary genepool have resulted in low genetic diversity that has remained a major bottleneck for genetic improvement of peanut. Harnessing the rich source of wild relatives has been negligible due to differences in ploidy level as well as genetic drag and undesirable alleles for low yield. Lack of appropriate genomic resources has severely hampered molecular breeding activities and this crop remains among the less-studied crops. The last five years, however, have witnessed accelerated development of genomic resources such as development of molecular markers, genetic and physical maps, generation of expressed sequenced tags (ESTs), development of mutant resources and functional genomics platforms that facilitate the identification of QTLs and discovery of genes associated with tolerance/resistance to abiotic and biotic stresses and agronomic traits. Molecular breeding has been initiated for several traits for development of superior genotypes. The genome or at least gene space sequence is expected to be available in near future and this will further accelerate use of biotechnological approaches for peanut improvement.

Shirasawa *et al.* (2012) used *in silico* analysis that increase the efficiency of polymorphic marker development by more than 3-fold. In total, 926 (34.2%) of 2,702 markers showed polymorphisms between parental lines of the mapping population. Linkage analysis of the 926 markers along with 253 polymorphic markers selected from 4,449 published markers generated 21 linkage groups covering 2,166.4 cM with 1,114 loci. Based on the map thus produced, 23 quantitative trait loci (QTLs) for 15 agronomical traits were detected.

Shoba *et al.* (2012) studied molecular markers in groundnut linked with Late Leaf Spot (LLS) resistant genotypes. LLS susceptible genotype (TMV 2) and the LLS resistant genotype (COG 0437) were crossed and their F_2 population was used for marker analysis. The phenotypic mean data on $F_{2:3}$ progenies were used for phenotypic analysis. Parents were surveyed with 77 SSR primers to identify polymorphic markers. Among SSR markers, nine primers were found polymorphic between the parents TMV 2 and COG 0437. These markers were utilized for Bulked Segregant Analysis (BSA). Three primers viz., PM 375/162, pPGPseq5D5/220 and PM 384/100 were polymorphic and able to distinguish the resistant and susceptible bulks and individuals for LLS. In single marker analysis, the markers PM 375, PM 384, pPGPseq5D5, PM 137, PM 3, PMc 588 and Ah 4-26 were linked with LLS severity score. The phenotypic variation explained by these markers ranged from 32-59%. The markers identified through BSA were also confirmed with single marker analysis. While validating the three primers over a set of resistant and susceptible genotypes, the primer PM 384/100 allele had association with resistance. Hence, PM 384 could be utilized in the marker assisted breeding programme over a wide range of genetic background.

Vasavirama and Kirti (2012) made a double gene construct with SniOLP (*Solanum nigrum* osmotinlike protein) and Rs-AFP2 (*Raphanus sativus* antifungal protein-2) genes under separate constitutive 35S promoters was used to transform peanut plants. Transgenic peanut plants expressing the SniOLP and Rs-AFP2 genes showed enhanced disease resistance to late leaf spot based on a reduction in number and size of lesions on leaves and delay in the onset of *Phaeoisariopsis personata* leaf spot disease. PCR, RT-PCR and Southern hybridization analysis

confirmed stable integration and expression of these genes in peanut transgenics. The results demonstrate the potential of SniOLP and Rs-AFP2 genes in developing late leaf spot disease resistance in transgenic peanut.

Wang *et al.* (2012) studied total 1,424 SSRs identified from 36,435 BAC-end sequences (BESs). Among these SSRs, dinucleotide (47.4%) and trinucleotide (37.1%) SSRs were predominant. The new set of 1,152 SSRs as well as about 4,000 published or unpublished SSRs were screened against two parents of a mapping population and found 385 as polymorphic. A genetic linkage map was constructed, consisting of 318 loci onto 21 linkage groups and covering a total of 1,674.4 cM, with an average distance of 5.3 cM between adjacent loci. Two markers related to Resistance Gene Homologs (RGH) were mapped to two different groups, thus anchoring 1 RGH-BAC contig and 1 singleton.

Zhao *et al.* (2012) detected 1,343 SSR markers as polymorphic (14.5%) from a total of 9,274 markers and found AG motif (36.5%) in abundant followed by AAG (12.1%), AAT (10.9%) and AT (10.3%).The mean length of SSR repeats in dinucleotide SSRs was significantly longer than that in trinucleotide SSRs. Dinucleotide SSRs showed higher polymorphism frequency for genomic SSRs when compared to trinucleotide SSRs, while for EST-SSRs, the frequency of polymorphic SSRs was higher in trinucleotide SSRs than in dinucleotide SSRs. The correlation of the length of SSR and the frequency of polymorphism revealed that the frequency of polymorphism was decreased as motif repeat number increased.

CHAPTER 3:

MATERIALS AND METHODS

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Plant material: Ninety five groundnut genotypes were fingerprinted at DNA level using 277 SSR and 10 ISSR primers to assess the genetic variability and relatedness among these genotypes. These genotypes are maintained as gene pool at Barani Agricultural Research Institute (BARI) Chakwal, Punjab, Pakistan. The list of genotypes and their origin are illustrated in Table 1.

Nucleic Acid Extraction: Total nucleic acid of 95 genotypes was extracted from young leaves using modified CTAB method (Doyle and Doyle, 1990) with slight modification in β-mercaptoethanol concentration. The proteins were removed by adding chloroform:isoamyl alcohol and precipitated by centrifugation. RNA was removed using RNA degrading enzyme called RNase. NaCl, together with CTAB was used to remove polysaccharides. The DNA concentration was determined on Nanodrop spectrophotometer (ND 2000, Thermo Scientific, USA) which measure absorbance at 260nm and quality was evaluated by loading 50 ng DNA from each genotype on 0.8% agarose gel prepared in 0.5xTE buffer with ethidium bromide staining. DNA dilution of each genotype was prepared separately for SSR and ISSR analysis by using the following formula:

$$C_1V_1=C_2V_2$$

Where, C_1 known concentration of DNA stock. V_1= Volume of DNA stock required for dilution making. C_2= Required concentration of working solution. V_2= Required volume of working solution.

Primers, reaction mixture and PCR program: The concentration of DNA, $MgCl_2$, *Taq* DNA Polymerase and dNTPs were optimized through a series of experiments. High resolution agarose, i.e., Metaphor gel and Polyacrylamide Gel (PAGE) were used in this experiment. For resolving DNA bands 287 microsatellite sequence primer pairs were selected from the reported polymorphic primers as revealed by different studies given in Table 2. The primer pairs got synthesized from GeneLink Company (USA).

Reaction volume of 20µL containing 30ng genomic DNA, 2.0µL of 10X PCR buffer {Tris (pH 8.3) 50mM KCl} 3mM of $MgCl_2$, 0.3mM each of dATP, dCTP, dGTP and dTTP (Fermentas, USA) 0.6µM of each forward and reverse primers (GeneLink, USA) and one unit of *Taq* DNA polymerase. All these concentrations were optimized through a series of experiments. The PCR program was set for 94°C for 5 min, 35 cycles of 94°C for 45 sec, 35 cycles at the annealing temperature (Table 3), 35 cycles at 72°C for 1 min, final extension at 72°C for 7 min and holding at 4°C. Ten micro litter of the PCR mixture was examined by agarose gel (2% high resolution agarose). If the reported size of segment do not appear, the PCR product were resolved again on 6% polyacrylamide (19:1 acrylamide:bis-acrylamide) gel on vertical gel electrophoreses system (BiocomDirect, UK) and visualized by silver nitrate staining.

Data analysis: Only the clear unambiguous DNA fragments were scored. Markers were scored for the presence and absence to corresponding DNA band among the genotypes. The score '1' and

'0' indicate the presence and absence of the bands, respectively. DNA band size was estimated by comparing DNA bands with 100bp DNA ladder in case of agarose gel and 50 bp in case of PAGE gel. For confirmation that the observed bands were amplified genomic DNA and not the primer artifacts, genomic DNA was omitted from control reaction. No amplification product was detected without genomic DNA in any PCR and a negative control was also run to confirm that the master/reaction mixture is correctly prepared or not. These analyses were performed using NTSYS-pc software, version 2.2 (Rohlf, 2005). The dendrogram was construction using Unweight Pair Group Method of Arithmetic Means (UPGMA).

Expected heterozygosity (He) or Nei's which is known as gene diversity or diversity index (DI) were computed on Microsoft Excel 2010 following formula Powell *et al*., (1996)

$$He=1-\Sigma(p_i)^2$$

Where pi is the frequency of the ith allele in the population/locus (if individual marker is considered).

The polymorphic information content (PIC) was computed according to the formula (De Riek *et al*., 2001):

$$PIC =1 – [f^2 + (1-f)^2]$$

Where f is the frequency of markers used in the data set.

To compare the efficiency of the markers in varietal identification, the Discrimination power (Dj) of each primer was estimated (Tessier *et al*., 1999). Cj (confusion probability for the j^{th} primer) is equal to the sum of the different c_i for all I patterns generated by the primer:

$$Cj = \sum_{i=1}^{I} Ci = \sum_{i=1}^{I} pi \frac{(Npi - 1)}{N - 1}$$

Where pi is the frequency of the i^{th} allele and N is the number of individuals scored. Thus, the discriminating power of the j^{th} primer is equal to:

$$D_j = 1 – C_j$$

The Resolving power (Rp) of each SSR marker was calculated using the formula (Prevost and Wilkinson, 1999):

$$Rp = \sum Ib$$

Where Ib represents allele information, which is computed by the formula $Ib = 1-(2\times \left| 0.5-M \right|)$. The M value is the proportion of the total genotypes containing the allele.

Effective Multiplex Ratio (EMR) and Marker Index (MI) were calculated for all the different series and ISSR independently to measure the usefulness of each series and marker system according to Powell *et al*. (1996). Average heterozygosity (He) was estimated by taking the average of PIC values obtained for all the markers. Marker index (MI) was calculated by multiplying the average heterozygosity (He) with EMR (Powell *et al*., 1996; Nagaraju *et al*., 2001).

Evaluation of groundnut genotypes for resistance to Cercospora leaf spot (CLS): Eighty two peanut genotypes collected from various sources were sown on 29-04-2009, 10-05-2010 and 21-4-2011 in already infected fields. Every year the Cercospora diseased leaves were collected at harvesting from farmers field, BARI Chakwal and kept under room conditions in craft paper bags. These Cercospora infested leaves were added in soil at time of sowing to enhance the disease pressure. CLS naturally infected spots were excised from infected leaves from diseased field and incubated under moist chamber lined with aluminum foil for 48 hours for sporulation. These sporulated excised spots were ground in Molinex grinder to obtain spore suspension. Inoculation with fresh inoculum was followed by creation artificial leaf wetness with water misting up till due at night. Data of disease incidence were recorded at pod development stage on 19-09-2009 during first year. It was suggested by project evaluation team to record disease incidence data more than one stage so data was recorded on 01-08-2010 at flowering stage, 17-09-2010 at pod development stage and 08-10-10, 30 days before harvesting. Project evaluation system during third year suggested to record data at more than one stages by more than one scientists so during third year disease data was recorded by three scientists on 30-07-2011 and 19-09-2011 using Florida Cercospora leaf spot scoring scale (Chitika *et al*., 1988) described elsewhere in text. Scale used for disease scoring is based on diseased leaves and defoliation characteristic of genotype due to disease.

Chemical control of Cercospora leaf spot of groundnut: Trials were conducted under field conditions at Barani Agricultural Research Institute Chakwal. A highly susceptible genotype Yh-14 was cultivated in randomized complete block design. CLS infected leaves collected from previous year crop were added in soil before sowing of crop. Fresh inoculum was used also. In case of prolonged dry weather misting with water was carried to provide favourable environmental conditions for disease development. Following treatments were included in three years experimentation:

2009	**2010**	**2011**
Mancozeb 4gL-1	Mancozeb 4gL-1	Mancozeb 4gL-1
Mancozeb 8gL-1	Mancozeb 8gL-1	Mancozeb 8gL-1
Mancozeb 12gL-1	Mancozeb 12gL-1	Mancozeb 12gL-1
Fostyl.Al 1.25ml L-1	Chlorothalonil 1gL-1	Chlorothalonil 1gL-1
Fostyl.Al 2.50ml L-1	Chlorothalonil 2gL-1	Chlorothalonil 2gL-1
Fostyl.Al 3.75ml L-1	Chlorothalonil 3gL-1	Chlorothalonil 3gL-1
Thiophanate methyl 0.5gL-1	Propineb 2.5gL-1	Propineb 2.5gL-1
Thiophanate methyl 1.0gL-1	Propineb5gL-1	Propineb5gL-1
Thiophanate methyl 1.5gL-1	Propineb7.5gL-1	Propineb7.5gL-1
Carbendazim 0.5gL-1	10. Tebuconazole +Trifloxystrobin (0.16g+0.16g)L-1	Tebuconazole +Trifloxystrobin 0.32gL-1
Carbendazim 1.0gL-1	Tebuconazole +Trifloxystrobin (0.32g+0.32g)L-1	Tebuconazole +Trifloxystrobin 0.65gL-1
Carbendazim 1.5gL-1	Tebuconazole 0.98gL-1 +Trifloxystrobin (0.98g +0.98g)L-1	Tebuconazole +Trifloxystrobin 1.97gL-1
Panaconazole 0.25gL-1	Triazole-+Chlorothalonil (1ml+1g)L-1	Triazole 1ml L-1

Panaconazole 0.5gL-1	Triazole-+Chlorothalonil (2ml+2g)L-1	Triazole 2ml L-1
Panaconazole 1.05gL-1	Triazole-+Chlorothalonil (3ml+3g)L-1	Triazole-+Chlorothalonil 3ml L-1
Control	Control was included in trial.	

These fungicides were sprayed at ten days interval. Disease severity was recorded before each spray and 10 days after last spray according to 1-10 Florida leaf spot scoring scale (Chitika *et al*., 1988) as given below:-

Score	**Disease symptoms**
1	No leaf spots
2	Very few lesions on the leaves; none on the upper canopy
3	Few lesions on the leaves; very few on the upper canopy
4	Some lesions with more on upper canopy ≈5% defoliation
5	Lesions noticeable even on upper canopy ≈20% defoliation
6	Lesions numerous and very evident even on upper canopy ≈50% defoliation
7	Lesions numerous on upper canopy ≈75% defoliation
8	Upper canopy covered with lesions ≈90% defoliation
9	Very few leaves remaining and those covered with lesions
10	Plants completely defoliated and killed by leaf spot

Significance of fungicide effect on leaf spot incidence was tested by analysis of variance and means of final disease severity data for each treatment was compared using Fisher's Least Significant Difference (LSD) with a level of significance at the $p \leq 0.05$ (Steel and Torri, 1980).

Hybridization for disease resistance high yielding traits: Hybridization was carried out between high yielding varieties: BARI-2011, BARI-2000, Golden with Cercospora leaf spot resistant varieties: ICGS-83 and Sudan in 2010 at Barani Agricultural Research Institute, Chakwal and pedigree method of breeding was carried out to develop high yielding and Cercospora leaf spot resistant variety. The hybrid population was evaluated for desired traits and now F3 generation has been sown.

Crosses made between high yielding and Cercospora leaf spot resistant varieties during 2010 and 2011 are as under:

1- BARI-2011 x Sudan
2- BM-14 x ICGS-83
3- BARI-2011 x ICGS-83
4- BM-14 x Sudan
5- 02CG005 x Sudan
6- BARI-2000 x ICGS-83
7- Golden x ICGS-83

Crosses between parental lines were made at the experimental field of Barani Agricultural Research Institute, Chakwal. For this purpose, the parental lines (male and female) were sown alternatively in April in crossing block/pots. The emasculation was done in the evening and

emasculated buds were covered to reduce the chances of crossing with unwanted pollens. Pollination was performed in the morning of next day by dusting pollens. The emasculated buds were marked with colored threads to differentiate between crosses of previous and next day crosses. Cross seed was harvested from female parent and stored for raising filial generations by space planting.

Chapter 4:

RESULTS AND DISCUSSION

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The assessment of genetic diversity or similarity is not only important for crop improvement but also for efficient management and protection of germplasm resources. This information can be obtained through DNA fingerprinting approaches capable of exhibiting large number of loci for extensive variability. Groundnut genotypes collected from diverse origins were analyzed using a highly repeatable PCR based fingerprinting assay known as Simple Sequence Repeats (SSR) and Inter Simple Sequence Repeats (ISSR) markers. Utilization of Simple Sequence Repeats (SSRs) have proven to be useful tools in plant genetic analysis (Weising *et al*., 1989; Condit and Hubbell, 1991; Akkaya *et al*., 1992; Morgante and Olivieri, 1993; Zhao and Kochert, 1993; Guilford *et al*., 1997; Kubik *et al*., 1999; Pinto *et al*., 2004; Duarte Filho *et al*., 2010) and have replaced isozymes, RAPDs and RFLPs due to their high polymorphism and versatility (Goldstein and Pollock, 1997). Several studies have compared polymorphism obtained from isozymes, RAPDs, or RFLPs to that obtained using SSRs (Wu and Tanksley, 1993; Terauchi and Konuma, 1994; Liu *et al*., 1995; Innan *et al*., 1997). In all cases, SSRs were more polymorphic per locus. This polymorphism is useful for distinguishing closely related varieties (Thomas and Scott, 1993; Rongwen *et al*., 1995; Olufowote *et al*., 1997), for assessing genetic relationships among individuals (Goldstein and Pollock, 1997) and identification of varieties (Molla *et al*., 2010; Han *et al*., 2011). Several studies have used SSR markers for genetic analysis in groundnut (Hopkins *et al*., 1999; Krishna *et al*., 2004) and found very little genetic diversity among the wild and cultivated groundnut species. This may be due to small number of SSR primers surveyed in the study because few hundred SSR primers were available up to 2005. In recent years, significant efforts have been made to develop the SSR markers in groundnut and more than 9000 SSR markers were developed in genus *Arachis* and they showed higher level of polymorphism over other DNA markers (Palmieri, 2002; Ferguson *et al*., 2004; Moretzsohn *et al*., 2004; He *et al*., 2005; Gimenes *et al*., 2007; Wang*et al*., 2007; Naito *et al*., 2008; Pandey *et al*., 2012).

Determination of genetic diversity by SSR and ISSR markers: Two hundred seventy seven SSR primer pairs and 10 ISSR primers were synthesize from GeneLink, USA and surveyed to estimate genetic diversity among 95 groundnut genotypes maintained at BARI Chakwal, Punjab Pakistan. Thirty two SSR and one ISSR were tried to amplify on different annealing temperature (48-64°C) but did not amplified. Out of 254 amplified primers 37 were monomorphic and 217 were polymorphic. The percentage of polymorphic primers was 85.4%. The 254 primers produced 506 alleles out of which 57 were monomorphic and 449 were polymorphic.The allelic polymorphism percentage was 88.7. The number of allele ranged from 1-6 with an average of 1.99 alleles per primers. The size of amplicon ranged from 50-950bp. The maximum six alleles were found in primer IPAHM-93 with amplicon size ranged 160-230bp followed by PM-3, PM-50, pPGSseq-18A5, pPGSseq-9A7, EM-132 and IPAHM-23, they have five alleles and DNA band size oscillated between 70-450bp (Table 3). The results of present study were in agreement with the earlier genetic diversity studies (Shoba *et al*., 2010) they reported six polymorphic SSRs primers out of 17 (24%) and produced 1 to 3 alleles per primer. Mondal and Badigannavar (2010)

observed that 26 SSR primers yielded 136 alleles and 104 of them were found polymorphic (76.5%). Kamburona (2007) studied 19 peanut lines and found that SSR primer Lee-4 produced four alleles (60-520bp), Ah4-4 yielded one allele and Ah4-20 produced two allele (200-270bp), Varshney *et al*. (2009) reported that 25 SSR markers produced 3 (Ah1TC6G09) to 20 (Ah1TC11H06) allele with an average of 10.6 alleles per locus. A little higher alleles per primer were obtained by some earlier scientist (He *et al*., 2003; Krishna *et al*., 2004; Moretzsohn *et al*., 2004; Cuc *et al*., 2008; Gautami *et al*., 2009; Liang *et al*., 2009; Molla *et al*., 2010) as they reported 2-8 alleles per marker. Mei *et al*. (2012) and Pandey *et al*. (2012) detected much higher number of alleles, i.e., 1-12 bands per primer pair with an average of 3.2 alleles per primer.

The comparison of two kinds of molecular markers (SSR and ISSR) was carried out on the basis of their levels of polymorphism, fraction of polymorphic loci, effective multiplex ratio and marker index (Table 4). The comparison was also made for these statistics between different series of SSR primers. Average number of polymorphic loci per assay unit was 1.778 in ISSR and 1.767 in SSR primers. Fraction of polymorphic loci was one and 0.884 in ISSR and SSR markers, respectively. The values of effective multiplex ratio were 1.778 for ISSR and 1.676 for SSR. The SSR series PM and IPAHM yielded 2.84 and 2.531 average number of polymorphic loci per assay, respectively. The Effective Multiplex Ratio (EMR) was 2.721 for PM and 2.126 for IPAHM SSR primer series respectively. The marker index was 0.980 for PM and 0.699 for IPAHM SSR primer series (Table 4).

The Polymorphic Information Content (PIC) values of the 217 polymorphic SSR and ISSR primers ranged from 0.021 for pPGPseq-13A10, pPGPseq-14A10, pPGPseq-3A4, pPGPseq-4G05, pPGPseq-16G4, pPGPseq-9D12, pPGPseq-8B11 pPGPseq-8C10 pPGPseq-3A4, pPGPseq-4G02, pPGPseq-3C2, pPGPseq-15G2, pPGPseq-18A8, pPGPseq-19H3, S-22, S13, S2, S20, S-1, S-9, S-26, PM-179, PM-73, PM-32, Ah4-20, Ah4-124, EM-18, UBC-829, UBC-808 to 0.50 for pPGPseq-1B09, pPGPseq-2A05, pPGPseq-13A7, pPGPseq-8E12, pPGPseq-4E8, pPGPseq-15E12, pPGPseq-7H6, pPGPseq-9E8, S-11, PM-238, Ah4-26, EM-132, IPAHM-171C, IPAHM-689, IPAHM-531 and IPAHM-23 with an average of 0.287. The SSR primers have higher average PIC values (0.289) as compared to ISSR primers (0.222). When comparing different series of groundnut the SSR primers PM series developed by He *et al*. (2003, 2005) was at the top with an average of 0.36 PIC value followed by IPAHM and pPGPseq primer series with mean PIC values 0.33 and 0.31, respectively. The Ah, EE, Lec and TC11H series were not included in comparison because they are in lesser numbers. The groundnut SSR primers series S gave minimum average PIC value (0.16) (Table 5).

Confusion probability (Cj) values ranged from 0.047 to 0.979 with an average of 0.579 in overall primers studied. The ISSR have Cj values (0.736) as compared to SSR (0.572). The SSR primer S series was at the top for Cj mean (0.77) followed by EM series (0.72). The lowest Cj values were recorded in pPGPseq series (0.33). In inclusive studies, the discrimination power (Dj) oscillated between 0.021 for primers pPGPseq-13A10, pPGPseq-14A10, pPGPseq-3A4, pPGPseq-4G05, pPGPseq-16G4, pPGPseq-9D12, pPGPseq-8B11 pPGPseq-8C10 pPGPseq-3A4, pPGPseq-4G02, pPGPseq-3C2, pPGPseq-15G2, pPGPseq-18A8, pPGPseq-19H3, S-22, S-13, S-2, S-20, S-1, S-9, S-26, PM-179, PM-73, PM-32, Ah4-20, Ah4-124, EM-18, UBC-829, UBC-808 to 0.953 for primer PM-50 with mean value of 0.421. On an average basis, the SSR yielded higher Dj values (0.428) as compare to ISSR (0.264). The SSR primer series PM was at the top in an average Dj value (0.63) followed by IPAHM (0.406) series. The lowest means Dj was yielded by SSR primer S series (0.23). The Resolving power (Rp) value for both SSR and ISSR polymorphic primers was calculated and it was observed that ISSR primers had greater Rp (2.45)

than SSR primers (2.38), however the number of ISSR (9) primers were much lower than SSR (208) and total Rp of SSR (497.4) was much higher than ISSR primers (24.484). In overall results of 217 primers, the Rp of the primers used in this study ranged between 1.116 (EM-129) to 5.811 (pPGPseq-12A7), with an average of 2.405 and a total Rp of 521.9. The Rp value of pPGPseq series ranged from 1.453 to 5.881 with an average of 2.16 per primer and total 222.05. Seventeen primers of series S developed by Wang *et al*. (2007) yielded Rp from 1.221 to 5.874 having mean value 2.27 and total value 38. 57. In PM series, the highest value of Rp (3.958) was scored by the primer PM-238 followed by 3.973 for the primer PM-201 and the lowest value (1.916) for the primer PM-35, PM-50 and PM-145. The primer EM-132 (5.158) was at the top in EM series for Rp value and EM-129 (1.116) was at the bottom with the average of 2.67 per primer. The total Rp of 28 SSR primers of IPAHM were 85.727 with an average of 3.297 per primer (Table 5).

