Identification of SSR Markers Associated with Resistance to Nematode *Tylenchorhynchus brevilineatus* Infection in Groundnut (*Arachis hypogaea* L.)

Vasanthi Raguru Pandu · Venkataramana Endla · Bhaskara Reddy Bommu Veera · Thanuja Rani Idemadakala · Sivaprasad Yeturu · Raja Reddy Kondreddy

ABSTRACT

Kalahasti malady is a disease caused by the soil-borne nematode, *Tylenchorhynchus brevilineatus* that occurs during the post-rainy season in Andhra Pradesh, India. Screening of segregating populations is very difficult as maintenance of a sick plot at the experimental farm is not possible. Hence, identification of molecular markers is a research priority. Among 35 SSR markers used for the study, the alleles of two primers (i.e., 225, 260, 275, 310 and 330 base pair (bp) alleles of pPGS seq14H6 and 210 bp allele of pPGS seq 16G8) showed an association with resistance to Kalahasti malady when seven parents (Tirupati 3, Kalahasti, Narayani, Kadiri 6, Tirupati 4, Prasuna and ICG (FDRS) 79), six F1s and 25 genotypes were screened. Confirmation of the association between resistance and markers has to be further carried out in mapping populations i.e. F2 populations and recombinant inbred lines, which are now being developed.

Keywords: alleles, groundnut, Kalahasti malady, nematode, SSR

INTRODUCTION

Groundnut is an important oil seed crop of India and in the state of Andhra Pradesh. In India, the crop is cultivated on 6.2 million ha with a production of 7.3 million tonnes (mt) in two major climatic situations i.e. 80% in rain-fed and 20% in *rabi* i.e., summer. In Andhra Pradesh, it is grown on 1.8 million ha with a production of 1.6 mt. In about 0.3 million hectares (m ha), it is cultivated during *rabi* with a productivity of 1932 kg/ha (Annual Report of Directorate of Groundnut Research 2009). Out of this, coastal sands along the sea coast and river belts of different rivers in Chittoor, Nellore, Kadapa and Prakasam districts constitute approximately 0.05 m ha.

A nematode disease of groundnut (*Arachis hypogaea* L.), termed ‘Kalahasti malady’, is a serious concern in the coastal sands. Among the plant parasitic nematodes isolated from the soil in the rhizosphere region of the affected groundnut crop, the nematode *Tylenchorhynchus brevilineatus* Williams was more abundant than others (Reddy et al. 1984; Sharma 1988; Prasad and Rangappa 1994; Naidu 1996). It was also found to be associated with the roots of sugarcane (*Saccharum officinarum* L.) and castor (*Ricinus communis* L.) in Haryana and Bihar States (Gupta 1986). It caused a significant reduction in plant growth in wheat (*Triticum aestivum* L.) var. ‘Sonalika’ wherein yellowing, stunting, reduced tillering with reduced grain yields were also reported (Thakar et al. 1986).

As a result of nematode infection, affected groundnut plants appeared stunted with greener-than-normal foliage (Reddy et al. 1984). The disease appears initially as small yellow to brown lesions on roots, pegs, pod stalks and young developing pods. As the disease progresses, the lesions turn brown with cracked margins. These lesions later turn from deep brown to black that coalesce and cover the whole pod surface (Fig. 1). The affected pods appear scabby after drying. In severe cases, a reduction in pod stalk length, pod size and kernel size also occur. Kernels from diseased pods apparently appear healthy. When the pegs are affected in initial stages, they fail to develop into pods, leading to severe yield losses. However, no symptoms on foliage (Ratna Kumar 1988; Naidu 1996), or differences in plant height were reported in diseased plants (Ratna Kumar 1988). Yield losses of 60-70% were reported in endemic areas (Rao et al. 1986).

