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Identification of rust resistance in groundnut using a validated SSR marker

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Abstract Groundnut (*Arachis hypogaea* L.) is an important crop cultivated in over 100 countries in the world. The rust disease of groundnut, caused by *Puccinia arachidis* Speg., can cause significant yield losses in tropical and subtropical areas. The disease affects not only seed yield but also fodder yield and quality. There are chemicals available to control rust; however, the development of resistant varieties is the most reasonable way to improve yield and quality, and to reduce the adverse effects of chemicals on the ecosystem. Characterization of germplasm diversity to identify resistant sources using traditional methods is a lengthy process and requires laborious field testing. Molecular marker-aided selection offers an alternative breeding method that is relatively easy, precise, and not affected by environmental fluctuation. In the present study, a validated SSR marker, GM1954, linked to the rust disease resistance gene was used for 256 groundnut genotypes to select rust resistance. This study reports the successful application of marker-assisted selection for further rust-resistant breeding programs in groundnut. Molecular analyses revealed that the banding pattern related to disease resistance was observed at high frequency in the variety *hypogaea* among the nine identified resistant genotypes in the collection. Approximately 3 % of the collection was selected for further field, greenhouse, and hybridization experiments.

Keywords Characterization · Fungal disease · Marker-aided selection · Molecular markers · *Puccinia arachidis* Speg.

Introduction

Cultivated groundnut or peanut (*Arachis hypogaea* L., $2n = 4 \times = 40$) is the only species that has been truly domesticated in the *Arachis* genus, which can be divided into nine sections and includes approximately 80 species (Krapovickas and Gregory 1994). It is native to South America and widely grown in more than 100 countries throughout tropical, subtropical, and warm temperate regions. There are many biotic and abiotic factors that constrain groundnut production in various eco-agricultural systems. Rust (*Puccinia arachidis* Speg.) is one of the important biotic stress factors that greatly affects groundnut yield quantity and quality. The disease causes yield losses in excess of 50 % in semi-arid tropical regions (Subrahmanyam et al. 1989; Waliyar 1991). The appearance of symptoms of peanut rust can be easily recognized when orange pustules (uredinia) first appear on the

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lower surface of the leaflet and rupture to release reddish brown urediniospores (Subrahmanyam et al. 1985). Infected leaves become lethal and completely dry (Mehan et al. 1994). Management of rust disease with fungicides is expensive and the application of chemicals increases the risk to global environmental safety. Development of disease-resistant cultivars seems to be the reasonable solution to control rust disease; however, the identification of resistant genotype processes requires particular, repeated, and comprehensive field and greenhouse screening under expected epidemical conditions, which is laborintensive and time consuming (Mondal et al. 2007). Insufficient disease incidence also complicates the selection of resistant plants in field experiments (Mondal et al. 2014). Although wild species offer high levels of resistance and even apparent immunity, undesirable agronomic traits prevent sustainable production (Leal-Bertioli et al. 2009).

Rust resistance mechanism is complex and has different inheritance patterns such as single gene, partially dominant, non-additive, additive × additive, and additive × dominance, which have been reported on the basis of genetic structure and types of resistance sources (Bromfield and Bailey 1972; Middleton and Shorter 1987; Varman et al. 1991; Mondal et al. 2007). Therefore, conventional breeding studies are insufficient without a genetic mechanism for effective selection. New genomic technologies provide an abundance of molecular markers to identify and follow resistance gene(s) (Varshney et al. 2014). These molecular tools increase the efficiency of selection and would likely be cost effective and faster than field studies. In the last decade, quantitative trait locus (QTL) and genetic mapping studies have been conducted to find markers that are strongly linked to a rust resistance gene. Mondal et al. (2007, 2012 and 2014) have identified two RAPD markers, two SSR markers, and two transposable element markers, respectively. A large number of QTLs have been identified in different mapping populations. Twelve QTLs were identified on the basis of genetic mapping of two RIL populations by Khedikar et al. (2010). Another QTL analysis was conducted by Sujay et al. (2012) and 15 QTLs were detected for rust resistance. A major QTL (82.96 % PVE) showed a significant association with the markers (IPAHM103, GM2009, GM1536, GM2301, GM1954, and GM2079). Recently, these identified markers have been compared with field rust disease scores and validated by Yeri et al. (2014), Gajjar et al. (2014), Varshney et al. (2014), and Sukruth et al. (2015). These validated markers would therefore be of great practical value to accelerate peanut breeding programs with high accuracy in selecting disease-resistant genotypes (Sukruth et al. 2015). In view of these developments, this study was aimed at identifying new genetic sources of rust resistance in 256 groundnut genotypes using molecular markers previously reported to be associated with resistance.

