

**SIMPLE SEQUENCE REPEAT POLYMORPHISM SCREENING FOR
RESISTANCE TO *CERCOSPORA ARACHIDICOLA* IN GROUNDNUT
(*Arachis hypogaea* L.) GENOTYPES**

BY

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DECLARATION

I, **Busisiwe Tamara Chali Ncube Kanyika**, declare that the work presented in this dissertation is my own and has never been submitted for a degree at this or any other university.

Signature:

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CERTIFICATE OF APPROVAL FORM

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ABSTRACT

Arachis sp. cultivation in Africa is largely by small holder farmers that lack the resources to counteract the negating effects of foliar disease Early Leaf Spot (ELS) caused by *Cercospora arachidicola*. *C. arachidicola* causes yield losses which on interaction with other pests and/ or diseases and favourable weather conditions aggravate yield losses in Sub-Saharan Africa. The utilisation of chemical control methods is not economically feasible for the small holder farmer and requires additional skill, a tedious regime for effective application and in addition, is toxic to the environment. Hence, reduced yields impinge on the income and nutrition of third world communities.

Resistant genotypes are available but have not been adequately accepted due to limited farmer preferred characteristics and other quality traits. The tediousness of handling a self pollinating species in gene introgression by interspecific hybridisation and the general mode of inheritance to disease resistance complicates the transfer of resistance. Resistance is a low heritability trait governed by quantitatively inherited recessive genes that are controlled by epistatic effects and are closely associated to the environment. Simple Sequence Repeat (SSR) molecular markers are environmentally stable and highly informative. These markers have therefore been identified as useful in hastening breeding programmes in *Arachis* sp.

The overall objective of this study was to identify highly informative SSR markers across 16 genotypes of farmer preferred and other improved African *Arachis* sp. germplasm traits that may be effectively linked to ELS disease resistance. The markers were graded on a scale of 1–4 according to ease of scoring. A total of 394 showed Polymorphic Information Content (PIC) values ranging from 0.06 to 0.86, giving a total of 1476 at an average of 3.7 alleles per locus and so distinguishing the 16 species. Markers of grade 1–3, high availability and PIC were carried forward to establish a dissimilarity matrix to highlight the most appropriate pair wise combinations for resistance breeding studies. The matrix ranged from the most closely related genotypes 0.34 (MGV5: ICGV 90704) to the most distantly related 0.66 (Chalimbana: 47–10). The mean dissimilarity matrix value was 0.51. A total of 139 (35 percent) polymorphic marker locations were ascertained with reference to pre-existing genome maps for future utilisation in Quantitative Trait Loci mapping.

The most informative SSR markers for 16 cultivated *Arachis* sp. indigenous to Sub-Saharan Africa were compiled. These can be used to improve the efficiency of introgression of ELS resistance into farmer preferred varieties and also other molecular marker assisted crop improvement studies for foliar disease resistance and quality traits of significance to the smallholder farmer in Sub-Saharan Africa.

This work is not only a stepping stone in Zambian research but has the potential to improve the livelihood of the whole Sub– Saharan community. I therefore, dedicate this work to all young African scientists willing to make a difference.

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LIST OF ABBREVIATIONS

\$	United States Dollar (currency)
%	percent
.AFT	almost free text file
.ARB	alphacam router vb macro file
.DIS	oracle discovery workbook file
.DON	textur editor file
.VAR	variable data file
µg	microgram
µl	microlitres
µM	micromolar
°C	degrees Celsius
AFLP	amplified fragment length polymorphism
Ag ⁺	silver ions
ANOVA	analysis of variance
BES	bacterial artificial chromosome end sequences
bp	base pairs
C ₂ H ₄	ethylene

CAPS	cleaved amplified polymorphic site
cDNA	complementary DNA
CoA	co enzyme A
CSO	central statistical office
CTAB	cetyl trimethyl ammonium bromide
cv.	cultivar
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotides
ELS	early leaf spot
eq 50%	equivalent concentration of 50% to be applied
EST	expressed sequence tag
<i>et al.</i>	and others
FAO	Food and Agriculture Organisation
GAM	genetic advance as percentage of mean
GCV	genotypic co-efficient of variation
GGC	guanine, g; cytosine, c
GRD	groundnut rosette disease
GRV	groundnut rosette virus

h^2	heritability
ha	hectare
Hg	hectograms
ICRISAT	International Crops Research Institute For The Semi-Arid Tropics
kg	kilogram
LAI	leaf area index
LCMS	living conditions monitoring survey
LG	linkage group
LLS	late leaf spot
LWR	leaf weight ratio
MAB/S	marker assisted backcross/ selection
Mbp	mega basepair
mM	millimolar
mm	millimetre
NA	not available
ng	nanograms
NJ	neighbour joining
nm	nanometer

O/L	oleic to linoleic acid ratio
of OTUs	operational taxonomic units
PCoA	principal coordinate analysis
PCR	polymerase chain reaction
PIC	polymorphic information content
QTL	quantitative trait loci
RAPD	randomly amplified polymorphic DNA
RFLP	restriction fragment length polymorphisms
RGH	resistance gene homologs
RIL	recombinant inbred line
rtp	room temperature and pressure
SADC	Southern African Development Community
SB	sclerotina blotch
SNP	single nucleotide polymorphisms
sp.	species
SSR	simple sequence repeats
subsp.	subspecies
ton(s)	tonnes

UV	ultraviolet
w/v	weight by volume
WP	wettable powder
μ	micro
ρ mol	picomoles

CHAPTER ONE

1.0 INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a cleistogamous annual crop belonging to the Family Leguminosae (Singh and Oswalt, 1991; Ntare, 2007). Cultivated groundnut is a self-pollinated allotetraploid of monophyletic origin (Bechara *et al.*, 2010). It has a genome of 2891 Mbp and chromosome length ranging from 1.4 to 3.9 μm (Holbrook and Stalker, 2003). It has been proposed to have arisen from a unique cross between the wild diploid species *A. duranensis* (A-genome) and *A. Ipaënsis* (B-genome) (Pandey *et al.*, 2012 b; Wang *et al.*, 2012; Upadhyaya *et al.*, 2011). Seijo *et al.* (2007) based on Genomic in Situ Hybridisation (GISH) proposed tetraploid *A. monticola*, as its immediate progenitor. Subspecies within *Arachis hypogaea* are subsp. *hypogaea* and subsp. *fastigiata*. Subsp. *hypogaea* ('runner type') includes the 'Virginia' type groundnut. It is characterised by a more prostrate growth habit without flowering branches on the main stem and with the cotyledonary lateral branches carrying alternate pairs of vegetative and reproductive secondary branches; it is usually late-maturing (Ntare, 2007). Subsp. *fastigiata* ('bunch type') includes the 'Spanish' and 'Valencia' types of groundnut. It is characterised by an erect growth habit with flowering branches on the main stem, and without a regular pattern in the sequence of vegetative and reproductive branches; it is early-maturing (Ntare, 2007).

A. hypogaea L. has been utilised as an important source of income as it is valued as an affordable source of edible oil rich in omega-3 fatty acids, high protein content (Izge *et al.*, 2007) and vitamin E (Pandey *et al.*, 2012 b). The ratio of Oleic to

Linoleic acid has an important bearing on the stability of the oil; the higher the ratio, the more stable the oil and shelf life. The ratio in mature seeds can range from less than 1.0 to greater than 3.0 (Ntare, 2007). The high dietary fibre, ash and α , α -diphenyl- β -picrylhydrazyl (antioxidant) in the shells and roots confer medicinal properties for use in prebiotic nutrition and pharmaceutical production (Sim *et al.*, 2012). Prebiotic nutrition varies from probiotic nutrition; a composition of living microorganisms favourable in stabilising and replenishing gut microbial flora. Prebiotic nutrition being dietary fibre that selectively stimulates favourable gut micro flora critical in conferring resistance to a variety of gastro-intestinal illnesses, colon cancer, obesity and Diabetes Mellitus Type II (Roberfroid *et al.*, 2010). Usefulness has also been shown in commercial production of dyes, plastics and resins (Pandey *et al.*, 2012 b). In Sub-Saharan Africa where inputs are costly, groundnuts are superior in that they fix nitrogen and so improve soil fertility and reduce dependence on nitrogenous fertilisers for their cultivation and for other crops such as maize (Monyo *et al.*, 2009) in crop rotation and most especially in an intercrop system.

Groundnuts are an affordable source of nutrition and so have a sustainable market demand and therefore, cardinal in overall poverty reduction. Assessment studies in Uganda (Alwang and Siegel, 2003; Moyo *et al.*, 2007; Kassie *et al.*, 2010) have shown the positive impact of improved groundnut yield on the income and nutrition of communities. Studies have shown that agricultural research and development improves productivity which is critical in sustainable health, nutrition and income of communities (Mausch and Bantilan, 2011, Monyo *et al.*, 2007).

The nutritional and commercial value is most significant in Africa where undernourishment from 2007–2008 increased by 10 percent with an increase in the

price of nutritious foods (FAO, 2011). With regards to Zambia, as of 2006, the poverty head count ratio at national poverty line (percent of population) was at 59.3 percent (World Bank, 2006) and malnutrition prevalence, weight for age (percent of children under five years of age) at 14.9 percent (World Bank, 2007). Fifty-four percent (54.2 percent) of children under five years of age exhibit stunted growth and are underweight (LCMS, CSO–Zambia, 2006). In rural areas, provincial poverty levels in Zambia range from 72 percent to 84 percent with a higher percent of malnourished children (LCMS, CSO–Zambia, 2006).