Presently, the disease is managed through the application of chemical insecticides. Soil application of carbofuran 3G or aldicarb 10G granules at 4-6 kg a.i/ha not only reduced the nematode population in the soil and the intensity of disease symptoms on the pods but also significantly enhanced pod yield (Rao et al. 1986). However, this method was found to be too expensive for wide-spread adoption by farmers. Hence, there is a need to develop alternate methods to control the disease. Groundnut germplasm was screened against nematode infection to identify accesses or cultivars that were resistant or tolerant to nematode infection. A total of 48 genotypes of different habit groups were screened for their reaction to nematode infection. The test entries exhibited wide variation (16.22 to 89.91%) in their susceptibility to nematode infection. Among the genotypes screened, ‘NC2’ was resistant with < 20% infection. The genotypes ‘NC1’ or TL-18-B’ (*T. CG 1706* group) recorded 30-40% and 61-70% infection, respectively while 71-80% infection was observed in 20 genotypes. Twenty five genotypes were highly susceptible with an 81-90% infection (Rao et al. 1986).

A total of 1599 groundnut germplasm accessions and breeding lines were screened in replicated trials on a farmer’s field in a hot spot location during 1985-1986 by Mehan et al. (1993). 22 genotypes were identified as resistant and they had disease scores of 2.0 or < 2 on a 1-5 disease scale. Of these, 14 were confirmed to be resistant in advanced screening trials in the 1986 rainy and the 1985/6, 1986/7 post-rainy seasons. Resistance to the nematode (*T. brevilineatus*) was stable in all three trial sites in the 1986/7 post-rainy season. Most of the resistant genotypes had undesirable pod/seed characteristics. One of the resistant genotypes was a high-yielding breeding line ’TCG 1518’. It

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a total of 23 advanced breeding lines developed in the breeding programme were evaluated in a hot spot location on the Andhra Pradesh State Seed Development Corporation Farm, and Srikalahasti was screened for nematode infection on a 1-5 scale at the time of harvest: 1-2 resistant (1-25% damage), 2-3 moderately resistant (26-50%), 3 to 4 susceptible (51-75% damage) and 4 to 5 highly susceptible (more than 75%). Naidu and Moses (2004) screened 38 genotypes derived from the crosses, ‘TCG 1716’× ‘TCG 1518’, ‘TCG 1717’× ‘TCG 1518’ and ‘TCG 273’× ‘TCG 1518’ in a hot spot location at the Andhra Pradesh State Seed Corporation Farm at Srikalahasti in Chittoor district of Andhra Pradesh during the post rainy season of 1992-93 and 1993-94. Two check varieties, one resistant (‘Tirupati 3’) and one susceptible (‘JL24’) were included in the screening trial. None of the genotypes were immune to nematode infection. Three genotypes, ‘TCG 328’ (41.7%), ‘TCGS 353’ (36.92%) and ‘TCGS 357’ (26.00%) and the resistant check variety, ‘Tirupati 3’ exhibited resistance to nematode attack with a score of 2.0. Total crude fibre content and total phenolic compounds in groundnut shells of resistant variety, ‘Tirupati 3’ were higher by 36.4 and 85.15%, respectively over the susceptible groundnut variety, ‘Tirupati 3’. Three genotypes, ‘TCG 332’ (43.50%), ‘TCGS 337’ (41.21%) and ‘TCGS 341’ (31.72%) – among moderately resistant genotypes – gave significantly higher pod yield over ‘JL 24’ (20.66 t/ha).

