IDENTIFICATION OF PCR-BASED DNA MARKERS LINKED WITH RESISTANCE TO RUST IN GROUNDNUT (*Arachis hypogaea* L.)

T. SIVANANDA VARMA

DEPARTMENT OF GENETICS AND PLANT BREEDING
COLLEGE OF AGRICULTURE, DHARWAD
UNIVERSITY OF AGRICULTURAL SCIENCES,
DHARWAD-580 005

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By

T. SIVANANDA VARMA

DEPARTMENT OF GENETICS AND PLANT BREEDING
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CERTIFICATE

This is to certify that the thesis entitled **IDENTIFICATION OF PCR-BASED DNA MARKERS LINKED WITH RESISTANCE TO RUST IN GROUNDNUT (*Arachis hypogaea* L.)"** *submitted* by **T. SIVANANDA VARMA** for the degree of MASTER OF SCIENCE (AGRICULTURE) *in GENETICS* AND *PLANT BREEDING, to the University of Agricultural Sciences Dharwad is a record of research work done by him during the period of his study in this university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree diploma associateship fellowship or other similar titles*

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Affectionately Dedicated

To

My Parents

and

Ramakrishna Seva Samithi,

Bapatla

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(T. S.N.VARMA)

CONTENTS

LIST OF TABLES

LIST OF PLATES

Introduction

I. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseeds crops in the world, grown throughout the tropical and warm temperate regions in an area of about 24 m hectares with the total production of 33.5 m tonnes (1998-2000 average, FAO data). It occupies 31.3 percent of the total cropped area under oilseeds and accounts for 36.1 percent of total oilseeds production in the world. Groundnut, the 'king' of oilseeds in India, occupies an area of about 7.8 m ha with a production of 9.0 m t. Groundnut production in the last three decades in India has increased considerably from 4.6 m t in 1968-69 to 9.0 m t. However, there has been marginal increase in groundnut area. A major driving force for increased production and productivity of groundnut has been the commissioning of technology mission on oil seeds in India.

The genus *Arachis* belongs to the family Leguminosaea, tribe Aeschynomeneae, and subtribe Stylosanthinae. It probably originated as a geocarpic form of Stylosanthinaes in Brazil or northeastern Paraguay (Krapovikas et al., 2000). The cultivated groundnut (*A hypogaea* L.) is classified into two subspecies based on the presence of flowers on the main axis: *hypogaea* Krap. et Rig (no flowers on the main axis) and *fastigiata* Waldron (flowers on the main axis). Susp. *fastigiata* has four botanical varieties, *fastigiata* Gregory et al, *peruviana* Krapov. and W. C. Gregory, *aequatoriana* Krapov. and W. C. Gregory, and *vulgaris* C. Hartz. The two botanical varieties in subsp. *hypogaea* are *hypogaea* Gregory et al and *hirusta* Kohler (Krapovikas and Gregory, 1994).

Genetics, the study of genes through their variation, has made a major contribution to improvement in agriculture. In spite of progress made through genetic enhancement, additional gains in agricultural productivity are demanded to cope up with the increasing population pressure. The science of molecular biology in recent years has provided tools suitable for rapid analysis of different organisms using DNA markers. The most wide spread application of molecular markers is in the construction of the genetic linkage maps to determine the chromosomal location of genes affecting both qualitative and quantitatively inherited traits. By knowing the map position of a gene, one can use nearby or flanking molecular markers to diagnose the presence of the gene without having to wait for the genes effects to be seen.

Marker-assisted selection (MAS) offers great scope for improving the efficiency of conventional plant breeding. Molecular markers are especially advantageous for traits with low heritability where traditional selection is difficult, expensive or lack accuracy or precision (Crouch, 2001). The essential requirements for developing MAS breeding programs include (i) availability of polymorphic germplasm with useful characteristics, (ii) identification of flanking markers closely linked on either side of the gene/quantitative trait loci, (iii) simple robust polymerase chain reaction (PCR)-based marker technology to facilitate rapid and cost effective screening of large breeding populations, and (iv) highly accurate and precise screening techniques for phenotyping of mapping populations. The molecular markers offer certain advantages over morphological markers as they are phenotypically neutral, occur throughout the genome, neither influenced by environments nor by pleotropic and epistatic interactions,

expression is not dependent on plant age, and often segregate into 1:1 ratio between marker expression and genetic constitution of the individual.

The main advantage of using molecular markers is the gain in time for introgression of resistance genes into cultivars (Tanksley et al., 1989; Melchinger, 1990). The use of DNA markers could speed up this process by three plant generations thus allowing selection of the resistant offspring that contain the lowest amounts of the donor genome in every generation (Tanksley et al., 1989). Molecular markers are particularly useful in disease resistance breeding as it (i) minimizes the need for screening of individuals once marker-trait relationships established, (ii) eases in identification and transfer of recessive genes, (iii) monitors alien gene introgression, (iv) reduces the linkage drag, and (v) facilitates map-based cloning of disease resistance genes.

Recent advances in development of marker protocols such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSRs) (also known as microsatellites) have revolutionized genetic analysis and opened new possibilities in the study of complex traits in crop plants. SSR belongs to the co-dominant marker class, are easy to manipulate, highly reproducible, and targets hypervariable regions of the genome. They are tandem repeats of DNA sequences of only a few base pairs (1-6 bp) in length, and (AT)n are the most abundant dinucleotide repeats in plants (Gupta et al., 1996). Variation in the number of repeated core sequence of nucleotides at a SSR locus among different genotypes provides the basis for polymorphism that can be used in plant

genetic studies. SSRs are therefore excellent choice of DNA markers for genetic mapping in plants. Unlike RFLPs, for instance, SSR technology is PCR-based, requires only minimal amounts of DNA, and is readily automatable. Unlike RAPDs, SSR markers have proven to be reliable and reproducible. Unlike AFLPs, they are co-dominant and species specific. Moreover, they are both size and sequence specific while RFLPs are sequence specific and RAPDs are size specific. SSRs can be used in pedigree analysis to determine kinship among individuals, fingerprinting, forensics, genetic mapping, and phylogenetic analysis (Gupta et al., 1996).

Michelmore et al (1991) developed bulked segregant analysis as a method for rapidly identifying markers linked to any specific gene or genomic region. The method involves comparing two pooled DNA samples of individuals from a segregating population originating from a single cross. Within each pool, the individuals are identical for the trait or gene of interest but are arbitrary for all other genes. Two pools contrasting for a trait are analyzed to identify markers that distinguish them. Markers that are polymorphic between the pools will be genetically linked to the loci determining the trait used to construct the pool. This procedure efficiently identifies markers linked to genes of interest, allowing their rapid placement on a genetic map. It also can be used to saturate genetic maps by identifying markers in sparsely populated regions and at the end of linkage groups.

Knowledge of the groundnut genome is very limited and only in recent years have molecular techniques been used to interpret the genome organisation. Extensive variation

for morphological and physiological traits has been observed in both wild *Arachis* and cultivated groundnut. Molecular tools such as DNA markers are increasingly becoming important and useful in groundnut breeding programs. This is necessitated by the presence of polymorphism at DNA level. Abundant polymorphism in wild *Arachis* species has been observed whereas little variation has been reported in cultivated groundnut (*Arachis hypogaea* L.) (Kochert et al., 1991; Halward et al., 1991, 1992; Paik-Ro et al., 1992; Stalker et al., 1994; He and prakash, 1997; Hopkins et al., 1999; Subramanian et al., 2000). A recent study at ICRISAT revealed, in contrast, up to 41% variation in genetic dissimilarity by RAPD analysis and grouped 26 cultivated germplasm accessions into five distinct clusters (Dwivedi et al., 2001). However, they could not relate differences in similarity to known biological information about the accessions falling into different clusters. Both RAPD and RFLP markers have been used to monitor introgression of wild *Arachis* chromosome segments into cultivated groundnut (Garcia et al., 1995), and few RAPD (RKN 229, RKN 410, and RKN 440) and RFLP (R2430E, R2545E, and S1137E) markers linked with root-knot nematode resistance in groundnut are reported (Burow et al., 1996; Choi et al., 1999).

Rust (*Puccinia arachidis* Speg.) is one of the important foliar diseases of groundnut causing substantial loss to production, and it also reduces the fodder and seed quality of groundnut. Although the disease can be controlled by application of fungicides, the adoption of resistant cultivars by the resource poor farmers is the best option to minimize losses and maintain good produce quality. Several sources of resistance to rust have been identified in groundnut (Singh et al., 1997). However, in majority of these

cases. the resistance is associated with undesirable pod and seed characteristics. Although few rust resistant cultivars have been released in India and elsewhere, they have not become popular mainly because of their (i) long duration, (ii) low shelling outturn, and (iii) inferior pod/seed characteristics as compared with otherwise locally adapted but susceptible cultivars. Molecular markers could play an important role in eliminating these undesirable traits in a much shorter time frame than those expected through conventional breeding techniques. The integration of molecular techniques into conventional breeding programs has therefore facilitated marker-assisted selection as an attractive strategy for simultaneously improving a multitude of complex agronomic traits.

The present experiment was initiated to

(i) study intra- and inter-accession polymorphic variation among rust resistant and susceptible mapping parents,

(ii) evaluate various generations (parents, F_1 F_2, $BC_1P_1F_1$, and $BC_2P_2F_1$) for rust resistance, and

(iii) identify SSR markers linked with resistance to rust in two crosses in groundnut (*Arachis hypogaea* L.).

Review of Literature

II. REVIEW OF LITERATURE

2.1 Effect of foliar diseases on pod yield and fodder and seed quality

Rust (*Puccinia arachidis* Speg.) is an economically important disease of groundnut in semi-arid tropics (Subrahmanyam and McDonald, 1983). It occurs in most of the groundnut growing states in India but predominantly in South Indian states as conditions favor the development and spread of the disease (Subrahmanyam and McDonald, 1982). Pod yield losses in excess of 50% have been reported due to rust in groundnut (Subrahmanyam and McDonald, 1983; Sandhikar et al., 1989). Foliar diseases control also causes changes in seed weight, total oil and protein contents, and fatty acid composition (Hammond et al., 1976; Worthington and Smith, 1974; Sanders et al., 1989; Dwivedi et al., 1993). Groundnut haulms are excellent forage for cattle as it is rich in protein and have better palatability than many other fodders (Cook and Crosthwaite, 1994).

2.2 Sources of resistance to rust in cultivated and wild *Arachis* species

There are over 15000 accessions of groundnut, representing 92 countries, and housed at ICRISAT Gene Bank at Patancheru, India. Resistance to rust has been reported to 169 accessions with a disease score of ≤ 5 (Subrahmanyam and McDonald, 1983; Subrahmanyam et al., 1982a, b, 1995; Ghewande et al., 1983; Waliyar et al., 1993; Singh et al., 1997; Jiang et al., 1998; Chen et al., 1999). However, most of these resistance

sources, which originated in South America and belong to subsp *fastigiata* var. *fastigiata*, are late maturing types, and possess undesirable pods (thick shell, highly reticulated and constricted pods, and low shelling outturn) and seeds (purple or blotched seed color). They are therefore not acceptable to commercial cultivation. Many of the wild *Arachis* species are reported to be immune or highly resistant to rust (Subrahmanyam et al., 1985; Wynne et al., 1991).

