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Review

Advancements in molecular marker development and their applications in the management of biotic stresses in peanuts

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ABSTRACT

Peanut is grown extensively in different parts of world, where various biotic and abiotic factors limit its productivity and quality. The major fungal biotic constraints to peanut production include rust (*Puccinia arachidis* Speg.), stem-rot (*Sclerotium rolfsii*), collar-rot (*Aspergillus niger* Van Teighem), afla-root (*Aspergillus flavus*), and late leaf spot (*Phaeoisariopsis personata* Ber. and M A Curtis), while viral disease constraints are peanut bud necrosis disease (PBND) caused by peanut bud necrosis virus (PBNV) and peanut stem necrosis disease (PSND) caused by tobacco streak virus (TSV). Since, only a few sources of resistance are available in cultivated peanut for some diseases, which has resulted in the limited success of conventional breeding programmes on disease resistance. Moreover, even marker assisted breeding in peanut is in the nascent stage and identification of some major quantitative trait loci (QTLs) for a few fungal disease resitance genes has only recently been reported. Substantial efforts are underway to develop PCR-based markers for the construction of high-density genetic linkage maps. This will enable the breeders to effectively pyramid various biotic stress resistance genes into different agronomically superior breeding populations, in a much shorter time. It is expected that the availability of various cost-effective genomic resources (SNPs, whole genome sequencing, KASPar, GBS etc.) and more effective mapping populations (NAM, MAGIC etc.) in the coming years will accelerate the mapping of complex traits in peanut. This review provides an overview of the current developments and future prospects of molecular marker development and their applications for improving biotic-stress resistance in peanut crop.

1. Introduction

Peanuts (*Arachis hypogaea* L.), also known as groundnuts, are grown in more than 120 countries with different agro-climatic zones between latitudes 40 °S and 40 °N on approximately 21–24 M ha of land annually (Sarkar et al., 2014). It is cultivated predominantly by small farms under low input conditions and ranks third and fourth as a source of protein and edible oil, respectively (Bhauso et al., 2014). Several biotic stresses are known to limit peanut productivity, and their severity and extent of distribution vary with the cropping system, growing season, and region. Among biotic stresses, several diseases including rust (*Puccinia arachidis* Speg.), early leaf spot (ELS, *Cercospora arachidicola*), late leaf spot (LLS, *Phaeoisariopsis personata* Ber. and M A Curtis), and aflatoxin contamination by *Aspergillus flavus* and *Aspergillus parasiticus* are global constraints against peanut production (Subrahmanyam et al., 1984; Waliyar, 1991). Rust, stem-rot (*Sclerotium rolfsii*), collar-rot (*Aspergillus niger* Van Teighem), and leaf spots are also quite serious and together may cause the loss of 50–60% of pod yield in India (Dwivedi et al., 2003; Subrahmanyam et al., 1985). In the peanut growing regions, high yielding, well-adapted cultivars contain multiple resistances to biotic stresses that can provide enhanced and sustainable peanut production (Dwivedi et al., 2003).

The world's largest peanut germplasm collection with more than 15,000 accessions is housed at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India (Gowda et al., 2013). These accessions have many differences in their vegetative, reproductive, physiological, and biochemical traits. The global *Arachis* gene pool possesses the source of resistance to many biotic stresses, including rust, ELS, LLS, Groundnut Rosette Disease [GRD, caused by a complex of three agents: groundnut

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rosette virus (GRV), its satellite RNA (sat RNA), and a groundnut rosette assistor virus (GRAV)], Peanut Bud Necrosis Virus (PBND), *A. flavus* induced aflatoxin contamination, bacterial wilt (*Ralstonia solanacearum*), leafminer (*Aproaerema modicella*), *Spodoptera,* jassids (*Empoasca kerri* Pruthi), thrips (*Frankliniella schultzei* Trybom) and termites (*Odontotermes* sp.) (Rao et al., 2002; Basu and Singh, 2004; Amin et al., 1985; Rao et al., 2014).

Since the 1960s, interspecific hybridization has received much attention in peanuts because several wild *Arachis* species show a very high level of resistance to many biotic stresses, such as rust, ELS, LLS, and stem rot (Holbrook and Stalker, 2003; Singh et al., 1984). However, success in transferring the resistance to cultivated peanuts has been limited mainly because of cross compatibility barriers, linkage drag, and long periods required for developing stable tetraploid interspecific derivatives (Wynne et al., 1991; Singh et al., 1997). Moreover, the partial and polygenic nature of biotic stresses makes the identification of resistant and susceptible lines very tedious using conventional screening techniques (Leal-Bertioli et al., 2009). Because of the frequent occurrence of multiple diseases, peanut yields are often significantly lower than their potential (Holbrook and Stalker, 2003). In the future, cultivars with multiple disease and pest resistances will be needed, which appears to be a very difficult endeavour for this crop species (Basu and Singh, 2004).