Two stabilized selections (‘TCG 1709’× ‘TCG 1518’)–54 and (‘TCG 1709’× ‘TCG 1518’)–8 were resistant to Kalahasti malady with a disease score of 2.0. These two selections matured in 110 days with a pod yield of 3.27 and 3.43 t ha\(^{-1}\), respectively. The susceptible control variety, ‘JL 24’ recorded a disease score of 4.0 with a pod yield of 2.76 t ha\(^{-1}\). Two selections (‘TCG 1709’× ‘TCG 1518’)–18 and (‘TCG 1709’× ‘TCG 1518’)–7, were moderately resistant with a pod yield of 4.00 and 3.94 t ha\(^{-1}\), respectively (Vasan
ti et al. 1998). In July 2002, one of these selections, ‘TCGS 320’ derived from ‘TCG 1709’× ‘TCG 1518’, was released as ‘Kalahasti’ (Fig. 3) by the Andhra Pradesh State Varietal Release Committee. Later on, a moderately susceptible line, ‘TCGS 341’, with a rose testa colour, was released as ‘Prasuna’ in 2006. To rectify the defects in these varieties and maintain high yield potential and resistance to Kalahasti malady, it is necessary to identify a robust screening tool. As Kalahasti malady is caused by soil-borne nematodes in specific types of soils in specific situations (e.g., sandy patches near river belts) the same type of situation cannot be created in experimental farms and screening has to be done in such hot spot locations. The appearance of Kalahasti malady is also influenced by various climate factors, even in hot spot locations. Studies of Naidu (1996) on the mode of spread of the disease clearly established that it occurs only through soil infestation with nematode and not through affected pods and seeds from diseased pods. Due to variations in climatic and soil conditions it would be difficult to precisely identify resistant plants/pro
genies, even in hot spot locations.

In this context, the identification of molecular markers associated with nematode resistance is essential to aid in the development of high-yielding Kalahasti malady resistant variety with desirable quality traits (Mehan et al. 1997). Simple sequence repeats (SSRs), also known as microsatell
tites, are a class of molecular markers based on tandem repeats of short (2-6 bp) DNA sequences (Litt and Lutty 1989). These repeat sequences are often highly polymorphic, even among closely related cultivars, due to slippage muta
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et al. 2007), AFLPs (Gimenes et al. 2002) and RAPDs (Dwivedi et al. 2001). Naito et al. (2008) reported that SSR provided the most abundant information and the highest reproducibility among the three DNA fingerprinting techniques i.e., RAPD, AFLP and SSR from their study which involved 116 groundnut genotypes comprising 97 germplasm accessions of A. hypogaea L. originating from 9 countries and 19 wild species of genus Arachis. Angelici et al. (2008) employed microsatellite markers to distinguish the species and accessions of section Rhizomatosae, detecting high variation among accessions. Cuc et al. (2008) and Wang et al. (2007) found SSR markers to be informative and useful for breeding applications in crops like groundnut, which originated through a single and recent polyploidization event followed by successive selection during breeding efforts and hence have a limited genetic background.

The advantages of RAPD and AFLP markers were circumvented by the development of SSR markers because such markers are detected by long primers which have increased specificity and consequently, repeatability, allowing the evaluation of individual loci (Yu et al. 1999). Hoshino et al. (2006) found high transferability and polymorphism of heterologous microsatellite markers from the genus Arachis. Selvaraj et al. (2009) identified SSR markers linked to pod and kernel traits through bulk segregant analysis. Hopkins et al. (1999) reported a primer pair amplifying an (AT)18 SSR located in the 3′ untranslated region of the lectin gene. A total of 14 fragments were amplified and 10 out of the 20 groundnut accessions were differentiated.

Varma et al. (2005) used SSR markers to study intra- and inter-accession polymorphic variation among rust resistant and susceptible parents and to identify SSR markers associated with resistance to rust in two crosses of groundnut. In their study, 14 primers showed inter-accession polymorphic variation. Five SSR primer pairs were common between resistant and susceptible parents between two crosses ‘ICGV 99005’×‘TMV2’ and ‘ICGV 99003’×‘TMV2’ but none of the markers of these five common SSRs were found to be associated with rust resistance; they concluded that the wild species A. stenosperma in the pedigree of resistant parents in ‘ICGV 99003’ and A. batizocoi in ‘ICGV 99005’ might be contributing different genes conferring resistance to rust.

The present study was undertaken to identify SSR markers associated with resistance to Kalahasti malady in a set of resistant and susceptible parents and to verify the association in a large number of genotypes with a known reaction to Kalahasti malady and F1s involving resistant and susceptible parents.