2.3 Exploiting rust resistance genes in applied breeding

A number of attempts have been made to introduce genes for resistance to rust into improved genetic backgrounds in groundnut. ICGV 87160 and ICGV 86590 in India (Reddy et al., 1992, 1993); ICGV-SM 86715 in Mauritius (Moss et al., 1998); Southern Runner in USA (Gorbet et al., 1987); and Yue You 223 in China (Liang et al., 1999) have been released for cultivation. However, rust resistant varieties in India have not become popular among farmers because of late maturity, low shelling outturn, and pod/seed characteristics still not comparable to locally adapted cultivars. Resistance to rust has also been transferred from wild *Arachis* to cultivated types. ICGV# 87157, 87165, and 86699 are some of the interspecific derivatives originated from interspecific crosses in India (Moss et al., 1997; Nigam et al., 1992; Reddy et al., 1996). They have not been released mainly due to late maturity and inferior pod/seed characteristics in comparison with commercially grown cultivars. However, they are extensively used in breeding programs in South and South East Asian countries mainly because of high resistance to rust.

2.4 Physiological barriers associated with disease resistance and implications in breeding

Duncan et al (1978) predicted that (i) partitioning of assimilates between vegetative and reproductive parts, (ii) the length of the pod filling period, and (iii) the rate of pod establishment are major physiological processes that explain most of the yield variation among groundnut cultivars. Of these, partitioning of assimilate had the greatest effect on pod yield. Williams et al (1987) observed low partitioning in rust resistant genotypes. Both genetic resistance and fungicidal control influenced crop growth rate, pod growth rate, and partitioning in groundnut (Williams et al., 1993). Varman et al (1995) reported higher crop growth rate, leaf area ratio, and leaf area index during pod filling and maturity stages in resistant and partially rust resistant genotypes indicating more partitioning of dry matter to leaf tissues than to pods.

2.5 Mechanism of rust resistance

Resistance to rust in groundnut is of "slow-rusting" type where resistant accessions have increased incubation period, decreased infection frequency, and reduced pustule size, spore production and viability (Subrahmanyam et al., 1983). Reduction in latent period, lesion size, and intensity of sporulation contribute to low disease progress when infection occurs early in growing season (Anderson et al., 1990). Reddy and Khare (1988) reported that rust resistant cultivars had longer incubation period, lower pustule densities, and small pustules than susceptible ones. Mehan et al (1994) studied the

components of rust resistance in 144 groundnut genotypes using the detached leaf technique. Infection frequency, incubation period, lesion diameter, % leaf area damaged, and sporulation index were significantly correlated with each other and with mean field rust scores. However, the greatest variability among accessions was observed for incubation period and sporulation index. Different components of resistance were not found in all genotypes. Complementation of components was evident in accession ICG 10890 and ICG 10881. They suggested use of incubation period and sporulation index in selecting for rust resistance. Dwivedi et al (2001) studied the genetic variability and relationships among components of resistance to rust in 14 interspecific derivatives and a susceptible cultivar TMV 2. Resistance to rust is due to longer incubation and latent periods, fewer pustules per leaf, smaller pustule diameter, lower sporulation index, and lesser leaf area damage and disease score. Rust resistant components appear to work additively, therefore, selection based on resistant components together with green leaf area retained on the plant should be the basis of selecting for resistance to rust in breeding programs.

Plants defend themselves against pathogenic fungi by producing fungi toxic substances such as phytoalexins, pathogenesis related (PR) proteins, oxidized phenols, and several other components. Peroxidase and polyphenol oxidase enzymes are reported to impart resistance to rust in groundnut (Ekbote and Mayee, 1984; Velazhahan and Vidhyasekaran, 1994). Differences in phytoalexin production were reported among foliar diseases resistant and susceptible genotypes (Subba Rao et al., 1996; Sankaran et al., 1996). Isoflavanones were found to be the major components of phytoalexin whereas

formononetin, diadzein, and medicarpin comprised a minor component. Phytoalexins such as hydroxy stilbenes, medicarpin, and alkyl bis phenyl ethers were isolated from groundnut leaves infected by rust (Subba Rao et al., 1988, Subba Rao et al., 1991). Edwards et al (1995) reported that rust resistant cultivars produced three times more medicarpin phytoalexin than susceptible cultivars, and emphasized the potential application of phytoalexins in screening germplasm.

2.6 Genetics of rust resistance

Resistance to rust in cultivated groundnut is controlled either by a few recessive genes in PI 298115 and unknown pollen donor (Bromfield and Bailey, 1972); PI 315608 and PI 314817 (Knauft, 1987); Phule Pragati and PI 259747 (Kalekar et al., 1984); CO 2, NC Ac 17090, PI 414331, and PI 414332 (Paramasivam et al., 1990); and Kadiri 1, EC 76446 (292), PI 393527B, PI 298115, and PI 41433 (Vasanthi and Reddy, 1997) or predominantly controlled by additive, dominance, and additive x additive and additive x dominance genetic effects in EC76446 (292), NC Ac 17090, PI 259747, J 11, and Gangapuri (Reddy et al., 1987); and CO 2, JL 24, NC Ac 17090, PI 414331, and PI 414332 (Varman et al., 1991). However, partial dominance is reported in F_1 hybrids of the cross *A. hypogaea* with *A. batizocoi* (Singh et al., 1984).

2.7 Exploiting the potential of genetic markers in applied breeding

2.7.1 Polymorphism

2.7.1.1 Biochemical markers

Identification of up to 17 polymorphic isozymes among wild species suggest that they may have the potential to follow gene introgression in interspecific hybrids and establish phylogenetic relationships in groundnut (Lacks et al., 1991; Lu and Pickersgill, 1993; Stalker et al., 1994). However, only aspertate amino transferase (AAT), glutamate oxalo transaminase (GOT), isocitrate dehydrogenase (IDH), and phospho hexose isomerase (PHI) were reported polymorphic in cultivated groundnut (Galgaro and Lopes, 1994; Grieshammer and Wynne, 1990; Lacks and Stalker, 1993). Low polymorphism shown by isozyme markers in cultivated groundnut reveals their limited utility in genetic enhancement in groundnut.

2.7.1.2 Molecular markers

Both RFLP- and PCR-based markers have been used to assess polymorphic variation in cultivated and wild *Arachis* species in groundnut. Halward et al (1991) studied genetic variation among wild *Arachis* species and unadapted germplasm resources of cultivated groundnuts from South America, Africa, and China following RAPD and RFLP assays. They reported high polymorphic varaiation among wild

Arachis species but very little among cultivated groundnut. Lanham et al (1992) detected 49 polymorphic loci between cultivated *Arachis hypogaea* (TMV 2) and a synthetic amphiploid (B x C)^2 created from *A. batizocoi* and *A. chacoense* cross. Of these, only SC10-35 and SC10-60 were polymorphic in *Arachis* germplasm. Paik-Ro et al (1992) evaluated RFLP variation, using 23 random genomic and seed cDNA probes, in six groundnut species within the section *Arachis* (*A. hypogaea, A. monticola, A. batizocoi, A. cardenasii, A. duranensis, and A. glandulifera*) They reported that most of the genomic probes detected the RFLP pattern of a few restriction fragment bands while more than half of the seed cDNA probes hybridized to multiple bands among the accessions of the tetraploid species. However, they could not detect any polymorphism within or between *A. hypogaea, A. monticola,* and interspecific derivatives. Halward et al (1992) evaluated two peanut cultivars, 25 unadapted *A. hypogaea* germplasm, the wild allotetraploid progenitors of cultivated groundnut (*A. monticola*), *A. glabrata* (a tetraploid species from section *A.* R*hizomatosae*), and 29 diploid wild species of A*rachis* for polymorphic variation using primers of arbitrary sequence to amplifying segments of genomic DNA. They did not find any polymorphism among cultivated groundnut germplasm but found considerable variation within *Arachis* species.

Bhagwat et al (1997) studied the variation in RAPD profiles between groundnut cultivar Spanish Improved and its mutants originating 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 O6 yielded high polymorphism among the mutants. He and Prakash (1997) assayed six

diverse accessions of cultivated groundnut from three botanical varieties (*hypogaea*, *fastigiata*, and *aequatoriana*) using DNA amplification fingerprinting (DAF) and AFLP assays. They reported 63 DAF polymorphic markers with an average of 3.7 polymorphic bands per primer. The AFLP analysis, in contrast, detected 111 polymorphic AFLP markers with an average of 6.7 polymorphic bands per primer. Hopkins et al (1999) reported 6 polymorphic SSR primers that together detected up to 10 putative SSR loci in cultivated groundnut. Further studies also revealed the presence of DNA polymorphism in cultivated groundnut using RAPD assay (Subramaniam et al., 2000; Dwivedi et al., 2001).

2.7.2 Gene introgression from wild *Arachis* to cultivated groundnut

Garcia et al (1995) analysed introgression of *A. cardenasii* chromosome segments into 46 lines derived from a cross between *Arachis hypogaea* and *A. cardenasii*. They used 73 RFLP probes and 70 RAPD primers to detect introgression. Thirty-four RFLP probes and 45 RAPD primers detected *A. cardenasii* segments in one or more introgression lines, and the total size of the introgressed segments represented approximately 360 cM of the diploid groundnut genome. They thus demonstrated the utility of molecular markers to tag and enhance the introgression of specific chromosome segments linked with desirable traits from wild *Arachis* to cultivated groundnut. Choi et al (1999) reported RFLP probes R2430E, S11137E, and R2545E linked with resistance to nematodes in BC_5F_2 populations of the cross Florunner x TxAG 7.

2.7.3 Genetic linkage map in groundnut

Halward et al (1993) reported first RFLP based genetic linkage map of groundnut using both random genomic and cDNA clones of DNA library constructed using groundnut cultivar GK 7 (subsp *hypogaea var hypogaea*). They evaluated 100 genomic and 300 cDNA clones on F_2 populations derived from the interspecific cross between *A stenosperma* and *A cardenasii*. Fifteen genomic and 190 cDNA clones revealed polymorphism among the mapping parents. Of the 132 markers analysed for seggregation, 117 were distributed into 11 linkage groups with a total map distance of approximately 1063 cM. Burow et al (2001) reported a RFLP based tetraploid genetic linkage map, originating from a cross between Florunner and a synthetic amphidiplod $\{[(A\ batizocoi$ K9484 x $(A\ cardenasii$ GKP 10017 x $A\ digoi$ GKP 10602)$]^{4X}\}$, consisting of 370 RFLP loci spread into 23 linkage groups with a total map distance of 2210 cM.

2.7.4 DNA markers associated with resistance to rust in other oilseeds crops

Haley et al (1993) identified two RAPD markers, OF10(970) and OI19(400), linked with rust (*Uromyces appendiculatus* Pevs.) resistance in bean (*Phaseolus vulgaris* L). Cheung et al (1998) identified co-segregating RFLP markers (X42 and X83) linked with *Acr* locus that confers resistance to white rust (*Albugo candida*) in *Brassica juncea*. Prabhu et al (1998) identified RAPD markers, WR2 and WR3, linked with resistance to white rust in an F_1 derived double haploid population in *B. juncea*. Hausner et al (1999)

developed co-dominant PCR/RFLP based markers linked to flax rust (*Melampsora lini*) resistant alleles L_6 and L_{11} of flax "L" locus that confers resistance to flax rust. Lawson et al (1998) reported that RAPD marker $OX20_{600}$ and $OO04_{950}$ were linked with resistance to rust (*Puccinia helianthi*) at 0.0 cM and 11 cM, respectively in sunflower (*Helianthus annuus* L.). From these RAPD markers, they developed sequence characterised amplified region (SCAR) markers ($SCX20_{600}$ and $SCO04_{950}$) that were linked at similar distances from their resistance locus as the RAPD markers. They also found that $SCO04_{950}$ co-segregate completely with rust resistance.