A group of 38 SSR primers (pPGSseq-16F1, pPGPseq-5D5, pPGPseq-2A06, pPGPseq-1B09, pPGPseq-4H11, pPGPseq-17E1, pPGPseq-2A05, pPGPseq-2C10, pPGPseq-14D1, pPGPseq-13A7, pPGPseq-2F5, pPGPseq-8E12, pPGPseq-14D11, pPGPseq-4E8, pPGPseq-16C7, pPGPseq-14G3, pPGPseq-13E6A, pPGPseq-18b8, pPGPseq-15E12, pPGPseq-7H6, pPGPseq-19B12, pPGPseq-9E8, pPGPseq-19F4, pPGPseq-19G7, pPGPseq-11E11, S-11, PM-238, PM-35, PM-65, Ah4-26, EM-132, EM-87, IPAHM-171C, IPAHM-689, IPAHM-123, IPAHM-475, IPAHM-531 and IPAHM-23) and one ISSR (UBC-808) primers were identified that yielded highest PIC values, ranged from 0.496 to 0.500, are highly polymorphic and application of these microsatellite primers are suggested when there are limited resources and researcher wants to reduce the cost and time of research for genetic diversity and genotype identification. The markers having PIC values ranged from 0.266-0.489 are moderately informative and markers having average PIC value 0.25 are slightly informative, whereas the markers having PIC value less than 0.25 are very low informative. The PIC for 72 primers ranged from 0.266-0.489, hence, are moderately polymorphic. The PIC values observed in this study are in agreement of earlier genetic diversity studies (Mace *et al*., 2006; Wang*et al*., 2007; Cuc *et al*., 2008; Gautami *et al*., 2009; Liang *et al*., 2009; Wang *et al*., 2012). Different PIC values were obtained from marker studies using different genetic materials in groundnut Shoba *et al*. (2010) reported PIC values ranging from 0.17 to 0.63 with an average of 0.41. Wang *et al*. (2012) observed that polymorphism values ranged from 0.21 to 0.87 and with an average of 0.450. Pandey *et al*. (2012) reported PIC values of groundnut ranged from 0.10 (GM-742) to 0.89 (S-009) with an average of 0.31 per marker and in total, only 15.67% markers had PIC value more than 0.50. Barkley *et al*. (2007) reported very high PIC values for SSR ranging from0.083 to 0.907 with an average of 0.687 per primers. Gautami *et al*. (2009) perceived low values of PIC, i.e., 0.13 to 0.36 with mean 0.25 while surveyed 14 SSR using 23 wild and cultivated groundnut lines. Molla *et al*. (2010) used three primers (PM-36, PM-50, PM-238) and reported very high PIC values ranged from 0.76-0.82. There was highly negative correlation between PIC and Confusion probability (Cj) where as highly positive correlation was observed between PIC and Discrimination power (Dj).

High PIC coupled with high Dj and low Cj make the primers more useful for identification of groundnut genotypes. We found 19 SSR primers pair (pPGSseq-16F1, pPGPseq-2A06, pPGPseq-17E1, pPGPseq-2A05, pPGPseq-14D1, pPGPseq-13A7, pPGPseq-16C7, pPGPseq-15E12, pPGPseq-7H6, pPGPseq-19B12, pPGPseq-9E8, pPGPseq-19G7, S-11, PM-238, PM-35, Ah4-26, EM-151, EM-119 and IPAHM-475) have high PIC and Dj whereas low Cj values hence, these primers may be useful for variety identification protection or registration program.Resolving power (Rp) is the capacity of a given primer to discriminate among different genotypes (Prevost and Wilkinson, 1999). In inclusive studied of SSR and ISSR, the mean value of Rp of ISSR were

higher than SSR primers surveyed in this study. The SSR primers S-21, pPGPseq-12A7, IPAHM-455, EM-132 and ISSR primer UBC-809 having high values of Rp these primers were more efficient and useful both in terms of producing more polymorphic bands and also having more equal proportion of present/absent bands among genotypes. The relatively low Rp values of SSR primers EM-129, S-24, pPGPseq-18G9 andpPGPseq-14C11 rendered these primers the least useful for discriminating among these set of groundnut genotypes.

Although, the value for average number of polymorphic loci per assay unit was higher in ISSR as compared to SSR which may be due to less number of ISSR primers used in this study as compared to SSR primers. Marker Index (MI) is the major statistic used to calculate the overall utility of a marker system, the value of MI was higher in SSR as compared to ISSR, which advocate the efficiency of SSR markers over ISSR markers in providing useful information about polymorphism and genetic diversity in groundnut .The higher EMR and MI values for PM series indicated that this SSR marker series is more effective for determining level of polymorphism and diversity in groundnut followed by IPAHM and pPGPseq SSR series.

Diversity among genotypes: Data obtained from SSR analysis was used to generate similarity matrix by Nei (1972) using data of 254 markers. The resulting similarity matrix showed mean genetic similarity coefficient 0.77 among 95 groundnut genotypes. As far as pair wise combinations are concerned genetic similarity ranged from 0.51 to 0.95. The two most closely related genotypes were from ICRISAT India i.e. ICG-2254 and ICG-2261 with the highest similarity index (0.95) followed by BARD-479 and Sudan, their similarity coefficient was 0.94. On the other hand, the two most distantly related cultivars were PG-1017 and Argentina-2 with low similarity index (0.51) followed by PW and Argentine-2 with similarity coefficient value 0.52. This shows that if, we cross these two extreme varieties, maximum genetic variability can be obtained in F_2 generation. Therefore, based upon our results, it would be a wise decision to use these genotypes for the future development of a new variety. On the basis of similarity matrix, a crossing program was suggested which is given in Table 6. In this table alleles of specific locus are given for identification of blood of each variety. For example in cross combination Argentina-2 X PG-1017 the two genotypes used in this cross combination are heterozygous for the loci S-11, PM-50, PM-42, pPGPseq-14H6, pPGPseq-8D9, PM-15, PM-137, pPGPseq-7H6, pPGPseq-2B10, PM-45, PM-201 and Ah4-26. The blood of Argentina-2 representing through the presence of allele S-11/160, PM-50/120, PM-42/200 pPGPseq-14H6/300, pPGPseq-8D9b/140, PM-15/180, PM-137/150, pPGPseq-7H6/305, pPGPseq-2B10/255, PM-45/100, PM-201/235 and Ah4-26/220, similarly the blood of PG-1017 representing by the presence of DNA bands produced by the SSR locus S-11/165, PM-50/105, PM-42/205, pPGPseq-14H6/350, pPGPseq-8D9/145, PM-15/190, PM-137/155, pPGPseq-7H6/295, pPGPseq-2B10/265, PM-45/105, PM-201/105 and Ah4-26/215. The scheme of molecular breeding for 21 crosses is given to use identification of hybrid and the percentage of contribution of each parent to their offspring.

The dendrogram generated with hierarchical UPGMA (Un-weighted Pair Group Method with Arithmetic mean) cluster analysis of the similarity matrix using 95 groundnut genotypes revealed 11 major clusters at different similarity percentages (Fig. 1). The first cluster was drawn at 84.5% similarity basis and it included 25 groundnut genotypes namely No. 334, Banki, ICG-485, ICGS-83, ICG-641, ICG-574, PK-964, ICGV-086128, Yh-4, 2KCG020, S-25, ICGV-015608, 96CG005, PG-015692, PG-015732, PG-986, ICGV-015603, ICG-015649, ICG-493, ICG-690, ICG-2742, ICG-540, PG-965, ICG-2254 and ICG-2261. Twenty genotypes were clustered in group II, when line was drawn at 84% similarity that are Chakori, BARD-479, Sudan,

ICG-635, NC-7, LICN, ICGS-17, PG-686, ICG-4, ICGS-6, ICG-488, PG-957, PG-668, PG-681, 04CG009, BARI-2000, ICG-4528, N-C5-1,Golden and PW. Group III contain six lines ICGS-015662, ICG-4523, ICG-4463, PG-978, Virginia and USA when similarity line was strained at 83.5%. Cluster IV included four genotypes 2KCG017, 2CG005, PG-1051 and 2CG002 when separated at 82.5% similarity level. In group V, there are nine strains, i.e., ICGS-38, PG-685, ICGV-960254, ICGV-89220, PG-1040, 08CG005, BARI-89, Runner and Yh-11 when similarity line was pinched at 79%. The cluster VI was stretched at 79.5% similarity level and contains five genotypes BARD-669, ICG-015779, PG-981, PG-977 and ICGV-015782. Cluster VII have six strains ICG-4747, 01CG009, PG-1052, 04CG004, ICGV-88316 and ICG(E)-18 and its similarity line was drawn at 78%. In cluster VIII, Chico, Chinese, Husta-J, ICGV-015668, PG-1017, ICGV-015592, Yh-9307, Yh-14, PG-690 and 93-7 ten genotypes grouped at 76.5% similarity level whereas in cluster IX two genotypes PG-699 and Spanish grouped each other at 76.5% similarity level. Group X has seven US-1, US-2, 04CG002, 04CG007, 02CG006, 07CG004, 07CG006SL-genotypes clustered at 78.5% similarity level. The cluster XI included only one genotype Argentina-2 when differentiated at 69% similarity level.

Although dendrogram grouped 95 genotypes in eleven groups but grouping pattern indicated that genotypes from same institute or origin tend to cluster each other in the homology tree, however, genotypes belongs to different institute have sufficient genetic diversity for initiation of viable groundnut breeding program. Mostly genotypes belong to ICRISAT (ICG, ICGS and ICGV) assembled in one group or neighboring group advocated the narrow genetic base of material collected from ICRISAT directly by BARI, Chakwal or through NARC, Islamabad. The ICG stands for ICRISAT groundnut, ICGV ICRISAT groundnut varieties and ICGS ICRISAT groundnut selection. The cluster I contain two obsolete commercial cultivars No.334 and Banki developed at Oilseed Research Institute, Faisalabad in 1970-71 before the development of BARI, Chakwal. Two lines from ICRISAT ICG-2254 and ICG-2261 in cluster I are 95% similar. The group II has four approved varieties at Pakistan three BARI-2000, Chakori and Golden developed at BARI, Chakwal and one BARD-479 developed by Barani Agriculture Research Development project. The variety Golden was evolved through gamma radiation (mutation) given to No.334. Group II have two genotypes BARD-479 and Sudan, they are 94% similar. The genotypes ICG-4, ICGS-6 and PG-668, PG-681 are 93% similar whereas NC-7 and LICN are 92%, similar. In group XI, there was one genotype Argentina-2 which is most diverse genotype in this study. It is suggested to select parents from distant cluster from different institute for harmonious combination of positive traits.

This study revealed a large degree of SSR polymorphisms among the genotypes, as only 12.7% of the SSR markers were monomorphic. High levels of polymorphism and heterozygosity were detected. This relationship will be beneficial to explore their potentiality in varietal improvement programs. The level of polymorphism indicates that distinction between any two varieties is possible with appropriate SSR primer pair. This supports to the use of SSR markers, as an excellent tool, for diversity analysis, loci mapping and variety improvement program. A number of scientists reported that microsatellite genotyping technology may be a good tool to ensure genetic diversity of gene pool of groundnut genotypes and molecular breeding (Palmieri, 2002; Ferguson *et al*., 2004; Krishna *et al*., 2004; Moretzsohn *et al*., 2004; He *et al*., 2005; Barkley *et al*., 2007; Gimenes *et al*., 2007; Wang*et al*., 2007; Cuc *et al*., 2008; Naito *et al*., 2008; Gautami *et al*., 2009; Liang *et al*., 2009; Mondal and Badigannavar, 2010; Shoba *et al*., 2010; Pandey *et al*., 2012; Mei *et al*., 2012; Wang *et al*., 2012; Zhao *et al*., 2012).

Candidate markers for Late Leaf Spot (LLS) resistance: Mace *et al*. (2006) surveyed 22 groundnut genotypes with markers the genotypes have varying levels of known resistance to rust and LLS and one genotype susceptible to both diseases were selected. He screened 23 SSRs developed by Ferguson *et al*. (2004). Three primers viz. pPGPseq-2B10$_{280}$, pPGPseq-13A7$_{305}$ and pPGPseq-2F5$_{280}$ proved their worth for identification of LLS resistant genotypes. Two primers pPGPseq-2B10$_{280}$ and pPGPseq-13A7$_{305}$ did not amplified the reported fragment size, however, pPGPseq-2F5 amplified reported fragment of 280bp in four genotypes namely Chico, Chinese, PG-690 and PG-1017. The SSR marker pPGPseq2F5 was surveyed and compared with phenotypic data (collected by BARI, Chakwal). The genotypic data did not coincide with the phenotypic data hence the marker was not validated. Mondal and Badigannavar (2010) screened 20 genotypes of cultivated groundnut against five (PM-179$_{120}$, PM-35$_{124}$, Ah4-24$_{300}$, TC11H06$_{200}$ and one ISSR marker UBC 810$_{500}$) LLS linked markers among them four markers had significant association with LLS resistant. Only one primer was amplified (Ah1TC11H06), however, the genotypic data did not agree with phenotypic data hence, the marker was not validated.

Shoba *et al*. (2012) utilized three markers (PPGPseq5D5, PM375 and PM 384) to distinguish resistant and susceptible individuals for LLS and recommended the utilization of PM384$_{100}$ for molecular breeding. All these three markers were surveyed using the material under study for identification of resistant and susceptible genotypes. The lowest LLS disease score of three year average was 3.3 for the variety ICG-574 it contain PM375$_{162}$ and PM384$_{100}$ resistant segments hence while using ICG-574 as resistant source the selection will be based on the presence of PM375$_{162}$ and PM384$_{100}$. The second lowest LLS disease score (3.7) was found BARI-89 and ICG-83 contain all the three markers linked with LLS hence, it is recommenced to use this variety for the development of LLS disease resistant groundnut genotypes and selection will be based on the presence of PPGPseq5D5$_{220}$, PM375$_{162}$ and PM384$_{100}$ DNA segments. The third lowest LLS disease score was observed 4.0 in nine genotypes. The three genotypes No.334, BARD-699 and S-25 contain all three resistant DNA bands hence all more reliable to utilize for LLS resistant breeding program. The gist of molecular marker and phenotypic disease score recommend the utilization of ICG-574, ICG-83, No.334, BARD-699 and S-25 as a LLS disease resistant parent for the development of LLS disease resistant groundnut breeding programs (Table 7).

Barani Agricultural Research Institute, Chakwal:

Screening of Groundnut Germplasm: Three scientists recorded Cercospora leaf spot disease reaction of 82 groundnut genotypes at two times during crop growth period during first year of experiment. The genotypes ICGS83, Sudan and BARI-89 showed minimum disease according to 1-10 Florida Cercospora leaf spot scoring scale. Two genotypes Yh-14, an exotic genotype and PG 1040 showed maximum disease score of 7.3. Cercospora leaf spot disease incidence was in lower scores because of unfavorable environmental conditions. PARB evaluation system advised to repeat experiment. Test genotypes exhibited inconsistent reaction during second year of experiment. There were highly conducive environmental conditions for disease development during third year and nature also helped in this regard. Test genotypes responded in higher scores but in line with first year trial although data were by three scientists independently. The genotypes ICGS-83, Sudan, ICG-574 and BARI-89 showed minimum disease of 3.00 under 1-10 Florida scoring scale. Six genotypes showed disease score of 8 and two genotypes exhibited maximum disease score of 9.

Most of times at early stages of crop growth lesser disease scores were encountered (Ijaz, 2011). In the studies, Yh-14 and Yh-11 showed maximum disease at crop growths. In germplasm screening studies, early maturing sequentially branched peanut cultivars were more susceptible to *Cercospora arachidicola* and *Cercosporium personatum* than were late maturing alternatively branched cultivars that exhibited various degrees of resistance (Ijaz, 2011; Gobbons, 1966). Mazzani *et al*. (1972) observed that leaf spot counts were higher on cultivars with large, light green leaves.

Chemical Control of disease: During 2009, five chemicals were tested. Among, which Penaconazole, Fostyl Al and Mancozeb performed better than others although the efficacy of all chemicals was at par statistically. However, only Mancozeb was tested during following year and other chemicals were replaced by the new ones. The selection was based on higher yields (Table 8). During the next year 2010, four new chemicals viz. Chlorothalonil, Propineb, Tebuconazole + Trifloxystrobin and Triazole + Chlorothalonil along with Mancozeb were tested for their efficacy against the CLS. Among the chemicals mentioned maximum percent decrease in disease was recorded in Tebuconazole + Trifloxystrobin and Triazole + Chlorothalonil. However the later combination showed phytotoxic effect on peanut plants even at lighter doses. During the year 2011, Triazole and Chlorothalonil were tested separately. The performance of Triazole was next to Tebuconazole + Trifloxystrobin, moreover, it was not injurious to crop as did it in mixture. The results are summarized in the Table (8-10). Chemical control of CLS has been practiced in advanced countries for a long time with varying degree of success (Backman *et al*., 1977; Smith and Littrel, 1980). Fungicides such as Benomyl were found acceptable in USA in the seventies and eighties until the resistance in the pathogen against these chemicals was detected (Littrell, 1974; Backman *et al*., 1977; Clark *et al*., 1974; Hagan *et al*., 2006). The Benomyl, a benzimidazole fungicide was very effective for controlling Cercospora leaf spot (Porter, 1970; Brenneman and Culbreath, 2000; Culbreath *et al*., 2002). In the southeastern America control of leaf spot diseases are largely dependent upon multiple applications of fungicides. Peanut growers in Albama, Georgia and Florida relied almost exclusively on formulation of Chlorothalonil, a broad spectrum protectant fungicide (LaPrade *et al*., 1999; Smith and Littrel, 1980). However, studies conducted by Culbreath *et al*. (2002) indicate that mixture and alternate application of Benomyl and Chlorothalonil were effective for management of leaf spot where Benomyl alone did not provide

season long leaf spot control. Moraes *et al*. (2001) reported that the Triazole fungicides were more effective than Chlorothalonil, resulting higher pod yields. It is concluded that systemic fungicides like Tebuconazole + Trifloxystrobin and Triazole is the more effective chemicals against CLS at lower doses than non-systemic fungicides. It was noted that the chemicals used at least dose found as much effective as was found at recommended or more than recommended doses.

The present study is an initiating step in rainfed situation and chances of success and failure will be obvious with the course of time. In the light of results of present study, however, authors are optimistic that foliar application of fungicides to manage the disease in locally adapted varieties would increase groundnut yield substantially. According to an estimate yield may be increased from 33-119% with appropriate improvement in disease management (Yaqoob *et al*., 1989). Work on this aspect has been reported 3500 Kg/ha yield by the usage of fungicides at 14 days interval (BARI, 1992).

Generations and advancement F_1, F_2 and F_3: The seeds of filial generations were sown with khurpa at Barani Agricultural Research Institute, Chakwal under rainfed conditions at space planting. All F_0 hybrid seeds obtained from successful crosses were sown in single row at significant space. F_1 seeds were harvested at the end of October and seed was kept for sowing of F_2 generation. Planting was done at 45 cm wide rows. Selection of plants with desirable characters (high yielding and Cercospora leaf spot tolerant) was done in filial generations. Now F_3 generation has been sown in field at Barani Agricultural Research Institute, Chakwal. Selection of plants with desirable characters will remain continued uptil F_7 generation and the selected entries will be included in preliminary, regular and micro yield trials and selected line will be sent in National trial for evaluation under wide environment. Successful candidate line will prove as Cercospora leaf spot tolerant variety and seed will be multiplied for general cultivation in the region.

National Institute for Genomics and Advanced Biotechnology (NIGAB):

Report from NIGAB, NARC Islamabad has not been submitted in spite of many reminders and letters. During the original gestation period of the project NIGAB was unable to achieve the target of EPSPS gene transformation in three groundnut varieties. On the request of NIGAB time period to complete the project activities was extended, but even after the expiry of extended period the task of gene transformation could not be completed. In this regard the activity regarding checking of NIGAB material by ABRI was also included and 12 putative EPSPS transgenic groundnut sample were tested through Real Time PCR and found that EPSPS gene not expressing in any transgenic plants. Detailed report is attached at the end with the conclusion that EPSPS gene is not expressing in any of the putative transgenic plants. Hence targets of the project were not achieved by the NIGAB.