MATERIALS AND METHODS

Plant materials

Seven parents, ‘Tirupati 3’ belonging to Arachis hypogaea subsp. hypogaea var. hypogaea, ‘Kalahasti’, ‘Narayani’, ‘Kadiri 6’, ‘Tirupati 4’ (Fig. 4), ‘Prasuna’ and ‘ICG (FDRS) 79’ belonging to subspecies fastigiatu variety vulgaris and six F1s and 25 genotypes whose reaction to Kalahasti malady is known through earlier studies, constituted the experimental material (Tables 2, 3). The reaction of F1s to Kalahasti malady was recorded by sowing them in a sick plot (in which Kalahasti malady regularly occurs naturally) during kharif, 2010 at the Regional Agricultural Research Station (RARS), Tirupati. Among the parents, ‘Tirupati 3’ (Mehan et al. 1993), ‘ICG (FDRS) 79’ (pers. obs.) and ‘Kalahasti’ (Vasanthis et al. 2011) are resistant to Kalahasti malady, ‘Prasuna’ is moderately resistant and ‘Narayani’, ‘Kadiri 6’ and ‘Tirupati 4’ are highly susceptible to Kalahasti malady (pers. obs.). ‘Tirupati 3’ is an isogenic selection with a solid red testa from ‘TMV10’, a variegated rust resistant accession with a solid red testa from ‘TMV10’, a variegated rust resistant accession with a solid red testa from ‘TMV10’, a variegated rust resistant accession with a solid red testa from ‘TMV10’, a variegated rust resistant accession with a solid red testa from ‘TMV10’. ‘Prasuna’ is derived from a double cross, (/g485ICGV 92069’×/g485ICGV 96246’×/g48592 R/75’). ‘Tirupati 4’ and ‘Kadiri 6’ are all derived from ‘JL 24’×‘Ah613/S’.

<table>
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<tr>
<th>Reference</th>
<th>Locus</th>
<th>Annealing temperatures of SSR markers (°C)</th>
<th>Nature of the marker</th>
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<td>Ferguson et al.</td>
<td>pGPS Seq 1D2F 57</td>
<td>pGPS Seq 12F7</td>
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<tr>
<td>2003</td>
<td>pGPS Seq 1F7E 58</td>
<td>pGPS Seq 17F6</td>
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<td></td>
<td>pGPS Seq 13A7 56</td>
<td>pGPS Seq 13A10</td>
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<td></td>
<td>pGPS Seq 2B10 57</td>
<td>pGPS Seq 3D9</td>
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<td></td>
<td>pGPS Seq 14H6 58</td>
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<td>pGPS Seq 4G2 59</td>
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<td></td>
<td>pGPS Seq 4H1L 60</td>
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<td>pGPS Seq 16G8 59</td>
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<td>pGPS Seq 8D9 62</td>
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<td>pGPS Seq 10D4 63</td>
<td>pGPS Seq 10D4</td>
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<td>pGPS Seq 33A9 61</td>
<td>pGPS Seq 3A1</td>
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<td>Cuc et al. 2008</td>
<td>IPAHM – 689</td>
<td>IPAHM – 103</td>
<td>no amplification</td>
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<tr>
<td></td>
<td>IPAHM – 165</td>
<td>IPAHM – 165</td>
<td>no amplification</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>IPAHM – 105</td>
<td>58</td>
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<td>Hopkins et al. 1999</td>
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<td>Ah – 191</td>
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<td>Ah – 229</td>
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<tr>
<td></td>
<td>Ah – 522</td>
<td>Ah – 522</td>
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<tr>
<td>He et al. 2003</td>
<td>PM – 3</td>
<td>PM – 36</td>
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<tr>
<td></td>
<td>PM – 45</td>
<td>PM – 36</td>
<td>no amplification</td>
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<tr>
<td>Selvaraj et al. 2009</td>
<td>PM – 375</td>
<td>PM – 375</td>
<td>no amplification</td>
</tr>
</tbody>
</table>

DNA isolation

Total genomic DNA was isolated from fresh leaves of 20-25-day-old plants following a modified CTAB method (Saghai-Maroof et al. 1984; Doyle and Doyle 1987) as reported previously in Mace et al. (2003). The quality and quantity of DNA was determined using a Nanodrop spectrophotometer (ND-1000 UV/Vis, Thermo Scientific, USA).