Marker-assisted selection (MAS) offers great promise for improving the efficiency of conventional plant breeding (Janila et al., 2013), including the potential to pyramid resistance genes in peanuts (Mishra et al., 2009; Varshney et al., 2014; Pandey et al., 2012). For any molecular breeding program, assessment of genetic diversity and development of genetic linkage maps are two very important steps (Dwivedi et al., 2003). Abundant polymorphisms in wild *Arachis* species have been observed, but progress in the molecular breeding of cultivated peanuts is greatly constrained due to the low level of detectable molecular genetic variation (Mondal et al., 2005; Herselman, 2003; Raina et al., 2001; He and Prakash, 2001). Therefore, the use of more robust assays such as single nucleotide polymorphisms (SNPs), competitive allele-specific PCR (KASPar) and genotyping by sequencing (GBS) approaches are needed. However, cost-effective SNP genotyping platforms are not readily available for tetraploid peanuts, but a large number of robust markers such as SSRs and SNPs (including KASPar) would be valuable. SSRs are still considered the marker of choice in peanuts (Pandey et al., 2012), and a wide range of genotypes have been used for mapping (Table 1) of many important biotic and abiotic traits using SSR markers (Table 2).

Despite being an important oilseed crop, very limited work in the area of molecular genetics and breeding of peanuts has been performed (Dwivedi et al., 2002; Raina et al., 2001). However, over the last decade, significant developments have been made in the use of various molecular approaches for biotic stress management in peanuts, and new efforts such as functional genomics are likely to play key roles in the future (Wang et al., 2011; Varshney et al., 2014; Gajjar et al., 2014). Recently, Kanyika et al. (2015) has identified 376 polymorphic SSR markers in 16 African groundnut cultivars with a wide range of disease resistance. These identified markers can be used to improve the efficiency of introgression of resistance to multiple important biotic constraints into farmer-preferred varieties of Sub-Saharan Africa. In this review, we made an attempt to capture the recent updates in molecular marker development and their applications in the management of various biotic stresses in peanut.

2. Markers associated with rust and LLS resistance gene(s)

Rust and leaf spots are economically very important foliar fungal diseases of peanuts that often occur together and not only reduce the yield but also adversely affect the fodder and seed quality (Subrahmanyam et al., 1985; Waliyar, 1991). Despite the economic importance of rust and LLS, very limited work has been carried out on host–fungus interaction, fungal genetic diversity, and physiological specialization (Mondal and Badigannavar, 2015). Several studies have emphasized the application of different types of molecular markers, construction of peanut linkage maps, or tagging of important agronomic traits, such as disease resistance (Wang et al., 2011; Gajjar et al., 2014). Recently, many DNA markers have been found to be putatively linked to rust and LLS resistance genes (Mondal et al., 2012a; Khedikar et al., 2010; Shoba et al., 2012; Sujay et al., 2012) (Table 2), a few of which have been validated and used in the breeding programme (Sujay et al., 2012; Gajjar et al., 2014; Varshney et al., 2014). Location of markers on the various linkage groups in Table 2, is derived after doing intensive meta-analysis of all the published literature, including the most comprehensive and consensus linkage maps available in peanut.

Validation of other linked markers will accelerate the process of introgression of disease resistance into preferred peanut genotypes (Sujay et al., 2012; Gajjar et al., 2014). Near isogenic lines (NILs) developed for rust resistance were thoroughly screened with both foreground and background molecular markers (Yeri et al., 2014). For the identification of LLS resistance, Luo et al. (2005b) identified genes in the resistant genotype that were more highly expressed than in the susceptible genotype (in response to *Cercosporidium personatum* infection) by microarray analysis and validated them by real-time PCR. In a recombinant inbred line (RIL) population (VG 9514 × TAG 24), two transposable element (TE) markers, TE 360 and TE 498, were found to be associated with the rust resistance gene. These two markers need further validation before they could be effectively applied for MAS of rust resistance in different backgrounds (Mondal et al., 2013).

3. Soil-borne fungal diseases and associated markers

(Collar rot, Stem rot, Aspergillus spp., Bacterial wilt and Sclerotinia blight)

Among soil-borne diseases, collar rot (*A. niger*) and stem-rot (*S. rolfsii*) are very important (Farr et al., 1989; Kolte, 1984). The search for peanut cultivars resistant to *S. rolfsii* originates all the way back

Table 1
List of a few genotypes, used for mapping of various resistance gene(s) (Dwivedi et al., 2003; Shoba et al., 2012; Sujay et al., 2012).

Traits	Genotypes
Early leaf spot	ICG 405, ICG 1705, ICG 6284, TMV 2
Late leaf spot	GPBD 4, ICGV 99001, ICGV 99004, COG 0437, TAG 24, TMV 2
Rust	GPBD 4, ICGV 99003, ICGV 99005, TG 26, TMV 2
Rosette disease	ICG 6323, ICG 6466, ICG 11044, JL 24
Bacterial wilt	ICG 7893, ICG 15222, and Chico
Aflatoxin production	U 4-7-5, 55-437, J 11