Materials and Methods

III. MATERIALS AND METHODS

3.1 Plant materials

ICGV 99003, ICGV 99005, and TMV 2 were selected for the study. ICGV 99003 and ICGV 99005 are phenotypically stable tetraploid interspecific derivatives. ICGV 99003 originates from the cross *A. hypogaea* x (*A. duranensis* x *A. stenosperma)* and ICGV 99005 from TMV 2 x (*A. hypogaea* x (*A. batizocoii* x *A. duranensis*). They are reported to be resistant to rust (Dwivedi et al., 2001). TMV 2 is a widely grown groundnut cultivar in southern India but highly susceptible to rust. ICGV# 99003 and 99005 belong to subsp *hypogaea* var. *hypogaea* whereas TMV 2 to subsp *fastigiata* var *vulgaris*. ICGV# 99003 and 99005 were crossed with TMV 2 to produce F_1, F_2, $BC_1P_1F_1$, and $BC_1P_2F_1$ populations of the two crosses in groundnut.

3.2 Evaluation of mapping populations for rust resistance

Experiments involving parents, F_1, F_2, $BC_1P_1F_1$, and $BC_1P_2F_1$ populations of the cross ICGV 99003 x TMV 2 and ICGV 99003 x TMV 2 were conducted under greenhouse conditions. Individual plants were grown in 15 cm diameter plastic pots containing autoclaved alfisol and farmyard manure (v/v 4:1 ratio). The rust inoculums were produced and maintained on incubated, inoculated detached leaves of the susceptible groundnut cultivar, TMV 2, in a Percival Plant Growth Chamber using a temperature of 23^0 C and 12 h photoperiod. The rust urediniospores were harvested with

a cyclone spore collector, and used for inoculation of experimental materials. Thirty-five day old plants were inoculated uniformly in the evening with rust inoculum, containing 20,000 uridospore ml^{-1}, with an automizer. Immediately after inoculation, the pots were shifted into dew chambers (Clifford, 1973) at 23 ^{0}C to ensure wetness of the leaf surface during the night. The pots were removed from the dew chambers on the morning of the following day and returned to the greenhouse to maintain a dry period during the day. This alternate wet (16 h) and dry (8 h) period treatments were repeated for 10 days to maximize the disease development (Butler et al., 1994). The pots were then kept permanently in the greenhouse till the completion of the experiment. Individual plant observation on rust disease score was recorded at 45 days after inoculation (DAI) on 1 to 9 scale where 1 = no disease and 9 ≥ 90% foliage damaged (Subba Rao et al., 1990).

3.3 Methods

3.3.1 DNA extraction

DNA was extracted based on a previously reported CTAB method (Saghai-Maroof et al., 1984) with some modification. Leaves were ground to fine powder in the presence of liquid nitrogen and transferred to a sterile tube containing 9 ml of pre-heated (65^{0} C) 2 X CTAB extraction buffer (100 mM Tris-HCl buffer pH 8, 700 mM NaCl, 20 mM EDTA pH 8, 2% hexadecyltrimethyl-ammonium bromide, 1% β-mercaptoethanol, and 1% sodium bisulphite). 200 mg polyvinylpyrrolidone (PVP) 10 per g of leaf tissue was added and mixed gently. The contents were incubated for 90 min at 65^{0} C in a water

bath with occasional shaking during incubation. The tubes were kept for 10 min to allow them to return to room temperature. An equal quantity (9 ml) of chloroform and isoamyl alcohol solution, prepared in a ratio of 24:1, was added to the tubes and they were rotated on a tube rotator for 10 min and centrifuged at 5000 rpm at 15^0 C for 20 min. The aqueous phase was transferred to a clean tube, and the chloroform and isoamyl alcohol solution step was repeated. Nucleic acids were precipitated by adding 0.6 ml chilled isopropanol to the aqueous phase and incubated at -20^0 C for 20 min. The DNA was spooled using glass pasteur pipettes and transferred to a new sterile tube containing 2 ml of $T_{50}E_{10}$ buffer (50 ml $T_{50}E_{10}$ + 1 ml Rnase 10 mg / ml) and left overnight at room temperature. Contents were later on incubated at 37^0 C for 30 min. 150 µl of 5 M NaCl was added to the tubes kept at 4^0 C. An equal volume (150 µl) of solution of phenol : chloroform : isoamyl alcohol, prepared in a ratio of 25:24:1, was added to the tube and mixed gently and the tube was centrifuged at 5000 rpm at 2^0 C. The clear phase was once again cleaned by another phenol: chloroform: isoamyl alcohol solution, washed and spun at 2^0 C. The aqueous phase was transferred to new tubes and DNA was precipitated using 2 to 4 ml of 100% chilled ethanol. Tubes were kept at -20^0 C for 10 min. The DNA precipitate was removed and washed with 2 ml of 0.2 M sodium acetate in 70% alcohol for 20 min followed by 1 ml of 10 mM ammonium acetate in 70% alcohol for 1 min. The DNA pellet was further washed with 70% alcohol for 30 min and re-centrifuged. The tubes were allowed to drain and dried at room temperature for 2 to 3 h and re-suspended in 200 to 500 µl of TE buffer.

The quality and concentration of DNA was assessed by a spectrophotometer and also by gel electrophoresis using 0.8% agarose with known concentrations of uncut lamda DNA.

$$\text{DNA } (\mu g/\mu l) = \frac{OD_{260} \times 50 \text{ (dilution factor)} \times 50 \ \mu l/ml}{1000}$$

OD_{260}/ OD_{280} ratio was used to assess the purity of DNA. A ratio of 1.6 or less indicates that there may be proteins and/or other UV absorbers in the sample whereas ratio higher than 2.0 indicates that the sample may be contaminated with chloroform or phenol.

3.3.2 Simple sequence repeats (SSRs) primer

Twenty-five SSR primer pairs, specific to cultivated groundnut, were selected for the study (Table 1). The primers were developed as a result of collaborative initiatives between University of Georgia, USA and ICRISAT, and Dr M E Ferguson of ICRISAT has been kind enough to provide the primers with sequence information that enabled us to conduct this study.

3.3.3 SSR analysis

The polymerase chain reactions (PCRs) were performed as described below. The reaction mixture (20µl) contained 1.0 µl (5 ng) of genomic DNA, 2.0 µl of 10 X PCR

Table 1 List of 25 SSR primers specific to cultivated groundnut used to identify SSR markers linked with resistance to rust in groundnut

Sequence Identity	Left Sequence	Right Sequence	Product Size (bp)	Annealing temp	SSR repeat family	# Loci
pPGPseq-3D09	TTCACCCGTACAAACCAGTG	CCTCGGCAGATCTGGAGTAA	292	63°C	ag/ac	1
pPGPseq-3C02	TCATCGCCGAGATTCTTTTC	CAAGGGAAATTGGTCAAGGA	281	57°C	ag	1
pPGPseq-3A01	ATCATTGTGCTGAGGGAAGG	CACCATTTTTCTTTTTCACCG	238	64°C	att	1
pPGPseq-2G04	TTCTTGGTTCCTTTGGCTTC	TGCTCAAGTGTCCTTATTGGTG	289	60°C	att	1
pPGPseq-2G03	ATTCACAAGGGGACAGTTGC	ATTCAAGCCTGGGAAACAGA	215	64°C	att	2
pPGPseq-2E06	TACAGCATTGCCTTCTGGTG	CCTGGGCTGGGGTATTATTT	250	60°C	ag	1
pPGPseq-2C11	TGACCTCAATTTTGGGGAAG	GCCACTATTCATCGCGGTA	264	58°C	att/cac	2
pPGPseq-2B10	AATGCATGAGCTTCCATCAA	AACCCCATCTTAAAATCTTACCAA	259	58°C	att	1
pPGPseq-4A06	CGCTTGCCCCACTACACTAT	AGCAGTGCTTTGCATGTACG	126	63°C	at	1
pPGPseq-2A05	GGGAATAGCGAGATACATGTCAG	CAGGAGAGAAGGATTGTGCC	252	60°C	att	2
pPGPseq-1B09	CGTTCTTTGCCGTTGATTCT	AGCACGCTCGTTCTCTCATT	282	64°C	ag	1
pPGPseq-3F01	AGCGATCAATCGGTTTCAAG	GAAACGAAACGAAGACCGAA	290	60°C	ctca	2
pPGPseq-3B10	GGTGATGCTCCCCTCTACAA	CCTGCGAAACACAACAGAAA	265	60°C	ag	1
pPGPseq-2F05	TGACCAAAGTGATGAAGGGA	AAGTTGTTTGTACATCTGTCATCG	262	58°C	att	1
pPGPseq-4D04	CGGCTGTTAGGTAATCAGTTCA	TCAACAGGAATAGCTGCACG	187	60°C	ag	2
pPGPseq-2D12B	AAGCTGAACGAACTCAAGGC	TGCAATGGGTACAATGCTAGA	265	60°C	att	1
pPGPseq-5D05	AAAAGAAAGACCTTCCCCGA	GCAGGTAATCTGCCGTGATT	274	64°C	ag	3
pPGPseq-3B06	TGCAGCCGTTTTTATGAATG	AGCAGTTTGCAAAGGAGCAT	244	61°C	ag	1
pPGPseq-3A08	ATACGTGACTTGGGCCAGAC	AGTGAAAAATACACCCAACGAA	152	64°C	att	1
pPGPseq-4G02	TCAACTTTGGCTGCTTCCTT	TCAACCGTTTTTCACTTCCA	285	60°C	ag	1
pPGPseq-6B08	ATCATCGTCATTGGCTCCAT	GAATCACAAGCAGCAACAGC	288	62°C	ccg	1
pPGPseq-4F09	ACGTGAAATCTGGCTGGAAA	ACAATCCACACGCCAACATA	290	63°C	ag	1
pPGPseq-4H02	AGGGAAAGAGTAGAGGGGCA	CTGTTAGCGATTCTCCGAGG	248	60°C	ag	1
pPGPseq-3A04	GCCGTGACTTGAGCCTTTAG	TTCTTCGGTTACATGGGCTT	200	58°C	att	1
pPGPseq-3F08	CTATTCAGGAACGCTTTCGG	CCTACAACAGCAACAACAACG	298	60°C	ag	1

buffer (Manufacturer) 4 0 µl of 10 mM MgCl2 1 5 µl of 2mM dNTPs 2 5 µl of 4 p moles SSR primer (both forward and reverse) 8 2 µl of double distilled water and 0 8 µl of 1 unit Taq DNA polymerase (Gibco BRL Life Technologies USA) Amplification was performed in 0 2 ml thin-walled tubes placed in a Thermal Cycler (DY XD Engine Peltier Thermal cycler MJO2451 USA) The samples were initially incubated to 94 0 ^{0}C for 2 min. and then subjected to 35 repeats of the following cycle 94 0 ^{0}C for 45 sec 60 0 ^{0}C for 1 min. 72 0 ^{0}C for 1 5 min 5 µl of orange dye (1g of orange dye powder was added into 100 ml of solution containing 10 ml of 0 5 M EDTA (pH 8 0) + 1 ml of 5 NACl + 50 ml glycerol + 39 ml distilled water) was added into PCR products prior to agarose and polyacrylmide gel electrophoresis for separating the amplified products