SSR marker genotyping

35 SSR primer pairs (Table 4) (Eurofins Genomics India Pvt. Ltd., whitefield, Bangalore, India and Sigma Aldrich®, Mahadevapura, Bangalore, India) developed by Ferguson et al. (2003), Cuc et al. (2008), Hopkins et al. (1999) and He et al. (2003) were used for screening three resistant, one moderately resistant and three susceptible genotypes. PCR amplifications were performed in a 25-μl reaction volume, which consisted of 1X Taq buffer F (Tris pH9, KCl, Triton-X100 – 10X), 1.5 μl of 25 mM MgCl₂ 0.5 μl of 10 mM dNTP mix, 0.6 μl of each forward and reverse primer, 0.4 μl of 3μl/μl Taq DNA polymerase (Genet, Bangalore, India), 100-250 ng of genomic DNA, and 14.67 μl of sterile milli Q water (Millipore, MilliQ Gradient Century, Millipore in Bangalore, India). The concentrations of primer pairs were optimized individually. Samples were subjected to the following thermal profile for amplification (Eppendorf programmable thermal controller, Hamsburg, Germany). The profile was modified from that of Varma et al. (2005) with an initial denaturation step of DNA at 94°C for 2 min, followed by 35 cycles of amplification consisting of 94°C for 45 sec, 55-65°C for 1 min, and 72°C for 1 min 30 sec.
RESULTS AND DISCUSSION

Among the 35 SSR markers used for screening three resistant, one moderately resistant and three susceptible genotypes to Kalahasti malady, 10 SSR primers were found to be polymorphic. Two primer pairs, pPGS Seq 14H6 and pPGS Seq 16G8 showed distinct polymorphism in resistant and susceptible genotypes to Kalahasti malady.

These two primers pairs were employed to screen 32 genotypes (including seven parents) and hybrids belonging to six crosses involving parents with resistance and susceptibility to Kalahasti malady. The amplification profiles resulting from pPGS seq14H6 primer pair are shown in Figs. 5 and 6. This primer was highly polymorphic among the primers used in the present study. Jiang et al. (2007) reported amplification of eight alleles among 31 genotypes comprising 27 bacterial-resistant genotypes and four highly susceptible genotypes to bacterial wilt with this primer. This primer pair produced 10 amplification products i.e., alleles with sizes from 225 to 800 bp in the material used in the present study. Alleles 225, 260, 275, 310 and 330 bp in size were associated with Kalahasti malady resistance. The amplification products of pPGS seq16G8 primer pair were 190, 210, 230, and 240 bp. Among the alleles, the allele 210 bp in size was associated with resistance to Kalahasti malady.

Polymorphism in parents and hybrids

Among the parents screened, ‘Tirupati 3’ possesses absolute resistance (i.e., almost immune) to Kalahasti malady (Mehan et al. 1993). In this genotype, alleles of pPGS seq14H6 primer at 225, 260, 275, 310, 330, 400 and 475 bp were observed. The genotype ‘Kalahasti’ is a derivative of TCG 1709 ‘×’ Tirupati 3 and is resistant to Kalahasti malady with a score of 1-2 on the 1-5 disease scale (Vasan-thi et al. 1998). In this genotype, alleles of pPGS seq14H6 primer were observed, except for the 310 bp allele, all other amplification products that were seen in ‘Tirupati 3’ were present. ‘JL24’ is highly susceptible to Kalahasti malady (Naidu 1996). The varieties, ‘Narayani’, ‘Kalahasti’, ‘Tirupati 4’ and ‘Tirupati 3’ are selections from the crosses, ‘JL24’×‘Ah316/s’. In ‘Narayani’, only one allele at 250 bp was seen while in ‘Tirupati 4’, while nine alleles were observed but some of them were similar with alleles observed in resistant genotypes, ‘Tirupati 3’ and ‘Kalahasti’. ‘Prasuna’, a moderately resistant genotype, showed amplification-
tion products of size 275, 260, 310, 375, 475 and 500 bp and some products not amplified in resistant/susceptible genotypes i.e., 525, 550, 600, 640, 700 and 800 bp. In total, 11 alleles were amplified in ‘Prasuna’ with pPGS seq14H6 primer. None of the resistant genotypes, ‘ICGV (FDRS) 79’ had alleles of size 225, 230, 345, 475, 500 and 650 bp as in var. ‘Kalahasti’.