CHAPTER 5:

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Table 1: Groundnut genotypes name, origin and botanical type used in study

#	Genotypes	Origin	Botanical Type	#	Genotypes	Origin	Botanical Type
1.	No. 334	Pakistan	Virginia	42.	02CG002	Pakistan	Virginia
2.	BANKI	Pakistan	Virginia	43.	02CG005	Pakistan	Valencia
3.	Chakori	Pakistan	Virginia	44.	04CG004	Pakistan	Virginia
4.	BARI-89	Pakistan	Virginia	45.	04CG009	Pakistan	Valencia
5.	Golden	Pakistan	Virginia	46.	08CG005	Pakistan	Valencia
6.	BARD-699	Pakistan	Virginia	47.	ICG-493	India	Virginia
7.	BARD-479	Pakistan	Virginia	48.	ICG-4	India	Unknown
8.	Chico	USA	Spanish	49.	ICGS-6	India	Virginia
9.	Chinese	Chinese	Spanish	50.	ICG-540	India	Virginia
10.	Sudan	Sudan	Spanish	51.	ICG-488	India	Virginia
11.	ICG-485	USA	Virginia	52.	ICG-690	India	Virginia
12.	NC-7	USA	Virginia	53.	ICG-2742	India	Unknown
13.	ICGS-83	India	Virginia	54.	ICG-4523	Uganda	Virginia
14.	LICN	USA	Virginia	55.	ICG-4463	India	Unknown
15.	PW	USA (PW)	Virginia	56.	PG-957	NARC	Spanish
16.	ICGS-17	India	Virginia	57.	PG-965	NARC	Spanish
17.	ICG(E)-18	India	Virginia	58.	PG-668	NARC	Spanish
18.	N-C 5-1	USA	Virginia	59.	PG-681	NARC	Spanish
19.	S-25	USA	Virginia	60.	PG-685	NARC	Spanish
20.	PG-686	NARC	Spanish	61.	PG-699	NARC	Spanish
21.	Husta-J	China	Spanish	62.	PG-977	NARC	Spanish
22.	ICG-574	India	Virginia	63.	BARI-2000	Pakistan	Virginia
23.	ICG-635	India	Virginia	64.	PG-978	NARC	Spanish
24.	ICG-641	India	Virginia	65.	PG-981	NARC	Spanish
25.	ICG-2254	India	Virginia	66.	PG-986	NARC	Spanish
26.	ICG-2261	India	Virginia	67.	PG-1017	NARC	Spanish
27.	ICG-4528	India	Virginia	68.	PG-1040	NARC	Spanish
28.	ICG-4747	Israel	Virginia	69.	PG-1051	NARC	Virginia
29.	PG-690	NARC	Spanish	70.	PG-1052	NARC	Spanish
30.	ICGS-38	India	Virginia	71.	ICGV-88316	India	Spanish
31.	Pk-90064	Pakistan	Virginia	72.	ICGV-015692	NARC	Virginia
32.	ICGV-86128	India	Virginia	73.	ICGV-015723	NARC	Unknown
33.	ICGV-89220	India	Virginia	74.	ICGV-015782	India	Virginia
34.	ICGV-015608	India	Virginia	75.	ICGV-960254	India	Unknown
35.	ICG-015662	India	Virginia	76.	ICGV-015592	India	Virginia
36.	ICGV-015668	India	Virginia	77.	ICGV-015603	India	Virginia
37.	ICG-015779	India	Virginia	78.	ICGV-015649	India	Unknown
38.	YH-9307	China	Spanish	79.	Spanish	USA	Spanish
39.	YH-14	China	Spanish	80.	Runner	USA	Unknown
40.	YH-11	China	Spanish	81.	2KCG-017	Pakistan	Virginia

41.	YH-4	China	Spanish	82.	2KCG-020	Pakistan	Virginia
#	**Genotypes**	**Origin**	**Botanical Type**	**#**	**Genotypes**	**Origin**	**Botanical Type**
83.	96CG005	Pakistan	Valencia	90.	07CG006	Pakistan	Virginia
84.	01CG009	Pakistan	Valencia	91.	02CG006	Pakistan	Virginia
85.	US-2	USA	Virginia	92.	Virginia	USA	Virginia
86.	Argentina-2	USA	Virginia	93.	USA	USA	Virginia
87.	04CG002	Pakistan	Virginia	94.	93-7	Unknown	Virginia
88.	04CG007	Pakistan	Virginia	95.	US-1	USA	Virginia
89.	07CG004	Pakistan	Virginia				

Table 2:List of SSR and ISSR primers along with polymorphic status surveyed for genetic diversity study

#	Primer	5' Left/Forward/Sense Seq 3'	5' Right/Reverse/Anti-Sense Seq 3'	Amplification status
1.	PM-3	GAAAGAAATTATACACTCCAATTATGC	CGGAGCTCTATGTCATGACT	Polymorphic
2.	PM-15	CCTTTTCTAACACATTCACACATGA	GGCTCCCTTCGATGATGAC	Polymorphic
3.	PM-32	AGTGTTGGGTGTGAAAGTGG	GGGACTCGGAACAGTGTTTATC	Monomorphic
4.	PM-35	TGTGAAACCAAATCACTTTCATTC	TGGTGAAAAGAAAGGGGAAA	Polymorphic
5.	PM-50	CAATTCATGATAGTATTTTATTGGACA	CTTTCTCCTCCCCAATTTGA	Polymorphic
6.	PM-53	CCTATCCTATGGGTCACTAGCC	GCTTGTGCTCATCTTGAGTTTT	Monomorphic
7.	PM-179	CTGATGCATGTTTAGCACACTT	TGAGTTGTGACGGCTTGTGT	Monomorphic
8.	PM-200	GCTATGTGGGAAAAATACTGCTT	CAGATGTGTGTGTGTGTGTGTG	Polymorphic
9.	PM-210	CCGCAGATCTTCTCCTGTGT	CCTCCTCATCCTCTAAACTCTGC	Polymorphic
10.	pPGPseq-1B09	CGTTCTTTGCCGTTGATTCT	AGCACGCTCGTTCTCTCATT	Polymorphic
11.	pPGPseq-2A05	GGGAATAGCGAGATACATGTCAG	CAGGAGAGAAGGATTGTGCC	Polymorphic
12.	pPGPseq-2D12B	AAGCTGAACGAACTCAAGGC	TGCAATGGGTACAATGCTAGA	Polymorphic
13.	pPGPseq-2G04	TTCTTGGTTCCTTTGGCTTC	TGCTCAAGTGTCCTTATTGGTG	Polymorphic
14.	pPGPseq-4G02	TCAACTTTGGCTGCTTCCTT	TCAACTTTGGCTGCTTCCTT	Monomorphic
15.	pPGPseq-4H11	ATCACCATCAGAACGATCCC	TTTGTAGCCTTCTGGCGAGT	Polymorphic
16.	pPGPseq-2B10	AATGCATGAGCTTCCATCAA	AACCCCATCTTAAAATCTTACCAA	Polymorphic
17.	pPGSseq-13A7	AATCCGACGCAATGATAAAAA	TCCCCTTATTGTTCCAGCAG	Polymorphic
18.	pPGPseq-2F5	TGACCAAAGTGATGAAGGGA	AAGTTGTTTGTACATCTGTCATCG	Polymorphic
19.	pPGPseq-8D9	TGCCAATTGCACTCTTCATC	TGAGGTGCTCTGCATGAAAC	Polymorphic
20.	pPGSseq-15C10	ATTCCCATGTCGTCAAGACC	GCGACGGTATTGGCTTTTAG	Polymorphic
21.	pPGPseq-3A4	GCCGTGACTTGAGCCTTTAG	TTCTTCGGTTACATGGGCTT	Monomorphic
22.	pPGPseq-2C10	GCAAGTCACATAGTTCAATTTTGG	GGCATAGCCATCCAAATCAT	Polymorphic
23.	pPGPseq-2A6	GCTTCTTCGTTGTTGCCTTC	TGCCAGTTGTTCATAGCTTCA	Polymorphic
24.	pPGPseq-3B10	GGTGATGCTCCCCTCTACAA	CCTGCGAAACACAACAGAAA	Monomorphic
25.	pPGPseq-3C2	TCATCGCCGAGATTCTTTTC	CAAGGGAAATTGGTCAAGGA	Monomorphic
26.	IPAHM-659	AAGTCACTGGCCAAAACTGC	CCCTCGATTTCGACTCAGAC	No amplification

27.	pPGPseq-4C11	GGTGCAGGAATTTCAATCCA	CTACTGCGTTTGAGCCCATT	Monomorphic
28.	pPGPseq-4D1	GGCAACAAGATGTGCTCAAA	TCCATGTCATTGCCTTTAACC	Monomorphic
29.	pPGPseq-5B2	GAGGTCGCCATAGTCATCGT	AAAATGTTGGGTGTTGGCAT	Monomorphic
30.	pPGPseq-5C5	TAGTGTTGCGTGCATGTTGA	TCCAGCTTTTTCCTTGCTGT	Polymorphic
31.	pPGPseq-5D5	AAAAGAAAGACCTTCCCCGA	GCAGGTAATCTGCCGTGATT	Polymorphic
32.	S-1	TGGACTAGACAAGGAACAACCA	GAGCCATGAGCACACAACAC	Monomorphic
33.	S-2	TTGTTGCTATTTAGGGTGATTGA	GTGGGACAAGGCTTTGTTGT	Monomorphic
34.	S-6	CCGGCTAGAGAATACACACACA	TCCTCCTTCCTCCTTGAACA	Polymorphic
35.	S-9	CGCTGTCCTTATCGAACCAT	CTCTCACTCGCGCTTTCTCT	Monomorphic
36.	S-17	CATTGGAAAGATCCGACGAT	GTTGCAACAACGACGATGG	Polymorphic
37.	S-18	CAATAAATTCGTCGTAT	GAGAGAAGAGAAGGTTAGAGA	Polymorphic
38.	S-20	CACGAACAGCCACTCAAAGA	CTCTGGGGGACTAGCTGTTG	Monomorphic
39.	S-22	CGTGACAAACATGTGCTGCT	TTTTGGAATCTGTTTATGGGAAA	Monomorphic
40.	S-23	CTGGAAGTGGTCCTGTTGGT	GCTGCTCCTGTCTCTGGAAT	Monomorphic
41.	S-25	GCTATGCTTTTACCACACCAAA	CCATTCATGGTCATCCCTTC	Monomorphic
42.	S-26	ACATGAGTGCCCAACTAGCC	TGCAGAGCTTCAACAACCAC	Monomorphic
43.	IPAHM-684	GCCCGAGTTTTGAAGACCTA	CAAGGCCTCAACTTCCCTAA	No amplification
44.	IPAHM-37	CGTATGCATTATAAGTGCTCGACAA	AATCCGATATCCGCTTCGAC	No amplification
45.	pPGSseq-11D4	CCCTTTTTCAAACAACCCAA	GGATTTTGCATTTGTAGTTGATAGC	Polymorphic
46.	pPGSseq-10C12	CAAACTTGGGCCAACAGACT	TTCACTTCTAACCGAGCTCTCTC	Polymorphic
47.	pPGSseq-12B6	GGCAGGCATGCTCAGATATT	AAAGAATGCTTGTGTATATCATCCC	Polymorphic
48.	pPGSseq-9D12	GCTTCACCATGTTGCTGCT	GATGCAAAGTTGCAAACGAA	Monomorphic
49.	pPGPseq-16E10	TGAAGATGATGGACGAGAAGG	TCATTTACATTCACGCGACC	Polymorphic
50.	pPGSseq-13D1A	TGGCACACACAACTTCGATT	TTGCAGAAGAGGCACAGTGA	Monomorphic
51.	pPGSseq-13C8	GATACAGCATTTTGGGCCTC	AAAGCTCATGAAAGCCGAGA	Polymorphic
52.	pPGSseq-14D1	TCGATTGGTTACGGCTAAAAA	GCTAAATACTCAAATGAAATTCAAAA	Polymorphic
53.	pPGSseq-15B1	TTTTAAAAGTGACAAACAACTCATGT	GTCGCAGGTTTGAGTTTCCT	Polymorphic
54.	pPGSseq-15D2	TATATTGTCCTGCAAGCCCG	TTTTGGCTACCTTTGCTGCT	Monomorphic
55.	pPGPseq-6B8	ATCATCGTCATTGGCTCCAT	GAATCACAAGCAGCAACAGC	Polymorphic
56.	pPGPseq-7B3	TCTGTTTTCTCGTTCGAGCTG	ACCCACCTAGCATCATTTGC	Monomorphic
57.	pPGPseq-8B11	AGGTGTTTGGGCCTAGCATA	AGAGCCTCAGCAAATTCCAA	Polymorphic
58.	pPGSseq-11F12	CACAGCCTTTGTGTTGCTGT	AACACACACAACACTCCACCA	Polymorphic
59.	pPGSseq-14A10	TTTGTTTGGTTCTTACCTTTTTCA	CCACAGGTATAGAGGTTCCTTTG	Monomorphic
60.	pPGSseq-16C3	TGATTCGTTCAACCACCAGA	CCCTCAAACAGCAAACAATG	Monomorphic
61.	pPGSseq-16F1	TGCTTCCATCAGCTTTTCCT	AAATGAGGGCCTCCAAAGTT	Polymorphic
62.	pPGSseq-15E11	ACATGACAGAGCACAATGGC	TTGCTCAAAGAGAACACCAA	Polymorphic
63.	pPGSseq-17C5	TTTTGCAAATGAATACACAAAATTA	GCCATGGCCTCATCTAATC	Polymorphic
64.	pPGSseq-17E1	TTCGTTGACGTGAGCGTTAC	TTAGGATTGTTCCAAGGCCA	Polymorphic
65.	pPGSseq-18A5	TGATTCGATTTACTCATGCACA	GAGGATTCTTGAGCCTCGAC	Polymorphic
66.	pPGSseq-18B11	GAAGAGGCAGAGATGATGGG	AAAATTGTGTGACCGACACG	Monomorphic
67.	pPGSseq-19A4	CCTCCAAGGTTGAAACCAGA	GAAAGCAAGGCAAGGATGAA	Polymorphic

68.	pPGSseq-18C2	TGAGTTCAACACTCACTTTTGTCA	GGGTACGAATTTGCCTACGA	Polymorphic
69.	pPGSseq-9B4	CATTGTTAATGCACCTTTGGAA	GGCAGATTTGGTTATTGCCT	Polymorphic
70.	pPGSseq-9A7	TCAGCCATTCTGATTATGTAAGTTTT	TCTCAGTTTCCACGTTGAGC	Polymorphic
71.	pPGPseq-7D9	TTAGACGACAGCTTTTCCCG	GAGATGGGAAGAGCACCTTG	Polymorphic
72.	pPGSseq-12C5	AAAAATTGCAGAATTAAGCGTT	CTTCACCTGAATTTTCACCAA	Polymorphic
73.	pPGSseq-13A4	TCCACAGCAAACCCAAAAAT	AAAATCCCCTGAGAGCCATT	Polymorphic
74.	pPGSseq-16C6	TTGCTACTAAGCCGAAAATGAAG	CTTGAAATTAACACATATGCACACA	Polymorphic
75.	pPGSseq-13A10	AACTCGCTTGTACCGGCTAA	AGGAATAATAACAATACCAACAGCA	Monomorphic
76.	pPGPseq-7E10A	AGGAAAGCCAAGCTCTAGCC	CTGAGGCTATTTGGGGTCAA	Polymorphic
77.	S-7	GCACCAATTTTGTCCCTGAT	CAGAAGGGGTTTGCACCTAA	Polymorphic
78.	S-8	AAGTCCAAAATGCATGCTCA	GGCTCTGTGTGGTAGGGTGT	Monomorphic
79.	S-11	ATGACGGCAGTAGCAGAAGC	TTGAGGAGAAGACGCTGTTG	Polymorphic
80.	S-12	TCATTGACCTAGCCGAATCC	GAGGGACCAATTGTTGGTTG	Polymorphic
81.	S-13	TTGTTGCTATTTAGGGTGATTGA	CGTCGTTTGATTCATGTAGCC	Monomorphic
82.	S-14	AGGCAAACCACTGCAAGAGT	CGCTTCCCTGGGATACTTAG	Monomorphic
83.	S-15	TGAACGAAAAATGCTAATGTGG	CGCAGAGACGTGTTGAAGAA	Polymorphic
84.	PM-36	ACTCGCCATAGCCAACAAAC	CATTCCCACAACTCCCACAT	Polymorphic
85.	PM-73	AGTGTTGGGTGTGAAAGTGG	GGGACTCGGAACAGTGTTTATC	Monomorphic
86.	Ah 4-20	ACCAAATAGGAGAGAGGGTTCT	CTCTCTTGCTGGTTCTTTATTAACTC	Monomorphic
87.	Ah 6-125	TCGTGTTCCCGATTGCC	GCTTTGAACATGAACATGCC	Polymorphic
88.	Ah 4-26	TGGAATCTATTGCTCATCGGCTCTG	CTCACCCATCATCATCGTCACATT	Polymorphic
89.	EM-97	TCTCCTTCTGCCTCCAACA	CGGAAAGTTGTGAGGAGGA	Polymorphic
90.	IPAHM- 282	AAGCCTTTGCGAATATAACCA	TGCAGGACTTGTATTTTGAGGA	No amplification
91.	IPAHM-540	TGGAGAACTAGGATCTCTTTTGTG	CCTAACTCAGCCTGCGAAAC	No amplification
92.	EM-145	GCTGGCGTGAATACAGTGAT	GCTCATGTAAAGGGAAGAAGAATA	Polymorphic
93.	EM-148	CTAATCTACCCATCACCTAAAGC	AAACAACCCAAGCACCTCTA	Polymorphic
94.	EM-140	TACACGCGGACAGATTTAGC	AAGTCGTTTGAATGTGAGGC	Polymorphic
95.	EM-155	GCAGATGGATTGAGGCATTG	CACGCGGACAGATTTAGCAG	Polymorphic
96.	EM-156	GATTACCTTGACGAGGATGAG	CACGCTGAGTTGAGTGAGTT	Polymorphic
97.	EM-100	TGATGCGGAAACTGAGATGG	CTCCATCCACGACAAACACC	Polymorphic
98.	EM-113	GCGAAGTATTATGCAGATGGG	GCTTCACTAGAACAACAGCCAC	Polymorphic
99.	EM-118	CAACGGCAACAACAAGAAGA	AACACTTGAAACGACGGAGG	Polymorphic
100.	EM-68	GATGAACTCCATTGCCAGTAA	GTCAACTTATCAGACCCTCCA	Polymorphic
101.	EM-23	GCCTCCCTTAAACACCTTCA	ATCCTCGTCCATGTCCAACT	Polymorphic
102.	EM-132	GTGAATTTCTGCCATTTGGG	CACCGTTGACTTATTTATCAGGAG	Polymorphic
103.	EE-73	GTTGGAGCAGGGCATCAGTT	AATCCAGCCACAAACATCAA	Polymorphic
104.	EE-89	CCTTCACAATCCCACCAGAG	GAAAGGCGATCATTCAAACG	Polymorphic
105.	pPGPseq-14E10	ACCTAGTGGGACAAGGCTTTA	TTGACAAAATAACCTCACTTCGAT	Monomorphic

106.	pPGPseq-18G10	TCTGGTTACTTCTGCTTTTGTTTC	CCCTTCTTTTTCAAAATCCTCA	Monomorphic
107.	pPGPseq-4D4	CGGCTGTTAGGTAATCAGTTCA	TCAACAGGAATAGCTGCACG	Polymorphic
108.	pPGPseq-19C3	TCATCGCCAAACTCTTCTCC	TCGAAGAGTGCATGTTGACC	Polymorphic
109.	pPGPseq-7H9	CACCTCAAATTCAACATTGGC	ATCCATGTTGCCTGGAGAAA	Polymorphic
110.	pPGPseq-17E3	TTTCCTTTCAACCCTTCGTG	AATGAGACCAGCCAAAATGC	Polymorphic
111.	pPGPseq-3F1	AGCGATCAATCGGTTTCAAG	GAAACGAAACGAAGACCGAA	Monomorphic
112.	pPGPseq-8H1	TTGTGACAACCTTCCCCTTC	CATCCGCTTTCTCTCACCAT	Polymorphic
113.	pPGPseq-7B9	TGCAGTTTACGATGAGGGTAGT	CAGCTTCTTGGTGTCCTTGA	Polymorphic
114.	pPGPseq-13E11	CCGAAAAAGCTCTGATACCC	TGTTGATGACGGCTCAGAAG	Polymorphic
115.	pPGPseq-4F10	TGCGAAACCCCTAACTGACT	TCTATGTTGCTGCCGTTGAC	Polymorphic
116.	PM-42	ACGGGCCAAGTGAAGTGAT	TCTTGCTTCTTTGGTGATTAGC	Polymorphic
117.	pPGSseq-19G5	GGAACTTCTTGTGCCAGACC	CAAACAGAAGATGGTTCGTGA	No amplification
118.	PM-65	GGACGTCTGGCTGCTAGAGA	TCGGCATCAAAACAGTGAGA	Polymorphic
119.	PM-137	AACCAATTCAACAAACCCAGT	GAAGATGGATGAAAACGGATG	Polymorphic
120.	PM-188	GGGCTTCACTGCTTTTGATT	TGCGACTTCTGAGAGGACAA	Polymorphic
121.	PM-201	CCTTTATAGAGGACCTTCCCTCT	GCCTATTTGGTATCGGCTCA	Polymorphic
122.	PM-204	TGGGCCTAAACCCAACCTAT	CCACAAACAGTGCAGCAATC	Polymorphic
123.	PM-375	CGGCAACAGTTTTGATGGTT	GAAAAATATGCCGCCGTTG	Polymorphic
124.	PM-377	ACGCTCACATGTTTGCTTTG	ACGCTCACATGTTTGCTTTG	Polymorphic
125.	PM-468	TCAAGCCATAATATGTTCCACA	AAAACAACCCAAGCACCTCT	Polymorphic
126.	pPGSseq-4E8	ACCATTGCACTTTGAAGCTCT	GCTTGGTTTGGGTTAGTTTGA	Polymorphic
127.	pPGSseq-14G3	GCTTGGTTTGGGTTAGTTTGA	TCGCAGTTCTCAAAGTTATCG	Polymorphic
128.	pPGSseq-16C7	TTGCTACTAAGCCGAAAATGAA	CTTGAAATTAACACATATGCACACA	Polymorphic
129.	PPGSseq-9G5	GGAACTTCTTGTGCCAGACC	CAAACAGAAGATGGTTCGTGA	Polymorphic
130.	IPAHM-569	ATTGGTGGATATCGGACTCG	CCTATCCTTCAAGGCTTCTCG	No amplification
131.	PPGSseq-13E6A	TGGCAATTTATTGATGCAGG	GTCACGTAATTGGATGCACG	Polymorphic
132.	PPGSseq-14F5	TGAACCACTTTTTCGAAATTTTTAT	GGGTCCTCTTGTCTTGTCCA	Polymorphic
133.	PPGSseq-15E12	GCAGAACTAAGGTCGGCAAG	TCCGCCCTTTATTTTTGTGT	Polymorphic
134.	PPGSseq-15G2	CCCGAAGGCTCATTTAGTACA	TCTTCAGCTCCCAAGGATTG	Monomorphic
135.	PPGSseq-16G4	CAAAAAGCTACGTGCCAAAAC	CAAACGGAAACCTCCCTTAT	Polymorphic
136.	PPGSseq-18B8	TGGGACAGCAAGGTCTATCA	CCGTGATTGATTTCAATTTTCA	Polymorphic
137.	PPGSseq-15E8	CTTTCGCGCTTGTTTGAAAT	AAGCTGCGTGTAAAAGGGTC	Polymorphic
138.	PPGSseq-18A8	TCAATTTCAAAATATTTCCACTTTCTT	GGGTTAGCATATGACCCCAA	Polymorphic
139.	PPGPseq-3E10	TCCCAAAAATAACAAACATGGA	ACGCTTTGAGACTCGTCGTT	Polymorphic
140.	pPGSseq-16F10	TGGAGGGAAAAACATTTTGG	CCTGGAGGGGTGAGAGGT	No amplification
141.	pPGSseq-12D5	GTGTGGGGAAGTTGGATTTG	GCTCTTTTTCTTTTCTTTTTCTCC	No amplification
142.	PPGPseq-3D9	TTCACCCGTACAAACCAGTG	CCTCGGCAGATCTGGAGTAA	Polymorphic
143.	PPGSseq-17H5	TCGTAAGTTCAACCTCGGCT	AATGGCGTCGTTTGATTCAT	Monomorphic
144.	PPGSseq-19B12	CGTCCTTGTCAAGCTGTATTG	CCAATGTGCAACAAAACAAGA	Polymorphic
145.	PPGSseq-19E9	ACTGCTTGCTCTCTTCCTCG	TTCCACCTATAAAATCAATGGTGA	Polymorphic
146.	PPGSseq-18C5	GGACAGCCGGATGCTATTTA	ACATGAGTCCCTTTTCCCTT	Polymorphic