Approximately 62 percent of the Zambian population is agricultural (FAOSTAT, 2012). With reference to Figure 1.1 and Table 1.1, the total land harvested for groundnut has increased from 2006 to 2010 (FAOSTAT, 2010). In spite of an increase in the total land harvested for groundnuts, there has been a reduction in total yield attained per hectare. Total yield output was highest in 2006 with the lowest area harvested. Overall production has increased from 2008 to 2010 from 70,527,000 kilograms to 163,733,000 kilograms (Figure 1.1; Table 1.1). In spite of this, the mean yield (633.6 Kg/Ha) over the five years is below that of the mean yield of Africa (949.2 Kg/Ha) and the World (1,578.4 Kg/Ha) (Figure 1.1; Table 1.1). With an estimated mean population increase of 2.7 percent per year from 2006 to 2012, production and yield are not sufficient to meet the demands of the growing Zambian population (FAOSTAT, 2012).

A major constraint in the high yielding potential of groundnuts in Eastern and Southern Africa (Singh and Oswalt, 1992; Monyo *et al.*, 2007) has been the prevalence of the fungal foliar disease Early Leaf Spot (ELS) caused by *Cercospora*

arachidicola Hori and teleomorph *Mycosphaerella arachidis* Deighton (asci and septated ascospores) (Haciwa *et al.*, 1990; Izge *et al.*, 2007).

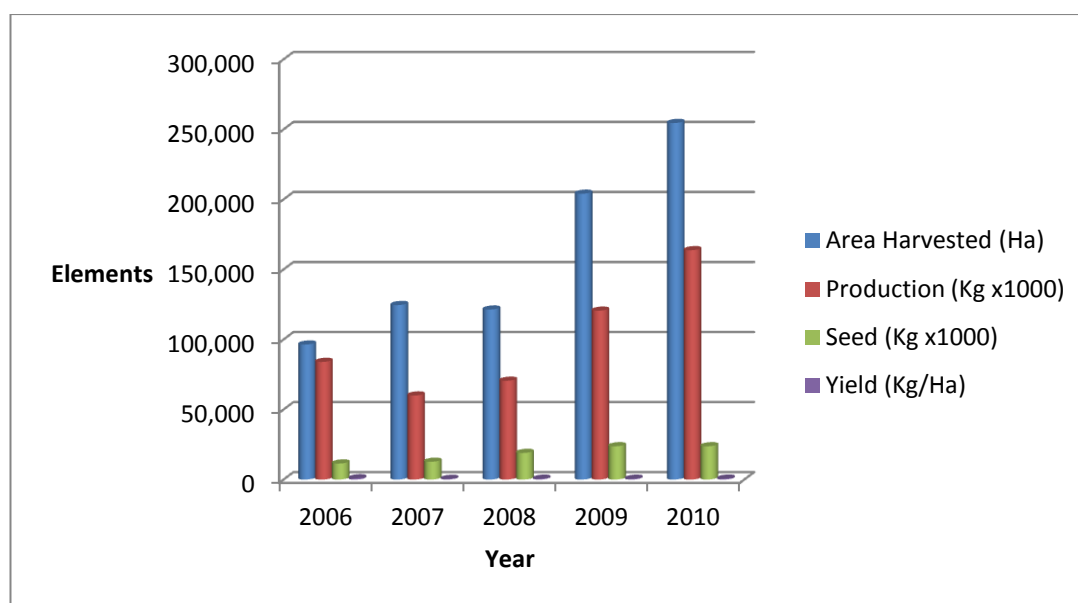


FIGURE 1.1: *Arachis* sp. production and yield trend in Zambia 2006 to 2010. (FAOSTAT, 2010)

TABLE 1.1: *Arachis* sp. production and yield in Zambia in comparison to Africa and World mean values 2006 to 2010.

Elements	Year (Zambia)					Mean Values		
	2006	2007	2008	2009	2010	Zambia	World	Africa
Area Harvested (Ha x 1000)	96.4	124.6	121.4	204.1	254.6	160.2	23,203.7	10,231.1
Production (Kg x 1000)	84,010	60,000	70,527	120,564	163,733	99,767	36,617,763	9,669,816
Seed (Kg x 1000)	11,440	12,690	19,019	23,760	23,760	18,134	1,750,577	622,661
Yield (Kg/Ha)	871.5	481.6	581.1	590.8	643.2	633.6	1,578.4	949.2

(FAOSTAT, 2010)

This has been found to cause 10 to 50 percent yield losses in combination with Groundnut Rosette Disease (GRD) (Babu *et al.*, 1995; McDonald *et al.*, 1985). Early Leaf Spot has resulted in 35 to 50 percent defoliation at peak flowering stage and yield losses may reach 20 to 25 percent (Mehan and Hong, 1994).

In advanced stages of ELS infection, necrosis and defoliation occur reducing the total Leaf Area Index (LAI) and Leaf Weight Ratio (LWR), these two attributes subsequently result in a drastic reduction in yield (Forrer and Zadocks, 1983). Furthermore, the additional stress reduces vigour and thereby aggravates cell death and is thus noted by the occurrence of peg rot and root deterioration (McDonald *et al.*, 1985; Cantonwine *et al.*, 2007).

C. arachidicola, the causative agent of ELS, is a soil borne pathogen that is dispersed directly from conidia that emerge from mycelia in conditions of high relative humidity and in temperatures ranging from 25 to 30°C (McDonald *et al.*, 1985). Factors that play a critical role in the harbouring of the pathogen are contaminated soil debris and seed/volunteer plants. Dispersal of conidia is aided by wind, splashing rain and insects play a critical role in dispersal of the conidia (McDonald *et al.*, 1985).

The pathogenicity of *C. arachidicola* Hori is linked to the production of photo activated Cercosporin which results in damage to nuclear membranes and cell organelles of the host plant enhancing symptoms and disease severity (Herrero *et al.*, 2007; Daub *et al.*, 1992; Daub and Chung, 2007).

Significant efforts have been made to prevent infection via conventional practices by use of fungicides, cultural practices and biological control. These have however, been limited in their overall efficacy. Chemical control by use of fungicides has high cost implications that the small holder and rural farmer are economically incapacitated to adopt (Coffelt and Porter, 1986). In Zambia, the minimum average cost is \$5.29 per kilogram of fungicide (Copper hydroxide eq 50 percent WP) (AMITSA, 2012). Cultural practices are limited in that overall efficiency is dictated

by interaction with other control methods (Royal *et al.*, 1997). Biological control by the use of mycoparasites, *Dicyma pulvinata* and *Verticillium lecanii*, have been shown to parasitize *Cercospora* sp. However, studies are largely limited to greenhouse experiments and have not been carried out under spontaneous or simulated farm conditions for evaluation of efficacy (Kishore and Podile, 2002).

The full success of a breeding programme requires that farmers accept an improved variety (Bucheyeki *et al.*, 2008; Monyo *et al.*, 2007).Therefore, in addition to pest and disease resistance, farmers consider traits of drought resistance, duration, quality characteristics and high yield. It has been found necessary to introgress resistance genes with respect to farmer preferences for successful adoption of improved varieties (DFID, 2002).

A number of varieties of accessions of wild diploid species that exhibit combined resistance and tolerance to abiotic and biotic stresses exist and these may vary among accessions of the same species (Singh and Oswalt, 1991). Natural polyploidisation and self pollination have resulted in a narrow genetic base of tetraploid varieties (Pandey *et al.*, 2012 b; Singh and Oswalt, 1991). The narrow genetic base limits the diversity of resistance traits and the variations in ploidy levels complicates the introgression of resistance traits by conventional breeding from wild to cultivated species. The only viable method of introgression is Interspecific Hybridisation. In addition, *A. hypogaea* being a self pollinated species requires additional time, labour and skill in emasculation and crossing. The simplest trait introgression has involved either the doubling of diploid hybrids or diploid species for genetic compatibility with the cultivated variety. *A. monticola* is currently the only identified tetraploid wild species that exhibits compatibility with *A. hypogaea* (Seijo *et al.*, 2007). Slight

complexity of trait introgression has involved the crossing of a diploid wild type with a tetraploid cultivated genotype (Singh and Oswalt, 1991). In spite of these limitations, varieties that have resistance to foliar diseases in groundnuts have been identified and with constraints, have still been successfully utilised for resistant gene/trait introgression by conventional breeding methods.

Resistance to ELS is a low heritability, quantitative and independently inherited recessive trait closely associated to environmental and epistatic effects (McDonald *et al.*, 1985; Kornegay *et al.*, 1980; Anderson *et al.*, 1986; Neville, 1982). Therefore the detection and transfer of resistance is rendered elusive. The detection of resistance at a molecular level is necessary for effective and hastened introgression of resistance to ELS. Various efforts have been made over the past two decades within *Arachis* sp. to detect polymorphic molecular markers associated with genes for disease resistance and grain quality (Stalker and Mazingo, 2001; Luo *et al.*, 2004; Semagn *et al.*, 2010) and differentiation of species (Halward *et al.*, 1992). Simple Sequence Repeats (SSR) molecular markers have gained broad applicability as opposed to other molecular markers. This is attributed to their co-dominance, simplicity, high polymorphism, repeatability and multi allelic nature (Kumar *et al.*, 2009). Their usability has been in phylogenetic (Raina *et al.*, 2001) and disease resistance studies (Leal-Bertioli *et al.*, 2009; Qin *et al.*, 2011). The detection of polymorphic Simple Sequence Repeats (SSRs) is critical in hastening the identification of appropriate parents in pre-breeding programmes and early identification of genotypes to carry forward in marker assisted resistance gene introgression studies (Semagn *et al.*, 2010). The detection of SSRs is therefore, a stepping stone to the biochemical and genetic appreciation of plant-pathogen interactions (Asins, 2002).

In summary, the main problem in groundnut cultivation is centred on the susceptibility to ELS of farmer preferred varieties inspite of resistant cultivars being available. This in turn reduces total yield output and reduces economic gain from the cultivated harvest. With respect to this particular study the key solution to this conundrum would be to speedily transfer resistance into varieties preferred by the farmer by use of marker assisted breeding as opposed to the lengthy transfer of resistance by conventional breeding methods. In this study SSR markers were employed as the initial phase in achieving resistant farmer preferred varieties within a reduced time frame.