Materials and methods

Plant material

The 256 groundnut accessions were used as a genetic stock in the present study. The list and details of the material were reported by Yol et al. (2015). This collection included the ICRISAT groundnut mini core collection (Upadhyaya et al. 2002), landraces, advanced breeding lines, and cultivated varieties. The seeds of 256 genotypes were grown at the West Mediterranean Agricultural Research Institute at Antalya, southern Turkey (36°52′N, 30°50′E, and altitude15 m), during 2013 and 2014. The genotypes ICG 4389 (resistant) and ICG 4750 (susceptible) were tested in the field by Sudini et al. (2015) and were used as controls in the present study to identify resistant and susceptible categories on the basis of validated marker profiles.

Molecular analysis

Genomic DNA was extracted from the fresh leaves of 256 groundnut genotypes using the CTAB method (Doyle and Doyle 1990). The quality and quantity of the extracted genomic DNA were estimated on 1 % (w/v) agarose gels by comparison with a DNA standard. The DNA extracts were diluted in Milli-Q PCR water and stored at −20 °C until use.

The validated SSR marker, GM1954 (Table 1) was used to screen the germplasm by a touchdown PCR protocol (Sujay et al. 2012; Sukruth et al. 2015). Reactions were performed in a total volume of 20 µl composed of 2 µl of 10× PCR buffer, 2.5 mM $MgCl_2$, 0.8 mM dNTP mix, 1 µM each of forward and reverse primers, 1 unit of *Taq* DNA polymerase (Fermentas Life

Table 1 Details of SSR marker linked to the rust resistance in groundnut

Marker	Primer sequence		Annealling Tm. (°C)	Resistance allele (bp)	Susceptible allele (bp)	References
	Forward (5′–3′)	Reverse (5′–3′)				
GM1954	GAGGAGTGTGAGGTTCTGACG	TGGTTCATTGCATTTGCATAC	59.7	120	123	Nagy et al. (2010)

Sciences, Burlington, Canada), and 1.5 µl of genomic DNA template and Milli-Q water to make up the final volume. Amplification of the SSR marker was carried out in a programmable thermocycler (BIONEER, MyGenieTM) under the following conditions: one cycle of 94 °C for 5 min, 35 cycles of 94 °C for 30 s, annealing (first 65 °C for 30 s and decreasing by 1 °C/cycle for the initial five cycles), extension (72 °C for 30 s), and one cycle of final elongation at 72 °C for 10 min (Sukruth et al. 2015). All reactions were performed twice. Amplification of PCR products was confirmed on 2 % agarose gels followed by automated capillary electrophoresis (Fragment Analyzer™, Advanced Analytical Technologies GmbH, Heidelberg, Germany) of amplified PCR products. In this capillary system, the 96-Capillary-33-55 Array-DNF-900 Reagent Kit Method was used for qualitative analysis of DNA fragments ranging from 35 to 500 bp, which were normalized by using the markers for 35 and 500 bp fragments. Raw data were analyzed using PROSize™ software (Version 1.2.1.1) (Advanced Analytical Technologies, AMES, IA, USA). Amplified bands were scored as resistant (R) or susceptible (S) according to a previous report by Sukruth et al. (2015).

Results and discussion

The 256 groundnut genotypes were screened for rust resistance using the validated SSR marker, GM1954. Molecular analyses indicated that almost all genotypes in the groundnut collection possessed a resistance or susceptible gene as revealed by the expected bands corresponding to different markers. The banding patterns were monitored using high-resolution biomonitoring technology (Fig. 1).

The marker GM1954 was shown to be related to rust resistance (Sujay et al. 2012; Sukruth et al. 2015), and was used to characterize available accessions in the groundnut collection. Molecular analysis showed that this resistance-related marker was observed in nine genotypes, which indicated the rust-resistant fragment of 120 bp (Fig. 1) (Table 2), while a 123-bp fragment identified in 243 genotypes was related to susceptibility. There was no amplification for four genotypes following PCR. Genotyping with the GM1954 marker revealed that only approximately 3 % of the collection was positive for resistance to rust in groundnut.