147.	PPGSseq-19F4	CCCATGATAAATTTGTATATCAAGCA	TCAACCACAGAAGACGACGA	Polymorphic
148.	PPGPseq-8E12	TCTGTTGAGAACCACCAGCA	GTGCTAGTTGCTTGACGCAC	Polymorphic
149.	pPGSseq-9H8	CTGGATACATCGACGCTGAG	GCGGTCCAATACTAACAAAATC	Polymorphic
150.	pPGSseq-14D11	ACGCTAGTCCCTTTGCTTTG	AAACAATCCTTTCTTTGGATGG	Polymorphic
151.	pPGSseq-16G8	CTCAAAAAGCGCTTAGCCAC	CTGCCTACTGCCTACTGCCT	Polymorphic
152.	S-5	CCGGCTAGAGAATACACACACA	CCGGCTAGAGAATACACACACA	No amplification
153.	S-29	CACCGCCGCCCGTTTCTTCTCCT	GGGCAACGGCTCGACGGTGGTATC	No amplification
154.	pPGSseq-11G7	CATGTCTCCATGAGCATTTCA	TGGATGTGGACAGCATATCG	Polymorphic
155.	pPGSseq-16H8	CCGTACCGGTGCCATTAT	CGCCAAAATGAATGCACTA	Polymorphic
156.	pPGSseq-19H3	TGGCAGGCAGTAAACATCAG	TTGAGGACGTGATGAACTGG	Polymorphic
157.	pPGSseq-9E8	TTCACTTTGGGGCATTTTGT	AAACCATATGCATTGGCACC	Polymorphic
158.	pPGSseq-9F1	GCCTAGTGTGCAAAGGTGCT	CACCTGATGTGTAGTGAGGCA	No amplification
159.	pPGSseq-12E10	TGCTTTTAGAGGCTTTGCCA	GAAACTGCAACAGCAACAGAA	No amplification
160.	pPGSseq-18G1	AATAGGTTGTGAAGCACGCA	TTCGGTGGTACTTTTAAGGCA	Polymorphic
161.	PM-145	GCTGTAATTAGGATCATTCCACA	CAACGGTTGGATCGATGA	Polymorphic
162.	PM-384	GGCGTGCCAATAGAGGTTTA	TGAAAACCAACAAGTTTAGTCTCTCT	Polymorphic
163.	PPGPseq-4A6	CGCTTGCCCCACTACACTAT	AGCAGTGCTTTGCATGTACG	Monomorphic
164.	PPGSseq-18G9	ATATCAGCGCCAATGACTCC	TCGCTCCTGGCACCTATATC	Polymorphic
165.	PPGSseq-19A5	ATTCGTCTCCTTCTTTTGGC	TTTTGCTTCCAAATGGCTTC	Polymorphic
166.	PPGPseq-3B8	GGAGAAAGATCAAACGAGAACA	TTCGAATATCTGACATTTGCTTTT	Polymorphic
167.	S-4	GAACGCCAGTTTACGTCGTC	TTGGGACACTTACCGAAGAGTT	No amplification
168.	S-16	TGGTAGTGGAGTCAGAGTGTGTG	GTTGCATTGCCCAACTCTTT	Monomorphic
169.	S-19	GCTCCACTAGTGCCGAAATC	CAGACACCCGGAGGCTTA	Polymorphic
170.	S-21	AGTCCTACTTGTGGGGGTTG	TCCCTTTTGCAGTGAAATCC	Monomorphic
171.	S-24	GGCAATGCACACGCTACTCT	CGTGAGGCGTGAGAGTTCAT	Polymorphic
172.	S-28	TTGCAAGATGTGCATCAAAA	TGACAAACCAACAAACGACA	Monomorphic
173.	EM-18	GAGGAGGAATCTTGCTTCGA	ACAATGGTGCTGTTCGTCTT	Monomorphic
174.	EM-22	TGAGCGGCTACTCGTATTCC	AAAGAGTGCGAGCGATGAGG	Polymorphic
175.	EM-31	AAAGTCCCATGAATGCTCTC	AGTAGAAAACACGGTAGCCA	Polymorphic
176.	EM-60	TCCACCTCACAACCTCTTCA	CCAGCAGGGACATCATCTAA	Monomorphic
177.	EM-69	ATCCACCGTAGCCAGAACCA	GTGGAAGGTTCGGTTGTTGC	Monomorphic
178.	pPGSseq-15C12	ACAATGCAATGACCGTTGTT	TTGTTGCATGAGAACGTGAA	No amplification
179.	EM-90	CACCAAATCTCCCACTTCCC	CGGCTATCAAATGGTGCTTC	Polymorphic
180.	EM-59	ACCATCACTACAGCCATCTC	ATCTTTGACATCACCGTTTC	Monomorphic
181.	EM-75	CAAAGACCTCAAACCCAATG	TCTTCAATGGTGTCGGATGT	Polymorphic
182.	EM-81	ATGCTTCGGTTCACCCAATT	CCAATGAAAGGCTCCACAAG	Polymorphic
183.	EM-82	TTCATCCTCCTGCTACCATT	GTAGACACGGTCACAAACGA	Polymorphic
184.	EM-154	TGAGTGAGCGAGTGAAACTACAT	AACCTGCTGTAAGGCATCTC	Monomorphic
185.	EM-151	ACCATTGATGCGGAGGGAC	CCTGCCTTCGCTGTTCCAT	Polymorphic
186.	IPAHM-171c	CAACACAAGCCCACAACAAA	TCCATCATCACCCTCATCAA	Polymorphic
187.	IPAHM-166	GGACAATTATGCCCCTCAGC	TCCTTCCTCTGAGCTTTTCG	Monomorphic

188.	IPAHM-171a	TTGGTTGTTCGTAGCTCTGC	AGCACGGCAAACACTAACACT	Polymorphic
189.	IPAHM-176	TTCAGCAAAAACATGCAAGG	TGCAATGAGTTATATTCACCTCTCC	Polymorphic
190.	pPGSseq-10D4	ATCCCTGATTAGTGCAACGC	CGTAGGTGGTTTTAGGAGGG	No amplification
191.	IPAHM-229	TCAGCCTGCGAAACTAAGGT	TGGAGAACTAGGATCTCTTTTGTG	Polymorphic
192.	IPAHM-395	CAGAGTCAATGGCAGCGTAG	TCCTTCCCTCATCTAAAACCAA	Monomorphic
193.	IPAHM-524	CAGAGTCAATGGCAGCGTAG	TCCTTCCCTCATCTAAAACCAA	Polymorphic
194.	IPAHM-689	GATGACAATAGCGACGAGCA	GTAAGCCTGCAGCAACAACA	Polymorphic
195.	EM-143	TGGATTTGTAGCCCAGTTTC	ACGCCTTTGAAGTTGTGATT	Monomorphic
196.	PPGPseq-2E6	TACAGCATTGCCTTCTGGTG	CCTGGGCTGGGGTATTATTT	Polymorphic
197.	Lec-1	CAAGCATCAACAACAACGA	GTCCGACCACATACAAGAGTT	Polymorphic
198.	PPGPseq-11H1	TTTGTGTTTAAGAAGGGGTGC	GCGGTCCAACATCCTTTTT	Polymorphic
199.	PPGPseq-14F4	ACGTTTAGTTGCTTGCGTGA	TGAATTCAAAGGAAAATGAAAAA	Polymorphic
200.	PPGPseq-17F6	CGTCGGATTTATCTGCCAGT	AGTAGGGGCAAGGGTTGATG	Polymorphic
201.	pPGSseq-4A11	ATTGAAGGCTACACTTGCGG	GAATCACGGGAGGGAAAGAT	No amplification
202.	IPAHM-219	TCTCTTTTGTGTATTTTGGGCTA	AGCCTGCGAAACTAAGGTTG	Monomorphic
203.	IPAHM-287	TCTAACCCTTCGGTTCATGG	TCACTATCCCATCCCTGCTC	Polymorphic
204.	IPAHM-475	GTGATTTCCTGGTTGGTGCT	AGCCTCAGCTGGTTTTGCT	Polymorphic
205.	IPAHM-165	CAACACGTTCGCTTCCAGAT	TCACTCTCATTTCCGCCATT	Polymorphic
206.	IPAHM-147	CCATTGTCTTCCTCCTCTATCTC	TGTTGATGCAGCAATTAGGC	Polymorphic
207.	IPAHM-283	GAAGACAAACCCCTCTGCTG	TCGGATAGCATGGATGTGAA	Monomorphic
208.	IPAHM-288	AAGTGAATTTGGGGATGCTG	CTCCACCACTGCCACTATCA	Polymorphic
209.	IPAHM-136	CCCCTTTCTCCACTACTACCA	TTCTCCTAGGGACTCCGATG	Polymorphic
210.	IPAHM-406	TGAAAGGGATTGGACCAAAA	TGTTGGACAGGATTTCACACA	Polymorphic
211.	IPAHM-407a	TTGGTTGTTCGTAGCTCTGC	AGCACGGCAAACACTAACACT	Polymorphic
212.	IPAHM-92	CTTCATGTCTGATTAGCAGAAGGT	CCCTGATATGGCCTCTTCAA	Monomorphic
213.	pPGSseq-2G03	ATTCACAAGGGGACAGTTGC	ATTCAAGCCTGGGAAACAGA	No amplification
214.	PM-238	CTCTCCTCTGCTCTGCACTG	ACAAGAACATGGGGATGAAGA	Polymorphic
215.	EM-119	CAGATGACTTAGTTGTTGGCATTA	TCGCCAGTCACATCATCAAC	Polymorphic
216.	EM-129	CTTCCACTCTTCGGAAACAA	AGTCATTTGGTTTGGCTGGT	Polymorphic
217.	IPAHM-290	CCACCGCTGATGTGTAATTGTA	GACGTGTAGTTGAAAACAACAGTATCA	Polymorphic
218.	EE-88	AAGATGGCTTCACGAAGGAT	CAGTCAGGATGGGAGGCAAA	No amplification
219.	IPAHM-531	TGCCAGGTTGCTGTAACAAA	CATACACGCTTTTCCCCTGT	Polymorphic
220.	IPAHM-455	TGCAGAGACTTGTATTTTGAGG	AAGCCTTTGCGAATATAACC	Polymorphic
221.	IPAHM-373	CAAGATCTTTCGTACATTCATTCAC	CACGCTCTTAGCAATTTCTGG	Polymorphic
222.	pPGSseq-2C11	TGACCTCAATTTTGGGGAAG	GCCACTATTCATCGCGGTA	No amplification
223.	IPAHM-356	TTGGGATTGGATCCCTAAGA	CAACTACCCTTCTCTCCACCA	Monomorphic
224.	pPGSseq-10H1A	TGACAATGGGGTGTTCTTCA	GTAAACAGACGCCGTTCCAT	Polymorphic
225.	PM-183	TTCTAATGAAAACCGACAAGTTT	CGTGCCAATAGAGTTTTATACGG	Polymorphic

226.	EE-91	GTCTTGGCAGTTGATGATAGTGT	GTAAATCATAGCCACTCATCCC	Polymorphic
227.	pPGSseq-11E11	CTGCTATATTCTGGGCGGAG	CGAGAAAACAGTTTGGGAGG	Polymorphic
228.	pPGSseq-14C11	CGTTGGGGACAAAAACGATA	TTTTCTTGAAACTCGTTGATATGG	Polymorphic
229.	pPGSseq-19B1	TTGGTGATGGTGTTGGAGAA	TTAAACCAGGCCAAAAGTGG	No amplification
230.	pPGSseq-19C10	TGTGAAAGGAAAATTTTGGG	TTTCAAGGTTGTAATGGGGC	No amplification
231.	IPAHM-23	GTGTCTTTTCGTTCGCGATT	CGACTCTTAGGGTGGATTATAGTGA	Polymorphic
232.	IPAHM-108	CTTGTCAAACTCTGTGACTTAGCA	CATGAACAATTACACCCAGTCA	Polymorphic
233.	Ah 4-24	TTCTGATTTTAGTAGTCTTCTTTCAC	CTCCTTAGCCACGGTTTCT	Polymorphic
234.	pPGSseq-19G7	ATTCAATTCCTCTCTCCCCC	TCAATCAATCAATCGCAGGA	Polymorphic
235.	pPGSseq-12A7	CACTGCCACACCGACCAC	GGGTAGATGGGTGATGATGC	Polymorphic
236.	S-10	CAAGCCAAAAGTGGAAAACC	TCCTTTTGCTAATGCGGTCT	Monomorphic
237.	EM-40	GCTTTCTACTATCTTCAGGACCAA	AAGTGCCTCCACATCCAAGT	Polymorphic
238.	IPAHM-177	TCAGCGGAGAAGAAAACTAAGG	GAGGTGTTTGGAGAACTAGGATTT	Polymorphic
239.	EM-78	CAATCAACATGATGGGAAGG	CCCGCATTTATTTGTTTACG	Polymorphic
240.	EE-30	AGGAAAGGAGGCAAAGGGAT	GGAAGTGAAGGAGGGTGGTG	No amplification
241.	IPAHM-123	CGGAGACAGAACACAAACCA	TACCCTGAGCCTCTCTCTCG	Polymorphic
242.	S-3	GCACCAATTTTGTCCCTGAT	AAGGGGTTTGCACGTAAATG	Polymorphic
243.	IPAHM-468	GGCTTTTGAAGTTCCCTTCC	TATGCCTCTTCCCCTTCCTT	Polymorphic
244.	EE-90	GTCCAGGTCTTCTTGTTTCCA	AGAAGTGGTGGGCATAGTCAG	Monomorphic
245.	IPAHM-718	AGATAGGGGCCGAGCTAGAG	ATGCTCCACCCCTTCATTTT	Polymorphic
246.	EM-94	CCTGTAACTGCCTCAAAGCC	TAGCCCTCATCATCACCCTC	No amplification
247.	PPGSseq-9G12B	CTGCCTCTGCTTCCTCTGTT	TTGGAAAACACACATGCACC	Monomorphic
248.	PM-45	TGAGTTGTGACGGCTTGTGT	GATGCATGTTTAGCACACTTGA	Polymorphic
249.	EM-106	TTCGTCGTCTCCGTACTACAC	TCATTACTGCTCCTGGCTTT	Polymorphic
250.	EM-135	TCATTACTGCTCCTGGCTTT	CTCAATCTCCTTCAGCCTCTT	Polymorphic
251.	S-27	ATCCGGCTCACAGTTCAATC	GCCAAGGCTGAAAAGAGTTG	Monomorphic
252.	pPGPseq-8C10	ACGCCAACGACGTTAATACC	GTGCAATGGATGAGCTGAAA	Monomorphic
253.	pPGSseq-3A01	AACTCGCTTGTACCGGCTAA	AGGAATAATAACAATACCAACAGCA	Polymorphic
254.	EM-92	GAGAATTGGTGGTGGCTATG	TTCCTCCACAATGTTCTCCA	No amplification
255.	EM-93	GAGGGATAGACAAGGAAGGA	GGTAAAGCCATAAGAGCACA	No amplification
256.	pPGPseq-7G2	ACTCCCGATGCACTTGAAAT	AACCTCTGTGCACTGTCCCT	Polymorphic
257.	pPGSseq-11G3	CCGCGTTGTTAAACCAGAAC	ATGGAGGATGTGAGTGGGAA	Polymorphic
258.	pPGSseq-17G6	AACGACAACGACAACGACAA	TCCACTATACAGTTGGGGGC	Polymorphic
259.	pPGSseq-18E7	AACGTGCGTGGAAAGAGTTC	TGAGAGTGGTTTTTGTTGGTG	Monomorphic
260.	EM-83	TAGTGAAAGAAGCATTGGGAGC	ACGCCTTTGAAGTTGTGATTGT	No amplification
261.	PPGPseq-3B6	TGCAGCCGTTTTTATGAATG	AGCAGTTTGCAAAGGAGCAT	Monomorphic
262.	PPGPseq-7H6	CATCCTCACGGGAGTCAGAT	ATACCTACGCGTTGTGGAGC	Polymorphic

263.	EM-56	TGTCTTATTCTGGGTTATGG	ATATGGGCAAAGTAAGTAGC	No amplification
264.	EM-63	AAGCCTTTGTTGGGAATGTG	GAACCTGGTGGAAGTAGTGG	No amplification
265.	pPGSseq-14H6	GCAACTAGGGTGTATGCCGT	CAACCCTATACACCGAGGGA	Polymorphic
266.	pPGSseq-15D3	CATGCCATCATCACAACACA	GGAGGAAGCAATGGTTTCAG	Polymorphic
267.	EM-11	TTTGGTGATGTCTCAAGGCTAT	CTGAGCATTGGGTATCTTTGAC	No amplification
268.	EM-37	GGGCTAAGGAATAAGGAGTGGA	TGAAAGTCTGCAAAGAAGTGCC	No amplification
269.	pPGSseq-19D6	TTTGTTATGCTCACACCCCA	AAAAATGAAGCAATATTTTGTTGTTAG	Polymorphic
270.	EM-96	AAATGGAGGGTCCTTAGTTCTC	TGTAGTTTCGCTCCTTCAGTTT	No amplification
271.	IPAHM-93	TCCATCGTTAGTGGCACTGT	GTCGACTCCTGCCCAATCTA	Polymorphic
272.	EE-84	CAGGGTGCTGAGGAAAGGG	TCTCGACCGACACGATGCT	Monomorphic
273.	Ah 4-4	CGATTTCTTTACTGAGTGAG	ATTTTTTTGCTCCACACA	Polymorphic
274.	EM-87	CATGCTCCTCCAATTTATTACG	CGAGACTTGAGTGCCTTGTTG	Polymorphic
275.	pPGPseq-1B09	CGTTCTTTGCCGTTGATTCT	AGCACGCTCGTTCTCTCATT	Polymorphic
276.	IPAHM-82	CCATATCATAGCCGCCAAGT	TACATCCACGATGCAGAAGG	Polymorphic
277.	Ah1TC11H06	CCATGTGAGGTATCAGTAAAGAAAGG	CCACCAACAACATTGGATGAAT	Polymorphic
		ISSR		
278.	UBC-810	GAGAGAGAGAGAGAGAT		Polymorphic
279.	UBC-809	AGAGAGAGAGAGAGAGG		Polymorphic
280.	UBC-814	CTCTCTCTCTCTCTCTA		Polymorphic
281.	UBC-816	CACACACACACACACAT		Monomorphic
282.	UBC-829	TGTGTGTGTGTGTGTGC		Monomorphic
283.	UBC-835	AGAGAGAGAGAGAGAGCTT		Polymorphic
284.	UBC-840	GAGAGAGAGAGAGAGACTT		Polymorphic
285.	UBC-848	CACACACACACACACAAGC		Monomorphic
286.	UBC-808	AGAGAGAGAGAGAGAGC		Polymorphic
287.	UBC824	TCTCTCTCTCTCTCTCG		No amplification

Table 3: Detail of number of alleles produced by each primer, polymorphic primers/alleles, size range, annealing temperature and citation of microsatellite primers used in diversity analysis.

#	Primers	Total No. of alleles	No. of polymorphic alleles	Sizerange ofAlleles(bp)	Annealing Temp (°C)	Citation
1.	PM-3	5	5	190-215	55	He *et al*., 2003
2.	PM-15	4	3	175-190	55	He *et al*., 2003
3.	PM-32	1	1	110	55	He *et al*., 2003
4.	PM-35	2	2	135-140	55	He *et al*., 2003
5.	PM-50	5	5	100-120	55	He *et al*., 2003
6.	PM-53	1	0	125	-	He *et al*., 2003
7.	PM-179	1	1	75	55	He *et al*., 2003
8.	PM-200	3	3	155-165	57	He *et al*., 2003
9.	PM-210	4	4	185-210	55	He *et al*., 2003

10.	pPGPseq-1B09	2	2	250-260	64	Ferguson *et al*., 2004a
11.	pPGPseq-2A05	2	2	250-255	60	Ferguson *et al*., 2004a
12.	pPGPseq-2D12B	3	3	290-300	60	Ferguson *et al*., 2004a
13.	pPGPseq-2G04	3	3	290-310	60	Ferguson *et al*., 2004a
14.	pPGPseq-4G02	1	1	280	60	Ferguson *et al*., 2004a
15.	pPGPseq-4H11	2	2	260-265	60	Ferguson *et al*., 2004a
16.	pPGPseq-2B10	3	3	255-265	58	Ferguson *et al*., 2004a
17.	pPGSseq-13A7	2	2	275-280	58	Ferguson *et al*., 2004
18.	pPGPseq-2F5	2	2	260-275	58	Ferguson *et al*., 2004a
19.	pPGPseq-8D9	3	3	135-150	61	Ferguson *et al*., 2004a
20.	pPGSseq-15C10	4	4	200-290	60	Ferguson *et al*., 2004
21.	pPGPseq-3A4	1	1	165	58	Ferguson *et al*., 2004a
22.	pPGPseq-2C10	2	2	170-175	58	Ferguson *et al*., 2004a
23.	pPGPseq-2A6	2	2	240-245	64	Ferguson *et al*., 2004a
24.	pPGPseq-3B10	3	0	240-300	-	Ferguson *et al*., 2004a
25.	pPGPseq-3C2	1	1	290	57	Ferguson *et al*., 2004a
26.	pPGSseq-3A01	4	4	220-245	60	Ferguson *et al*., 2004
27.	pPGPseq-4C11	1	0	280	-	Ferguson *et al*., 2004a
28.	pPGPseq-4D1	1	0	170	-	Ferguson *et al*., 2004a
29.	pPGPseq-5B2	1	0	240	-	Ferguson *et al*., 2004a
30.	pPGPseq-5C5	1	1	120	62	Ferguson *et al*., 2004a
31.	pPGPseq-5D5	2	2	250-260	64	Ferguson *et al*., 2004a
32.	S-1	1	1	200	55	Wang *et al*., 2007
33.	S-2	1	1	220	55	Wang *et al*., 2007
34.	S-6	1	1	170	55	Wang *et al*., 2007
35.	S-9	1	1	190	55	Wang *et al*., 2007
36.	S-17	2	2	205-210	55	Wang *et al*., 2007
37.	S-18	1	1	220	55	Wang *et al*., 2007
38.	S-20	1	1	190	55	Wang *et al*., 2007
39.	S-22	1	1	180	55	Wang *et al*., 2007
40.	S-23	1	0	180	-	Wang *et al*., 2007
41.	S-25	1	0	150	-	Wang *et al*., 2007
42.	S-26	1	1	230	55	Wang *et al*., 2007
43.	S-27	1	0	190	-	Wang *et al*., 2007
44.	pPGPseq-8C10	1	1	170	64	Ferguson *et al*., 2004a
45.	pPGSseq-11D4	3	3	225-250	61	Ferguson *et al*., 2004b
46.	pPGSseq-10C12	1	1	175	64	Ferguson *et al*., 2004b
47.	pPGSseq-12B6	1	1	250	62	Ferguson *et al*., 2004b
48.	pPGSseq-9D12	1	1	300	62	Ferguson *et al*., 2004b
49.	pPGPseq-16E10	2	2	210-320	62	Ferguson *et al*., 2004a
50.	pPGSseq-13D1A	1	0	205	-	Ferguson *et al*., 2004b
51.	pPGSseq-13C8	1	1	205	65	Ferguson *et al*., 2004b
52.	pPGSseq-14D1	4	4	280-330	60	Ferguson *et al*., 2004b
53.	pPGSseq-15B1	1	1	120	60	Ferguson *et al*., 2004b
54.	pPGSseq-15D2	1	0	120	-	Ferguson *et al*., 2004b
55.	pPGPseq-6B8	1	1	290	62	Ferguson *et al*., 2004a
56.	pPGPseq-7B3	1	0	276	-	Ferguson *et al*., 2004a
57.	pPGPseq-8B11	2	2	240-290	64	Ferguson *et al*., 2004a
58.	pPGSseq-11F12	1	1	145	56	Ferguson *et al*., 2004b
59.	pPGSseq-14A10	1	1	275	60	Ferguson *et al*., 2004b
60.	pPGSseq-16C3	1	0	150	60	Ferguson *et al*., 2004b
61.	pPGSseq-16F1	2	2	280-300	-	Ferguson *et al*., 2004b
62.	pPGSseq-15E11	1	1	300	60	Ferguson *et al*., 2004b