3.3.4 Agarose gel (2%) electrophoresis

The amplification products along with Lamda marker (1µl 50bp marker (Manufacturer), 3µl dye and 6µl $T_{10}E_{1}$ buffer) were initially analysed by electrophoresis in 2% agarose gels stained in ethidium bromide (10mg/ml) and run in 1 X Tris borate EDTA (TBE) buffer at a constant voltage (100 v) for 2-3 h The gels were photographed under UV illumination using UVI Tech (DOL-008 XD, ENGLAND) gel documentation system

3.3.5 Polyacrylamide gel (6%) electrophoresis (PGE)

The amplification products were also run on polyacrylamide gel for better separation of the fragments as PGE gives a higher resolution than agarose gels. The polyacrylmide gels were prepared with the following recipe

Component	Quantity
Acrylamide/Bisacrylamide 29 1 (W/W) (Manufacturer)	15 ml
TBE 10 X (Tris Borate FDTA Buffer)	7 5 ml
Distilled water	53 ml
Temed (nnnn-tetramethylethylnediamine)	90 µl
10 % Ammoniumpersulphate (APS)	350 µl

Acrylamide/Bisacrylamide was prepared by dissolving 29 0 g of acrylamide and 1 0 g of bisacrylamide in 100 ml of water The solution was then filtered through Whatman No 1 filter paper and stored at 4 0 ^{0}C in dark bottles TBE 10 X was prepared by dissolving 109 g of Tris base and 55 g of Boric acid one by one in 800 ml of double distilled water containing 40 ml of 0 5M EDTA The final volume was adjusted to 1 0 l with double distilled water The solution was then sterilised by autoclaving and stored at 4 0 ^{0}C 0 5 X TAE (Tris acetate Buffer) was prepared by dissolving 242 g of Tris base in 500 ml of double distilled water and then added 100 ml of 0 5M EDTA and 57 1 ml of

glacial acetic acid The final volume was adjusted to 1 0 l with double distilled water The solution was then sterilised by autoclaving and stored at 4 0 ^{0}C This solution was diluted 100 times to 0 5 X TAE buffer Ammoniumpersulphate (10%) was prepared by dissolving 10 g of ammoniumpersulphate in 100 ml of water and stored at 4 0 ^{0}C Bindsilane buffer was prepared by dissolving 1 5 µl bindsilane (Manufacturer) in 5 ml of acetic acid and 993 5 ml ethanol and stored at 4 0 ^{0}C

Few drops of repulsone (Manufacturer) and bindsilane were used to clean glass plates (38 cm x 32 5 cm x 0 4cm) Glass plate sandwich was then prepared by using clean glass plates with spacers (0 4 microns) and clamps Polyacrylamide gel mixture was prepared by mixing correct volumes of all components except TEMED and APS that were added just before pouring the mixture into the gelcasting unit The assembled unit was placed horizontally on a plane surface and the polyacrylamide gel mixture was poured into the glass plates with the help of syringe, and then comb was inserted at the top position in reverse direction to form wells for loading the PCR amplified products The assembly was left undisturbed for about 30 – 60 minutes for polymerization to occur After polymerization, the comb was carefully removed and wells were washed with 0 5 X TBE and fixed to electrophoresis apparatus

The lower tank and upper reservoir of electrophoresis apparatus was filled with 0 5 X TBE buffer The wells were then cleaned by aspirating the TBE buffer using a pasteur pipette to remove small fragments of gel and tiny bubbles Finally comb tips were inserted up to 1 mm into the gel The gel was pre-run to warm it for at least 10

minutes at 400v and 9W 3-5 µl of PCR product were then loaded on each gel well After loading of samples, voltage clamps were attached and the gel apparatus was connected to power pack set at 400v and 9W The gel was run for 3-4 h for migration of DNA fragments to desired resolution

3.3.6 Silver staining of the polyacrylmide gel

The following reagents were prepared for silver staining the polyacrylmide gel

CTAB (0.1%)

It was prepared by dissolving 2 g of CTAB in 2 litres of double distilled water

Liquid ammonia (0.3%)

It was prepared by dissolving 26 ml ammonia in 2 litres of double distilled water

Staining solution

0 2 g $AgNO_3$ was dissolved in 125 ml of double distilled water and then 0 5 ml of freshly prepared 1M NaOH solution (40 g of NaOH in 1000 ml dH_2O) was added that turned the solution brownish and cloudy Following this 0 5 to 0 6 ml of 25% ammonia was added drop by drop until the solution became transparent

Developer

30 g of sodium carbonate (1 5% Na_2CO_3) was dissolved with intense stirring in 2 l of distilled water and 0 4 ml formaldehyde (0 02%)

Fixer

30 ml of glycerol (1 5%) was added to 2 l of double distilled water

Silver staining the gel

The gel was first rinsed in water for 3 to 5 minutes, soaked in 0 1% CTAB and gently agitated for 20 minutes, and incubated in 0 3 % ammonia for 15 minutes The gel was then incubated in silver staining solution (2 g silver nitrate, 8 ml of 1M NaOH, 6-8 ml 25% ammonia) for about 15 minutes, and transferred to 2 l of double distilled water for about 3 seconds The gel was then developed by gently agitating it in developer solution for about 8-15 minutes, and then rinsed in 2 litres of distilled water for about 3 seconds Finally, the gel was placed in fixer solution for about 10-15 minutes The gel was dried overnight before scanning

3.3.7 Scoring amplified products

The amplified fragments were scored as '1' for the presence and '0' for the absence of a alleles from higher to lower molecular weight products, and approximate base pair (bp) determined

Results

IV. RESULTS

4.1 Polymorphic variability among mapping parents

4.1.1 Intra-accession variation

Ten individual plants of each of ICGV 99003, ICGV 99005, and TMV 2 were evaluated for intra-accession variation using 25 SSR primer pairs specific to cultivated groundnut. None of the primers showed intra-accession variability among the three mapping parents. Figure 1 and 2 respectively show the size of alleles detected among individual plants of the parents and F_1 hybrids of the cross ICGV 99003 x TMV 2 and ICGV 99005 x TMV2 with primer 5D05 Eighteen primer pairs amplified one locus, 6 primer pairs amplified two loci, and one primer pairs amplified 3 loci (Table 2)

4.1.2 Inter-accession variation

Of the 25 primer pairs evaluated for polymorphism among mapping parents, only 28% primer pairs (3A01, 5D05, 1B09, 3A08, 2G04, 2G03, and 2D12B) between ICGV 99003 and TMV2 and 20% primer pairs (5D05, 1B09, 3A08, 2G04, and 2G03) between ICGV 99005 and TMV 2 showed polymorphism These primers produced alleles of the size of 5 to 25 base pair (bp) differences between ICGV# 99003 and TMV 2 and of 5 to 23 bp differences between ICGV 99005 and TMV 2 (Table 2). The primer pairs 3A01, 5D05, and 2D12B in ICGV 99003 and TMV 2 and 5D05 in ICGV 99005 and TMV 2

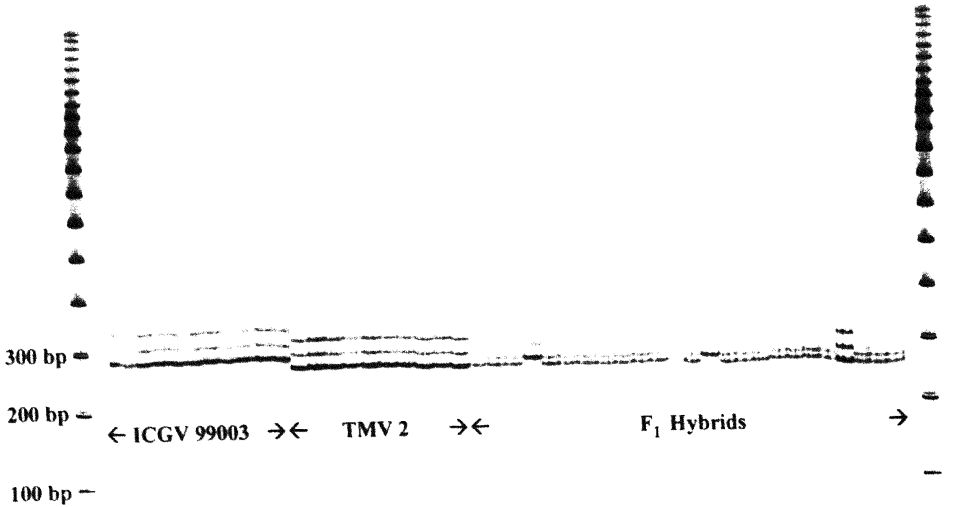


Plate 1. Intra-and inter-accession variability as revealed by primer 5D05 in parents and F_1 hybrids of the cross ICGV 99003 x TMV 2 in groundnut

Table 2. **Intra- and inter-accessions polymorphic variation among rust mapping parents in groundnut**

Primer pairs identity	Allele size (bp) among mapping parents			Intra-accession variation	Inter-accession variation	No. of loci
	ICGV 99003	ICGV 99005	TMV 2			
2E06	275	275	275	No	Monomorphic	1
2F05	260	260	260	No	Monomorphic	1
4A06	120	120	120	No	Monomorphic	2
	260	260	260			
4F09	285	285	285	No	Monomorphic	2
	320	320	320			
6B08	290	290	290	No	Monomorphic	1
4D04	120	120	120	No	Monomorphic	2
	160	160	160			
4H02	290	290	290	No	Monomorphic	2
	330	330	330			
2B10	295	295	295	No	Monomorphic	1
3F01	295	295	295	No	Monomorphic	1
2C11	305.	305	305	No	Monomorphic	1
3B10	245	245	245	No	Monomorphic	2
	280	280	280			
3C02	275	275	275	No	Monomorphic	1
3B06	255	255	255	No	Monomorphic	1
3D09	285	285	285	No	Monomorphic	1
3A04	240	240	240	No	Monomorphic	1
2A05	255	255	255	No	Monomorphic	1
4G02	275	275	275	No	Monomorphic	1
3F08	280	280	280	No	Monomorphic	1
2D12B	260	240	240	No	Polymorphic	1
2G04	285	280	275	No	Polymorphic	1
5D05	275	270	260	No	Polymorphic	3
	285	295	288			
	330	335	312			
3A01	275	293	293	No	Polymorphic	2
	387	412	412			
3A08	190	190	180	No	Polymorphic	1
1B09	275	270	287	No	Polymorphic	1
2G03	250	250	250	No	Polymorphic	2
	270	270	275			

showed greater differences in allele size An example of DNA polymorphism among the mapping parents with primer pairs 5D05 is shown in figure 1 and 2

4.2 F_1 heterozygosity as detected by SSR analysis

Twenty five F_1 plants of the cross ICGV 99003 x TMV 2 and 15 F_1 plants of the cross ICGV 99005 x TMV 2 were assessed for heterozygosity using 7 polymorphic SSR markers in the former and 5 polymorphic SSR markers in the latter cross Of the 25 F_1 plants studied in the cross ICGV 99003 x TMV 2, 22 plants were heterozygous had allele from both the parental genotypes, 2 plants homozygous for ICGV 99003 (designated as P_1), and 1 plant homozygous for TMV 2 (designated as P_2) (Table 3) The plants with P_1 allele resulted from the selfing of ICGV 99003 rather than a controlled cross between ICGV 99003 and TMV 2 P_2 (TMV 2) allele resulted from accidental mixture (TMV 2) in the F_1 population of the cross ICGV 99003 x TMV 2 In cross ICGV 99005 x TMV 2 14 F_1 plants were heterozygous and 1 plant homozygous for P_1 allele (ICGV 99005) (Table 4) A F_1 plant with P_1 allele is self The F_1 heterozygosity as revealed by SSR analysis of the cross ICGV 99003 x TMV 2 and ICGV 99005 x TMV 2 with the primer pair 5D05 is shown in Figure 1 and 2, respectively