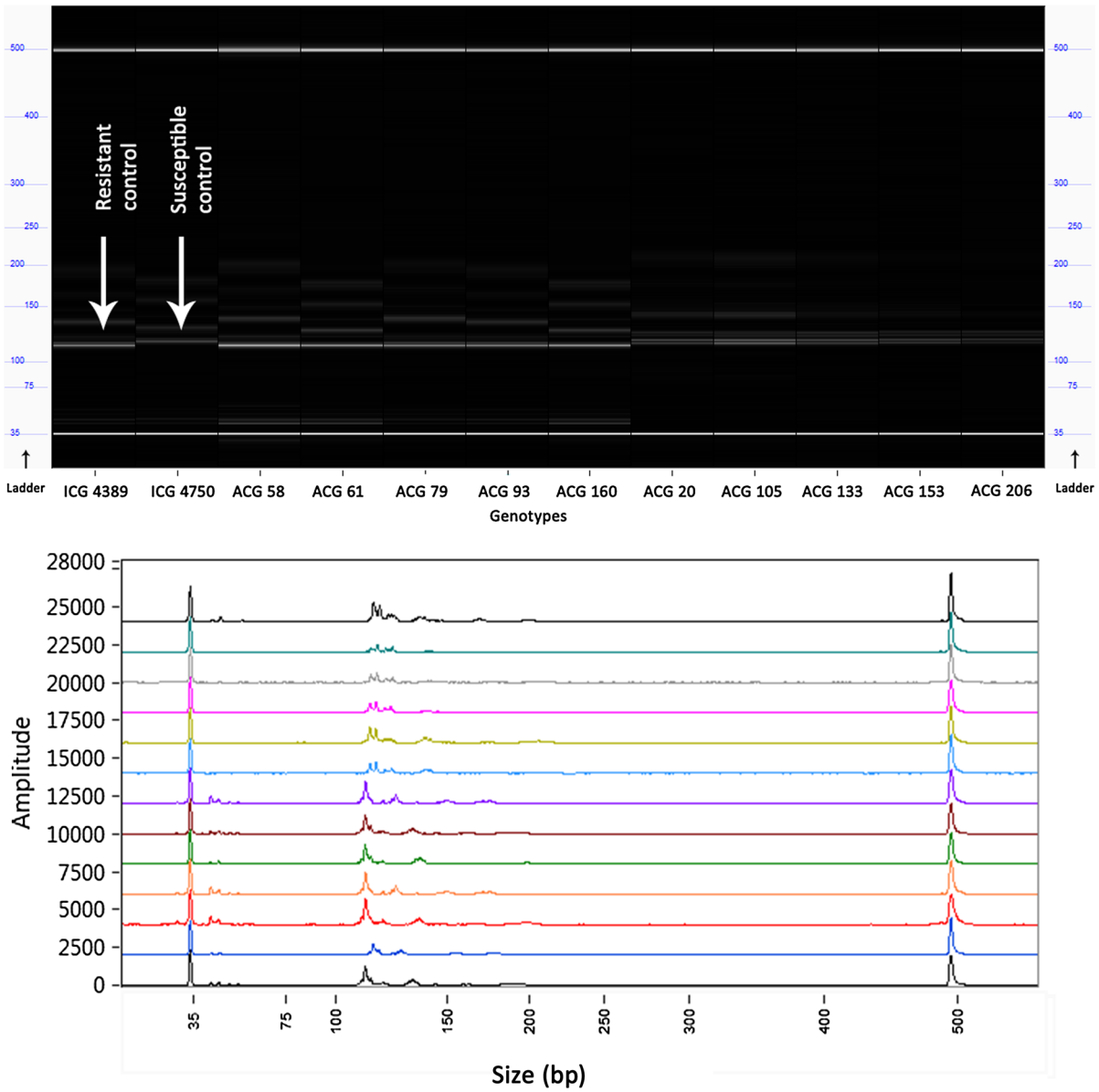

Fig. 1 Fragment Analyzer™ shows the gel picture and peak analysis graphic for the selected resistant/susceptible genotypes amplified by validated rust resistance associated marker, GM1954. Resistant and susceptible controls are ICG 4389 and ICG 4750, respectively