63.	pPGSseq-17C5	1	1	250	58	Ferguson *et al*., 2004b
64.	pPGSseq-17E1	2	2	300-310	60	Ferguson *et al*., 2004b
65.	pPGSseq-18A5	5	5	270-330	60	Ferguson *et al*., 2004b
66.	pPGSseq-18B11	1	1	300	60	Ferguson *et al*., 2004b
67.	pPGSseq-19A4	1	1	175	60	Ferguson *et al*., 2004b
68.	pPGSseq-18C2	3	3	140-190	60	Ferguson *et al*., 2004b
69.	pPGSseq-9B4	2	2	230-260	60	Ferguson *et al*., 2004b
70.	pPGSseq-9A7	5	5	250-300	60	Ferguson *et al*., 2004a
71.	pPGPseq-7D9	1	1	300	55	Ferguson *et al*., 2004a
72.	pPGSseq-12C5	1	1	260	58	Ferguson *et al*., 2004b
73.	pPGSseq-13A4	2	2	130-145	60	Ferguson *et al*., 2004b
74.	pPGSseq-16C6	1	1	240	62	Ferguson *et al*., 2004b
75.	pPGSseq-13A10	1	1	270	62	Ferguson *et al*., 2004b
76.	pPGPseq-7E10A	1	1	300	54	Ferguson *et al*., 2004a
77.	S-7	1	1	175	55	Wang *et al*., 2007
78.	S-8	1	0	190	-	Wang *et al*., 2007
79.	S-11	2	2	160-165	55	Wang *et al*., 2007
80.	S-12	2	2	230-235	55	Wang *et al*., 2007
81.	S-13	1	1	250	55	Wang *et al*., 2007
82.	S-14	2	0	300-350	-	Wang *et al*., 2007
83.	S-15	2	1	240-320	59	Wang *et al*., 2007
84.	PM-36	3	3	260-290	55	He *et al*., 2003
85.	PM-73	1	1	105	55	He *et al*., 2003
86.	Ah 4-20	1	1	210	55	Hopkins *et al*., 1999
87.	Ah 6-125	1	1	190	55	Hopkins *et al*., 1999
88.	Ah 4-26	3	2	160-220	55	Hopkins *et al*., 1999
89.	EM-97	1	1	210	55	Liang *et al*., 2009
90.	EM-106	1	1	390	55	Liang *et al*., 2009
91.	EM-135	1	1	420	55	Liang *et al*., 2009
92.	EM-145	1	1	160	55	Liang *et al*., 2009
93.	EM-148	3	2	350-400	55	Liang *et al*., 2009
94.	EM-140	2	2	220-240	62	Liang *et al*., 2009
95.	EM-155	2	2	320-260	55	Liang *et al*., 2009
96.	EM-156	1	1	340	55	Liang *et al*., 2009
97.	EM-100	1	1	250	57	Liang *et al*., 2009
98.	EM-113	2	2	180-210	60	Liang *et al*., 2009
99.	EM-118	2	2	160-200	60	Liang *et al*., 2009
100.	EM-68	2	2	220-240	55	Liang *et al*., 2009
101.	EM-23	1	1	190	55	Liang *et al*., 2009
102.	EM-132	5	5	70-450	51	Liang *et al*., 2009
103.	EE-73	1	1	210	55	Liang *et al*., 2009
104.	EE-89	1	1	330	55	Liang *et al*., 2009
105.	pPGPseq-14E10	1	0	160	-	Ferguson *et al*., 2004a
106.	pPGPseq-18G10	1	0	265	-	Ferguson *et al*., 2004a
107.	pPGPseq-4D4	1	1	200	60	Ferguson *et al*., 2004a
108.	pPGPseq-19C3	4	4	220-290	60	Ferguson *et al*., 2004a
109.	pPGPseq-7H9	1	1	220	60	Ferguson *et al*., 2004a
110.	pPGPseq-17E3	3	3	180-200	57	Ferguson *et al*., 2004a
111.	pPGPseq-3F1	1	1	280	57	Ferguson *et al*., 2004a
112.	pPGPseq-8H1	1	1	290	62	Ferguson *et al*., 2004a
113.	pPGPseq-7B9	1	1	260	62	Ferguson *et al*., 2004a
114.	pPGPseq-13E11	1	1	290	62	Ferguson *et al*., 2004a
115.	pPGPseq-4F10	3	3	160-250	54	Ferguson *et al*., 2004a

116.	PM-42	3	3	160-200	50	He *et al*., 2003
117.	PM-45	3	3	100-110	60	He *et al*., 2003
118.	PM-65	3	3	225-280	55	He *et al*., 2003
119.	PM-137	3	3	140-155	55	He *et al*., 2003
120.	PM-188	3	2	100-115	55	He *et al*., 2003
121.	PM-201	3	3	140-240	55	He *et al*., 2003
122.	PM-204	3	3	220-230	55	He *et al*., 2003
123.	PM-375	3	3	110-150	55	He *et al*., 2005
124.	PM-377	4	3	145-175	55	He *et al*., 2005
125.	PM-468	1	1	200	55	He *et al*., 2005
126.	pPGSseq-4E8	2	2	280-290	57	Ferguson *et al*., 2004b
127.	pPGSseq-14G3	4	4	255-310	52	Ferguson *et al*., 2004b
128.	pPGSseq-16C7	2	2	240-245	57	Ferguson *et al*., 2004b
129.	PPGSseq-9G5	3	3	240-280	63	Ferguson *et al*., 2004b
130.	PPGSseq-9G12B	1	0	120	-	Ferguson *et al*., 2004b
131.	PPGSseq-13E6A	2	2	265-270	63	Ferguson *et al*., 2004b
132.	PPGSseq-14F5	1	1	250	60	Ferguson *et al*., 2004b
133.	PPGSseq-15E12	2	2	130-135	63	Ferguson *et al*., 2004b
134.	PPGSseq-15G2	1	1	280	60	Ferguson *et al*., 2004b
135.	PPGSseq-16G4	1	1	275	61	Ferguson *et al*., 2004b
136.	PPGSseq-18B8	2	2	265-270	57	Ferguson *et al*., 2004b
137.	PPGSseq-15E8	2	2	220-230	55	Ferguson *et al*., 2004b
138.	PPGSseq-18A8	1	1	190	57	Ferguson *et al*., 2004b
139.	PPGPseq-3E10	4	4	280-390	53	Ferguson *et al*., 2004a
140.	PPGPseq-3B6	3	0	230-270	-	Ferguson *et al*., 2004a
141.	PPGPseq-7H6	2	2	295-305	60	Ferguson *et al*., 2004a
142.	PPGPseq-3D9	1	1	280	63	Ferguson *et al*., 2004a
143.	PPGSseq-17H5	1	0	150	-	Ferguson *et al*., 2004b
144.	PPGSseq-19B12	2	2	180-185	59	Ferguson *et al*., 2004b
145.	PPGSseq-19E9	3	3	265-275	62	Ferguson *et al*., 2004b
146.	PPGSseq-18C5	3	3	280-300	51	Ferguson *et al*., 2004b
147.	PPGSseq-19F4	2	2	270-280	60	Ferguson *et al*., 2004b
148.	PPGPseq-8E12	2	2	195-200	60-50	Ferguson *et al*., 2004a
149.	pPGSseq-9H8	3	2	250-310	57	Ferguson *et al*., 2004b
150.	pPGSseq-14D11	2	2	160-175	60	Ferguson *et al*., 2004b
151.	pPGSseq-16G8	2	2	200-210	59	Ferguson *et al*., 2004b
152.	pPGPseq-7G2	3	3	235-250	63	Ferguson *et al*., 2004a
153.	pPGSseq-11G3	3	3	215-230	59	Ferguson *et al*., 2004b
154.	pPGSseq-11G7	3	3	230-275	63	Ferguson *et al*., 2004b
155.	pPGSseq-16H8	1	1	320	63	Ferguson *et al*., 2004b
156.	pPGSseq-19H3	1	1	290	53	Ferguson *et al*., 2004b
157.	pPGSseq-9E8	2	2	300-310	63	Ferguson *et al*., 2004b
158.	pPGSseq-14H6	3	3	280-350	63	Ferguson *et al*., 2004b
159.	pPGSseq-15D3	3	3	290-305	60	Ferguson *et al*., 2004b
160.	pPGSseq-18G1	3	3	245-265	60	Ferguson *et al*., 2004b
161.	PM-145	1	1	180	55	He *et al*., 2003
162.	PM-384	3	3	80-120	59	He *et al*., 2005
163.	PPGPseq-4A6	1	0	120	-	Ferguson *et al*., 2004a
164.	PPGSseq-18G9	4	3	130-180	60	Ferguson *et al*., 2004b
165.	PPGSseq-19A5	1	1	300	57	Ferguson *et al*., 2004b
166.	PPGPseq-3B8	1	1	275	61	Ferguson *et al*., 2004a
167.	S-3	3	0	180-220	-	Wang *et al*., 2007
168.	S-16	1	0	70	-	Wang *et al*., 2007

169.	S-19	2	2	100-200	55	Wang *et al*., 2007
170.	S-21	3	3	230-280	55	Wang *et al*., 2007
171.	S-24	2	2	300-500	55	Wang *et al*., 2007
172.	S-28	1	0	250	-	Wang *et al*., 2007
173.	EM-18	1	1	300	47	Liang *et al*., 2009
174.	EM-22	1	1	130	47	Liang *et al*., 2009
175.	EM-31	2	2	110-140	57	Liang *et al*., 2009
176.	EM-60	1	0	450	-	Liang *et al*., 2009
177.	EM-69	1	0	180	-	Liang *et al*., 2009
178.	EM-78	2	2	360-320	57	Liang *et al*., 2009
179.	EM-90	2	1	380-410	55	Liang *et al*., 2009
180.	EM-59	1	0	200	-	Liang *et al*., 2009
181.	EM-75	1	1	150	62	Liang *et al*., 2009
182.	EM-81	2	2	280-850	62	Liang *et al*., 2009
183.	EM-82	1	1	180	62	Liang *et al*., 2009
184.	EM-154	1	0	100	-	Liang *et al*., 2009
185.	EM-151	3	3	200-310	57	Liang *et al*., 2009
186.	IPAHM-171c	2	2	140-150	65-60	Cuc *et al*., 2008
187.	IPAHM-166	1	0	180	-	Cuc *et al*., 2008
188.	IPAHM-171a	4	4	190-260	65-60	Cuc *et al*., 2008
189.	IPAHM-176	3	3	140-200	65-60	Cuc *et al*., 2008
190.	IPAHM-177	2	2	200-250	65-60	Cuc *et al*., 2008
191.	IPAHM-229	2	2	100-150	65-60	Cuc *et al*., 2008
192.	IPAHM-395	1	0	200	-	Cuc *et al*., 2008
193.	IPAHM-524	1	1	300	65-60	Cuc *et al*., 2008
194.	IPAHM-689	2	2	250-270	65-60	Cuc *et al*., 2008
195.	EM-143	2	1	100-130	55	Liang *et al*., 2009
196.	PPGPseq-2E6	2	2	240-250	62	Ferguson *et al*., 2004a
197.	Lee-1	3	3	210-300	57	Hopkins *et al*., 1999
198.	PPGPseq-11H1	1	1	190	55	Ferguson *et al*., 2004a
199.	PPGPseq-14F4	3	3	180-210	50	Ferguson *et al*., 2004a
200.	PPGPseq-17F6	1	1	850	47	Ferguson *et al*., 2004a
201.	IPAHM-123	4	4	135-185	65-60	Cuc *et al*., 2008
202.	IPAHM-219	2	0	120-130	-	Cuc *et al*., 2008
203.	IPAHM-287	4	4	180-215	65-60	Cuc *et al*., 2008
204.	IPAHM-475	4	4	265-300	65-60	Cuc *et al*., 2008
205.	IPAHM-165	1	1	230	65-60	Cuc *et al*., 2008
206.	IPAHM-147	2	1	400-440	47	Cuc *et al*., 2008
207.	IPAHM-283	1	0	120	-	Cuc *et al*., 2008
208.	IPAHM-288	1	1	490	57	Cuc *et al*., 2008
209.	IPAHM-136	3	3	110-140	65-60	Cuc *et al*., 2008
210.	IPAHM-406	2	2	280-300	55	Cuc *et al*., 2008
211.	IPAHM-407	4	3	190-260	59	Cuc *et al*., 2008
212.	IPAHM-92	2	0	150-180	-	Cuc *et al*., 2008
213.	IPAHM-468	5	4	175-320	50	Cuc *et al*., 2008
214.	PM-238	4	4	150-170	48	He *et al*., 2003
215.	EM-119	3	3	70-350	47	Liang *et al*., 2009
216.	EM-129	2	1	70-200	48	Liang *et al*., 2009
217.	IPAHM-290	1	1	290	55	Cuc *et al*., 2008
218.	IPAHM-718	1	1	500	48	Cuc *et al*., 2008
219.	IPAHM-531	3	2	265-300	50	Cuc *et al*., 2008
220.	IPAHM-455	4	2	140-170	50	Cuc *et al*., 2008
221.	IPAHM-373	2	2	180-430	48	Cuc *et al*., 2008

222.	EE-90	1	0	50	-	Liang *et al*., 2009
223.	IPAHM-356	1	0	50	-	Cuc *et al*., 2008
224.	pPGSseq-10H1A	1	1	205	62	Ferguson *et al*., 2004b
225.	PM-183	4	4	120-145	55	He *et al*., 2003
226.	UBC-810	2	2	195-410	50	Mondal *et al*., 2009
227.	UBC-809	3	3	200-380	50	Mondal *et al*., 2009
228.	UBC-814	2	2	310-350	50	Mondal *et al*., 2009
229.	UBC-816	1	1	490	50	Mondal *et al*., 2009
230.	UBC-829	1	1	350	50	Mondal *et al*., 2009
231.	UBC-835	2	2	450-500	55	Mondal *et al*., 2009
232.	UBC-840	1	1	500	50	Mondal *et al*., 2009
233.	UBC-848	1	1	460	50	Mondal *et al*., 2009
234.	UBC-808	3	3	290-400	50	Mondal *et al*., 2009
235.	EE-91	1	1	260	57	Liang *et al*., 2009
236.	pPGSseq-11E11	2	2	200-240	62	Ferguson *et al*., 2004b
237.	pPGSseq-14C11	2	2	200-220	64	Ferguson *et al*., 2004b
238.	pPGSseq-17G6	2	2	470-500	62	Ferguson *et al*., 2004b
239.	pPGSseq-18E7	1	0	50	-	Ferguson *et al*., 2004b
240.	IPAHM-23	5	5	120-150	47	Cuc *et al*., 2008
241.	IPAHM-108	3	3	220-290	55	Cuc *et al*., 2008
242.	Ah 4-24	1	1	60	50	Hopkins *et al*., 1999
243.	pPGSseq-19G7	2	2	350-400	60	Ferguson *et al*., 2004b
244.	pPGSseq-12A7	3	2	195-300	63	Ferguson *et al*., 2004b
245.	S-10	1	0	75	-	Wang *et al*., 2007
246.	EM-40	1	1	950	62	Liang *et al*., 2009
247.	pPGPseq2A06	2	2	240-250	64	Ferguson *et al*., 2004a
248.	pPGSseq-19D6	3	3	210-240	60	Ferguson *et al*., 2004b
249.	IPAHM-93	6	6	160-230	62	Cuc *et al*., 2008
250.	EE-84	1	0	50	-	Liang *et al*., 2009
251.	Ah 4-4	3	3	85-100	55	Hopkins *et al*., 1999
252.	EM-87	2	2	220-230	55	Liang *et al*., 2009
253.	IPAHM-82	2	2	280-290	60	Cuc *et al*., 2008
254.	Ah1TC11H06	4	4	200-230	55	Mondal and Badigannavar, 2010

Table 4: Polymorphism level and discriminating capacity of SSRs, ISSRs and different series of SSR

Indexes	Abbreviation	pPGPseq	S	PM	Ah	EM	IPAHM	EE	Lec	TC11H	SSR Total	UBC (ISSR)
Total Primer Synthesize	#	130	29	25	5	42	38	6	1	1	277	10
Not Amplified	#	12	3	0	0	10	6	1	0	0	32	1
Amplification %age	%	90.8	89.7	100	100	76.2	84.2	83.3	100	100	824.2	90
Monomorphic Primer	#	15	9	1	0	4	6	2	0	0	37	0
Polymorphic Primer	#	103	17	24	5	28	26	3	1	1	208	9
Polymorphic Primer %age	%	87.3	65.4	96	100	87.5	81.3	60	100	100	777.5	100
No. of Assay Unit (Primers amplified)	U	118	26	25	5	32	32	5	1	1	245	9
Total No. of Loci	L	226	37	71	9	54	81	5	3	4	490	16
No. of Polymorphic Loci	n_p	205	25	68	8	49	68	3	3	4	433	16
Polymorphic Loci %age	%	90.7	67.6	95.8	88.9	90.7	84	60	100	100	777.7	100
No. of MONOMORPHIC LOCI	n_m	21	12	3	1	5	13	2	0	0	57	0
Average No. of Polymorphic Loci/Assay Unit	n_p/U	1.737	0.962	2.720	1.600	1.531	2.125	0.600	3.000	4.000	1.767	1.778
No. of Loci/Assay Unit	$n_u=L/U$	1.915	1.423	2.840	1.800	1.688	2.531	1.000	3.000	4.000	2.00	1.778
Expected Heterozygosity	H_e= PIC mean	0.31	0.16	0.36	0.23	0.23	0.329	0.188	0.440	0.367	0.289	0.222
Fraction of Polymorphic Loci	$\beta=n_p/L$	0.907	0.676	0.958	0.889	0.907	0.840	0.600	1	1	0.884	1
Effective Multiplex Ratio	$E=n_u\beta$	1.737	0.962	2.721	1.600	1.531	2.126	0.600	3.000	4.000	1.676	1.778
Marker Index	$MI=EH_e$	0.538	0.154	0.980	0.368	0.352	0.699	0.113	1.320	1.468	0.511	0.395

Table No. 5: Polymorphic Information Content (PIC), Confusion probability (Cj), Discriminating power (Dj) and Resolving power (Rp) of polymorphic SSRs and ISSRs primers

Sr. No.	Primers Name	PIC	Cj	Dj	Rp
	pPGSseq-16F1	0.499	0.248	0.752	1.895
1.	pPGPseq-16C6	0.061	0.938	0.062	1.937
2.	pPGPseq-13A10	0.021	0.979	0.021	1.979
3.	pPGPseq-12C5	0.100	0.897	0.103	1.895
4.	pPGPseq-7E10A	0.172	0.819	0.181	1.811
5.	pPGPseq-5D5	0.498	0.222	0.778	1.874
6.	pPGPseq-15E11	0.236	0.744	0.256	1.726
7.	pPGPseq-2A06	0.499	0.227	0.773	1.916
8.	pPGPseq-9B4	0.491	0.313	0.687	1.726
9.	pPGPseq-19A4	0.041	0.958	0.042	1.958
10.	pPGPseq-7D9	0.172	0.819	0.181	1.811
11.	pPGPseq-1B09	0.500	0.304	0.696	2.000
12.	pPGPseq-4H11	0.496	0.292	0.708	1.832
13.	pPGPseq-17C5	0.041	0.958	0.042	1.958
14.	pPGPseq-9D12	0.021	0.979	0.021	1.979
15.	pPGPseq-8D9	0.451	0.200	0.800	2.063
16.	pPGPseq-8B11	0.021	0.979	0.021	3.958
17.	pPGPseq-17E1	0.499	0.223	0.777	1.895
18.	pPGPseq-2B10	0.444	0.156	0.844	2.000
19.	pPGPseq-14A10	0.021	0.979	0.021	1.979
20.	pPGPseq-2D12B	0.440	0.174	0.826	1.958
21.	pPGPseq-2A05	0.500	0.264	0.736	1.937
22.	pPGPseq-8C10	0.021	0.979	0.021	1.979
23.	pPGPseq-5C5	0.041	0.958	0.042	1.958
24.	pPGPseq-18B11	0.021	0.979	0.021	1.979
25.	pPGPseq-13C8	0.061	0.938	0.062	1.937
26.	pPGPseq-12B6	0.154	0.838	0.162	1.832
27.	pPGPseq-10C12	0.100	0.897	0.103	1.895
28.	pPGPseq-18A5	0.320	0.115	0.885	2.000
29.	pPGPseq-3A4	0.021	0.979	0.021	1.979
30.	pPGPseq-13A4	0.100	0.897	0.103	3.789
31.	pPGPseq-15B1	0.061	0.938	0.062	1.937
32.	pPGPseq-4G02	0.021	0.979	0.021	1.979
33.	pPGPseq-11D4	0.444	0.200	0.800	2.000
34.	pPGPseq-2C10	0.499	0.331	0.669	1.916
35.	pPGPseq-18C2	0.442	0.218	0.782	1.979
36.	pPGPseq-3A01	0.385	0.193	0.807	2.084
37.	pPGPseq-9A7	0.315	0.063	0.937	1.958
38.	pPGPseq-6B8	0.041	0.958	0.042	1.958
39.	pPGPseq-3C2	0.021	0.979	0.021	1.979
40.	pPGPseq-11F12	0.320	0.638	0.362	1.600
41.	pPGPseq-14D1	0.499	0.228	0.772	3.832
42.	pPGPseq-2G04	0.424	0.098	0.902	1.832
43.	pPGPseq-15C10	0.385	0.099	0.901	2.084
44.	pPGPseq-16E10	0.486	0.480	0.520	2.337
45.	pPGPseq-13A7	0.500	0.237	0.763	1.937
46.	pPGPseq-2F5	0.498	0.366	0.634	1.874
47.	pPGPseq-15G2	0.021	0.979	0.021	1.979
48.	pPGPseq-16G4	0.137	0.857	0.143	1.853
49.	pPGPseq-18A8	0.021	0.979	0.021	1.979
50.	pPGPseq-3F1	0.061	0.938	0.062	1.937
51.	pPGPseq-3E10	0.488	0.217	0.783	3.389
52.	pPGPseq-18C5	0.430	0.109	0.891	1.874
53.	pPGPseq-8E12	0.500	0.341	0.659	1.958

54.	pPGPseq-14D11	0.499	0.471	0.529	2.084
55.	pPGPseq-19H3	0.021	0.979	0.021	1.979
56.	pPGPseq-4E8	0.500	0.341	0.659	1.958
57.	pPGPseq-7H9	0.061	0.938	0.062	1.937
58.	pPGPseq-7B9	0.100	0.897	0.103	1.895
59.	pPGPseq-13E11	0.061	0.938	0.062	1.937
60.	pPGPseq-16C7	0.498	0.227	0.773	1.874
61.	pPGPseq-4F10	0.465	0.512	0.488	3.789
62.	pPGPseq-14G3	0.498	0.291	0.709	3.726
63.	pPGPseq-14F5	0.154	0.838	0.162	1.832
64.	pPGPseq-13E6A	0.496	0.409	0.591	1.832
65.	pPGPseq-18b8	0.497	0.319	0.681	1.853
66.	pPGPseq-18G1	0.444	0.144	0.856	2.000
67.	pPGPseq-18G9	0.367	0.097	0.903	1.453
68.	pPGPseq-19C3	0.441	0.596	0.404	5.368
69.	pPGPseq-8H1	0.041	0.958	0.042	1.958
70.	pPGPseq-4D4	0.251	0.726	0.274	1.705
71.	pPGPseq-15E12	0.500	0.260	0.740	1.958
72.	pPGPseq-7H6	0.500	0.244	0.756	1.979
73.	pPGPseq-3D9	0.118	0.877	0.123	1.874
74.	pPGPseq-19E9	0.424	0.126	0.874	1.832
75.	pPGPseq-9H8	0.484	0.267	0.733	1.642
76.	pPGPseq-16G8	0.361	0.589	0.411	3.053
77.	pPGPseq-19A5	0.307	0.655	0.345	1.621
78.	pPGPseq-7G2	0.432	0.165	0.835	1.979
79.	pPGPseq-11G3	0.404	0.085	0.915	1.684
80.	pPGPseq-19B12	0.498	0.240	0.760	1.874
81.	pPGPseq-9E8	0.500	0.271	0.729	1.979
82.	pPGPseq-14H6	0.442	0.213	0.787	1.979
83.	pPGPseq-15D3	0.437	0.108	0.892	1.937
84.	pPGPseq-15E8	0.489	0.283	0.717	1.705
85.	pPGPseq-16H8	0.172	0.819	0.181	1.811
86.	pPGPseq-3B8	0.221	0.762	0.238	1.747
87.	pPGPseq-19F4	0.496	0.347	0.653	1.811
88.	pPGPseq-11G7	0.493	0.386	0.614	3.347
89.	pPGPseq-17E3	0.416	0.147	0.853	1.768
90.	pPGPseq-9G5	0.460	0.585	0.415	3.853
91.	pPGPseq-11H1	0.137	0.857	0.143	1.853
92.	pPGPseq-14F4	0.464	0.530	0.470	3.811
93.	pPGPseq-17F6	0.061	0.938	0.062	1.937
94.	pPGPseq-13A4	0.100	0.897	0.103	3.789
95.	pPGPseq-10H1A	0.221	0.762	0.238	1.747
96.	pPGPseq-2E6	0.480	0.279	0.721	1.600
97.	pPGPseq-19G7	0.499	0.271	0.729	1.895
98.	pPGPseq-12A7	0.061	0.938	0.062	5.811
99.	pPGPseq-19D6	0.435	0.149	0.851	1.916
100.	pPGPseq-11E11	0.497	0.356	0.644	2.147
101.	pPGPseq-14C11	0.465	0.174	0.826	1.474
102.	pPGPseq-17G6	0.493	0.390	0.610	1.768
	Total	**31.52**	**55.33**	**47.67**	**222.05**
	Average	**0.31**	**0.54**	**0.46**	**2.16**
	Standard Deviation	**0.19**	**0.33**	**0.33**	**0.75**
	Range	**0.021-0.5**	**0.085-0.979**	**0.021-0.937**	**1.453-5.811**
1.	S-12	0.473	0.227	0.773	1.916
2.	S-22	0.021	0.979	0.021	1.979
3.	S-15	0.041	0.958	0.042	3.916
4.	S-13	0.021	0.979	0.021	1.979