4.3 Evaluation of mapping populations for resistance to rust

Parents, F_1 F_2, $BC_1P_1F_1$, and $BC_1P_2F_1$ populations of the cross ICGV 99003 x TMV 2 and ICGV 99005 x TMV 2 were evaluated on 1 to 9 scale where 1 = no disease,

Table 3. **F_1 heterozygosity as revealed by SSR primer in the cross ICGV 99003 x TMV 2 in groundnut**

Primer pairs identity	Allele size (bp) in ICGV 99003 (P_1)	Allele size (bp) in TMV 2 (P_2)	# F_1 plant heterozygous for P_1 and P_2 alleles	# F_1 plant homozygous for ICGV 99003	# F_1 plant homozygous for TMV 2
3A01	275	293	22	2	1
	387	412			
5D05	275	260	22	2	1
	285	288	22	2	1
	330	312	22	2	1
2G04	285	275	22	2	1
3A08	190	180	22	2	1
1B09	275	287	22	2	1
2D12B	260	240	22	2	1
2G03	250	250	22	2	1
	270	275	22	2	1

Table 4. **F_1 heterozygosity as revealed by SSR primer in the cross ICGV 99005 x TMV 2 in groundnut**

Primer pairs identity	Allele size (bp) in ICGV 99005 (P_1)	Allele size (bp) in TMV 2 (P_2)	# F_1 plant heterozygous for P_1 and P_2 alleles	# F_1 plant homozygous for ICGV 99005
5D05	270	260	14	1
	295	288	14	1
	335	312	14	1
3A08	190	180	14	1
1B09	270	287	14	1
2G04	280	275	14	1
2G03	250	250	14	1
	270	275	14	1

and 9 = ≥ 90% foliage damaged, for resistance to rust (Table 5). The average disease score is 3.3 for ICGV 99003, 7.2 for TMV 2, 4.9 for F_1 5.4 for F_2, 4.5 for $BC_1P_1F_1$, and 5.9 for $BC_1P_2F_1$ in ICGV 99003 x TMV 2 and 3.1 for ICGV 99005, 7.5 for TMV 2, 5.7 for F_1 5.8 for F_2, 5.6 for $BC_1P_1F_1$, and 6.3 for $BC_1P_2F_1$ in ICGV 99005 x TMV 2. F_1's of both the crosses showed partial dominance for resistance to rust. However, F_1's of the cross ICGV 99003 x TMV 2 showed greater resistance to rust than F_1 of the cross ICGV 99005 x TMV 2.

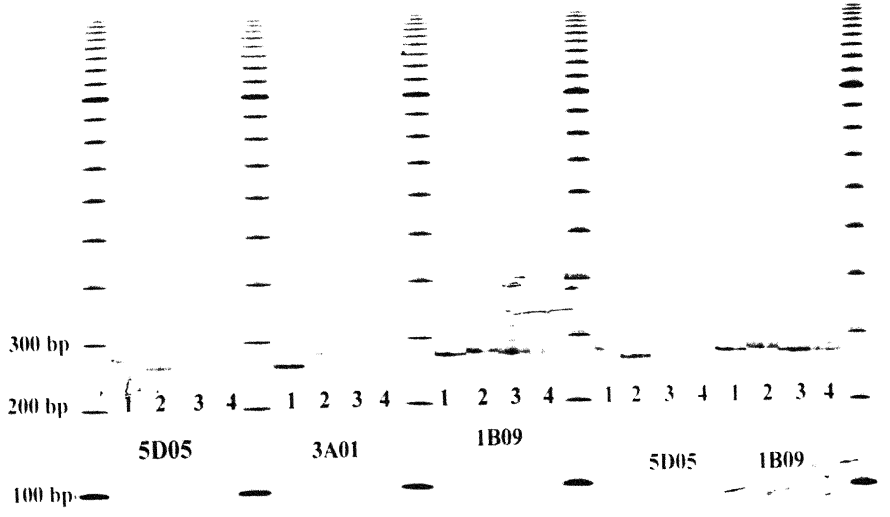
4.4 DNA markers associated with resistance to rust

4.4.1 Bulked segregant analysis

It was performed on four DNA bulks (resistant parent bulk, susceptible parent bulk, highly resistant F_2 bulk, and highly susceptible F_2 bulk) in both the crosses. The DNA from the individual plants was pooled to form four bulks. The SSR markers 3A01, 5D05, 1B09, 3A08, 2G04, 2G03, and 2D12B in cross ICGV 99003 x TMV 2 and 5D05, 1B09, 3A08, 2G04, and 2G03 in cross ICGV 99005 x TMV 2 were evaluated to identify markers linked with resistance to rust. None of the markers showed linkages with resistance or susceptibility to rust in resistant and susceptible F_2 bulks as both the parental alleles were found present in these bulks at the same position as in case of resistant and susceptible parents in both the crosses (Figure 3).

Table 5. Rust disease score among parents, F_2, $BC_1P_1F_1$, and $BC_2P_2F_1$ populations of the two crosses in groundnut

Cross	Generation	Disease score and number of plants in each class							Average disease score
		2	3	4	5	6	7	8	
ICGV 99003 x TMV 2	ICGV 99003	-	7	3	-	-	-	-	3.3
	TMV 2	-	-	-	-	1	4	3	7.2
	F_1	1	2	4	8	7	1	-	4.9
	F_2	1	8	23	21	29	19	6	5.4
	$BC_1P_1F_1$	3	8	17	9	13	1	1	4.5
	$BC_1P_2F_1$	-	2	11	5	12	24	1	5.9
ICGV 99005 x TMV 2	ICGV 99005	1	6	-	1	-	-	-	3.1
	TMV 2	-	-	-	-	-	5	5	7.5
	F_1	-	1	3	5	5	8	-	5.7
	F_2	3	6	17	11	27	24	13	5.8
	$BC_1P_1F_1$	2	2	8	2	21	9	2	5.6
	$BC_1P_2F_1$	1	-	3	5	20	24	3	6.3

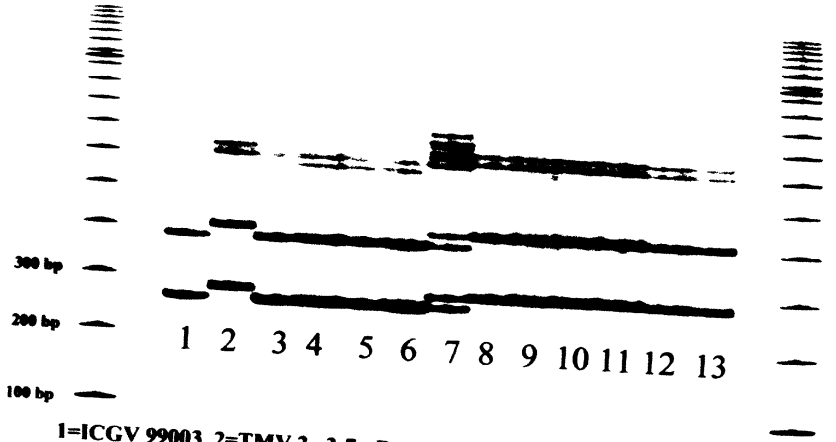


1 = Resistant parent bulk, 2 = Susceptible parent bulk, 3 =Resistant F_2 bulk, 4 = Susceptible F_2 bulk.

Plate 3.Bulked segregant analysis of the resistant and susceptible F_2 bulks along with the parents in two crosses in groundnut

4.4.2 Individual plant analysis

Highly resistant and susceptible F_2 plants of the cross ICGV 99003 x TMV 2 and ICGV 99005 x TMV 2 were analyzed for marker-trait relationships using 7 polymorphic SSR markers in the former and 5 polymorphic SSR markers in the latter cross SSR alleles $3A01_{275}$ and $3A01_{387}$ in cross ICGV 99003 x TMV 2 (Figure 4 and Table 6) and $5D05_{270}$, $5D05_{295}$, and $5D05_{335}$ in cross ICGV 99005 x TMV 2 (Figure 5 and Table 7) were associated with resistance to rust A resistant F_2 plant (sample identity 1391 in Table 7 and lane number 7 in figure 4) of the cross ICGV 99003 x TMV 2 has four alleles of the same size as detected in ICGV 99003 and TMV 2 Susceptibility to rust is associated with alleles $3A01_{293}$ and $3A01_{412}$ in cross ICGV 99003 x TMV 2 and $5D05_{260}$, $5D05_{288}$, and $5D05_{312}$ in cross ICGV 99005 x TMV 2 None of the other primer included in this study showed definite pattern linking either with resistance or susceptibility to rust in both the crosses

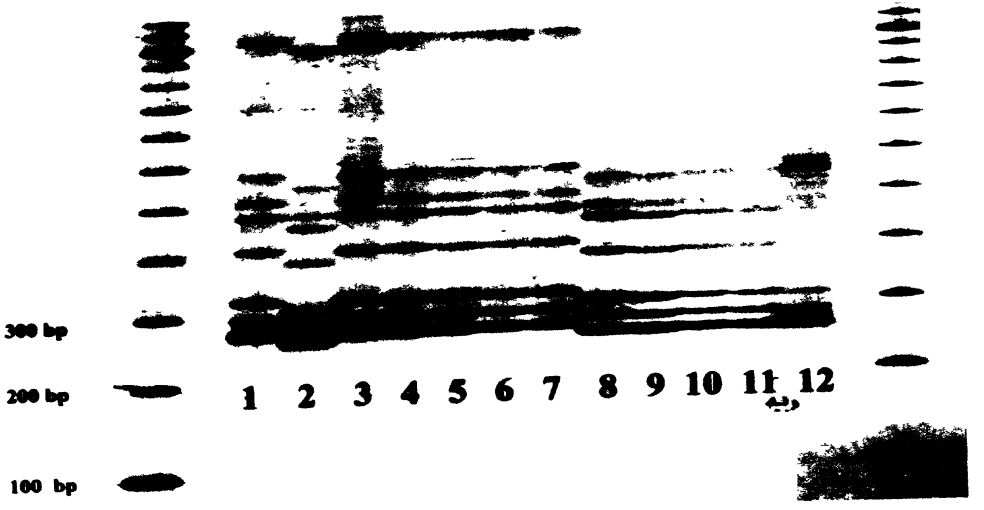


1=ICGV 99003, 2=TMV 2 , 3-7= Resistant F_2plants, 8-13= Susceptible F_2plants

Plate 4. SSR marker 3A01 associated with resistance to rust in F_2 population of the cross ICGV 99003 x TMV 2 in groundnut

Table 6. SSR allele of the marker 3A01 associated either with resistance or susceptibility to rust in F_2 population of the cross between ICGV 99003 and TMV 2 in groundnut