The cultivated groundnut is divided into two subspecies, ssp. *hypogaea* and ssp. *fastigiata* (Gregory et al. 1980), and six botanical varieties *hirsuta, hypogaea*, *fastigiata*, *aequatoriana, peruviana,* and *vulgaris*. This classification is important because commercially grown market types (Runner, Virginia, Spanish, and Valencia) were derived from these botanical varieties (Krapovickas and Gregory 1994). In this study, eight identified resistant genotypes belonged to subsp. *hypogaea* var. *hypogaea* while one resistant genotype, ACG 58, was from subsp. *fastigiata* var. *fastigiata* (Table 2). Generally, available rust-resistant genotypes belong to var. *fastigiata,* which mostly originated from Peru, a secondary gene center of primitive *fastigiata* types (Subrahmanyam et al. 1993). Previously, more than 13,000 genotypes were handled and 169 genotypes were scored as rust resistant (Subrahmanyam et al. 1995), 80 % of the

Table 2 Association of rust resistant marker with the genotypes of groundnut collection

Accession No.	ICRISAT Genebank entry (ICG)/Cultivar Name	Subspecies	Botanical variety	Marker GM1954
ACG 55	ICG 4389	*hypogaea*	*hypogaea*	R*
ACG 58	ICG 4538	*hypogaea*	*hypogaea*	R
ACG 61	ICG 4670	*fastigiata*	*fastigiata*	R
ACG 79	ICG 5663	*hypogaea*	*hypogaea*	R
ACG 93	ICG 6667	*hypogaea*	*hypogaea*	R
ACG 95	ICG 9766	*hypogaea*	*hypogaea*	R
ACG 160	ICG 13099	*hypogaea*	*hypogaea*	R
ACG 212	Swallow	*hypogaea*	*hypogaea*	R
ACG 216	Osmaniye	*hypogaea*	*hypogaea*	R

* R is resistant

identified resistant genotypes belonging to subsp. *fastigiata* var. *peruviana* (Singh et al. 1997). Liao (2003) screened 5700 accessions and 92 of them showed rust resistance, most of the resistance sources belonging to var. *fastigiata* and var. *peruviana*. However, these genotypes had undesirable agro-morphological characters, including low yield, thick pods, and noncommercial coat colors. The present study therefore reports different varieties for rust resistance with different agronomical backgrounds.

The collection including the ICRISAT groundnut mini core collection (Upadhyaya et al. 2002) was also evaluated using the validated marker. The present study identified nine rust resistant genotypes, seven of them being part of the mini core collection (approximately 4 %, 7 out of 184 accessions). The resistant banding pattern was observed at a high frequency in the variety *hypogaea*. The presence of *Sclerotinia* blight resistance in the mini core collection was also investigated by Yol et al. (2015), who found that the resistant banding pattern was more evenly distributed among the variety *vulgaris* and less distributed among the variety *hypogaea*. Of the nine rust resistance genotypes identified in the current investigation, the genotype ACG 61 was also associated with *Sclerotinia* resistance, as reported earlier by Yol et al. (2015), which would be highly useful for pyramiding in groundnut.

The correct identification of a marker linked to a specific trait is critical for marker-assisted breeding because large numbers of genotypes are eliminated after molecular screening. In this molecular analysis, approximately 3 % of the collection was selected for further field, greenhouse, and hybridization studies. The marker used in this study was validated using RILs and elite and popular varieties, and a positive correlation was observed between field and molecular analyses with regard to rust resistance (Sujay et al. 2012; Varshney et al. 2014; Sukruth et al. 2015). This marker could therefore be directly used for marker-aided selection in breeding studies. However, marker GM1954 has moderate phenotypic variance (Sujay et al. 2012) and further field studies are needed to validate its use and to determine the different environmental effects on rust resistance, the mechanism of which is complex, as are the QTL-environment interactions that control the effects of disease resistance (Mondal and Badigannavar 2015). Agronomical selection for the selected genotypes will also be conducted in the fields because groundnut is an industrial crop and the genotypes should meet the expectations for optimal commercial exploitation.

Conclusion

The validated SSR marker employed for screening of rust resistance is well established and effective. The obtained results showed nine rust resistant genotypes, eight of them (subspecies *hypogaea*, botanical variety *hypogaea*) belonging to Virginia or Runner market types, which are frequently used in the food industry. These selected genetic materials may therefore be used as a gene pool to obtain superior commercial types and to improve rust resistance in groundnut using marker-assisted or conventional breeding.

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