As SSR markers are co-dominant in nature, true hybrid plants can be identified through an amplification profile in hybrids. True hybrid plants should possess alleles from both parents i.e., heterozygous condition. In the F1 of ‘Narayani’×‘Tirupati 3’, a hybrid pattern, i.e., alleles from susceptible and resistant genotypes, ‘Narayani’ and ‘Tirupati 3’, were observed. F1 was resistant to Kalahasti malady. F1s of other crosses involving susceptible and resistant genotypes, ‘Tirupati 4’×‘Tirupati 3’ and ‘Kadiri 6’×‘Tirupati 3’, also exhibited heterozygosity with respect to the markers. In F1s of ‘Prasuna’×‘Tirupati 3’, ‘Kalahasti’×‘Tirupati 3’ and ‘Tirupati 4’×‘ICG (FDRS) 79’, heterozygosity was clearly observed confirming hybridity; moreover, one or two alleles not seen in either of the genotypes involved were also observed in F1s.

With 16G8 primer pair, four alleles were amplified in the material studied. Among the alleles, the allele 210 bp in size was associated with resistance to Kalahasti malady. In susceptible genotypes, there was either no amplification or alleles of size 190, 230, and 240 bp were observed. In all the F1s, the allele 210 bp in size was amplified. In ‘Tirupati 4’×‘Tirupati 3’, a hybrid pattern as was found in both parents i.e., alleles of size, 210 and 230 bp, was recorded.

Validation of pPGS seq14H6 and pPGS seq16G8 markers in other genotypes

In addition to screening parents and F1s, these two primer pairs were used for further validation of 25 other genotypes whose reaction to Kalahasti malady is known. In moderately resistant genotypes i.e., ‘TLG 45’, ‘TCGS 876’, ‘ICGS 584’, ‘ICVS 2’, ‘ICG 06138’, either all or some of the alleles found to be associated with resistance i.e., 225, 260, 275, 310, 330 bp, were present (Table 1). In amplification products through pPGS seq14H6 in susceptible genotypes, alleles not found associated with resistance i.e., 225, 275, 310, 330 bp, were present. The F1s of ‘Prasuna’×‘Tirupati 3’ and ‘Kalahasti’×‘Tirupati 3’ were resistant to Kalahasti malady. F1s of ‘Prasuna’×‘Tirupati 3’ and ‘Kalahasti’×‘Tirupati 3’ combination, two alleles were amplified in ‘Prasuna’ with pPGS seq14H6 primer only amplification products of size 190, 230, and 240 bp were detected. In the ‘Prasuna’×‘Tirupati 3’ combination, two alleles 210 and 230 bp in size were amplified.

Screening segregating populations is very difficult as maintenance of a sick plot at the experimental farm is not possible. Hence, the mode of inheritance has not been deciphered so far. In 2002-2003 rabi, there was a moderate incidence of Kalahasti malady in one of the fields of RARS, ‘Tirupati 4’. In the cross, ‘Kalahasti’×‘Tirupati 3’, the F1 segregation was a good fit to a phenotypic ratio of 13 moderately resistant: 3 resistant, a typical ratio indicating the involvement of inhibitory factors, duplicate - dominance kind of interaction (pers. obs.). The screening results of the present study, F1 was resistant indicating that resistance to Kalahasti malady is dominant.