Sample identity	Mapping parents and F_2 plants identity	Rust disease score	3A01 allele (bp)
1400	ICGV 99003	3	275, 387
1401	TMV 2	8	293, 412
1445	F_2 resistant plant	3	275, 387
1454	F_2 resistant plant	3	275, 387
1419	F_2 resistant plant	3	275, 387
1462	F_2 resistant plant	3	275, 387
1391	F_2 resistant plant	3	275, 387
			293, 412
1398	F_2 susceptible plant	8	293, 412
1477	F_2 susceptible plant	8	293, 412
1529	F_2 susceptible plant	8	293, 412
1602	F_2 susceptible plant	8	293, 412
1611	F_2 susceptible plant	8	293, 412
1517	F_2 susceptible plant	8	293, 412



1=ICGV 99005, 2=TMV 2 , 3-7= Resistant F_2plants, 8-12= Susceptible F_2plants

Plate 5.SSR marker 5D05 associated with resistance to rust in F_2 population of the cross ICGV 99005 x TMV 2 in groundnut

Table 7. SSR allele of the marker 5D05 associated either with resistance or susceptibility to rust in F_2 population of the cross between ICGV 99005 and TMV 2 in groundnut

Sample identity	Mapping parents and F_2 plants identity	Rust disease score	5D05 allele (bp)
1001	ICGV 99005	3	270, 295, 335
1002	TMV 2	8	260, 288, 312
1288	F_2 resistant plant	2	270, 295, 335
1359	F_2 resistant plant	2	270, 295, 335
1157	F_2 resistant plant	3	270, 295, 335
1296	F_2 resistant plant	3	270, 295, 335
1357	F_2 resistant plant	3	270, 295, 335
1095	F_2 susceptible plant	8	260, 288, 312
1112	F_2 susceptible plant	8	260, 288, 312
1121	F_2 susceptible plant	8	260, 288, 312
1127	F_2 susceptible plant	7	260, 288, 312
1134	F_2 susceptible plant	8	260, 288, 312

Discussion

V. DISCUSSION

Groundnut is the most important oilseeds crop in India. There are several biotic and abiotic stresses that adversely affect groundnut production at farm level. Among them, rust is one of the major foliar diseases that not only reduce pod yield but also adversely influence fodder and seed quality in groundnut. Although there are fungicides that provide good control of rust disease, this increases the cultivation cost and frequently it is not feasible to implement foliar diseases control due to continuous bad weather conditions which can often prevail during the rainy season. Use of resistant cultivars by the farmers is the best strategy to minimize losses due to foliar diseases. Several sources of resistance to rust have been reported in cultivated and wild *Arachis* germplasm. They are not suitable for commercial cultivation mainly because of the undesirable pod/seed characteristics. Using these resistant sources, a few foliar diseases resistant cultivars have been released in India. However, these cultivars have not become popular among farmers mainly because of late maturity, low shelling outturn, and inferior pod/seed characteristics compared to locally adapted cultivars preferred by the farmers.

The discovery of DNA markers have revolutionized the genetic analysis and opened up new vistas in crop improvement that can be achieved in a much shorter time frame than expected through conventional breeding techniques. To accelerate the genetic gain through marker assisted selection (MAS), it is essential to (i) discover polymorphism using appropriate DNA marker assay, (ii) develop effective techniques to evaluate traits of interest, (iii) know the marker-trait relationships, and (iv) construct

genetic linkage map to identify flanking markers closely linked with useful traits The researchers in the past reported very low level of polymorphism in cultivated groundnut in contrast abundant polymorphism in wild *Arachis* species The lack of polymorphism in cultivated groundnut is attributed to its origin from a single polyploidization event that occurred relatively recently on an evolutionary time scale (Young et al , 1996) However, recent studies revealed evidence of molecular diversity in cultivated groundnut germplasm (He and Prakash, 1997, Hopkins et al , 1999, Subramanian et al , 2000, Dwivedi et al , 2001, Morage Ferguson unpublished data at ICRISAT) The utility of RFLP- and RAPD-based assays has been demonstrated to monitor gene introgression (Garcia et al , 1995) and to identify markers linked with resistance to nematodes in groundnut (Burrow et al , 1996, Choi et al , 1999) An RFLP-based tetraploid genetic linkage map consisting of 370 RFLP loci spread into 23 linkage groups with a total map distance to 2213 cM has been reported (Burow et al , 2001) This discovery provides the roadmap for targeted genetic enhancement in groundnut The RFLP technology, however, is very laborious, time demanding, uses radioactivity, and requires large amount of DNA It has therefore limited value for bringing marker-assisted genetic enhancement in groundnut Recently, collaborative efforts between the University of Georgia (USA) and ICRISAT have succeeded in the discovery of a large number of SSR primer pairs from a cDNA library of groundnut cultivar, Florunner, and many of them have shown polymorphism among diverse germplasm in cultivated groundnut

To facilitate the marker-assisted selection for disease resistance breeding, the present experiment was initiated to identify SSR markers linked with resistance to rust in

groundnut ICGV 99003 and ICGV 99005, the interspecific derivatives highly resistant to rust, were crossed with a highly susceptible cultivar TMV 2 and various generations (F_1 F_2 $BC_1P_1F_1$ and BC_1P_2 F_1) were evaluated along with the parents for resistance to rust under greenhouse conditions Twenty-five SSR primer pairs were screened for polymorphism among mapping parents Seven primer pairs showed polymorphism between ICGV 99003 and TMV 2 and 5 between ICGV 99005 and TMV 2 None of the primer pairs showed intra-accession variability among mapping parents F_1 heterozygosity was also established by SSR analysis Both the parental alleles were found in F_1 hybrids at the same positions as detected in parents SSR analysis of the F_1 hybrids could detect two selfed plants in the cross ICGV 99003 x TMV 2 and one plant in cross ICGV 99005 x TMV 2 as they had alleles of the female parents only However, these F_1 plants were not included for advancing F_2 populations used for phenotyping as well marker genotyping for resistance to rust Highly resistant and susceptible F_2 plants were selected for marker genotyping using bulked segregant analysis (Michelmore et al , 1991) for identifying markers linked with resistance to rust This approach, however, did not produce any meaningful relationships as alleles from both the parents were present in both the resistant and susceptible F_2 bulks at the same position as in case of resistant and susceptible parents Using bulked segregant analysis, Michelmore et al (1991) demonstrated that markers can be reliably identified in a 25 cM window on either side of the targeted locus, and loci further away will be detected with decreasing frequency as genetic distance increases The width of the genetic window also depends on the nature of segregating populations to construct the bulks Any segregating population originating from a single cross can be used bulks made from backcross populations would provide

greater focus around the region of interest than F_2 population The genetic control of the trait might have also influenced the outcome of the bulked segregant analysis The resistance to rust is reported to be from recessive to partial dominant with few genes (1 to 3 genes) to quantitatively inherited traits with modifying effects (Bromfield and Bailey, 1972, Kaleker et al , 1984, Singh et al , 1984, Knauft, 1987, Reddy et al , 1987, Paramasivam et al , 1990, Varman et al , 1991, Vasanthi and Reddy, 1997)

The selected F_2 plants used in bulk segregant analysis were later on genotyped individually along with mapping parents The alleles at 275 and 387 base pair (bp) of the marker 3A01 were shown associated with resistance and therefore designated as $3A01_{275}$ and $3A01_{387}$ markers linked with resistance to rust in cross ICGV 99003 x TMV 2 Similarly susceptibility to rust in this cross is associated with alleles at $3A01_{293}$ and $3A01_{412}$ markers Of the 6 resistant F_2 plants analyzed individually, one plant showed the presence of both the parental alleles One possible reason could be that this plant showed false resistance to rust as it might have escaped from the disease pressure due to low inoculum falling on this plant at the time of inoculation It is also possible that this plant may not have the same level of resistance to rust as with the other plants The alleles of the primer 5D05 were found linked with resistance or susceptibility to rust in cross ICGV 99005 x TMV 2 The designated markers for resistance to rust are $5D05_{270}$, $5D05_{295}$, and $5D05_{335}$ Similarly markers associated with susceptibility to rust are $5D05_{260}$, $5D05_{288}$, and $5D05_{312}$ Further studies are necessary to confirm these preliminary observations At ICRISAT, efforts are on to develop recombinant inbred lines (RILs) that may be tested in replicated trials in hotspot locations for resistance to rust, and later on these could

genotyped using high-throughput assay to identify SSR flanking markers linked with genes/QTLs for resistance to rust in groundnut

Identification of SSR markers linked with genes/QTLs for resistance to rust should facilitate the rapid recovery and transfer of chromosomal region associated with resistance to rust into elite groundnut genotypes by using marker-assisted back cross breeding (MAB) It is expected that MAB should overcome the problem of linkage drag, that often a problem in gene introgression through conventional breeding techniques, and minimize the need for field testing of breeding populations for resistance to rust

Summary

VI. SUMMARY

Rust (*Puccinia arachidis* Speg.) is one of the important foliar diseases of groundnut that causes substantial yield loss as well reduces the fodder and seed quality. Use of resistant cultivars by the farmers is the best strategy to minimize losses due to foliar diseases. Few foliar diseases resistant cultivars have been released in India however, they are not popular among farmers mainly because of late maturity, low shelling outturn, and inferior pod/seed characteristics compared to locally adapted cultivars. The resistant sources possess many undesirable pod/seed characteristics that are difficult to eliminate due to linkage drag through conventional breeding techniques.

DNA markers have revolutionized the genetic analysis of plant germplasm and opened up new vistas in crop improvement that can be achieved in a much shorter time frame than expected through conventional breeding techniques. The present experiment was initiated to identify SSR markers linked with resistance to rust in two crosses in groundnut. The parents, F_1, F_2, $BC_1P_1F_1$, and BC_1P_2 F_1 populations were evaluated for resistance to rust under greenhouse conditions. Of the 25 SSR primer pairs screened for polymorphism, 7 primer pairs detected variation between ICGV 99003 and TMV 2 and 5 primer pairs between ICGV 99005 and TMV 2. None of the primers showed intra-accession variability among mapping parents. Highly resistant and susceptible F_2 plants were selected to form bulks, and analyzed using bulked segregant analysis to identify markers linked with resistance to rust. The bulked segregant analysis did not provide useful results as in many cases parental alleles of the same size were found in both the

resistant and susceptible F_2 bulks. Later on resistant and susceptible F_2 plants were individually analyzed for marker-trait relationships along with the parents. Rust resistance is associated with markers $3A01_{275}$ and $3A01_{387}$ in cross ICGV 99003 x TMV 2 and with markers $5D05_{270}$, $5D05_{295}$, and $5D05_{335}$ in cross ICGV 99005 x TMV 2. The susceptibility to rust, on contrary, is associated with markers $3A01_{293}$ and $3A01_{412}$ in cross ICGV 99003 x TMV 2 and with markers $5D05_{260}$, $5D05_{288}$, and $5D05_{312}$ in cross ICGV 99005 x TMV 2. Further studies are necessary to confirm these observations in later generations.

References

VII. REFERENCES

ANDERSON, W.F., BEUTE, M.K., WYNNE, J.C. AND WONGKAEW, S., 1990, Statistical procedures for assessment of resistance in a multiple foliar disease complex of peanut. *Phytopathology*, **80**: 1451-1459.

BHAGWAT, A., KRISHNA, T.G. AND BHATIA, C.R., 1997, RAPD analysis of induced mutants of groundnut (*Arachis hypogaea* L.). *Journal of Genetics*, **76**: 201-208.