1.1 RESEARCH OBJECTIVES

1.1.1 Overall Objective

The overall objective of this project was to identify Simple Sequence Repeat (SSR) markers linked to Early Leaf Spot (ELS) resistance in farmer preferred and other improved African *Arachis* sp. germplasm.

1.1.2 Specific Objectives

The objectives of this study were,

1. To assess the genetic diversity among 16 groundnut genotypes using SSR markers.
2. To compile a dissimilarity matrix of the analysed groundnut genotypes that can serve as a tool kit for breeders to identify suitable parents for mapping populations or marker–assisted introgression.

3. To select a subset of SSR markers evenly spread across the groundnut genome for future ELS resistance QTL mapping.

CHAPTER TWO

A REVIEW OF EARLY LEAF SPOT DISEASE IN *ARACHIS* *HYPOGAEA* L.

2.1 GROUNDNUT FOLIAR DISEASES

A major constraint in the yielding potential of groundnuts in Eastern and Southern Africa (Singh and Oswalt, 1992) has been the prevalence of viral Groundnut Rosette disease (GRD) and fungal diseases Rust and Early Leaf Spot (ELS). The successful transmission of GRD by *Aphis leguminosae* and *Aphis craccivora* is achieved by the transfer of a complex of three components i.e., Umbravirus Groundnut Rosette Virus (GRV), Groundnut Assistor Luteovirus (GRAV) and satellite RNA (Sat-RNA) (Storey and Bottomley, 1928; Murant *et al.*, 1988; Demler *et al.*, 1996; Murant, 1990). The causative agents of fungal diseases are: Rust– *Puccinia arachidis* Speg and Early Leaf Spot (ELS) – *Cercospora arachidicola* Hori, teleomorph *Mycosphaerella arachidis* Deighton (asci and septated ascospores) (Haciwa *et al.*, 1990; Izge *et al.*, 2007). An additional fungus of significance is the contaminating *Aspergillus flavus/parasiticus* which has resulted in aflatoxin contamination that causes death (Wu *et al.*, 2011) and severe post harvest economic losses to farmers (Richard and Payne, 2003).

2.2 ECONOMIC SIGNIFICANCE OF ELS

Early Leaf Spot (ELS) is an endemic fungal disease which has been of highest prevalence for over 15 years in Eastern and Southern Africa (Singh and Oswalt, 1992).

With Zambia's neighbour Malawi, on station and on farm losses of 34.3 percent and 18.9 percent respectively were recorded in 1995 totalling \$4.79 million in mean annual loss (Babu *et al.*, 1995). Overall, yield losses vary from 10 to 50 percent depending on the agro-ecological location, season and interaction with other diseases. That is, GRD and ELS (10 to 50 percent) (Babu *et al.*, 1995; McDonald *et al.*, 1985), ELS (20 to 25 percent) (Mehan and Hong, 1994) and Rust and ELS (10 to 50 percent) (McDonald *et al.*, 1985). The prevention in certain instances may play a counteractive role in the onset a related fungal disease Late Leaf Spot (LLS) and so contribute to counteracting additional prevalence of LLS.

2.3 OCCURENCE AND CHARACTERISTICS OF ELS DISEASE

Early Leaf Spot (ELS) disease is caused by *Cercospora arachidicola* a soil borne intracellular (Daub and Ehrenshaft, 2000) pathogen that is dispersed directly from conidia that emerge from mycelia in conditions of high relative humidity and warm temperatures ranging from 25–30°C (McDonald *et al.*, 1985). A high relative humidity could be due to an extended rainy season, night dew and frequent irrigation (µMETOS User Manual, 2001). Contaminated soil debris and seed/volunteer plants, wind, splashing rain and insects play a critical role as agents of dispersal of the conidia and ascospores. Initial infection proceeds after ten days from the basal leaves and is characterised by mild chlorotic circles of penetrating mycelia on the adaxial

surface which after five days develop into one to ten millimetre subcircular lesions. The lesions are dark brown to reddish brown and light brown to orange on the adaxial and abaxial leaflet surfaces respectively. The change in colour intensity is as a result of maturation into septated conidiophores. Distinctly margined oval–elongate lesions are formed on the stems, petioles and pegs (McDonald *et al.*, 1985). A yellow halo borders the lesions of mature leaves. The number of lesions is inversely proportional to temperature (Waliyar *et al.*, 1994) and directly proportional to wetness in susceptible and partially resistant varieties (Wu *et al.*, 1999). Figure 2.1 below shows a simple schematic pathway of the disease cycle for ELS.

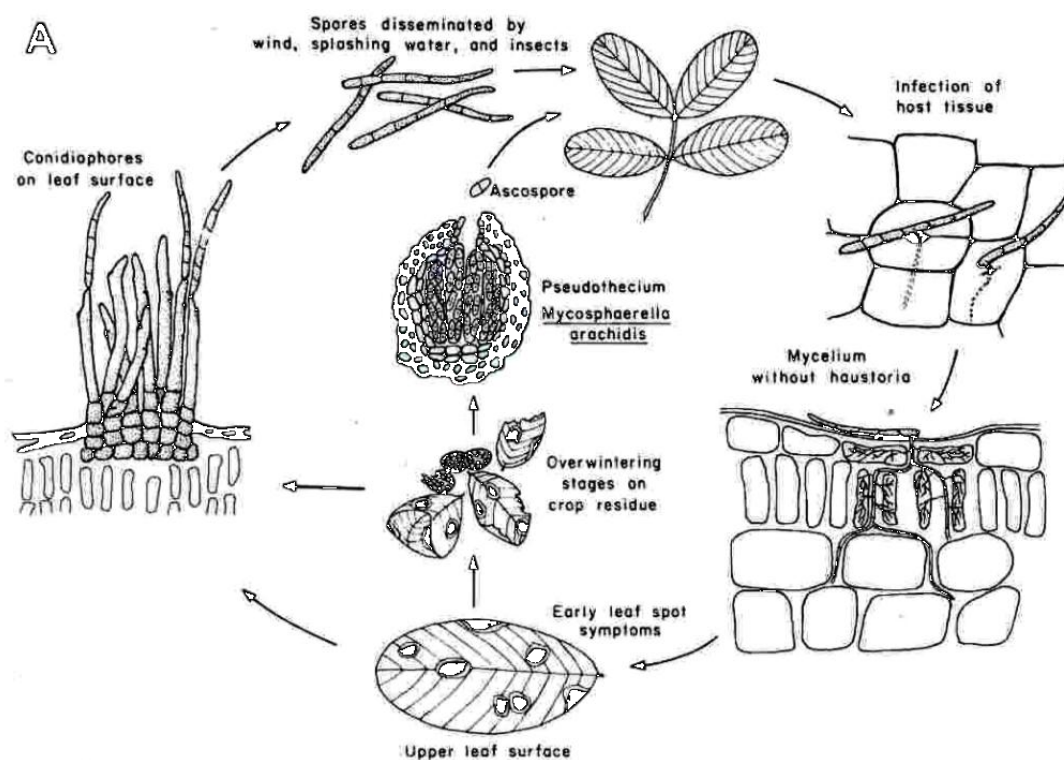


FIGURE 2.1: Disease cycle for Early Leaf Spot caused by *Cercospora arachidicola*. (McDonald *et al.*, 1985)

In advanced stages of ELS infection, necrosis and defoliation occur reducing the total Leaf Area Index (LAI) and Leaf Weight Ratio (LWR), subsequently reducing yield (McDonald *et al.*, 1985; Cantonwine *et al.*, 2007). The invasion of *C. arachidicola*

results in a phytohormone response facilitating the early onset of plant senescence. This host– pathogen interaction was early established in a study by Ketring and Melouk (1982) that confirms that enhanced defoliation in susceptible varieties arises from the active release and action of Ethylene (C₂H₄). This additional stress reduces the plants ability to maintain itself due to a loss in vigour which further aggravates cell death. This is noted by peg rot and root deterioration (McDonald *et al.*, 1985; Cantonwine *et al.*, 2007).

2.4 PATHOGENICITY OF *C. ARACHIDICOLA* HORI

The pathogenicity of *C. arachidicola* Hori is linked to the production of cercosporin. It is a perylenequinone synthesised from initial condensation of Acetyl CoA with 6– Malonyl CoA in a complex six step biosynthetic pathway. Regulation is by positive feedback of the zinc cluster transcription factor, CRG1, CTB gene cluster and the regulator *CTB8* (Daub and Chung, 2007). Photoactivation facilitates the provision of cell nutrients for fungal nutrition (Daub and Ehrenshaft, 2000). It leads to the production of highly toxic reactive oxygen species (singlet and superoxide) that damage nuclear membranes and cell organelles of the host plant for fungal nutrition enhancing symptoms and disease severity (Herrero *et al.*, 2007; Daub *et al.*, 1992). The molecular structure of cercosporin is shown below in Figure 2.2.

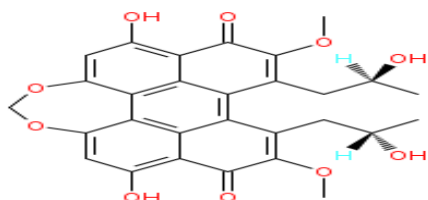


FIGURE 2.2: Cercosporin– C₂₉H₂₆O₁₀.
(<http://www.chemspider.com/chemical-structure.10188562.html> accessed 07/05/2014)

2.5 PLANT RESISTANCE MECHANISMS TO *C. ARACHIDICOLA*

Resistance after penetration is associated with the formation of a barrier in advance of and around the infection site. Defence is in the form of cell wall swelling and thickening and the deposition of pectic substances on the cell walls and in intercellular spaces (Abdou *et al.*, 1974).