In the present study, alleles of size 225, 260, 275, 310 and 330 bp were amplified by pPGS seq14H6 primer only in genotypes with resistance i.e., ‘Tirupati 3’, ‘Kalahasti’ and moderately resistant genotypes, ‘TLG 45’, ‘ICGS 876’, ‘ICVS 2’.

### Table 4: Sequences of SSR primers used for screening.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5' - 3')</th>
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<tr>
<td>pPGSeq15C10-F</td>
<td>ATGCCAATGTGCATCACCGAC</td>
</tr>
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<td>pPGSeq15C10-R</td>
<td>TCGCAGTCTGACCTGTCAGTT</td>
</tr>
<tr>
<td>pPGSeq3A8-F</td>
<td>ATGGAAGTCCAAGGAACACA</td>
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<tr>
<td>pPGSeq3A8-R</td>
<td>CTGGTGCTGACCTGTCAGTT</td>
</tr>
<tr>
<td>pPGSeq4G2-F</td>
<td>TACGGGCGTCAAGGAACACA</td>
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<tr>
<td>pPGSeq4G2-R</td>
<td>AGTGAAAAATACACCCAACGAA</td>
</tr>
</tbody>
</table>

**Identification of SSR markers associated with resistance to nematode T. brevilineatus infection in groundnut. Vasanthi et al.**
‘Tirupati 2’, ‘Prasuna’, etc. In susceptible genotypes, alleles of different sizes i.e., 240 and 250 bp were amplified with this primer pair. As distinctly different alleles were amplified in resistant and susceptible genotypes, this primer seems to be useful to distinguish resistant and susceptible genotypes. This would help in marker-assisted breeding in the development of high-yielding genotypes with resistance to Kalahasti malady as the markers would be helpful in screening resistant plants in advanced generations of crosses for Kalahasti malady resistance in an experimental farm at the seedling stage. If markers are not available, segregating advanced generations of crosses made to select for resistance to Kalahasti malady have to be grown in hot spot areas in farmers’ fields to identify resistant plants or progenies. Due to variation in climatic and soil conditions it would be difficult to precisely identify resistant plants or progenies. There is a hope for selection of susceptible plants as resistant as the population build-up of nematodes may not be that uniform at different places, even in hot spot locations. The creation of a sick plot at an experimental farm is not safe as the transmission of nematodes is through infested soil and it is also very difficult to maintain a uniform load of nematodes throughout the plot.

Another SSR primer pair, pPGS seq16G8 amplification pattern in resistant and susceptible genotypes was also distinct. Only alleles of size 210 bp were found in resistant and moderately resistant genotypes while alleles of size 190 and 230 bp were found in susceptible genotypes. In F₁s of crosses involving resistant and susceptible genotypes, alleles found in both resistant and susceptible genotypes were found and hybridity could be clearly established through the observed profile. From the results, it is obvious that the pattern observed in resistant and susceptible genotypes has a clear association with resistance and susceptibility respectively with the two primer pairs, pPGS seq14H6 and pPGS seq16G8. The heterozygous pattern of the markers in F₁s and the resistance reaction of F₁s to Kalahasti malady further confirmed the association. However, co-segregation analysis of the markers and phenotypic data i.e. reaction to Kalahasti malady has to be further studied in mapping populations i.e. F₂ populations and recombinant inbred lines which are being developed. On further validation and confirmation of association in mapping populations, these markers would be very useful for routine screening for Kalahasti malady resistance.

As groundnut is an important oilseed crop with high food value, its area under well-managed rabi-summer situation is likely to increase in future. Kalahasti malady would also become a major problem in this situation. As chemical control is not cost-effective, only the development of resistant varieties offers the best solution to the problem. In this context, identification of molecular markers associated with resistance would greatly aid as robust screening tools in the development of high yielding Kalahasti malady resistant varieties with acceptable quality attributes.

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