BROMFIELD, K.R. AND BAILEY, W.K.,1972, Inheritance of resistance to *Puccinia arachidis* in peanut. *Phytopathology*, **62**: 748.

BUROW, M.D., SIMPSON, C.E. AND STARR, J.L., 1996, Identification of peanut (*Arachis hypogaea* L.) RAPD markers diagnostic of root-knot nematode (*Meloidogyne arvenaria* (Neal) Chitwood) resistance. *Molecular Breeding*, **2**: 369-379.

BUROW, M.D., SIMPSON, C.E., STARR, J.L., PATERSON, A.H.,2001, Transmissiongenetics of chromatin from a synthetic amphiploid in cultivated peanut (*A.hypogaea* L.): Broadening the gene pool of a monophyletic polyploid species.*Genetics*, **159**: 823-837.

BUTLER, D R , WADIA, K D AND JADHAV, D R ,1994, Effects of leaf wetness and temperature on late leaf spot infection of groundnut *Plant Pathology* **43** 112-120

CHEN, Y S , YE, Y X , HONG, D Z AND ZHUANG, W D ,1999, Characterization and evaluation of groundnut germplasm in Fuji *Journal of Fujian Academy of Agricultural Sciences*, **14** 12-16

CHEUNG, W Y , GUGEL, R K , AND LANDRY, B S ,1998, Identification of RFLP markers linked to the white rust resistance gene (Acr) in mustard (*Brassica juncea* (L) Czern and Coss) *Genome* **41** 626-628

CHOI, K , BOROW,M D , CHORCH, G , BOROW, G , PATERSON, A H ,SIMPSON, C E , AND STARR, J L ,1999, Genetics and mechanism of resistance to *Meloidogyne arenaria* in peanut germplasm *Journal of Nematology* **31** 283-290

CLIFORD, B C ,1973, The construction and operation of a dew simulation chamber *New Phytology* **77** 76-81

COOK, B G AND CROSTHWAITE I C ,1994, Utilization of *Arachis* species as forage p 624–663 In J Smart (ed), The Groundnut Crop A Scientific Basis forImprovement, Chapman and Hall, London

CROUCH, J.H.,2001, Molecular marker-assisted breeding: a perspective for small to medium-sized plant breeding companies. Asia and Pacific Seed Association Technical Report No. **30**: 1-14.

DUNCAN, W.G., McDONALD, D., McGRAW, R.L. AND BOOTE, K.J.,1978, Physiological aspects of peanut yield improvement. *Crop Science*, **18**: 1015-1020.

DWIVEDI, S.L., GURTU, S., CHANDRA, S., YUEJIN, W., AND NIGAM, S.N.,2001, Assessment of genetic diversity among selected groundnut germplasm. 1: RAPD analysis. *Plant Breeding*, **120**: 345-349.

DWIVEDI, S.L., NIGAM, S.N., JAMBUNATHAN, R., SAHRAWAT, K.L., NAGABHUSHANAM, G.V.S., AND RAGHUNATH, K. ,1993, Effects of genotypes and environments on oil contentand oil quality parameters and their correlation in peanut (*Arachis hypogaea* L.).*Peanut Science*, **20**: 84–89.

DWIVEDI, S.L., PANDE, S., RAO, J.N. AND NIGAM, S.N.,2001, Components of resistance tolate leaf spot and rust among interspecific derivatives and their significance in afoliar disease resistance breeding in groundnut (*Arachis hypogaea* L.). *Euphytica*(in print).

EDWARDS, C , STRANGE, R M AND COLE, D L 1995 Accumulation of isoflavonoid phytoalexins in leaves of *Arachis hypogaea* L differing in reaction to rust (*Puccinia arachidis*) and early leaf spot (*Cercospora arachidicola*) *Plant Pathology (Oxford)* **44** 573-579

EKBOTE, A U AND MAYEE C D ,1984, Biochemical changes due to rust in resistant and susceptible groundnuts I Changes in oxidative enzymes *Indian Journal of Plant Pathology*, **2** 21-28

GALAGARO, M L AND LOPES, C R , 1994, Isoenzymatic variability among five peanut cultivars *Bragantia* **53** 135-140

GARCIA, G M , STALKER, H T , AND KOCHERT, G ,1995, Introgression analysis of an interspecific hybrid population in peanuts (*Arachis hypogaea* L) using RFLP and RAPD markers *Genome* **38:** 166-176

GHEWANDE, M P , PANDEY, R N , SHUKLA, A K AND MISHRA, D P , 1983, Source of resistance to late leaf spot and rust of groundnut *Indian Botanical Reporter*, **2** 174

GORBET, D W , NORDEN, A J , SHOKES, F M AND KNAUFT D A ,1987, Registration ofSouthern Runner peanut *Crop Science* **27** 817

GRIESHAMMER, U., AND WYNNE, J.C.,1990, Isozyme variability in mature seeds of U.S. peanut cultivars and collection. *Peanut Science*, **18**: 72-75.

GUPTA, P.K., BALYAN, H.S., SHARMA, P.C. AND RAMESH, B.,1996, Microsatellites in plants: A new class of molecular markers. *Current Science*, **70**: 45-54.

HALEY, S.D., MIKLAS, P.N., STAVELY, J.R., BYRUM, J., AND KELLY J.D.,1993, Identification of RAPD markers linked to a major rust resistance gene block in common bean. *Theoritical and Applied Genetics*, **86**: 505-512.

HALWARD, T.M., STALKER, H.T., LARUE, E. AND KOCHERT, G.,1991, Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild species. *Genome*, **34**: 1013-1020.

HALWARD, T.M., STALKER, H.T., AND KOCHERT, G.,1993, Development of RFLP linkage map in diploid peanut species. *Theoritical and Applied Genetics*, **87**: 379-384.

HALWARD, T.M., STALKER, H.T., LARUE, E. AND KOCHERT, G., 1992, Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.). *Plant Molecular Biology*, **18**: 315-325.

HAMMOND, J.M., BACKMAN, P.A., AND LYLE, J.A.,1976, Peanut foliar fungicides: relationships between leaf spot control and kernel quality. *Peanut Science*, **3**: 70-72.

HAUSNER, G., RASHID, K.Y., KENASCHUK, E.O., PROCUNIER, J.D.,1999, The develoment of codominant PCR/RFLP based markers for the flax rust-resistance alleles at the L locus. *Genome*, **42**: 1-8.

HE, G. AND PRAKASH, C.S.,1997, Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). *Euphytica*, **97**: 143–149.

HOPKINS, M.S., CASA, A.M., WANG, T., MICHELL, S.E., DEAN, R.E., KOCHERT, G.D. AND KRESOVICH, S., 1999, Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut. *Crop Science*, **39**: 1243-1247.

JIANG, H. F., DVAN, N.X. AND REN, X.P., 1998, Comprehensive evaluation of groundnut (*Arachis hypogaea* L.) germplasm. *Chinese Journal of Oil Crop Sciences*, **20**: 31-35.

KALEKAR, A. R., PATIL, R. C. AND DEOKAR, A. B.,1984, Inheritance of resistance to rust in groundnut. *Madras Agriculture Journal*, **71**: 125-126.

KNAUFT, D A ,1987, Inheritance of rust resistance in groundnut *In Groundnut Rust Disease Proceedings of Discussion Group Meeting* International crops Research Institute for Semi Arid Tropics Patancheru India 24-28 September 1984 pp 183-187

KOCHERT, G , HALWARD, T , BRANCH, W D AND SIMPSON, C E ,1991, RFLP variability in peanut (*Arachis hypogaea* L) cultivars and wild species *Theoretical and Applied Genetics*, **81** 565-570

KRAPOVIKAS, A , AND GREGORY, W C ,1994, Taxonomy of genus *Arachis* (Legumonisae) *Bonplandia* **8** 1-186

KRAPOVIKAS, A , VALLS, J F M AND SIMPSON, C E ,2000, History of *Arachis* includingevidence of *A hypogaea* L progenitors p 46 In J R Sholar (ed), *American Peanut Research Educational Society*, USA

LACKS, G D AND STALKER, H T ,1993, Isozyme analyses of *Arachis* species and interspecific hybrids *Peanut Science* **20** 76-82

LACKS, G D , STALKER, H T AND MURTHY, J P , 1991, Patterns of isozyme variation among *Arachis* species In *Symposium on Plant Breeding in the 1990* Eds Stalker, H T and Murphy, J P Department of Crop Science, North Carolina State University, Research Report No 103 pp 71

LANHAM, P.G., FENNELL, S., MOSS, J.P.AND POWELL. W.,1992. Detection of polymorphic loci in *Arachis* germplasm using random amplified polymorphic DNAs. *Genome*, **35**: 885-889.

LAWSON, W.R., GOULTER K.C., HENRY, R.J., KONG, G.A. AND KOCHMAN. J.K.,1998, Marker-assisted selection for two rust resistance genes in sunflower. *Molecular Breeding*, **4**: 227-234.

LIANG, X.Q., LI, Y.C., LI, S.X. AND ZHOU. G.Y.,1999, Yueyou 223; a high-yielding Chinese cultivar with good resistance to rust. *International Arachis Newsletter*, **19**: 16-17.

LU, J. AND PICKERSGILL, B.,1993, Isozyme variation and species relationships in peanut and its wild relatives (*Arachis hypogaea* L.). *Theoretical and Applied Genetics*, **85**: 550-560.

MEHAN, V.K., REDDY, P.M., RAO, K.V. AND McDONALD, D.,1994, Components of rust resistance in peanut genotypes. *Phytopathology*, **84**: 1421-1426.

MELCHINGER, A.E.,1990, Use of molecular markers in breeding for oligogenic disease resistance. *Plant breeding*, **104**: 1-19.

MOSS, J.P., SINGH, A.K., Nigam, S.N., Hildebrand, G.L., Govinden, N., Ismael, F.M., Surbrahmanyam, P., and Reddy, L.J.,1998, Registration of ICGV-SM 86715 Peanut Germplasm. *Crop Science*, **38**: 572.

MOSS, J.P., SINGH, A.K., REDDY, L.J., NIGAM, S.N., SUBRAHMANYAM, P., MCDONALD, D. AND REDDY, A.G.S.,1997, Registration of ICGV 87165 peanut germplasm with multipleresistance. *Crop Science*, **37**: 1028.

NIGAM, S.N., REDDY, L.J., SUBRAHMANYAM, P., REDDY, A.G.S., MCDONALD, D., AND GIBBONS, R.W.,1992, Registration of ICGV 87157, an elite peanut germplasm with multiple resistance to diseases. *Crop Science,* **32**: 837.

PAIK-RO, O.G., SMITH, R.L. AND KHAUFT, D.A., 1992, Restriction length polymorphism evaluation of six peanut species within the *Arachis* section. *Theoretical and Applied Genetics,* **84**: 201-208.

PARAMASIVAM, J.M., JAYASEKHAR, M., RAJASEKHARAN, R. AND VEERAABADHIRAN, P., 1990, Inheritance of rust resistance in groundnut(*Arachis hypogaea* L.). *Madras Agricultural Journal,* **77**: 50-52.

PRABHU, K.V., SOMERS, D.J., RAKOW, G. AND GUGEL, R.K.,1998, Molecular markers linked to white rust resistance in mustard *Brassica juncea. Theoritical and Applied Genetics,* **97**: 865-870.