2.6 THE SCREENING OF ELS IN GROUNDNUTS

Preliminary and advanced screening of early leaf spot is on a one to nine scoring scale (Table 2.1) for disease development. Disease development is recorded 28 to 42 days after inoculation against the parameters; defoliation, leaf area damage, infection frequency, lesion diameter and duration to sporulation or latent period (Singh and Oswalt, 1992).

TABLE 2.1: Description of leaf spot disease severity rating scale (1 to 9) (McDonald *et al.*, 1985)

Score	Description Disease severity	(%)
1	No disease	0
2	Lesions largely on lower leaves; no defoliation.	1–5
3	Lesions largely on lower leaves; very few lesions on middle leaves; defoliation of some leaflets evident on lower leaves.	6– 10
4	Lesions on lower and middle leaves, but severe on lower leaves; defoliation of some leaflets evident on lower leaves.	11– 20
5	Lesions on all lower and middle leaves; over 50 percent defoliation of lower leaves.	21– 30
6	Lesions severe on lower and middle leaves; lesions on top leaves but less severe; extensive defoliation of lower leaves; defoliation of some leaflets evident on middle leaves	31– 40
7	Lesions on all leaves but less severe on top leaves; defoliation of all lower and some middle leaves.	41– 60
8	Defoliation of all lower and middle leaves; lesions severe on top leaves and some defoliation of top leaves evident.	61– 80
9	Defoliation of almost all leaves leaving bare stems; some leaflets may be present, but with severe leaf spots	81–100

These parameters are largely dependent on temperature and the degree of genotype susceptibility (Waliyar *et al.*, 1994).

2.7 CONVENTIONAL METHODS ADOPTED TO MANAGE EARLY LEAF SPOT

2.7.1 Fungicides

Commonly used fungicides are Azoxystrobin, Chlorothalonil, Flutoloni, Penthiopyrad, Pyraclostrobin, Prothioconazole, Tebuconazole, Propiconazole and Fentin hydroxide. These may be supplied in combination or as sole fungicide products (Shew, 2012). Sulphur/ Copper (Coffelt and Porter, 1986) dust has proved effective when applied on early morning dew (Ntare, 2007).

Fungicide usage has proved variable in its effectiveness and adoption. Small scale farmers have not adopted fungicides due to the increase in production cost against low yielding varieties (Coffelt and Porter, 1986). In addition to the cost implication is the need for necessary skills, tools and lengthy protocols that vary with respect to fungicide supplier recommendations and weather conditions. It is further noted with regards to fungicide supplier recommendations that an effective fungicide program under farmland conditions requires a minimum of five sprays and three fungicides (Shew, 2012). Such a regime may be modified only with reference to a certified advisory programme. This regime therefore, further puts a high minimum restriction on the overall cost of effective fungicide usage. The dependence on ideal weather conditions also plays a limiting role in efficient usage of fungicides as this is reliant on rainfall and an effective irrigation facility. The unavailability of well established clean water sources also limits effective usage (Culbreath *et al.*, 2002).

The level of toxicity is also a limiting factor in fungicide usage. Fentin hydroxide an affordable fungicide is an organotin compound that is an acutely toxic chemical (Kegley *et al.*, 2010). It is a carcinogen and may result in reproductive abnormalities in humans and other mammals (Kimbrough, 1976).

The use of Pyraclostrobin a strobilurin–type fungicide applied at a lower rate showed efficiency equivalent to that of existing fungicides applied at higher rates (Culbreath *et al.*, 2002). This showed continuous improvement in available fungicides by total reduction in applied concentrations (Culbreath *et al.*, 2002). However, chemical control still impacts the environment negatively and presents the risk of resistant pathogens (Brent and Holloman, 2007). Azoxystrobin, Pyraclostrobin and Tebuconazole pose a high risk of resistance and so usage must be coupled with a structured regime/ protocol. Chlorothalonil a low cost fungicide has been shown to aggravate spider mites and make Sclerotinia Blight (SB) worse (Shew, 2012).

Cell damage by *C. arachidicola* further increases the production of stress ethylene which further increases lesion diameter. The use of 150 mg/l of Silver (Ag^+) has been shown to block ethylene biosynthesis and thus counteract defoliation (Ketrang and Melouk, 1982). The delay in defoliation by application Ag^+ is practical in delaying disease severity and therefore valuable in giving a time allowance for the effective use of fungicide treatment (Biles *et al.*, 1990).

Hence, fungicides must be critically balanced in terms of consistent cultural practice, ease and scheduling of application, toxicity, environmental sustainability and affordability for profit and eventual price of the commodity. Commercial farmer systems are able to meet the financial demand for use of chemical control by

fungicide application as opposed to the majority smallholder and rural farmers of Sub-Saharan Africa that function on a low input resource farming system (Subrahmanyam *et al.*, 1997). In order to curb yield losses and counteract the unavailability of fungicides, cultural practices have been adopted to limit infection.

2.7.2 Cultural Practices

Key cultural activities in limiting infection are; four year crop rotation with nonleguminous crops, well fertilised crops, late planting and removal of excess debris by deep burying and burning or disposal as animal feed. During crop growth, removal of volunteer crops and consistent weeding to prevent alteration of the crop microclimate is critical as this may favour fungal development (McKenzie *et al.*, 1997) enhancing disease prevalence (McDonald *et al.*, 1985). For credence of cultural practices, it was found by Royal *et al.* (1997) that the presence of weeds reduced fungicide deposition by 20 to 40 percent, and increased the incidence of ELS by 10–20 percent. The predicted groundnut yield loss ranged from 16 to 39 percent depending on the weed species used in the study. Furthermore, slow decaying broken leaves from plants that demonstrated a level of tolerance to ELS infection were shown to have a higher sporulation rate and so increased the risk of enhanced infection of healthy intact leaves (Ricker *et al.*, 1985). The use of herbicides regimes incorporating Ethalfluralin and Paraquat as opposed to manual weeding have shown reduction in yield and stunted growth (Grichar and Dotray, 2010) and so showing that cultural practices are not detrimental to overall crop physiology. The mechanical method of Strip-tillage which may be classified as a modern cultural technique retains soil nutrients and delays the onset of ELS by a minimum of seven days and so reduces the total fungicide usage (Cantonwine *et al.*, 2007).

Thus, cultural activities are mandatory in all cultivation practices. Variation in disease onset and severity with similar practices in locations across the Southern Africa Development Community (SADC) was shown in a study by Subrahmanyam *et al.* (1997). Cultural practices must therefore, be optimised to each location. This therefore reduces the overall sole efficiency of a single cultural regime across the Sub– Saharan region.

2.7.3 Biological Control

Biological control may provide plant protection through induction of host plant defence systems, elimination of plant signals that trigger pathogen development or competition for nutrients. They may also confer antibiotics, siderophores, ammonia and hydrolytic enzymes antagonistic to the fungal pathogen species (Lam and Gaffney, 1993).

Bacillus cereus strain 304, a chitinolytic bacterial antagonist showed great potential by significantly reducing the severity of ELS in two of three field trials of chitin–amended leaves and on leaves treated with chitin plus the chitinolytic *B. cereus* (Kokalis–Burelle *et al.*, 1992).

The use of mycoparasites, *Dicyma pulvinata* and *Verticillium lecanii*, have been shown to parasitize *Cercospora* sp. However, studies are limited to greenhouse experiments (Kishore and Podile, 2002).

Notably, significant efforts have been made to prevent infection via conventional practices. However, thoroughly economical and reliable solutions have been ambiguous. The most effective and economical control is the development and use of resistant varieties. Conferred pre–harvest host resistance is economical to growers,

leaves no harmful residue in the environment and is compatible with other control measures (Brown *et al.*, 1999).

2.8 BREEDING FOR RESISTANCE TO EARLY LEAF SPOT DISEASE

Cultivated varieties of *Arachis* sp. are tetraploid whilst wild varieties are diploid and possess genes for resistance to fungal foliar diseases, drought tolerance and nematodes. There is combined resistance to fungal foliar diseases in many accessions of wild species and these resistances may be different among accessions of the same species. The variation in ploidy levels complicates the introgression of resistance traits by conventional breeding from wild diploid species to tetraploid cultivars by any method other than Interspecific Hybridisation (Pandey *et al.*, 2012 b). This limits the diversity of resistance traits in cultivated groundnut. In addition, *A. hypogaea* being a self pollinated species requires additional time, labour and skill in emasculation and crossing (Singh and Oswalt, 1991). Natural polyploidisation and self pollination have resulted in a narrow genetic base of tetraploid varieties (Pandey *et al.*, 2012 b). In spite of these limitations, varieties that have resistance to foliar diseases in groundnuts have been identified, and with constraints, have still been successfully utilized for resistant gene/trait introgression by conventional breeding methods. The chromosomal mechanism of gene introgression arises from reciprocal recombination and/ or translocation (Garcia *et al.*, 2006).

Overall success of breeding for resistance is achieved by Participatory Breeding programmes that focus on both disease resistance and farmer preferred traits for final selection and development of an ideal variety (Waliyar *et al.*, 2007; Monyo *et al.*, 2007). In general, farmers base good quality on high yield, large fully developed, bold and spotless pods, clear colour, flavour and oil content of kernels, a high

shelling percentage, early maturity, drought tolerance and low production costs (Waliyar *et al.*, 2007; Monyo *et al.*, 2007). It has been noted that the incorporation of resistance traits from wild to cultivated species does not alter quality traits such as flavour and so the modified cultivar (cv.) is not unfavourably modified (Tallury *et al.*, 2008).