5.	S-11	0.500	0.271	0.729	1.979
6.	S-2	0.021	0.979	0.021	1.979
7.	S-17	0.150	0.355	0.645	1.958
8.	S-18	0.221	0.762	0.238	1.747
9.	S-20	0.021	0.979	0.021	1.979
10.	S-1	0.021	0.979	0.021	1.979
11.	S-6	0.154	0.838	0.162	1.832
12.	S-9	0.021	0.979	0.021	1.979
13.	S-26	0.021	0.979	0.021	1.979
14.	S-7	0.061	0.938	0.062	1.937
15.	S-19	0.486	0.464	0.536	2.337
16.	S-21	0.041	0.958	0.042	5.874
17.	S-24	0.402	0.530	0.470	1.221
	Total	**2.68**	**13.15**	**3.85**	**38.57**
	Average	**0.16**	**0.77**	**0.23**	**2.27**
	Standard Deviation	**0.18**	**0.27**	**0.27**	**1.04**
	Range	**0.021-0.5**	**0.227-0.979**	**0.021-0.773**	**1.221-5.874**
1.	PM-238	0.500	0.264	0.736	3.958
2.	PM-35	0.499	0.235	0.765	1.916
3.	PM-179	0.021	0.979	0.021	1.979
4.	PM-200	0.440	0.134	0.866	1.958
5.	PM-73	0.021	0.979	0.021	1.979
6.	PM-32	0.021	0.979	0.021	1.979
7.	PM-15	0.492	0.238	0.762	2.611
8.	PM-50	0.310	0.047	0.953	1.916
9.	PM-36	0.437	0.130	0.870	1.937
10.	PM-3	0.344	0.061	0.939	2.211
11.	PM-210	0.375	0.091	0.909	2.000
12.	PM-65	0.499	0.363	0.637	2.884
13.	PM 42	0.447	0.544	0.456	3.979
14.	PM 137	0.449	0.124	0.876	2.042
15.	PM 45	0.456	0.133	0.867	2.105
16.	PM-204	0.442	0.141	0.859	1.979
17.	PM-201	0.451	0.572	0.428	3.937
18.	PM-375	0.447	0.212	0.788	2.021
19.	PM-468	0.061	0.938	0.062	1.937
20.	PM-377	0.442	0.115	0.885	1.979
21.	PM-145	0.081	0.917	0.083	1.916
22.	PM-183	0.370	0.092	0.908	1.958
23.	PM-384	0.491	0.202	0.798	2.589
24.	PM 188	0.476	0.489	0.511	3.663
	Total	**8.57**	**8.98**	**15.02**	**57.43**
	Average	**0.36**	**0.37**	**0.63**	**2.39**
	Standard Deviation	**0.17**	**0.33**	**0.33**	**0.71**
	Range	**0.021-0.5**	**0.047-0.979**	**0.021-0.953**	**1.916-3.958**
1.	Ah4-20	0.021	0.979	0.021	1.979
2.	Ah6-125	0.172	0.819	0.181	1.811
3.	Ah4-24	0.021	0.979	0.021	1.979
4.	Ah4-4	0.442	0.150	0.850	1.979
5.	Ah4-26	0.500	0.249	0.751	2.000
	Total	**1.16**	**3.18**	**1.82**	**9.75**
	Average	**0.23**	**0.64**	**0.36**	**1.95**
	Standard Deviation	**0.20**	**0.36**	**0.36**	**0.07**
	Range	**0.021-0.5**	**0.105-0.979**	**0.021-0.850**	**1.811-2.000**
1.	EM-97	0.205	0.781	0.219	1.768
2.	EM-106	0.221	0.762	0.238	1.747
3.	EM-135	0.100	0.897	0.103	1.895

4.	EM-148	0.481	0.439	0.561	3.579
5.	EM-145	0.118	0.877	0.123	1.874
6.	EM-156	0.154	0.838	0.162	1.832
7.	EM-113	0.109	0.887	0.113	3.768
8.	EM-100	0.100	0.897	0.103	1.895
9.	EM-118	0.137	0.857	0.143	3.705
10.	EM-68	0.154	0.838	0.162	3.663
11.	EM-23	0.221	0.762	0.238	1.747
12.	EM-18	0.021	0.979	0.021	1.979
13.	EM-22	0.081	0.917	0.083	1.916
14.	EM-31	0.197	0.794	0.206	3.558
15.	EM-78	0.432	0.506	0.494	2.737
16.	EM-75	0.100	0.897	0.103	1.895
17.	EM-81	0.188	0.805	0.195	3.579
18.	EM-82	0.081	0.917	0.083	1.916
19.	EM-151	0.449	0.241	0.759	2.042
20.	EM 155	0.100	0.897	0.103	3.789
21.	EM-132	0.500	0.387	0.613	5.158
22.	EM 140	0.172	0.819	0.181	3.621
23.	EM-90	0.398	0.595	0.405	2.905
24.	EM-143	0.021	0.979	0.021	3.958
25.	EM-119	0.494	0.226	0.774	3.326
26.	EM-129	0.493	0.309	0.691	1.116
27.	EM-40	0.154	0.838	0.162	1.832
28.	EM-87	0.496	0.318	0.682	1.832
	Total	**6.38**	**20.26**	**7.74**	**74.63**
	Average	**0.23**	**0.72**	**0.28**	**2.67**
	Standard Deviation	**0.16**	**0.23**	**0.23**	**0.99**
	Range	**0.021-0.5**	**0.226-0.979**	**0.021-0.774**	**1.116-5.158**
1.	IPAHM-171C	0.500	0.479	0.521	1.979
2.	IPAHM-171A	0.432	0.580	0.420	5.474
3.	IPAHM-176	0.280	0.694	0.306	4.989
4.	IPAHM-177	0.061	0.938	0.062	3.874
5.	IPAHM-229	0.100	0.899	0.101	3.789
6.	IPAHM-524	0.100	0.897	0.103	1.895
7.	IPAHM-689	0.500	0.440	0.560	2.000
8.	IPAHM-123	0.499	0.310	0.690	3.789
9.	IPAHM-287	0.476	0.204	0.796	3.116
10.	IPAHM-475	0.499	0.269	0.731	1.916
11.	IPAHM-165	0.041	0.958	0.042	1.958
12.	IPAHM-147	0.221	0.762	0.238	1.747
13.	IPAHM-288	0.266	0.708	0.292	1.684
14.	IPAHM-136	0.458	0.475	0.525	3.874
15.	IPAHM-406	0.172	0.823	0.177	3.621
16.	IPAHM-407A	0.430	0.517	0.483	5.495
17.	IPAHM-468	0.480	0.342	0.658	4.000
18.	IPAHM-290	0.041	0.958	0.042	1.958
19.	IPAHM-718	0.205	0.781	0.219	1.768
20.	IPAHM-531	0.500	0.397	0.603	2.000
21.	IPAHM-455	0.398	0.642	0.358	5.811
22.	IPAHM-373	0.154	0.841	0.159	3.663
23.	IPAHM-23	0.500	0.316	0.684	5.158
24.	IPAHM-108	0.460	0.501	0.499	3.853
25.	IPAHM-82	0.350	0.605	0.395	3.095
26.	IPAHM-93	0.433	0.113	0.887	3.221
	Total	**8.556**	**15.449**	**10.551**	**85.727**
	Average	**0.329**	**0.594**	**0.406**	**3.297**

	Standard Deviation	**0.167**	**0.247**	**0.247**	**1.307**
	Range	**0.041-0.5**	**0.113-0.958**	**0.042-887**	**1.684-5.811**
1.	EE-89	0.137	0.857	0.143	1.853
2.	EE-73	0.221	0.762	0.238	1.747
3.	EE-91	0.205	0.781	0.219	1.768
	Total	**0.563**	**2.400**	**0.600**	**5.368**
	Average	**0.188**	**0.800**	**0.200**	**1.789**
	Standard Deviation	**0.036**	**0.041**	**0.041**	**0.046**
	Range	**0.137-0.221**	**0.762-0.857**	**0.143-0.238**	**1.747-1.853**
1.	Lec-1	0.440	0.186	0.814	1.958
	Total	0.440	0.186	0.814	1.958
	Average	-	-	-	-
	Standard Deviation	-	-	-	-
1.	TC11H06	0.367	0.076	0.924	1.937
	Total	0.367	0.076	0.924	1.937
	Average	-	-	-	-
	Standard Deviation	-	-	-	-
	Total SSR	60.23	119.007	88.993	497.424
	Mean SSR	0.290	0.572	0.428	2.391
1.	UBC-810	0.109	0.889	0.111	3.768
2.	UBC-809	0.061	0.938	0.062	5.811
3.	UBC-814	0.494	0.309	0.691	1.789
4.	UBC-816	0.041	0.958	0.042	1.958
5.	UBC-829	0.021	0.979	0.021	1.979
6.	UBC-835	0.482	0.462	0.538	2.379
7.	UBC-840	0.266	0.708	0.292	1.684
8.	UBC-848	0.021	0.979	0.021	1.979
9.	UBC-808	0.499	0.406	0.594	3.137
10.	**Total**	**1.994**	**6.628**	**2.372**	**24.484**
11.	**Average**	**0.222**	**0.736**	**0.264**	**2.720**
12.	**standard deviation**	**0.203**	**0.258**	**0.258**	**1.272**
13.	**Range**	**0.021-0.499**	**0.309-0.979**	**0.021-0.691**	1.684-5.811
	Overall Total	**62.224**	**125.635**	**91.365**	**521.908**
	Average	**0.287**	**0.579**	**0.421**	**2.405**
	Standard deviation	**0.189**	**0.325**	**0.325**	**0.977**
	Range	**0.021-0.5**	**0.047-0.979**	**0.021-0.953**	**1.116-5.811**

Table 6: Cross combination, Locus and allele size for using in molecular breeding of groundnut

Cross Combination	Parents	Locus with allele size
Argentina-2 X PG-1017	Argentina-2	S-11/160, PM-50/120 PM-42/200 pPGPseq-14H6/300 pPGPseq-8D9/140 PM-15/180 PM-137/150 pPGPseq-7H6/305 pPGPseq-2B10/255, PM-45/100, PM-201/235 Ah4-26/220
	PG-1017	S-11/165 PM-50/105 PM-42/205 pPGPseq-14H6/350 pPGPseq-8D9/145 PM-15/190 PM-137/155 pPGPseq-7H6/295 pPGPseq-2B10/265 PM-45/105 PM-201/105 Ah4-26/215
Argentina-2 X PW	Argentina-2	S-11/160, pPGPseq-1B09/260, PM-15/180, PM-50/110, pPGPseq-4E8/286 PM-137/150 pPGPseq-14H6/300 pPGPseq-15D3/290 PM-377/170 IPAHM-171C/140 IPAHM-136/110 PM-238/170 PM-183/120 IPAHM-93/180 Ah4-4/95 Ah4-26/220
	PW	S-11/165, pPGPseq-1B09/250, PM-15/190, PM-50/105, pPGPseq-4E8/274 PM-137/140 pPGPseq-14H6/280 pPGPseq-15D3/295 PM-377/160 IPAHM-171C/150 IPAHM-136/120 PM-238/165 PM-183/125 IPAHM-93/225 Ah4-4/85 Ah4-26/215
Argentina-2 X Chico	Argentina-2	S-11/160, pPGPseq-8D9/145 pPGPseq-2B10/255 PM-50/120 PM-188/100 PM-42/200 PM-137/150 PM-45/100 pPGPseq-7H6/305 pPGPseq-14H6/300 pPGPseq-15D3/290 IPAHM-136/120 PM-238/150 IPAHM-531/270
	Chico	S-11/165, pPGPseq-8D9/140 pPGPseq-2B10/260 PM-50/105 EM-148/350 PM-42/205 PM-137/155 PM-45/105 pPGPseq-7H6/295 pPGPseq-14H6/350 pPGPseq-15D3/295 IPAHM-136/140 PM-238/155 IPAHM-531/265
Argentina-2 X Sudan	Argentina-2	PM-137/150 EM-132/75 PM-375/110 pPGPseq-7H6/305 pPGPseq-9E8/140 PM-238/165 EM-119/75
	Sudan	PM-137/140 EM-132/420 PM-375/120 pPGPseq-7H6/295 pPGPseq-9E8/150 PM-238/170 EM-119/70
Argentina-2 X ICG-4747	Argentina-2	pPGPseq-1B09/260 pPGPseq-2B10/255 pPGPseq-4E8/274 PM-42/200 pPGPseq-15E12/130 pPGPseq-7H6/305
	ICG-4747	pPGPseq-1B09/250 pPGPseq-2B10/265 pPGPseq-4E8/286 PM-42/205 pPGPseq-15E12/135 pPGPseq-7H6/295
Argentina-2 X ICGV-015592	Argentina-2	pPGPseq-8D9/145 pPGPseq-2B10/255 PM-42/200 PM-137/150 PM-201/235 pPGPseq-15E12/130 pPGPseq-7H6/305 pPGPseq-14H6/300 PM-377/160 IPAHM-136/120 PM-238/150 Ah4-26/220
	ICGV-015592	pPGPseq-8D9/140 pPGPseq-2B10/265 PM-42/205 PM-137/155 PM-201/105 pPGPseq-15E12/135 pPGPseq-7H6/295 pPGPseq-14H6/350 PM-377/170 IPAHM-136/140 PM-238/155 Ah4-26/215
Chico X 04CG007	Chico	pPGPseq-5D5/250 PM-200/160 S-11/165, pPGPseq-8D9/140 pPGPseq-17E1/300 pPGPseq-2B10/260 PM-15/185 PM-50/105 pPGPseq-11D4/225 pPGPseq-2C10/175 pPGPseq-3A01/245 pPGPseq-9A7/250 PM-36/290 PM-3/190 pPGPseq-14D1/330 pPGPseq-14D1/280 pPGPseq-13A7/280pPGPseq-2F5/275 pPGPseq-3E10/300 pPGPseq-8E12/200 EM-148/405 pPGPseq-4E8/286 PM-42/205 pPGPseq-16C7/245 PM-137/155 PM-377/160 EM-31/140 pPGPseq-11G7/275 IPAHM-171A/260 Lec-1/250 IPAHM-123/140 IPAHM-287/205 IPAHM-136/140 IPAHM-531/265 PM-183/135 pPGPseq-14C11/220 PM-384/165 Ah4-26/215 TC11H06/200
	04CG007	pPGPseq-5D5/260 PM-200/400 S-11/160, pPGPseq-8D9/145 pPGPseq-17E1/310 pPGPseq-2B10/255 PM-15/190 PM-50/110 pPGPseq-11D4/230 pPGPseq-2C10/170 pPGPseq-18C2/140 pPGPseq-9A7/300 PM-36/280 PM-3/200 pPGPseq-14D1/320 pPGPseq-14D1/285 pPGPseq-13A7/275 pPGPseq-2F5/260 pPGPseq-3E10/280 pPGPseq-8E12/195 EM-148/400 pPGPseq-4E8/274 PM-42/200 pPGPseq-16C7/240 PM-137/150 PM-377/170 EM-31/110 pPGPseq-11G7/270 IPAHM-171A/240 Lec-1/300 IPAHM-123/135 IPAHM-287/215 IPAHM-136/120 IPAHM-531/270 PM-183/125 pPGPseq-14C11/200 PM-384/165 Ah4-26/220 TC11H06/210
Chico X 04CG002	Chico	pPGPseq-5D5/250 PM-200/160 pPGPseq-2A06/240 S-11/165, pPGPseq-2B10/260 pPGPseq-2A05/250 PM-15/185 PM-50/105 pPGPseq-11D4/225 pPGPseq-3A01/245 pPGPseq-9A7/250 PM-36/290 PM-3/190 pPGPseq-14D1/330 pPGPseq-14D1/280 pPGPseq-13A7/280 pPGPseq-2F5/275 pPGPseq-3E10/300 pPGPseq-8E12/200 PM-42/205 PM-137/155 PM-375/110 pPGPseq-15E12/135 pPGPseq-7H6/295 pPGPseq-7G2/235 pPGPseq-14H6/350 PM-377/160 pPGPseq-11G7/275 IPAHM-689/250 IPAHM-123/140 IPAHM-136/140 IPAHM-468/320 PM-238/155 IPAHM-531/265 PM-183/135 pPGPseq-19G7/350 pPGPseq-11E11/200 PM-384/165
	04CG002	pPGPseq-5D5/260 PM-200/400 pPGPseq-2A06/245 S-11/160, pPGPseq-2B10/255 pPGPseq-2A05/255 PM-15/190 PM-50/110 pPGPseq-11D4/230 pPGPseq-18C2/140 pPGPseq-9A7/300 PM-36/280 PM-3/200 pPGPseq-14D1/320 pPGPseq-14D1/285 pPGPseq-13A7/275 pPGPseq-2F5/260 pPGPseq-3E10/280 pPGPseq-8E12/195 PM-42/200 PM-137/150 PM-375/120 pPGPseq-15E12/130 pPGPseq-7H6/305 pPGPseq-7G2/240 pPGPseq-14H6/300 PM-377/170 pPGPseq-11G7/270 IPAHM-689/270 IPAHM-123/135 IPAHM-136/120 IPAHM-468/310 PM-238/150 IPAHM-531/270 PM-183/125 pPGPseq-19G7/400 pPGPseq-11E11/240 PM-384/170
Chines X 04CG002	Chines	pPGPseq-5D5/250 PM-200/160 S-12/235 pPGPseq-8D9/140 pPGPseq-2B10/260 pPGPseq-2D12B/300 pPGPseq-2A05/250 PM-15/185 pPGPseq-11D4/250 pPGPseq-11D4/225 pPGPseq-9A7/250 PM-36/260 PM-3/190 pPGPseq-14D1/330 pPGPseq-14D1/280 PM-210/205 PM-35/140 pPGPseq-13A7/280 pPGPseq-2F5/275 pPGPseq-3E10/300 pPGPseq-8E12/200 PM-42/205 PM-137/155 pPGPseq-14G3/255 PM-201/105 pPGPseq-15E12/135 pPGPseq-14H6/350 pPGPseq-15D3/290 PM-377/160 pPGPseq-11G7/275 IPAHM-689/250 IPAHM-136/140 IPAHM-468/320 PM-238/155 IPAHM-531/265 UBC-808/400 pPGPseq-19G7/350 pPGPseq-19D6/240 pPGPseq-11E11/200 PM-384/165
	04CG002	pPGPseq-5D5/260 PM-200/400 S-12/230 pPGPseq-8D9/145 pPGPseq-2B10/255 pPGPseq-2D12B/295 pPGPseq-2A05/255 PM-15/190 pPGPseq-11D4/230 pPGPseq-2C10/175 pPGPseq-9A7/300 PM-36/280 PM-3/200 pPGPseq-14D1/320 pPGPseq-14D1/285 PM-210/200 PM-35/135 pPGPseq-13A7/275 pPGPseq-2F5/260 pPGPseq-3E10/280 pPGPseq-8E12/195 PM-42/200 PM-137/150 pPGPseq-14G3/250 PM-201/235 pPGPseq-15E12/130 pPGPseq-14H6/300 pPGPseq-15D3/295 PM-377/170 pPGPseq-11G7/270 IPAHM-689/270 IPAHM-136/120 IPAHM-468/310 PM-238/150 IPAHM-531/270 UBC-808/350 pPGPseq-19G7/400 pPGPseq-19D6/220 pPGPseq-11E11/240 PM-384/170