REDDY, L.J., NIGAM, S.N., DWIVEDI, S.L. AND GIBBONS, R.W., 1987. Breeding groundnut cultivars resistant to rust (*Puccinia arachidis* Speg.). In: *Groundnut rust disease; Proceedings of a Discussion Group Meeting,* 24-28 September 1984, ICRISAT. Patancheru, Andhra Pradesh, India. pp. 17-25.

REDDY, L.J., NIGAM, S.N., MOSS, J.P., SINGH, A.K., SUBRAHMANYAM, P., MCDONALD, D. AND REDDY, A.G.S.,1996, Registration of ICGV 86699 peanut germplasm linewith multiple disease and insect resistance. *Crop Science,* **36**: 821.

REDDY, L.J., NIGAM, S.N., SUBRAHMANYAM, P., REDDY, A.G.S., MCDONALD, D., GIBBONS, R.W. AND PENTAIAH, V.,1993, Registration of ICGV 86590 peanut cultivar.*Crop Science,* **33**: 357–358.

REDDY, L.J., NIGAM, S.N., SUBRAHMANYAM, P., REDDY, A.G.S., MCDONALD, D., GIBBONS, R.W. AND PENTAIAH, V., 1992, Registration of ICGV 87160 peanut. *Crop Science*, **32**: 1075.

REDDY, P.N. AND KHARE, M.N., 1988, Components of resistance in groundnut cultivars to *Puccinia arachidis* Speg. *Journal of Oilseeds Research,* **5**: 153-154.

SAGHAI-MAROOF, M A , SOLIMAN, K M , JORGENSEN, R A AND ALLARD, R W , 1984, RibosomalDNA spacer length polymorphism in barley Mendelian inheritance, chromosallocation and population dynamics *Proceedings of National Acadamy of Sciences* USA **81** 8014-8018

SANDERS, T H , GORBET, D W , SHOKES, F M , WILLIAMS, E J AND MCMEANS, J L ,1989,Effect of chlorothalonil application frequency on quality factors of peanut(*Arachis hypogaea*) *Journal of Science Food and Agriculture* **49** 281-290

SANDHIKAR, R N , BULBULE, S V AND MAYEE, C D ,1989, Prediction models for rust epidemic in groundnut *Indian Journal of Mycology and Plant Pathology* **19** 60-67

SANKARAN, S A , SUBBA RAO, P V AND STRANGE, R N , 1996, Differential accumulation of phytoalexins in leaves of susceptible and resistant genotypes of groundnut (*Arachis hypogaea* L) inoculated with *Puccinia arachidis* Speg *Phytopathology*, **144** 527-532

SINGH, A K , MEHAN, V K AND NIGAM, S N , 1997, Sources of resistance to groundnut fungal and bacterial diseases an update and appraisal *Information Bulletin No 50* International Crops Research Institute for Semi Arid Tropics, Patancheru, Andhra Pradesh, India pp 48

SINGH, A.K., SUBRAHMANYAM, P. AND MOSS, J.P., 1984, The dominant nature of resistance to *Puccinia arachidis* in certain wild *Arachis* species. *Oleagineux*, **39**: 535-537.

STALKER. H.T., PHILLIPS, T.D., MURPHY, J.P. AND JONES, T. M.,1994, Variation of isozyme patterns among Arachis species. *Theoretical and Applied Genetics*, **87**: 746-755.

SUBBA RAO, P.B., EINHORN, J., GEIGER, J.P., MALOSSE, C., RIO, B. AND RAVISE, A., 1991, Alkyl bis phenyl ethers, new phytoalexins produced by *Arachis hypogaea* L. infected with *Puccinia arachidis* Speg. *Oleagineux*, **46**: 501-507.

SUBBA RAO, P.V., GEIGER, J.P., EINHORN, J., RIO, B., MALOSSE, C., NICOLE, M., SOVARY, S. AND RAVISE, A.,1988, Host defense mechanisms against groundnut rust. *International Arachis Newsletter*, **4**: 16-18.

SUBBA RAO, P.V., SUBRAHAMANYAM, P. AND REDDY, P.M.,1990, A modified nine point disease scale for assessment of rust and late leaf spot of groundnut. In: *Second International Congress of French Phytopathological Society*, Montpellier, France, 28-30 November 1990.

SUBBA RAO, P.V., WADIA, K.D.R. AND STRANGE, R.N., 1996, Biotic and abiotic elicitation of phytoalexins in leaves of groundnut (*Arachis hypogaea* L.). *Physiological and Molecular Plant Pathology*, **49**: 343-357.

SUBRAHMANYAM, P., GHANEKHAR, A. M., NOLT, B.T., REDDY, D.V.R. AND McDONALD, D.,1985, Resistance to groundnut diseases in wild *Arachis* species. In: *Proceedings of International Workshop on Cytogenetics of Arachis*, 31 October- 1 November 1983, International Crops Research Institute for Semi Arid Tropics, Patancheru, Andhra Pradesh, India, pp. 49-55.

SUBRAHMANYAM, P., GIBBONS, R. W., NIGAM, S. N. AND RAO, V. R.,1982b, Screening methods and future sources of resistance to peanut rust. *Peanut Science*, **7**: 10-12.

SUBRAHMANYAM, P., AND McDONALD, D.,1982, Groundnut rust, its survival and carry over in India. *Proceedings of Indian Academy of Science (Plant Science)*, **91**: 93-100.

SUBRAHMANYAM, P. AND McDONALD. D., 1983, *Rust disease of groundnut. Information Bulletin No. 13*, International Crops Research Institute for Semi-Arid Tropics, Patanchera, Andhra Pradesh, India. pp 15.

SUBRAHMANYAM, P., McDONALD. D. GIBBONS, R.W., NIGAM, S.N. AND NEVILL, D.J.,1982a, Resistance to rust and late leaf spot diseases in some genotypes of *Arachis hypogaea*. *Peanut Science*, **9**: 6-10.

SUBRAHMANYAM, P., McDONALD, D. AND SUBBA RAO, P. V.,1983, Influence of host genotype on uredospore production and germinability in *Puccinia arachidis*. *Phytopathology*, **73**: 726-729.

SUBRAHMANYAM, P., McDONALD, D., WALIYAR, F., REDDY, L.J., NIGAM, S.N., GOBBONS, R.W., RAO, V.R., SINGH, A. K., PANDE, S., REDDY, P.M. AND RAO, P.V.S.,1995, *Screening methods and sources of resistance to rust and late leaf spot of groundnut*.Information Bulletin No. 47, International Crops Research Institute for Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. pp. 20.

SUBRAMANIAN, V., GURTU, S., RAO, R.C.N., AND NIGAM, S.N.,2000, Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. *Genome*, **43**: 656-660.

TANKSLEY, S.D., YOUNG, N.D., PATERSON, A.H., AND BONIERBAL M.W., 1989. RFLP mapping in plant breeding –new tools for an old science. Bio/Technol. 7: 257-264.

VARMAN, P.V., LAKSHMI, V.G. AND RAVEENDRAN, T.S.,1995, Analysis of growth pattern in rust resistant genotypes of groundnut. *Madras Agricultural Journal*, **82**: 235-238.

VARMAN, P.V., RAVEENDRAN, T.S. AND GANPATHI, T.,1991, Genetic analysis of rust resistance in groundnut (*Arachis hypogaea* L.). *Journal of Oilseeds Research*, **8**: 35-39.

VASANTHI, R.P. AND REDDY, C.R.,1997, Inheritance of testa color and resistance to late leaf spot and rust in groundnut (*Arachis hypogaea* L.).*Journal of Oilseeds Research*, **14**: 244-248.

VELAZHAHAN, R. AND VIDHYSEKARAN, P.,1994, Role of phenolic compounds, peroxidase and polyphenol oxidase in resistance of groundnut to rust. *Acta Phytopathlogica et Entomologica Hungarica*, **29**: 23-29.

WALIYAR, F., BOSC, J.P. AND BONKOUNGOU, S.,1993, Sources of resistance to foliar diseases of groundnut and their stability in West Africa. *Oleagineux*, **48**: 283-286.

WILLIAMS, J.H., RAMRAJ, V.M. AND PAL, M., 1987, Physiological studies on foliar disease: varietal differences in response to use of fungicides. In: *Groundnut Rust Disease; Proceedings of a Discussion Group Meeting*, 24-28 September 1984, International Crops Research Institute for Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. pp. 49-53.

WILLIAMS, J.H., WALIYAR, F. AND SUBRAHMANYAM, P., 1993, Crop physiology response to fungicides by cultivars differing in foliar disease resistance. In: *Summary of Proceeding of the third ICRISAT Regional Groundnut Meeting.* 14-17 September 1992, West Africa, Ouagadougou, Burkina Fago.p. 48.

WORTHINGTON, R.E., AND SMITH, D.H.,1974, Modification of peanut oil fatty acid composition by foliar applications of 2', 2'-dimethylsuccinohydrazide (kylar). *Journal ofAgricultural Food Chemistry,* **22**: 507-508.

WYNNE, J.C., BEUTE, M.K. AND NIGAM, S.N.,1991, Breeding for disease resistance in peanut (*Arachis hypogaea* L.). *Annual Review of Phytopathology,* **29**: 279-303.

YOUNG, N.D., WEEDEN, N.F. AND KOCHERT, G.,1996, Genome mapping in legumes (Fam.*Fabaceae*). In: A.H. Paterson (ed.), Genome Mapping in Plants, 211-227. LandesC0., Austin, TX.

IDENTIFICATION OF PCR-BASED DNA MARKERS LINKED WITH RESISTANCE TO RUST IN GROUNDNUT (*Arachis hypogaea* L.)

T. Sivananda varma **2002** **Dr Chennabyre Gowda**
(Major Advisor)

ABSTRACT

Rust (*Puccinia arachidis* Speg.) is one of the important foliar diseases of groundnut that cause substantial yield loss as well reduces the fodder and seed quality. Few foliar diseases resistant cultivars have been released in India, however, they are not popular among farmers mainly because of late maturity, low shelling outturn, and inferior pod/seed characteristics compared to locally adapted cultivars. The resistant sources possess many undesirable pod/seed characteristics that were difficult to eliminate because of linkage drag by conventional breeding techniques.

The DNA markers have revolutionized the genetic analysis and opened up new vistas in crop improvement. The present experiment was initiated to identify SSR markers linked with resistance to rust in two crosses in groundnut. The parents, F_1, F_2, $BC_1P_1F_1$, and BC_1P_2 F_1 populations were evaluated for resistance to rust under greenhouse conditions. Of the 25 SSR primers screened for polymorphism, 7 primer detected variation between ICGV 99003 and TMV 2 and 5 between ICGV 99005 and TMV 2. None of the primers showed intra-accession variability among mapping parents. Highly resistant and susceptible F_2 plants were bulked and analyzed using bulk segregant analysis to identify markers linked with resistance to rust. The bulk segregant analysis did not provide useful results as in many cases both the parental bands were present in the resistant and susceptible F_2 bulks. Later on the individual resistant and susceptible F_2 plants were analyzed for marker-trait relationships. Resistance to rust in cross ICGV 99003 x TMV 2 is associated with SSR markers $3A01_{275}$ and $3A01_{387}$ while susceptibility with $3A01_{293}$ and $3A01_{412}$. Resistance to rust in cross ICGV 99005 x TMV 2 is associated with markers $5D05_{270}$ and $5D05_{335}$ and susceptibility with markers $5D05_{260}$ and $5D05_{312}$ Further studies are necessary to confirm these observations in later generations.