Sources of resistance are also available from various research institutions (McDonald *et al.*, 1985). Continual breeding projects at ICRISAT Malawi, have identified ICGV–SM 07502, ICGV–SM 07505, and ICGV–SM 07508 as promising materials for ELS resistance whilst ICGV–SM 95714 continues to be used for ELS resistance introgression (Monyo *et al.*, 2009) and varieties ICGV–1S–96805 and ICGV–SM–93534 are resistant to ELS, LLS and Groundnut Rosette Disease (GRD) (Iwo and Olorunju, 2009). Abdou *et al.* (1974) classified *Arachis* sp. into highly resistant, moderately and highly susceptible based on pathogen germ tube penetration. Ricker *et al.* (1985) evaluated 20 *Arachis* genotypes identifying NC 3033 and NC–GP 343 with heritable resistance to *Cercospora arachidicola*. However, transferability was limited in that offspring arising from NC 3033 x NC–GP 343 performed below that of the individual parents and therefore rendering the hybrids invalid for commercial use and highlighting the complications associated with breeding for resistance to *Cercospora arachidicola* in groundnuts.

The modified Bulk–pedigree method with Single Seed Selection has shown success in the development of ICGV86699 (*Arachis batizocoi* x *A. duranensis*) x *A. hypogaea* (CV NC2) in four subsequent generations. This variety has shown tolerance to ELS and tolerance/resistance to other diseases and insect pests (ICRISAT, 1996). Triploid interspecific hybridisation has been used in crosses

involving trait introgression from *A. cardenasii* and *A. batizocoi* into *A. hypogaea* (cv. NC 6 and Argentine) (Garcia *et al.*, 2006). Cultivar 91 PA 150 derived from *A. cardenasii* has been ranked highly resistant to *C. arachidicola* in temperatures ranging from 24 to 38 °C (Waliyar *et al.*, 1994). The use of *A. batizocoi* in crosses has been successful in trait introgression but has raised concern in that this particular species also carries susceptibility to LLS and other diseases and may hence carry the risk of incorporation of unfavourable traits into the cultivated variety (Upadhyaya *et al.*, 2011). There is, therefore, a need for a highly intensive breeding strategy to minimise on selection for varieties susceptible to LLS in breeding with *A. batizocoi*. High resistance to *C. arachidicola* have been shown in varieties ICGV–SM–93531, ICGV–IS–96802, ICGV–IS–96827 and ICGV–IS–96808 in a study by Izge *et al.* (2007) based on levels of disease incidence for selection and in 22 wild and cultivated varieties (Fávero *et al.*, 2009). Iwo and Olorunju (2009) evaluated 23 advanced lines, selecting nine varieties of composite resistance and high pod yield.

It has been noted that the early maturing lines show positive and direct correlation to high pod yielding ability, kernel yield and leaf spot tolerance (Iwo and Olorunju, 2009; Izge *et al.*, 2007). This allows for an indirectly integrated trait breeding approach for ideal agronomic traits and resistance. Vishnuvardhan *et al.* (2012) estimated high Genotypic Co-efficient of Variation (GCV) accompanied by high heritability (h^2) and Genetic Advance as percentage of Mean (GAM) for disease severity (susceptibility) and number of immature pods per plant indicated amenability for phenotypic selection in early generations.

2.9 GENETIC ENGINEERING

The transformation of plants to confer resistance to Cercosporin at a transcriptional level would be most ideal either by enhancing synthesis of antioxidants or by use of gene silencing technologies. The incorporation of genes coding for synthesis of lytic enzymes for degradation of the fungal cell wall in fungal disease resistance is an option worth exploring. Site specific synthesis would most certainly have to be put into consideration to slacken pathogen resistance (Brown, 2006).

Daub and Chung (2007) studies on the biosynthetic pathway of Cercosporin production and the resistance of *Cercospora* sp. elucidated the complex interaction among environmental factors (nutrients, Pyridoxine biosynthesis, temperature and Light: 450 to 490 nm) and other genetic factors. Their study revealed the complexity of understanding of the genetic modification of crop tissue for resistance.

2.10 THE MODE OF INHERITANCE OF EARLY LEAF SPOT RESISTANCE

Studies have been conducted to determine the mode of inheritance of resistance. Late and Early Leaf Spot genetics are very similar. Resistance to ELS is a low heritability, quantitative and independently inherited recessive trait closely associated to environmental and epistatic effects (Anderson *et al.*, 1986; Kornegay *et al.*, 1980; McDonald *et al.*, 1985; Neville, 1982).

The close association of susceptibility to environmental conditions, low heritability, quantitative inheritance and epistasis show a need to identify gene interaction at the molecular level and to link secondary traits for selection and development of resistant high yielding varieties. This must further be coupled to resistance gene pyramiding for stable yield. There is a need to understand genetic resistance

mechanisms at the molecular level and use specific molecular techniques to hasten and enhance the output of cultivar resistance breeding programs (Luo *et al.*, 2005; Semagn *et al.*, 2010).

2.11 GENETIC MARKERS AND QTL ANALYSIS IN PLANT BREEDING

There are two distinct classes of genetic markers i.e. classical and DNA markers. The former comprise morphological, cytological and biochemical markers while the latter are broadly termed as molecular markers (Jiang, 2012; Bagali *et al.*, 2010).

Morphological markers are easily discerned visually. However, they are most often influenced by the environment, limited in number and not ideal for selection of economically important quantitative traits such as yield (Jiang, 2012; Farooq and Azam, 2002 a). Cytological markers enable the physical identification of chromosome patterns by karyotyping techniques. These are useful in physical mapping and linkage group identification. However, their sole use in genetic mapping and plant breeding is limited and requires combination with other molecular techniques (Jiang, 2012). Biochemical markers are co-dominant markers limited in number, low in polymorphism and may vary with respect to tissue location (Farooq and Azam, 2002 a; Kumar *et al.*, 2009). Their usability is centred largely on isozyme chemistry as a basis for differentiation of gene products by analysis of electrophoretic mobility as a result of amino acid substitution (Jiang, 2012). The utilisation of classical markers is largely reliant on the environment and developmental growth phase of the plant most especially biased to the mature or reproductive phase.

Molecular markers are DNA marker sequences that result from mutations (such as base deletion, insertion and substitution) and so exhibit variation among individuals of a species (Jiang, 2012). Molecular markers are superior to classical markers in that they are: heritable, polymorphic, phenotypically neutral, developmentally and environmentally stable. Furthermore, they are detectable in young juvenile plants and are not concealed by morphological genotype–environment interactions (Park *et al.*, 2009; Kumar *et al.*, 2009).

The most ideal DNA markers must meet the following criteria as highlighted by Jiang (2012) and Kumar *et al.* (2009): high polymorphism, well distributed across genome, co–dominance, distinct alleles, single copy and no pleiotropic effect, cost–efficient, amenable to automation, neutral to environmental conditions, high reproducibility, specific and not detrimental to the phenotype. Molecular markers are identified by methods of Southern Hybridisation, Polymerase Chain Reactions (PCR) and Gene Sequencing and are therefore classified with respect to the stated methods (Jiang, 2012; Bagali *et al.*, 2010). That is: Southern Hybridisation markers–Restriction Fragment Length Polymorphisms (RFLP); PCR based markers–Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Microsatellites/ Simple Sequence Repeats (SSR); Gene Sequencing Markers–Single Nucleotide Polymorphisms (SNP).

Restriction Fragment Length Polymorphism (RFLP) is the earliest of DNA marker types (Botstein *et al.*, 1980). This method relies on deletions or insertions at specific endonuclease restriction sites resulting in length variations of restriction enzyme digestion products of homologous chromosomes. Detection of polymorphism is by use of gel electrophoresis and DNA probe hybridisation (Jiang, 2012). The markers

are co-dominant and are useful for synteny and comparative mapping and studies in disease linkage. The method is robust, reliable, simple and transferrable between laboratories (Farooq and Azam, 2002). Shortfalls are the requirement for large amounts of quality DNA, low amenability to automation and low genotyping throughput (Jiang, 2012).

Randomly Amplified Polymorphic DNA (RAPD) is based on enzymatic amplification of target or random DNA segments with arbitrary primers and visualisation by gel electrophoresis from which presence/ absence scoring is done (Kumar *et al.*, 2009). These markers have shown applicability in genetic characterisation (Bagali *et al.*, 2010). These markers are highly polymorphic and do not require prior sequence information. The overall method is simple and amenable to automation. Shortfalls are a lack of co-dominance and low reproducibility (Jiang, 2012).

Amplified Fragment Length Polymorphism (AFLP) relies on the enzymatic digestion and amplification of restriction fragments (Farooq and Azam, 2002). Applicability has been in phylogenetic studies in distinguishing genotypes, genetic relatedness and genetic map construction. The applicability is attributed to its' generation of highly informative fingerprinting profiles (Kumar *et al.*, 2009). The method has a high multiplex ratio and genotyping throughput and is reproducible across laboratories. However, shortfalls are the lack of co-dominance, requirement for large amounts of quality DNA, poor chromosome distribution and the method is complicated and expensive (Jiang, 2012).

Microsatellites/ Simple Sequence Repeats (SSR) are conserved regions on transcribed and untranscribed locations in both the nuclear and cytoplasmic genome.

They are characterised by tandem repeats of nucleotide motifs ranging from one to seven base pairs (adenine, guanine, cytosine and thymine) per nucleotides long. The most common repeats are di-, tri- and tetra-nucleotide repeats. The variability in the number of repeats between and within individuals of a species confers polymorphism which is detectable by PCR based methods (Jiang, 2012; Farooq and Azam, 2002; Kumar *et al.*, 2009).