Husta-J X 04CG002	Husta-J	pPGPseq-2A06/240 S-11/165, pPGPseq-2D12B/290 pPGPseq-2A05/250 PM-15/185 PM-50/105 pPGPseq-3A01/245 pPGPseq-9A7/250 PM-36/290 pPGPseq-14D1/330 pPGPseq-14D1/280 pPGPseq-13A7/280 pPGPseq-3E10/390 PM-42/205 PM-201/105 pPGPseq-15E12/135 pPGPseq-7H6/295 pPGPseq-7G2/235 pPGPseq-14H6/350 pPGPseq-15D3/290 PM-377/160 EM-81/850 IPAHM-689/250 IPAHM-123/140 IPAHM-136/140 IPAHM-468/320 PM-238/170 PM-238/155 IPAHM-531/265 pPGPseq-19G7/350 pPGPseq-11E11/200 PM-384/165 Ah4-26/220
	04CG002	pPGPseq-2A06/245 S-11/160, pPGPseq-2D12B/295 pPGPseq-2A05/255 PM-15/190 PM-50/110 pPGPseq-3A01/240 pPGPseq-9A7/300 PM-36/280 pPGPseq-14D1/320 pPGPseq-14D1/285 pPGPseq-13A7/275 pPGPseq-3E10/385 PM-42/200 PM-201/235 pPGPseq-15E12/130 pPGPseq-7H6/305 pPGPseq-7G2/240 pPGPseq-14H6/300 pPGPseq-15D3/295 PM-377/170 EM-81/280 IPAHM-689/270 IPAHM-123/135 IPAHM-136/120 IPAHM-468/310 PM-238/165 PM-238/150 IPAHM-531/270 pPGPseq-19G7/400 pPGPseq-11E11/240 PM-384/170 Ah4-26/215
Chines X 04CG007	Chines	pPGPseq-5D5/250 PM-200/160 S-12/235 pPGPseq-2A06/245 pPGPseq-8D9/140 pPGPseq-17E1/300 pPGPseq-2B10/260 pPGPseq-2D12B/300 pPGPseq-2A05/250 PM-15/185 pPGPseq-11D4/250 pPGPseq-9A7/250 PM-36/260 PM-3/190 pPGPseq-14D1/330 pPGPseq-14D1/280 PM-210/205 PM-35/140 pPGPseq-3E10/300 pPGPseq-13A7/280 pPGPseq-2F5/275 pPGPseq-8E12/200 pPGPseq-4E8/286 PM-42/205 pPGPseq-16C7/245 PM-137/155 PM-201/105 pPGPseq-15E12/135 pPGPseq-7H6/295 pPGPseq-19B12/180 pPGPseq-14H6/350 PM-377/160 EM-31/140 pPGPseq-11G7/275 IPAHM-171A/260 Lec-1/250 IPAHM-287/205 PM-238/165 PM-238/155 EM-119/75 IPAHM-531/265 pPGPseq-11E11/200 pPGPseq-14C11/220 PM-384/165 TC11H06/200
	04CG007	pPGPseq-5D5/260 PM-200/400 S-12/230 pPGPseq-2A06/240 pPGPseq-8D9/145 pPGPseq-17E1/310 pPGPseq-2B10/255 pPGPseq-2D12B/295 pPGPseq-2A05/255 PM-15/190 pPGPseq-11D4/230 pPGPseq-9A7/300 PM-36/280 PM-3/200 pPGPseq-14D1/320 pPGPseq-14D1/285 PM-210/200 PM-35/135 pPGPseq-13A7/275 pPGPseq-13A7/275 pPGPseq-3E10/385 pPGPseq-8E12/195 pPGPseq-4E8/274 PM-42/205 pPGPseq-16C7/245 PM-137/150 PM-201/235 pPGPseq-15E12/130 pPGPseq-7H6/305 pPGPseq-19B12/185 pPGPseq-14H6/300 PM-377/170 EM-31/110 pPGPseq-11G7/270 IPAHM-171A/240 Lec-1/300 IPAHM-287/215 PM-238/170 PM-238/150 EM-119/70 IPAHM-531/270 pPGPseq-11E11/240 pPGPseq-14C11/200 PM-384/170 TC11H06/210
04CG002 X ICGV-015668	04CG002	pPGPseq-5D5/260 pPGPseq-2A06/245 S-11/160, pPGPseq-8D9/145 pPGPseq-2B10/255 pPGPseq-2A05/255 PM-15/190 PM-50/110 pPGPseq-11D4/230 pPGPseq-2C10/175 pPGPseq-3A01/240 pPGPseq-9A7/300 PM-36/280 PM-3/200 pPGPseq-14D1/320 pPGPseq-14D1/285 pPGPseq-15C10/220 pPGPseq-13A7/275 pPGPseq-2F5/260 pPGPseq-3E10/385 pPGPseq-8E12/195 PM-42/200 PM-137/150 PM-375/120 pPGPseq-15E12/130 pPGPseq-7H6/305 pPGPseq-7G2/240 pPGPseq-14H6/300 pPGPseq-15D3/295 PM-377/170 EM-78/360 IPAHM-689/270 pPGPseq-14F4/200 IPAHM-475/290 IPAHM-136/120 PM-238/165 PM-238/150 pPGPseq-19G7/400 IPAHM-93/225
	ICGV-015668	pPGPseq-5D5/250 pPGPseq-2A06/240 S-11/165, pPGPseq-8D9/140 pPGPseq-2B10/260 pPGPseq-2A05/250 PM-15/185 PM-50/115 pPGPseq-11D4/250 pPGPseq-11D4/225 pPGPseq-3A01/245 pPGPseq-9A7/260 PM-36/260 PM-3/190 pPGPseq-14D1/330 pPGPseq-14D1/280 pPGPseq-15C10/200 pPGPseq-13A7/280 pPGPseq-2F5/275 pPGPseq-3E10/300 pPGPseq-8E12/200 PM-42/205 PM-137/140 pPGPseq-15E12/135 pPGPseq-7H6/295 pPGPseq-7G2/250 pPGPseq-14H6/350 pPGPseq-15D3/290 PM-377/160 EM-78/320 IPAHM-689/250 pPGPseq-14F4/210 IPAHM-475/300 IPAHM-136/140 PM-238/170 PM-238/155 pPGPseq-19G7/350 IPAHM-93/180
US-1 X PG-1017	US-1	pPGSseq-16F1/300 S-12/235 pPGPseq-2A06/240 S-11/160, S-17/205 pPGPseq-8D9/145 pPGPseq-2B10/255 pPGPseq-2C10/170 pPGPseq-9A7/300 PM-36/280 PM-3/205 pPGPseq-14D1/285 PM-210/185 pPGPseq-2F5/260 pPGPseq-3E10/300 pPGPseq-4E8/274 pPGPseq-14G3/300 EM-132/70 PM-375/120 pPGPseq-7H6/305 IPAHM-136/120 IPAHM-407A/250 IPAHM-468/200 PM-238/170 PM-238/150 IPAHM-455/160 PM-183/120 UBC-808/350 pPGPseq-19D6/220 pPGPseq-11E11/240 IPAHM-108/290 PM-384/165 Ah4-4/85 Ah4-26/220 TC11H06/210
	PG-1017	pPGSseq-16F1/280 S-12/230 pPGPseq-2A06/245 S-11/165, S-17/210 pPGPseq-8D9/140 pPGPseq-2B10/265 pPGPseq-2C10/175 pPGPseq-9A7/250 PM-36/290 PM-3/210 pPGPseq-14D1/280 PM-210/200 pPGPseq-2F5/275 pPGPseq-3E10/280 pPGPseq-4E8/286 pPGPseq-14G3/310 EM-132/75 PM-375/110 pPGPseq-7H6/295 IPAHM-136/110 IPAHM-407A/260 IPAHM-468/190 PM-238/165 PM-238/155 IPAHM-455/170 PM-183/125 UBC-808/400 pPGPseq-19D6/240 pPGPseq-11E11/200 IPAHM-108/280 PM-384/80 Ah4-4/95 Ah4-26/215 TC11H06/215
04CG002 X PG-1017	04CG002	pPGPseq-5D5/260 S-11/160, pPGPseq-2B10/255 pPGPseq-2D12B/295 PM-50/110 pPGPseq-9A7/300 PM-36/280 PM-3/200 pPGPseq-14D1/320 pPGPseq-14D1/285 pPGPseq-2F5/260 PM-42/200 PM-137/150 pPGPseq-14G3/300 PM-201/235 PM-375/120 pPGPseq-15E12/130 pPGPseq-7H6/305 pPGPseq-15D3/295 PM-377/170 IPAHM-689/270 PM-65/280 IPAHM-136/120 PM-238/150 UBC-808/350 pPGPseq-19G7/400 pPGPseq-11E11/240 IPAHM-23/145 IPAHM-108/290
	PG-1017	pPGPseq-5D5/250 S-11/165, pPGPseq-2B10/265 pPGPseq-2D12B/290 PM-50/105 pPGPseq-9A7/250 PM-36/290 PM-3/190 pPGPseq-14D1/330 pPGPseq-14D1/280 pPGPseq-2F5/275 PM-42/205 PM-137/155 pPGPseq-14G3/310 PM-201/105 PM-375/150 pPGPseq-15E12/135 pPGPseq-7H6/295 pPGPseq-15D3/290 PM-377/160 IPAHM-689/250 PM-65/280 IPAHM-136/110 PM-238/155 UBC-808/400 pPGPseq-19G7/350 pPGPseq-11E11/200 IPAHM-23/150 IPAHM-108/280
04CG007 X PG-699	04CG007	PM-200/400 S-11/160, pPGPseq-2D12B/295 PM-3/200 PM-210/200 PM-35/135 pPGPseq-13A7/275 pPGPseq-2F5/260 pPGPseq-3E10/385 pPGPseq-4E8/274 pPGPseq-16C7/240 PM-137/150 pPGPseq-14G3/250 pPGPseq-19B12/185 pPGPseq-14H6/300 pPGPseq-18G1/245 PM-377/170 EM-31/110 IPAHM-287/215 IPAHM-475/290 IPAHM-136/120 PM-238/150 EM-119/70 pPGPseq-11E11/240 Ah4-4/95 Ah4-26/220
	PG-699	PM-200/160 S-11/165, pPGPseq-2D12B/300 PM-3/190 PM-210/205 PM-35/140 pPGPseq-13A7/280 pPGPseq-2F5/275 pPGPseq-3E10/300 pPGPseq-4E8/286 pPGPseq-16C7/245 PM-137/140 pPGPseq-14G3/255 pPGPseq-14H6/350 pPGPseq-18G1/260 PM-377/160 EM-31/140 IPAHM-287/205 IPAHM-475/300 IPAHM-136/140 EM-119/350 EM-119/75 pPGPseq-11E11/200 Ah4-4/85 Ah4-26/215
04CG007 X Spanish	04CG007	pPGPseq-5D5/260 S-11/160, S-17/205 pPGPseq-8D9/145 pPGPseq-17E1/310 pPGPseq-2D12B/295 pPGPseq-11D4/230 pPGPseq-18C2/140 PM-3/200 pPGPseq-14D1/320 pPGPseq-14D1/285 PM-210/200 pPGPseq-13A7/275 pPGPseq-2F5/260 pPGPseq-3E10/385 pPGPseq-4E8/274 PM-42/200 pPGPseq-16C7/240 PM-137/150 pPGPseq-14G3/250 PM-201/235 PM-375/120 pPGPseq-7H6/305 pPGPseq-7G2/240 pPGPseq-19B12/185 pPGPseq-18G1/245 PM-377/170 EM-

		31/110 IPAHM-287/215 IPAHM-136/120 IPAHM-468/190 PM-238/170 PM-238/150 EM-119/70 pPGPseq-11E11/240 TC11H06/210
	Spanish	pPGPseq-5D5/250 S-11/165, S-17/210 pPGPseq-8D9/140 pPGPseq-17E1/300 pPGPseq-2D12B/300 pPGPseq-11D4/250 pPGPseq-18C2/145 PM-3/190 pPGPseq-14D1/330 pPGPseq-14D1/280 PM-210/205 pPGPseq-13A7/280 pPGPseq-2F5/275 pPGPseq-3E10/300 pPGPseq-4E8/286 PM-42/205 pPGPseq-16C7/245 PM-137/155 pPGPseq-14G3/255 PM-201/105 PM-375/110 pPGPseq-7H6/295 pPGPseq-7G2/235 pPGPseq-19B12/180 pPGPseq-18G1/260 PM-377/160 EM-31/140 IPAHM-287/205 IPAHM-136/140 IPAHM-468/200 PM-238/165 PM-238/155 EM-119/75 pPGPseq-11E11/200 TC11H06/200
02CG005 X ICGV-015592	02CG005	S-12/235 pPGPseq-2A06/245 S-11/160, pPGPseq-9B4/230 S-17/205 pPGPseq-4H11/260 pPGPseq-8D9/140 pPGPseq-2B10/255 pPGPseq-2A05/255 pPGPseq-18A5/330 pPGPseq-11D4/225 PM-3/200 pPGPseq-2G04/300 pPGPseq-8E12/195 pPGPseq-4E8/274 PM-42/200 pPGPseq-16C7/240 PM-137/150 pPGPseq-4F10/250 pPGPseq-14G3/255 pPGPseq-14G3/300 PM-201/235 PM-375/120 pPGPseq-7G2/235 pPGPseq-19B12/185 pPGPseq-14H6/300 pPGPseq-18G1/265 PM-377/175 pPGPseq-11G7/275 EM-151/295 Lec-1/300 IPAHM-123/185 IPAHM-123/140 IPAHM-407A/260 PM-238/170 PM-238/150 IPAHM-455/170 PM-183/135 UBC-814/310 IPAHM-93/225 pPGPseq-11E11/200 IPAHM-23/145 IPAHM-23/120 IPAHM-108/290 Ah4-4/85 TC11H06/210
	ICGV-015592	S-12/230 pPGPseq-2A06/240 S-11/165, pPGPseq-9B4/260 S-17/210 pPGPseq-4H11/265 pPGPseq-8D9/140 pPGPseq-2B10/265 pPGPseq-2A05/250 pPGPseq-18A5/320 pPGPseq-11D4/230 PM-3/190 pPGPseq-2G04/310 pPGPseq-8E12/200 pPGPseq-4E8/286 PM-42/205 pPGPseq-16C7/245 PM-137/155 pPGPseq-4F10/240 pPGPseq-14G3/250 pPGPseq-14G3/310 PM-201/105 PM-375/110 pPGPseq-7G2/240 pPGPseq-19B12/180 pPGPseq-14H6/350 pPGPseq-18G1/260 PM-377/170 pPGPseq-11G7/270 EM-151/310 Lec-1/250 IPAHM-123/175 IPAHM-123/135 IPAHM-407A/250 PM-238/165 PM-238/155 IPAHM-455/160 PM-183/145 UBC-814/350 IPAHM-93/230 pPGPseq-11E11/240 IPAHM-23/150 IPAHM-23/130 IPAHM-108/280 Ah4-4/95 TC11H06/215
07C006 X Chico	07C006	S-12/235 S-11/160, pPGPseq-17E1/310 pPGPseq-2D12B/295 pPGPseq-2A05/255 PM-15/190 pPGPseq-18C2/140 pPGPseq-9A7/300 PM-36/280 pPGPseq-14D1/320 pPGPseq-14D1/285 pPGPseq-15C10/240 pPGPseq-2F5/260 pPGPseq-3E10/390 pPGPseq-3E10/280 pPGPseq-8E12/195 pPGPseq-4E8/274 PM-42/200 pPGPseq-16C7/240 pPGPseq-4F10/250 PM-375/120 pPGPseq-15E12/130 pPGPseq-7H6/305 pPGPseq-7G2/240 pPGPseq-14H6/300 pPGPseq-18G1/260 PM-377/170 Lec-1/300 IPAHM-123/135 IPAHM-287/215 IPAHM-136/120 IPAHM-407A/250 IPAHM-468/310 PM-238/150 pPGPseq-11E11/240 IPAHM-108/280 Ah4-26/220 TC11H06/210
	Chico	S-12/230 S-11/165, pPGPseq-17E1/300 pPGPseq-2D12B/300 pPGPseq-2A05/250 PM-15/185 pPGPseq-3A01/245 pPGPseq-9A7/250 PM-36/290 pPGPseq-14D1/330 pPGPseq-4D1/280 pPGPseq-15C10/219 pPGPseq-2F5/275 pPGPseq-3E10/385 pPGPseq-3E10/300 pPGPseq-8E12/200 pPGPseq-4E8/286 PM-42/205 pPGPseq-16C7/245 pPGPseq-4F10/240 PM-375/110 pPGPseq-15E12/135 pPGPseq-7H6/295 pPGPseq-7G2/235 pPGPseq-14H6/350 pPGPseq-18G1/265 PM-377/160 Lec-1/250 IPAHM-123/140 IPAHM-287/205 IPAHM-136/140 IPAHM-407A/260 IPAHM-468/320 PM-238/155 pPGPseq-11E11/200 IPAHM-108/290 Ah4-26/215 TC11H06/200
07C006 X Yh-14	07C006	PM-200/160 S-12/235 S-11/160, S-17/205 pPGPseq-17E1/310 pPGPseq-2D12B/295 pPGPseq-2A05/255 PM-15/190 pPGPseq-18A5/330 PM-3/205 pPGPseq-14D1/320 pPGPseq-14D1/285 pPGPseq-2F5/260 pPGPseq-3E10/390 pPGPseq-8E12/195 pPGPseq-4E8/274 PM-42/200 pPGPseq-4F10/250 PM-375/120 pPGPseq-7H6/305 pPGPseq-7G2/240 pPGPseq-14H6/300 pPGPseq-15D3/295 pPGPseq-18G1/260 IPAHM-287/215 IPAHM-136/120 IPAHM-407A/250 PM-238/150 IPAHM-93/225 pPGPseq-11E11/240 IPAHM-108/280 PM-384/165 Ah4-26/220
	Yh-14	PM-200/400 S-12/230 S-11/165, S-17/210 pPGPseq-17E1/300 pPGPseq-2D12B/300 pPGPseq-2A05/250 PM-15/185 pPGPseq-18A5/320 PM-3/210 pPGPseq-14D1/330 pPGPseq-14D1/280 pPGPseq-2F5/275 pPGPseq-3E10/385 pPGPseq-8E12/200 pPGPseq-4E8/286 PM-42/205 pPGPseq-4F10/240 PM-375/110 pPGPseq-7H6/295 pPGPseq-7G2/235 pPGPseq-14H6/350 pPGPseq-15D3/290 pPGPseq-18G1/265 IPAHM-287/205 IPAHM-136/140 IPAHM-407A/260 IPAHM-468/320 PM-238/155 IPAHM-93/180 pPGPseq-11E11/200 IPAHM-108/290 PM-384/80 Ah4-26/215 pPGPseq-2B10/255 pPGPseq-2D12B/295 PM-15/190
07CG006 X PG-699	07CG006	pPGPseq-5D5/250 S-12/235 S-11/160, PM-3/205 pPGPseq-14D1/320 PM-35/135 pPGPseq-2F5/260 pPGPseq-3E10/280 pPGPseq-4E8/274 PM-42/200 pPGPseq-16C7/240 pPGPseq-4F10/250 PM-42/200 pPGPseq-16C7/240 pPGPseq-4F10/250 PM-375/120 pPGPseq-7G2/240 pPGPseq-14H6/300 pPGPseq-15D3/295 PM-377/170 IPAHM-287/215 IPAHM-136/120 IPAHM-407A/250 PM-238/165 PM-238/150 IPAHM-455/170 pPGPseq-11E11/240 IPAHM-108/280 PM-384/165 Ah4-26/220
	PG-699	pPGPseq-5D5/260 S-12/230 S-11/165, pPGPseq-2B10/265 pPGPseq-2D12B/300 PM-15/185 PM-3/210 pPGPseq-14D1/330 PM-35/140 pPGPseq-2F5/275 pPGPseq-3E10/300 pPGPseq-4E8/286 PM-42/205 pPGPseq-16C7/245 pPGPseq-4F10/240 PM-375/110 pPGPseq-7G2/235 pPGPseq-14H6/350 pPGPseq-15D3/290 PM-377/160 IPAHM-287/205 IPAHM-136/140 IPAHM-407A/260 PM-238/170 PM-238/155 IPAHM-455/160 pPGPseq-11E11/200 IPAHM-108/290 PM-384/80 Ah4-26/215
07CG006 X PG-1017	07CG006	PM-200/160 S-12/235 S-11/160, S-17/205 pPGPseq-17E1/310 pPGPseq-2B10/255 PM-15/190 pPGPseq-18A5/330 pPGPseq-9A7/300 PM-36/280 PM-3/205 pPGPseq-14D1/320 pPGPseq-14D1/285 pPGPseq-15C10/240 PM-35/135 pPGPseq-2F5/260 pPGPseq-4E8/274 PM-42/200 pPGPseq-16C7/240 PM-375/120 pPGPseq-15E12/130 pPGPseq-7H6/305 pPGPseq-14H6/300 pPGPseq-15D3/295 pPGPseq-18G1/260 PM-377/170 Lec-1/300 IPAHM-287/215 IPAHM-136/120 IPAHM-407A/250 PM-238/150 IPAHM-93/225 pPGPseq-11E11/240 IPAHM-23/145 IPAHM-108/280 PM-384/165 Ah4-26/220
	PG-1017	PM-200/400 S-12/230 S-11/165, S-17/210 pPGPseq-17E1/300 pPGPseq-2B10/265 PM-15/185 pPGPseq-18A5/320 pPGPseq-9A7/250 PM-36/290 PM-3/210 pPGPseq-14D1/330 pPGPseq-14D1/280 pPGPseq-15C10/219 PM-35/140 pPGPseq-2F5/275 pPGPseq-4E8/286 PM-42/205 pPGPseq-16C7/245 PM-375/110 pPGPseq-15E12/135 pPGPseq-7H6/295 pPGPseq-14H6/350 pPGPseq-15D3/290 pPGPseq-18G1/265 PM-377/160 Lec-1/250 IPAHM-287/205 IPAHM-136/140 IPAHM-407A/260 PM-238/155 IPAHM-93/180 pPGPseq-11E11/200 IPAHM-23/150 IPAHM-108/290 PM-384/80 Ah4-26/215

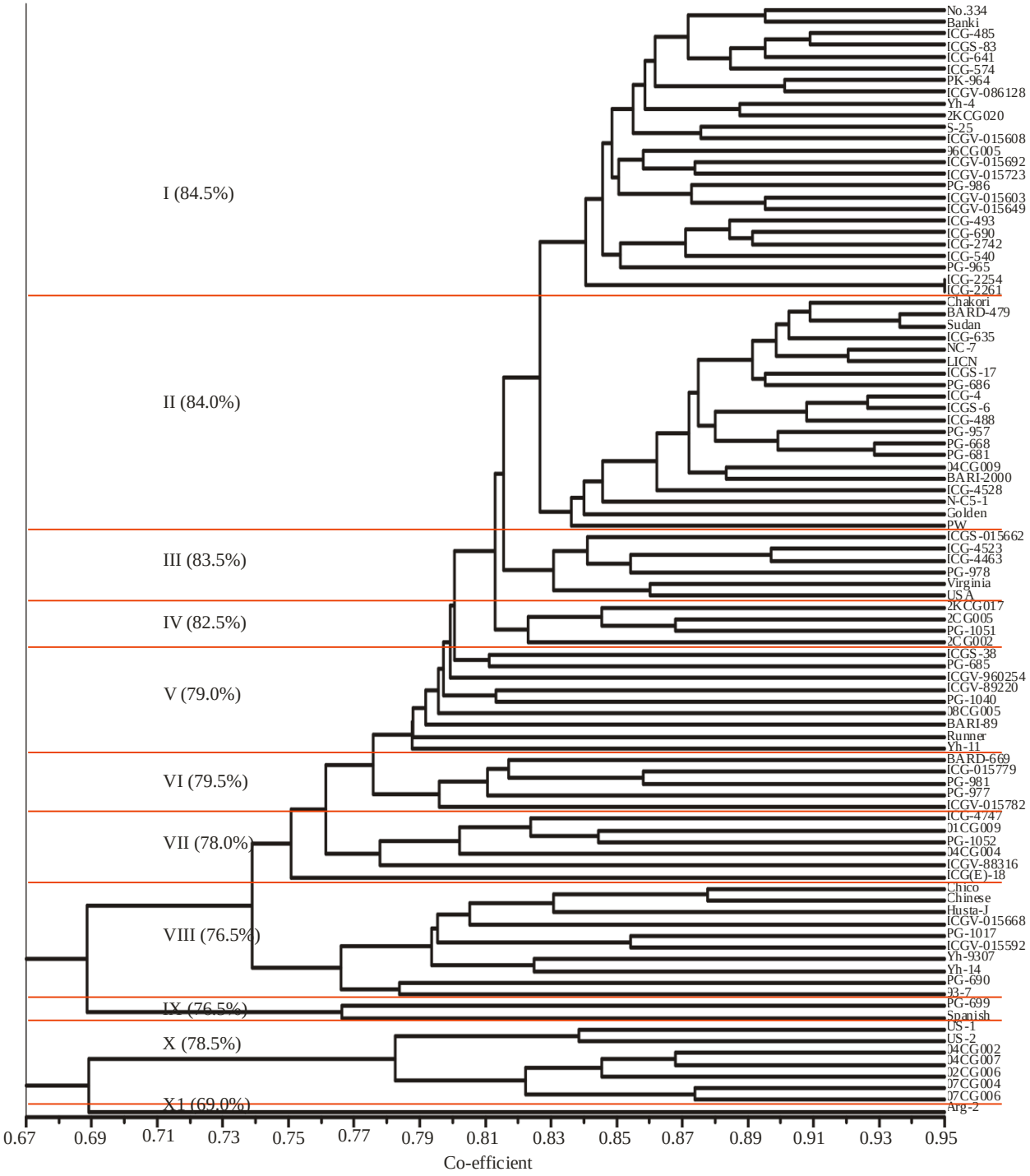

Figure 1:Dendrogram representing the relationship among 95 varieties of groundnut using UPGMA cluster analysis I to XI groups of groundnut.