SSRs are currently the most popular of molecular markers with broad applicability in phylogenetic studies, genetic mapping studies, diversity studies and Quantitative Trait Loci (QTL) mapping (Jiang, 2012). The method is favourable in that it requires low quantities of DNA due to the high copy number conferred by PCR, is easy to use, highly amenable to automation and highly reproducible. The overall cost of analysis in large studies is reduced by the multiplexing and high throughput genotyping applicability of SSRs (Kumar *et al.*, 2009). They are co-dominant; highly polymorphic, highly abundant and well distributed across eukaryotic genomes with favourable bias in low-copy regions (Kumar *et al.*, 2009). The use of SSR markers in this particular study/ project was based on these attributes to screen for ELS resistance. Furthermore, the SSR marker database in *Arachis* sp currently exceeds 10, 000 of which those chosen for this study were as compiled by a study that created a main, all inclusive and descriptive database of polymorphic SSR markers in *Arachis* sp. (Zhao *et al.*, 2012).

Single Nucleotide Polymorphisms (SNPs) are the most recent of automated genotyping based markers. The name is derived from its ability to detect a single nucleotide base substitution. This may also be referred to as a point mutation and occurs very commonly in eukaryotes hence increasing the usability of SNPs. They

are highly useful in phylogenetic studies, gene mapping studies and have increased usefulness as genome sequences for various species become more available. The favourability of attributes in methodology are ranked as highly as that of SSRs with the exception of high start up costs, the need for high quality DNA and cost effectiveness is largely restricted to highly comprehensive studies (Jiang, 2012).

Molecular markers in quantitative loci studies have proved effective in enhancing breeding of foliar disease resistant groundnuts and other crops (Park *et al.*, 2009; Kumar *et al.*, 2009).

Marker Assisted Breeding (MAB) is defined as the application of molecular biotechnologies, specifically molecular markers, in combination with linkage maps and genomics, to alter and improve plant or animal traits on the basis of genotypic assays (Jiang, 2012). The use of molecular markers is critical in the selection of parents and identification of resistant varieties/cultivars early in a breeding programme. Using molecular markers to select by associating to Quantitative Trait Loci (QTL) maps has the potential to hasten the intensity of selection and therefore advance the breeding populations within a reduced timeframe.

QTL mapping requires that the researcher (i) select and/or develop an appropriate mapping population (experimental populations for linkage-based mapping or natural/breeding populations for association mapping); (ii) phenotype the population for the trait(s) of interest (morphological characters, agronomic traits, disease and pest scores, drought resistance etc.) under greenhouse, screen-house and/or field conditions; (iii) decide the type of molecular marker(s), the genotyping approach (entire population, selective genotyping or bulk segregant analysis) and generate the molecular data for adequate number of uniformly-spaced polymorphic markers; (iv)

identify molecular markers linked to the trait(s) of interest using statistical programs (linkage-based QTL mapping methods requires construction of genetic linkage map) and; (v) test the applicability and reliability of the markers associated with major QTLs in predicting the trait(s) in related families (marker validation or verification) for QTLs of medium to large effect (Semagn *et al.*, 2010).

There are over 30,000 accessions of groundnut cultivars available globally that may be used in QTL studies (Pandey *et al.*, 2012 b).

2.12 MOLECULAR MARKERS STUDIED IN *ARACHIS* sp.

Detection of polymorphic (Hildebrand *et al.*, 1992) molecular markers associated with genes conditioning disease and insect resistance have been progressive and are critical in hastening the output of cultivar resistant breeding programmes for enhanced yield and grain quality (Stalker and Mozingo, 2001; Luo *et al.*, 2004; Semagn *et al.*, 2010).

Polymorphism in RAPDs has been sufficient in the differentiation of species as studied by Halward *et al.* (1992). However, the information obtained could not be used in genetic mapping due to the complexity of the banding patterns and lack of co-dominance. Garcia *et al.* (1995) characterised alien gene introgression in 40-chromosome hybrid derivatives (*A. hypogaea* x *A. cardenasii*) of groundnut at the molecular level as a step towards associating desirable genes from wild *Arachis* species with molecular markers. Both RFLP and RAPD markers were efficient in the detection of alien chromosome introgression. The use of existing RFLP linkage maps to assign introgressed segments to specific linkage groups with the speed and efficiency of using RAPD markers was demonstrated by Garcia *et al.* (1995). RAPD

markers have also effectively identified the chromosomal mechanisms of reciprocal combination and/ or translocation in overall introgression of genes into cultivars (Garcia *et al.*, 2006). In 1998, the need to reveal polymorphisms by use of SSRs and AFLP at the molecular level was demonstrated by Singh *et al.* (1998). RAPDs and Simple Sequence Repeats (SSRs) have been utilised in the assessment of genetic diversity, varietal identification and phylogenetic relationships (Raina *et al.*, 2001) and AFLPs in linkage mapping to aphid resistance in GRD (Herselman *et al.*, 2004).

Simple Sequence Repeat (SSR) markers have gained precedence in molecular research as opposed to other markers due to the co-dominance, simplicity, high polymorphism, repeatability, multi-allelic nature and transferability within the genus *Arachis*. Significant polymorphism was initially identified in novel SSRs by He *et al.* (2003). As of 2003, 110 SSR markers detected genetic variation in a diverse array of 24 groundnut land races (Dwivedi *et al.*, 2003). Anderson *et al.* (2004) showed that it is possible to identify molecular markers to tag resistance for use in conventional breeding by gene pyramiding into high yielding cultivars.

A significant breakthrough was made by Mace *et al.* (2006) in reporting high levels of genetic polymorphism in cultivated groundnut. Twelve of 23 SSRs (52 percent) showed polymorphism with Polymorphism Information Content (PIC) > 0.5. Tang *et al.* (2007) studied 34 SSR markers that showed polymorphism ranging from 0.67 to 1.00 for intra-variety differentiation among *A. hypogaea* subspecies. Cuc *et al.* (2008) developed 104 SSRs of average PIC 0.46 in cultivated groundnut and therefore increasing available SSRs useful in germplasm analysis, linkage mapping, diversity studies and phylogenetic relationships in *A. hypogaea* and related species.

Varshney *et al.* (2010) screened parents with 1089 SSRs of which 6.15 percent were polymorphic, facilitating the development of a partial linkage map with 56 SSR loci from which 11 QTLs for LLS were identified. Also in LLS, cDNA from Expressed Sequence Tag (EST) and real time PCR were used to link the functionality of 20 up-regulated genes to the disease resistant trait in susceptible and resistant gene pools (Luo *et al.*, 2005). Transcriptome resources such as the ESTs may further be applied to identify transcriptional changes during plant immunity responses (Pandey *et al.*, 2012 b).

Song *et al.* (2010) developed 610 EST SSRs classified into 14 functional categories of which 6.4 percent were for genes related to disease resistance mechanisms. EST sequencing plays a critical role in *Arachis* genomics providing information on gene functionality and facilitating the development of molecular markers (Feng *et al.*, 2012). There are 253,274 *Arachis* EST sequences; *A. hypogaea*–178,490, *A. hypogaea* subsp. *fastigiata*– 745 and diploid species–74,342 (NCBI, dbEST, 2012).

Hong *et al.* (2010 b) constructed a 175 SSR based tetraploid genetic map. Wang *et al.* (2012) constructed a genetic linkage map from SSR derived Bacterial Artificial Chromosome End Sequences (BES), facilitating the identification of markers linked to Resistance Gene Homologs (RGH) and for map-based cloning. There are currently over 6,000 SSR markers (Pandey *et al.*, 2012 b) of which 199 are highly informative SSRs with PIC values >0.50 and 946 are novel SSRs respectively. The former are anticipated to rapidly advance molecular genetics and breeding studies in cultivated peanut.

Yuan *et al.* (2010) developed (GGC)_n SSRs of low polymorphism that play a contributing role in enhancing the total available SSRs in wild species for

construction of genetic linkage maps and so transfer of ideal traits into cultivated groundnut.

SSRs have enhanced phylogenetic studies of the *Arachis* species, for pre-breeding parent determination and integration of SSR based maps in both diploid and tetraploid species (Moretzsohn *et al.*, 2004; Moretzsohn *et al.*, 2005; Asif *et al.*, 2009, Molla *et al.*, 2010), comprehensive QTL analysis for linkage to disease resistance (Sujay *et al.*, 2011; Qin *et al.*, 2011), comparative mapping studies (Hong *et al.*, 2010 b; Koppolu *et al.*, 2010) and as a footstool in identification of candidate genome regions controlling disease resistance (Leal-Bertioli *et al.*, 2009; Tang *et al.*, 2007).

Studies carried out with respect to ELS are limited. However, as highlighted above, studies linked to the overall groundnut genome have been relevant in creating a backbone for ELS gene analysis. Studies by Bulk Segregation analysis on the association of SSR markers with pod and kernel related traits in cultivated groundnut identified three QTLs. These SSR markers identified were positively correlated to traits for seed length, pod length and 100 seed weight (Selvaraj *et al.*, 2009). According to Iwo and Olorunju (2009) these traits are linked to ELS disease resistance.

CHAPTER THREE

MATERIALS AND METHODS

3.1 PLANT MATERIALS

The plant materials in this study comprised 16 genotypes that varied in yield and quality traits, susceptibility and resistance to biotic stresses and are broadly categorised in Table 3.1.

3.1.1 Disease Resistant/ Tolerant Genotypes

ICGV–SM 95342: This variety was developed at ICRISAT and is resistant to LLS and Rust.

ICGV 94114: This is an ICRISAT improved variety that is used as a parent for introgression of Rust resistance traits in farmer preferred varieties (Monyo *et al.*, 2007).

ICG 12991: This is a short duration (90 to 110 days to maturation), drought–tolerant, Spanish type peanut (*Arachis hypogaea* L. subsp. *fastigiata*) germplasm line with a high level of field resistance to GRD vector (*Aphis* sp.) and ELS (Deom *et al.*, 2006). It was developed in India and is cultivated in Malawi, Mozambique, Uganda and Zambia.

ICGV–SM 90704: This is a high–yielding medium–duration Virginia bunch type variety (*Arachis hypogaea* subsp. *Hypogaea*) with resistance to GRV but susceptible to aphid vector (*Aphis craccivora*) for GRV transmission (van der Merwe *et al.*, 2001; Monyo *et al.*, 2009). A high yield of 1500 kg/ha has been recorded (Monyo *et al.*, 2009). A concern has been on the difficulty in shelling the harvested seed (Monyo *et al.*, 2009). It was developed in Malawi

TABLE 3.1: Sixteen sources of African *Arachis* germplasm in three categories: Disease tolerance/ resistance, high yielding and quality traits and farmer preferred traits.

Category	Genotype	Essential traits		Location of cultivation
		Disease Resistance/ Susceptibility	Other agronomic traits	
Disease Resistance/ Tolerance	ICGV–SM 95342	Resistance; LLS and Rust.	–	Malawi.
	ICGV 94114	Resistance; Rust (Useful parent in resistance introgression).	–	Malawi.
	ICG 12991	Resistance; <i>Aphis</i> sp. (Groundnut Rosette disease)	Short duration, drought–tolerant, Spanish type variety	India, Malawi, Mozambique, Uganda and Zambia
	ICGV–SM 90704	Resistance; GRV Susceptibility; <i>Aphis</i> sp.	High–yielding (~1500 kg/ha), medium–duration, Virginia bunch type variety, difficulty in shelling	Malawi, Uganda, Mozambique and Zambia
	ICG 7878	Resistance; LLS and tolerance to ELS	Virginia bunch type variety, high–yielding (1500 to 1800 kg/ha), amenable to technology, large seed	–
	ICGV 95714	Resistance; ELS (Useful parent in introgression of resistance) and Web Blotch (WB)	Short duration variety	–
	55–437	Tolerance; Aflatoxin contamination	Drought resistant, high oil content (49%), yield (1000 to 1500 kg/ha)	West Africa
High Yielding And Quality Traits	FLEUR II	Susceptibility; <i>ELS</i> . and <i>Aspergillus flavus</i>	Non dormant, yield (>1500 kg/ha)	–
	CG 7 (MGV4)	Susceptibility; GRD, ELS and Rust	Late maturing, favourable taste, short cooking duration, drought tolerance, uniform kernels, high oil content (~ 50%), yield (>2000 kg/ha)	Malawi and Zambia

	MGV5		Virginia runner type variety, suitable for confectionery, high oil content (~ 50%),, roasts ‘evenly–softer’, attractive tan–coloured kernels, yield (>2000 kg/ha)	Zambia
	Chalimbana	Susceptibility; GRD, ELS and Rust	Virginia runner type variety, large seed size, high oil content (~45%), ease of shelling, favourable taste, pre–harvest dormancy, late maturing	Malawi and Zambia
Farmer Preferred	ICGV–SM 99557		High–yielding (> 1400 kg/ha)	Malawi
Traits	Pendo		High–yielding, large seeded	Tanzania
	ICGV 86124	Resistance; Rust sp. and tolerance to LLS	Early–maturing, high–yielding, Spanish type variety	Senegal and Mali.
	47–10	Highly susceptible to Rust	–	Senegal and Mali
	JL 24 (Luena)	Susceptibility; GRD, ELS and Rust	Spanish type variety, early–maturing, high–yielding (1500 to 2000 kg/ha),, drought tolerance, no seed dormancy	India , Malawi, Mali, Philippines, Congo, Zambia, South Africa and Zimbabwe

and has been introduced to Uganda, Mozambique and Zambia. Average seed yield reported from demonstration plots at 22 sites in Malawi was 1262 kg/ha for CG 7, 1260 kg/ha for ICGV–SM 90704 and 1087 kg/ha for JL 24 (Freeman *et al.*, 2002).

ICGV–SM 95714: This is a short duration variety resistant to ELS and Web Blotch (WB). It is used for introgression of ELS resistance (Monyo *et al.*, 2009).

ICG 7878: This is a Virginia bunch type variety with large seed. Its average pod yield of 1500 to 1800 kg/ha is achievable with good irrigation. It produces good haulm and is preferred by farmers for its good technological qualities and it exhibits resistance to LLS and tolerance to ELS (ICRISAT, Groundnut Seed Project, 2002).

55–437: This is a drought tolerant variety that exhibits tolerance to aflatoxin contamination. It was developed in Senegal and is very common in West Africa and is well adapted to low rainfall areas. It has a compact fruiting habit and an average pod yield of 1000 to 1500 kg/ha. This variety has a medium plant development. For optimal yield plant population density must be respected to achieve good yield. Its oil content is 49 percent (ICRISAT, Groundnut Seed Project, 2002).

3.1.2 High Yielding and Quality Traits Genotypes

FLEUR II: This is a non dormant variety and so has the inherent capacity to germinate readily in the field and in storage. It has been shown to exhibit high yield in comparison to 55–437 (Cisse *et al.*, 2004). It is susceptible to *Cercospora sp.* and *Aspergillus flavus*. Yield losses occur with regards to the former and the latter may require a significant financial investment to ensure storage from the field to the consumer does not elevate *Aspergillus flavus* contamination. The need for education of the farmer and sensitisation to cost effective

measures to reduce *Cercospora sp.* and *Aspergillus flavus* is a necessary measure to be considered by agricultural institutions (Waliyar *et al.*, 2007).

CG 7 (MGV 4): This is a high yielding, late maturing (120 to 140 days) Virginia bunch variety. It is highly preferred by farmers because of its high yield, good taste, short cooking duration, and drought tolerance (Thakur *et al.*, 2000). The kernels are red, uniform and medium in size, contain 48 to 50 percent oil and have an Oleic/Linoleic (O/L) ratio of 1.5 indicating a good shelf life (EPFC–Zambia, 2012). Studies in Malawi showed yield and economic benefit of CG 7 superior to that of JL 24 and Chalimbana with predictions of an increase in profit of up to 17 million dollars per year with sole cultivation of CG 7 (Subrahmanyam *et al.*, 2000). It is susceptible to all major diseases; GRD, ELS and Rust (Monyo *et al.*, 2007). Average seed yield reported in demonstration plots at 22 sites in Malawi was 1262 kg/ha for CG 7, 1260 kg/ha for ICGV–SM 90704 and 1087 kg/ha for JL 24 (Freeman *et al.*, 2002).

MGV 5: This is an early duration (120 days) high yielding Virginia runner type variety. It was recently released in Zambia and is particularly suitable for confectionery with 48 percent oil content, an O/L ratio of 1.5 and roasts ‘evenly–softer’ in texture. It is well adapted for production in the plateau regions of Zambia and has a kernel yield of about 2000 kg/ha with smallholder farmers. It has large attractive tan–coloured kernels that are an excellent high–yielding substitute for Chalimbana a highly farmer preferred variety (EPFC–Zambia, 2012).

CHALIMBANA: This is a high yielding Virginia runner type variety. It is highly preferred by farmers specifically for its large seed size, ease of shelling, taste and pre–harvest dormancy (Monyo *et al.*, 2009). It is largely preferred by farmers in Malawi. It matures in 140 to 150 days and has large seed with 45 percent oil content. Average seed yield reported

in demonstration plots at 22 sites in the country was 623 kg/ha (Freeman *et al.*, 2002). It is susceptible to all major diseases; GRD, ELS and Rust (Monyo *et al.*, 2007).

3.1.3 Farmer Preferred Genotypes

ICGV–SM 99557: This is a farmer preferred variety. It has been identified as a high yielding variety, achieving pod yield output of 742 kg/ha higher than that of farmer preferred variety, Pendo (697 kg/ha) (Monyo *et al.*, 2009).

PENDO: This is a farmer preferred high yielding, large seeded variety with farmer preferred traits. Research in Tanzania showed it out yielded local varieties with a yield range of 924.67 kg/ha to 937 kg/ha. This was high in comparison to the national average yield of 500 kg/ha (Bucheyeki *et al.*, 2008). In southern Tanzania, Pendo was identified to be among the top three yielding varieties (in the order ICGV 99557, Pendo and ICGV 99555) at pod yield of 697 kg/ha (Monyo *et al.*, 2009).

ICGV 86124: This is a farmer preferred early–maturing, high–yielding Spanish breeding line. It is resistant to Rust and tolerant to LLS. It was developed at ICRISAT for rain season cultivation from a cross between Ah 65, a Spanish germplasm, and a Rust resistant Valencia germplasm NC Ac 17090 (Upadhyaya *et al.*, 2001). It is cultivated in Senegal and Mali.

47–10: This is a farmer preferred groundnut variety developed in Senegal. This variety accounts for 40 percent of Mali’s total groundnut cultivation (Abate, 2012).

JL 24 (LUENA): This is a farmer preferred Spanish type, early–maturing (90 to 110 days) and high–yielding (1500 to 2000 kg/ha) with a bunch growth habit. It is preferred largely due to its drought tolerance and early maturing habit over other varieties such as CG 7 and has been preferred over ICGV–SM 90704 (Freeman *et al.*, 2002). It has no seed dormancy

(Freeman *et al.*, 2002). It was developed in India and is cultivated in Malawi, Mali, Philippines, Congo, Zambia, South Africa and Zimbabwe. It is susceptible to all major diseases; GRD, ELS and Rust (Monyo *et al.*, 2007). Average seed yield reported in demonstration plots at 22 sites in the country was 1262 kg/ha for CG 7, 1260 kg/ha for ICGV–SM 90704 and 1087 kg/ha for JL 24 (Freeman *et al.*, 2002).