Table 7: Characterization of groundnut genotypes for LLS with published markers

Sr. No.	Genotypes	PPGPseq5D5	PM375	PM 384	Ah1TC11H06	pPGPseq2F5	BARI observation (2009)	BARI observation (2010)	BARI observation (2011)	Avg
1.	No.334	R	R	R	S	S	4	6	2	4.0
2.	BANKI	R	R	R	S	S	5	6	2	4.3
3.	CHAKORI	S	R	S	R	S	4	7	2	4.3
4.	BARI-89	S	R	S	S	S	3	6	2	3.7
5.	GOLDEN	S	R	R	S	S	5	8	3	5.3
6.	BARD-699	R	R	R	S	S	4	6	2	4.0
7.	BARD-479	S	R	S	R	S	4	7	2	4.3
8.	CHICO	S	S	S	R	R	5	6	2	4.3
9.	CHINESE	S	S	S	R	R	6	8	3	5.7
10.	SUDAN	S	R	S	R	S	3	8	2	4.3
11.	ICG-485	R	R	R	S	S	4	7	2	4.3
12.	NC-7	S	R	S	R	S	4	6	2	4.0
13.	ICGS-83	R	R	R	S	S	3	6	2	3.7
14.	LICN	S	R	S	R	S	4	7	2	4.3
15.	PW	R	R	H	R	S	5	7	3	5.0
16.	ICGS-17	S	R	S	R	S	4	6	3	4.3
17.	ICG(E)-18	S	R	R	R	S	5	7	3	5.0
18.	N-C 5-1	S	R	S	R	S	5	7	3	5.0
19.	S-25	R	R	R	S	S	4	6	2	4.0
20.	PG-686	S	R	S	R	S	4	7	2	4.3
21.	HUSTA-J	S	R	H	R	S	5	6	2	4.3
22.	ICG-574	S	R	R	S	S	3	5	2	3.3
23.	ICG-635	S	R	H	R	S	4	6	2	4.0
24.	ICG-641	S	R	R	S	S	4	6	2	4.0
25.	ICG-2254	S	R	R	R	S	5	6	2	4.3
26.	ICG-2261	S	R	R	R	S	5	6	2	4.3
27.	ICG-4528	S	R	H	R	S	4	7	3	4.7
28.	ICG-4747	S	R	R	S	S	5	6	2	4.3
29.	PG-690	S	S	R	S	R	6	7	3	5.3
30.	ICGS-38	S	-	-	R	S	6	5	2	4.3
31.	PK-90064	R	R	R	S	S	6	9	2	5.7
32.	ICGV-86128	S	R	R	S	S	7	7	2	5.3
33.	ICGV-89220	S	R	H	S	S	5	7	2	4.7
34.	ICGV-01560	S	R	R	S	S	5	6	3	4.7
35.	ICG-015662	S	S	-	S	S	6	7	3	5.3
36.	ICGV-01566	S	S	S	S	S	6	7	3	5.3
37.	ICG-015779	S	R	R	S	S	5	7	3	5.0
38.	YH-9307	S	R	R	S	S	4	9	2	5.0
39.	YH-14	S	S	H	S	S	9	9	2	6.7
40.	YH-11	S	R	R	S	S	9	9	3	7.0
41.	YH-4	S	R	R	S	S	8	9	3	6.7
42.	2KCG017	S	R	R	S	S	5	7	2	4.7
43.	2KCG020	S	R	R	S	S	4	5	3	4.0
44.	96CG005	S	R	R	S	S	5	8	2	5.0
45.	01CG009	S	R	R	S	S	6	6	3	5.0
46.	02CG002	S	R	R	R	S	7	5	3	5.0
47.	02CG005	S	R	R	S	S	5	6	2	4.3
48.	04CG004	S	R	R	S	S	7	5	3	5.0
49.	04CG009	S	R	R	R	S	7	8	3	6.0
50.	08CG005	S	R	R	S	S	7	6	3	5.3
51.	ICG-493	S	R	R	S	S	5	6	3	4.7
52.	ICG-4	S	R	H	R	S	5	7	3	5.0
53.	ICGS-6	S	R	R	R	S	5	7	2	4.7

54.	ICG-540	S	R	R	S	S	5	6	3	4.7
55.	ICG-488	S	R	R	S	S	6	6	3	5.0
56.	ICG-690	S	R	R	S	S	6	6	3	5.0
57.	ICG-2742	S	R	R	S	S	4	6	2	4.0
58.	ICG-4523	S	S	R	S	S	7	6	2	5.0
59.	ICG-4463	S	H	R	R	S	6	7	3	5.3
60.	PG-957	S	R	R	R	S	6	6	3	5.0
61.	PG-965	S	R	R	S	S	7	7	2	5.3
62.	PG-668	S	R	H	S	S	8	8	2	6.0
63.	PG-681	S	R	H	S	S	8	6	2	5.3
64.	PG-685	S	R	R	R	S	5	8	2	5.0
65.	PG-699	-	-	-	S	S	8	8	2	6.0
66.	PG-977	S	R	R	S	S	7	8	3	6.0
67.	BARI-2000	S	R	H	S	S	4	6	2	4.0
68.	PG-978	S	R	R	S	S	5	7	2	4.7
69.	PG-981	S	R	R	S	S	5	6	2	4.3
70.	PG-986	S	R	R	S	S	4	7	2	4.3
71.	PG-1017	S	H	H	S	R	5	6	3	4.7
72.	PG-1040	S	H	H	S	S	4	6	2	4.0
73.	PG-1051	S	R	R	S	S	4	7	2	4.3
74.	PG-1052	S	R	R	S	S	4	6	3	4.3
75.	ICGV-88316	S	R	S	S	S	8	6	2	5.3
76.	PG-015692	S	R	R	S	S	5	6	2	4.3
77.	PG-015723	S	R	R	S	S	5	6	2	4.3
78.	ICGV-015782	S	H	R	S	S	6	6	2	4.7
79.	ICGV-960254	S	H	R	S	S	5	6	2	4.3
80.	ICGV-015592	S	S	S	S	S	8	6	2	5.3
81.	ICGV-015603	S	R	R	S	S	5	8	3	5.3
82.	ICG-015649	S	R	R	S	S	5	8	2	5.0
83.	SPANISH	-	-	-	R	S				
84.	RUNNER	S	R	-	S	S				
85.	VIRGINIA	S	R	R	R	S				
86.	USA	S	H	R	R	S				
87.	93-7	S	H	R	S	S				
88.	US-1	S	H	R	S	S				
89.	US-2	S	R	R	S	S				
90.	Argentina-2	S	H	R	S	S				
91.	04CG002	S	H	R	S	S				
92.	04CG007	R	H	R	S	S				
93.	02CG005	S	H	H	S	S				
94.	07CG004	S	H	R	S	S				
95.	07CG006	S	H	R	S	S				

Table 8: Screening of 82 Groundnut Genotypes against Cercospora leaf spot under PARB Project No.16 during 2009

S. No	Entry	2009*	S. No	Entry	2009
1	No. 334	4	42	Yh-4	8
2	Banki	5	43	2KCG017	5
3	Chakori	4	44	2KCG020	4
4	BARI-89	3	45	96CG005	5
5	BARI-2000	4	46	01CG009	6
6	Golden	5	47	02CG002	7
7	BARD-699	4	48	02C005	5
8	BARD-479	4	49	04CG004	7
9	Chico	5	50	04CG009	7
10	Chinese	6	51	08CG005	7
11	Sudan**	3	52	ICG-493	5
12	ICG-485	4	53	ICG-4	5
13	NC-7	4	54	ICGS-6	5
14	ICGS-83**	3	55	ICG-540	5
15	LICN	4	56	ICG-488	6
16	PW	5	57	ICG-690	6
17	ICGS-17	4	58	ICG-2742	4
18	ICG(E)-18	5	59	ICG-4523	7
19	N-C 5-1	5	60	ICG-4463	6
20	S-25	4	61	PG-957	6
21	PG-686	4	62	PG-965	7
22	Husta-J	5	63	PG-668	8
23	ICG-574	3	64	PG-68	8
24	ICG-635	4	65	PG-685	5
25	ICG-641	4	66	PG-699	8
26	ICG-2254	5	67	PG-977	7
27	ICG-2261	5	68	PG-978	5
28	ICG-4528	4	69	PG-981	5
29	ICG-4747	5	70	PG-986	4
30	PG-690	6	71	PG-1017	5
31	ICGS-38	6	72	PG-1040	4
32	PK-90064	6	73	PG-1051	4
33	ICGV-86128	7	74	PG-1052	4
34	ICGV-89220	5	75	ICGV-88316	8
35	ICGV-015608	5	76	PG-015692	5
36	ICG-015662	6	77	PG-015723	5
37	ICGV-015668	6	78	ICGV-015782	6
38	ICG-015779	5	79	ICGV-960254	5
39	Yh-9307	4	80	ICGV-015592	8
40	Yh-14	9	81	ICGV-015603	5
41	Yh-11	9	82	ICG-015649	5

Table 9:Response of groundnut genotypes to Cercospora leaf spot under artificial epidemiological conditions during Kharif 2010

S. No	Entry	Mean CLS Scoring	S. No	Entry	Mean CLS Scoring	S. No	Entry	Mean CLS Scoring
1	01CG009	6.0	31	ICG-4747	6.0	61	PG-1051	7.0
2	02C005	6.0	32	ICG-485	7.0	62	PG-1052	6.0
3	**02CG002**	**5.0**	33	ICG-488	6.0	63	PG-668	8.0
4	**04CG004**	**5.0**	34	ICG-493	6.0	64	PG-681	6.0
5	04CG009	8.0	35	ICG-540	6.0	65	PG-685	8.0
6	08CG005	6.0	36	**ICG-574**	**5.0**	66	PG-686	7.0
7	2KCG017	7.0	37	ICG-635	6.0	67	PG-690	7.0
8	**2KCG020**	**5.0**	38	ICG-641	6.0	68	PG-699	8.0
9	96CG005	8.0	39	ICG-690	6.0	69	PG-957	6.0
10	Banki	6.0	40	ICGS-17	6.0	70	PG-965	7.0
11	BARD-479	7.0	41	ICGS-38	6.0	71	PG-977	8.0
12	BARD-699	6.0	42	ICGS-6	7.0	72	PG-978	7.0
13	BARI-2000	6.0	43	**ICGS-83**	**5.0**	73	PG-981	6.0
14	BARI-89	6.0	44	ICGV-15592	6.0	74	PG-986	7.0
15	Chakori	7.0	45	ICGV-15603	8.0	75	PK-90064	9.0
16	Chico	6.0	46	ICGV-15608	6.0	76	PW	7.0
17	Chinese	8.0	47	ICGV-15668	7.0	77	S-25	6.0
18	GOLDEN	8.0	48	ICGV-15782	6.0	78	**Sudan**	**8.0**
19	Husta-J	6.0	49	ICGV-86128	7.0	79	Yh-11	9.0
20	ICG(E)-18	7.0	50	ICGV-88316	6.0	80	Yh-14	9.0
21	ICG-015649	8.0	51	ICGV-89220	7.0	81	Yh-4	9.0
22	ICG-015662	7.0	52	ICGV-60254	6.0	82	Yh-9307	9.0
23	ICG-015779	7.0	53	LICN	7.0			
24	ICG-2254	6.0	54	N-C 5-1	7.0			
25	ICG-2261	6.0	55	NC-7	6.0			
26	ICG-2742	6.0	56	No. 334	6.0			
27	ICG-4	7.0	57	PG-015692	6.0			
28	ICG-4463	7.0	58	PG-015723	6.0			
29	ICG-4523	6.0	59	PG-1017	6.0			
30	ICG-4528	7.0	60	PG-1040	6.0			

Table 10.Screening of 82 Groundnut Genotypes against Cercospora leaf spot under PARB Project No.16- Date of Observation: 29-7.2011-Name of scientist: Madiha (RA)

S. No	Entry	Score	S. No	Entry	Score	S. No	Entry	Score
1	No.334	2	31	BARI-2000	2	61	ICG-488	3
2	Banki	2	32	ICG-4747	2	62	ICG-690	3
3	Chakori	2	33	PG-690	3	63	Golden	2
4	Bari-89	2	34	ICG-538	2	64	ICG-2742	2
5	BARI-2000	3	35	PK-90064	2	65	ICG-4523	2
6	Golden	3	36	ICGV-86128	2	66	ICG-4463	3
7	BARD-699	2	37	ICGV-89220	2	67	PG-957	3
8	BARD-479	2	38	ICGV-015608	3	68	PG-965	2
9	Chico	2	39	ICGV-015662	3	69	PG-668	2
10	Chinese	3	40	ICGV-015668	2	70	PG-681	2
11	Sudan	2	41	Golden	3	71	PG-685	2
12	BARI-2000	2	42	ICG-015779	3	72	PG-699	2
13	ICG-485	2	43	YH-9307	2	73	PG-977	3
14	NC-7	3	44	YH-14	2	74	BARI-2000	2
15	ICG-83	2	45	YH-11	3	75	PG-978	2
16	LICN	2	46	YH-4	3	76	PG-981	2
17	PW	3	47	2KCG017	2	77	PG-986	2
18	ICGSE-17	3	48	2KCG020	3	78	PG-1017	3
19	ICGSE-18	3	49	96CG005	2	79	PG-1040	2
20	NC-5-1	3	50	01CG009	3	80	PG-1051	2
21	S-25	2	51	02CG002	3	81	PG-1052	3
22	Golden	3	52	BARI-2000	2	82	ICGV-88316	2
23	PG-686	2	53	02CG005	2	83	PG-015692	2
24	Husta-J	2	54	04CG004	3	84	PG-015723	2
25	ICG-574	2	55	04CG009	3	85	Golden	2
26	ICG-635	2	56	08CG005	3	86	ICGV-15782	2
27	ICG-641	2	57	ICG-493	3	87	ICGV-960254	2
28	ICG-2254	2	58	ICG-4	3	88	ICGV-015592	2
29	ICG-2261	2	59	ICGS-6	2	89	ICGV-015603	3
30	ICG-4528	3	60	ICG-540	3	90	ICGV-015649	2

PHYSICAL PROGRESS REPORT FOR PARB CGS PROJECT

(Physical progress upto 14-05-2013)

A. Basic Information

1.	**Name of the project**	Genetic Improvement of Groundnut for herbicide and disease resistance
2.	**Project No.**	16
3.	**Total Project cost**	26.23 Million Rupees
4.	**Total project duration**	Three years (2009-2010 to 2011-2012) one year extension
5.	**Funds released so far**	8.167 Million Rupees
6.	**Project commencement Date**	15 May, 2009
7.	**Name of the Project Manager or Team Leader with designation**	Dr. Muhammad Zaffar Iqbal

B. Physical Research Achievements

REVISE ACTIVITIES				
Output-2	Transformation of EPSP groundnut varieties (Golden and BARI 2000, BARD 497)	December 30, 2012	Heterozygous transgenic groundnut lines available for evaluation	▪ Visited NIGAB extracted RNA, synthesize cDNA for expression analysis on Real Time PCR.
Activity 2.3	PCR based analysis of transgenic and transfer of technology to ABRI along with the construct DNA sequence	June 30, 2012 September 30, 2012 December 31, 2012	Confirmation of EPSP gene integration accomplished	▪ According to Email dated 13-05-2013 following transgenic material is available Plants obtained through embryo transformation 1. Shoot multiplication =77 2. Root induction =91 3. Glass house =51 Plants obtained through callus transformation 1. Shoot multiplication = 108 2. Root induction = 40
Activity 2.5	PCR based transgene confirmation and determination of EPSP expression (qPCR) in coordination with ABRI	December 30, 2012	Transgene confirmation and expression level confirmed in all the transgenic	▪ 12 putative EPSPS transgenic groundnut sample were tested through Real Time PCR and found that EPSPS gene not expressing in any transgenic plants. Detailed report attached.

GENE EXPRESSION ANALYSIS OF SYNTHETIC EPSP GENE IN PUTATIVE TRANSGENIC GROUNDNUT PLANTS DEVELOPED BY NIGAB (PARB PROJECT-16)

Transcript level of EPSPS gene in putative transgenic groundnut was assayed through qRT-PCR. Total RNA isolation and first strand cDNA synthesis was conducted at National Institute for Genomics & Advanced Biotechnology (NIGAB). For transcript analysis, qRT-PCR was performed at Agricultural Biotechnology Research Institute, Faisalabad.

Methodology

Total RNA from leaf tissue of 12 putative EPSPS transgenic groundnut plants along with wild type was isolated using TRIZOLE method as detailed below. Around 200 mg leaf tissue from *in-vitro* grown plants were ground in liquid nitrogen using pre-sterilized pestle and mortar. The powder was shifted to chilled 1.5ml eppendorf tube, and supplemented with 1 ml of Trizol (ice cold) and 200µl of chloroform. The tube was shacked vigorously for 10-15s and incubated at room temperature for 2-3 min. Following incubation, tubes were centrifuged 12500rpm for 15 min. Supernatant was transferred into new 1.5ml eppendorf tube and500µl of chilled isopropanol was added, followed by10 min incubation at room temperature. Samples were again centrifuged for 10 min. at 12500 rpm. Flow through was discarded and pellet was dried for 5 min at room temperature. The pellet was dissolved in 30µl sterile double distilled PCR water and an aliquot of 4µl was run on 1% agarose gel to determine quality of isolated RNA.

Uniform amount of isolated RNA was converted into cDNA using RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas) following manufacturer's instructions. In brief, 7.5µl of water, 1µl of oligo dT primer and 6µl of RNA was heated at 65^{o}C for 5 min and immediately chilled on ice for 3 min. Then, 4µl of RT buffer, 1µl of dNTPs and 0.5µl RT enzyme were added to the mixture. The final reaction mixture was incubated at 42^{o}C for 1 hour following the 72^{o}C for 10min. Incubations were performed in Veriti® Thermal Cycler (Applied Biosystems).

For gene expression analysis TaqMan probes and primers were designed using Primer Express software. Transcripts analysis was conducted on ABI7500 Real time PCR System through comparative Ct method. Total reaction volume of 25µl contained 12.5µl 2x Maxima Probe/ROX qPCR Master Mix (Fermentas), 1.0µl of cDNA, 0.6µl each of 5.0µM reverse and forward primers, 0.4µl of 5µM probe and 9.9µl of PCR water. Each reaction was repeated thrice. The qRT-PCR reactions were carried out following the recommended thermal profile: 50°C for 2 min, 95°C for10 min, followed by 40 cycles of 95°C for 15 s and 60°Cfor 1 min. A groundnut housekeeping gene, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) was used to normalize qRT-PCR data. Primer efficiency for GAPDH and EPSPS was determined using 10x serial dilutions of cDNA and the plasmid, respectively.

Results

Gel analysis showed significant amount of good quality RNA in 8 out 12 samples which were further proceeded for cDNA (Figure 1). TaqMan probes and primers were designed from nucleotide sequence of *Archis hypogea* GAPDH mRNA (GenBank ID: JF957835.1.) downloaded from NCBI, and EPSPS sequence provided by NIGAB. Optimization of both primers through PCR showed a single band of expected size.

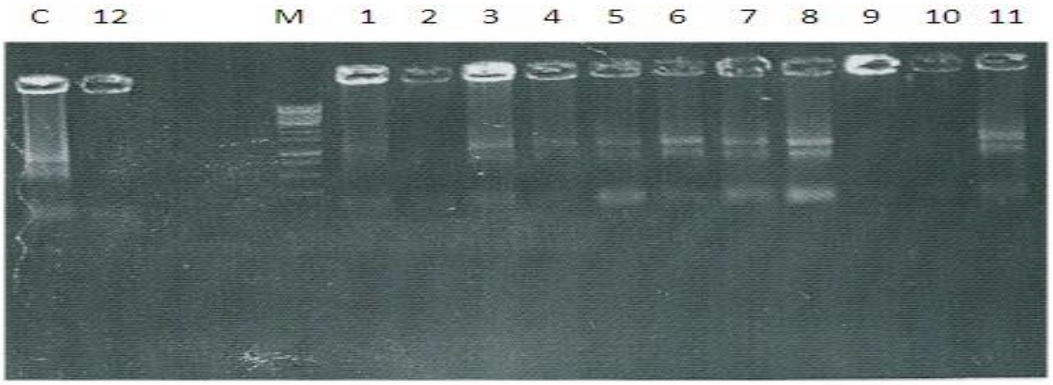

Figure 1: Total RNA isolated from putative transgenic groundnut plants as well as control.

M = 1kb DNA ladder, lanes 1-12 =RNA isolated from EPSPS transgenic plants 1-12, C = wild type control plant.

Table 1: Primer and probes used in this study

Name	Sequence (5-3)	Tm	Product Size
A. hyp EPSPS-F	AAGCTGGAATGCCACACGATA	58	127
A. hyp EPSPS-R	ACATGAAAGCCCTGTGGCTAA	59	
A. hyp EPSPS-P	6-FAM 5'-CCACAGCAGGCCAGCTACCGCT-3' TAMRA	68	
A. hyp GAPDH-F	GGCCTCTCACCTTCTCAAGTATG	58	70
A. hyp GAPDH-R	CCTCACCGACAGGCTTAACG	59	

A. hyp GAPDH-P	6-FAM 5'-CTCCACCCTTGGCATATTCGACGC-3' TAMRA	68	

In QPCR, standard curves constructed using 10x serial dilutions of templates showed primer efficiency of 0.92 and 0.99, and r^2 value of 0.99 for EPSPS and GAPDH, respectively. Transcript analysis through qRT-PCR revealed Ct value ranged from 14-18 for reference (GAPDH) gene; however no amplification was found for any of the putative transgenic plants except in positive control (Ct value 14) where plasmid containing the synthetic EPSPS gene was used as template.

Conclusion

EPSPS gene is not expressing in any of the putative transgenic plants.

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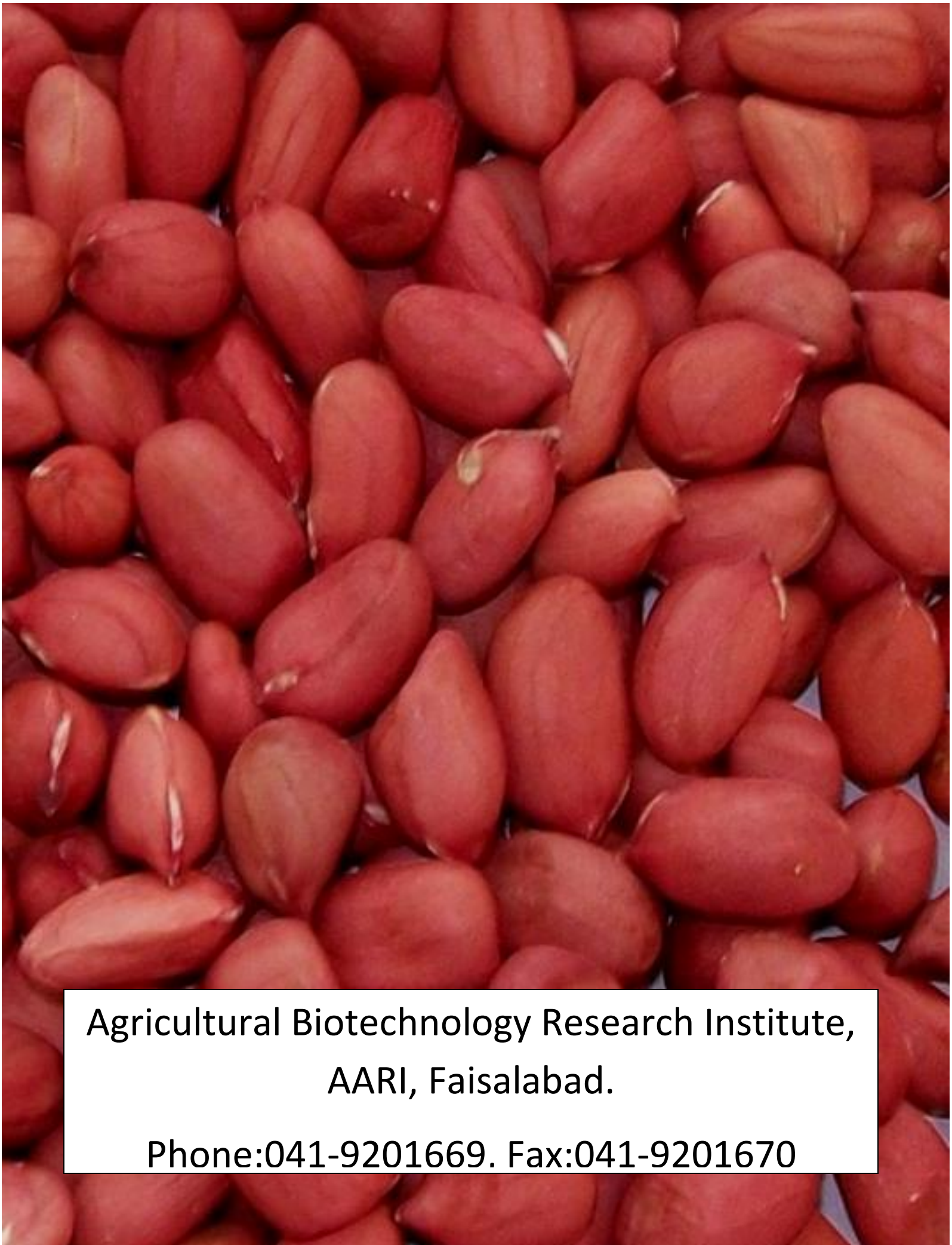

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