3.2 DNA EXTRACTION

3.2.1 Sample Preparation.

Due to the vastness of SSR markers studied in *Arachis* sp. and the ambiguity in the naming of the markers from one study to the other, it was necessary to utilise markers from an explicit and uniform database. Therefore, 799 SSR primers comprising of di- and tri-nucleotide motifs from both genomic and Expression Sequence Tag (EST) SSRs compiled in a comprehensive study by Zhao *et al.* (2012) were utilised to screen across a total of 16 cultivated *Arachis* sp. genotypes indigenous to Africa. Information on the forward, reverse sequence (5'–3') and repeat motif for each primer are as stated in Appendix A. For each genotype, DNA was extracted from fresh leaves of 14-day-old greenhouse seedlings. Each genotype was bulked from three individuals into a single sample. The genomic DNA was extracted according to the CTAB method of Mace *et al.* (2003) with the exclusion of the phenol–chloroform extraction step.

3.2.2 Assessment of resultant genomic DNA

To each dried pellet of DNA, 100 µl of TE Buffer was added and the samples incubated in a water bath at 45 °C for 20 minutes. The quality of the extracted genomic DNA was evaluated by using gel electrophoresis on a 0.8 percent Agarose gel (a 0.8 percent gel has a larger matrix and so suitable for large genomic DNA fragments approximating 800 to 12000 bp

(Promega, 2013)). Electrophoresis was at 80 volts powered by Pharmacia Biotech Electrophoresis Power Supply EPS-600 in 1X TE Buffer for 60 minutes. Electrophoresis was against a 100 bp ladder. The DNA quality was further evaluated at 260 and 280 nm respectively by use of the Thermo Scientific Nanodrop 2000 Spectrophotometer with TE buffer as a blank. The gel was viewed under Ultraviolet light (UV Transilluminator, UVITEC, United Kingdom).

The DNA was adjusted to a concentration of 100 ng/ μ l and stored at -20°C .

3.3 SAMPLE PREPARATION FOR SSR ANALYSIS

3.3.1 Amplification

Analysis was in batches of 96 well plates at a concentration of 2 μ M. The DNA concentration was adjusted to 10 ng/ μ l. Each batch of primers required four and two 384 and 96 well plates (Applied Biosystems, USA) respectively for all 16 genotypes. The 384 well plates were aliquoted respective to each fluorescent dye in the sequence of FAM (blue), VIC (Green), NED (yellow), and PET (red) whilst the 96 well plates were halved to fit two fluorescent dye samples per half plate. Each dye fluoresces maximally at a different wavelength allowing for distinction of amplification products. DNA amplification was performed by use of the nested PCR with M13-tag (5'- CACGACGTTGTAAAACGAC -3') on the 5' end that was fluorescently labelled to allow detection of amplification products (Scheulke, 2000). The reaction was carried out in a total volume of 10 μ l containing 1x PCR Buffer (SibEnzyme Ltd, Russia), 2 mM MgCl_2 , 0.16 mM dNTPs, 0.04 pmol forward primer, 0.2 pmol reverse primer, 0.2 U Amplitaq (Applied Biosystems, USA), 3 μ l of DNA template and 2.36 μ l sterile water, 0.16 pmol of each fluorescent label (FAM, VIC, NED PET) were added to 24 primers respectively. The thermo cycler (Applied Biosystems GeneAmp[®] PCR System 9700) incubation conditions were as follows: Initial denaturation for 5 minutes at 94°C followed

by, 35 cycles for 94 °C, 59 °C and 72 °C (30 seconds, 1 minute and 2 minutes respectively) and finally 72 °C (20 minutes).

3.3.2 Gel Electrophoresis

Four micro litres of each amplified product were mixed with 2 µl of loading dye (Bromophenol blue, Sucrose/Glycerol and Xylene) and analysed on two percent agarose gel. Samples were evaluated against a 50 bp ladder for confirmation of amplification. All results were visualised under UV light and photographed.

3.4 SSR MARKER ANALYSIS

3.4.1 Capillary Electrophoresis

The 384 well plates of amplified PCR products were aliquoted into a single 384 well plate with each well comprising 2 µl of NED, 2 µl PET, 1 µl of FAM and 1 µl of VIC. The same was followed for the 96 well plate amplified products into 48 wells.

A mix of Hidi (Formamide) and LIZ–500 standard (orange) was prepared at a ratio of 2:3 and mixed thoroughly by vortexing. Twenty micro litres of the mixture was pipetted into each well. The samples were thereafter denatured in the thermocycler at 94 °C for 5 minutes and rapidly cooled in an ice bucket. Capillary electrophoresis was carried out in an ABI 3500 Genetic Analyzer for allele fragment analysis.

3.4.2 SSR Analysis

Gene Mapper Software (Version 4.0) was used for allele scoring. Allelic variation to compile a dissimilarity matrix was performed by use of PowerMarker (Version 3.25) and DARwin software (Version 5.0.158 2009–07–06) (Perrier and Jacquemoud– Collet, 2003).

The quality of alleles in Gene Mapper was classified on a scale of one to four. That is, 1: Clear single peaks, 2: Clear peaks with multiple elevations, 3: Peaks not well defined, scored and 4: Difficult to score with certainty due to noise/ multiple loci binding/low availability. Markers with a grading of four were considered anomalous and excluded from the final dataset. Markers graded one to three were carried forward. PowerMarker was used to extract Summary statistics on each allele scored. The main data obtained was Polymorphic Information Content (PIC), Heterozygosity, Number of Alleles, Availability and Major Allele Frequency.

Darwin was used to establish a relationship matrix.

3.5 PRINCIPLE AND METHODOLOGY OF MAIN PARAMETERS OF ANALYSIS

3.5.1 Polymorphic Information Content (PIC)

This ranges from zero to one and is used to measure the Informativeness of a genetic marker for linkage analysis studies (Gou and Elston, 1999; Elston, 2005; Hildebrand *et al.*, 1992). The higher the PIC value the more effective a marker is in detecting polymorphisms within a population or among individuals. It is dependent on the number of detectable alleles and the distribution of their frequency and is equivalent to gene diversity. The formula for PIC of co-dominant markers (Li *et al.*, 2011) is as follows,

$$PIC = 1 - \sum_{u=1}^k P_{lu}^2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^k 2P_{lu}^2 P_{lv}^2$$

Where, l =index for marker ' l '; P_{lu} = proportion of marker ' l ' alleles which are of allele type ' u '; P_{lv} = proportion of marker ' l ' alleles which are of allele type ' v ' and k = number of alleles types present for marker ' l '.

3.5.2 Expected Heterozygosity

This ranges from zero to one and is the proportion of heterozygous individuals in the population (Liu and Muse, 2005). It is therefore, the probability that any randomly chosen individual is heterozygous for any two alleles at a marker locus having allele frequencies p (Hildebrand *et al.*, 1992). It is defined by the following formula:

$$He = 1 - \sum (p^i)^2$$

Where, p^i = frequency of the i^{th} allele in the population/ locus (if individual marker is considered) (Powell *et al.*, 1996) and $\sum (p^i)^2$ = homozygosity (Hildebrand *et al.*, 1992).

3.5.3 Availability

This is defined as follows:

$$1 - Obs/n$$

Where, ‘Obs’ is the number of observations and n is the number of individuals sampled (Liu and Muse, 2005). This parameter aids in evaluating the overall performance of a marker across genotypes and therefore, its reliability and future usefulness. Markers that have low availability may be reanalysed to confirm performance. In this study markers of availability below 0.44 were not carried forward in analysis.

3.5.4 Number of alleles

This is the total number of alleles present in a current sample for selected number of alleles present in a sample for the selected loci (Liu and Muse, 2005).

3.5.5 Major Allele Frequency

This refers to the measure of the relative frequency of the most common allele in a given sample of genotypes. Allele frequencies between 0.5 and 0.8 provide information to detect

linkage of alleles. It is inversely proportional to PIC. Markers with allele frequency greater than 0.8 have a low level of information content (Goddard *et al.*, 2000).

3.5.6 Dissimilarity Matrix

According to Perrier *et al.* (2003), the dissimilarity matrix (d) ranges from zero to one. It is a distance matrix that distinguishes M objects in a pair-wise manner. It is calculated as the value one less the similarity value. It is a square symmetrical (M x M) matrix with the (ij)th element equal to the value of a chosen measure of distinction between the (i)th and the (j)th object such that,

$$d_{ij} = d_{ji}.$$

Therefore, the distinction value between objects is inversely proportional to the degree of genetic relatedness and so the distinction between an object and itself is postulated as zero. Allelic data of polymorphic markers were input into DARwin software with missing data represented with integer code –999 and the data was computed into a .VAR file as input data and stored in a .DIS file as output data. Pair-wise locus deletion was set at 90 percent valid data for removal of missing data per current unit pair and bootstrap analysis was set at a maximum of 30,000 (Perrier *et al.*, 2003). Bootstrapping is a computer-based method for assigning measures of accuracy to sample estimates and allows estimation of the sample distribution of almost any statistic using only very simple methods (Weisstein *et al.*, 2012). The formula for Dissimilarity is as follows:

$$d_{ij} = 1 - \frac{1}{L} \sum_{l=1}^L \frac{m_l}{\pi}$$

The principle of any tree construction method is to approach as closely as possible the dissimilarity d , chosen for its relevance in describing the relationships between units, by a distance d that can be represented as a tree (Perrier *et al.*, 2003). Neighbour Joining (NJ) method was presented by Saitou and Nei (1987) and relies on distance matrix data to construct phylogenetic trees. According to Saitou and Nei (1987) the principle of this method is to find pairs of Operational Taxonomic Units (OTUs [= neighbours]) that minimise the total branch length at each stage of clustering of OTUs starting with a star like tree. In the present research weighted neighbour joining was used. It gives significantly less weight to the longer distances in the distance matrix and so reduces error as opposed to NJ (Bruno *et al.*, 2000). The data was computed in DARwin from the .DIS file into an output .ARB file which stores the structure and the parameters of the tree. A radial tree was constructed was done the by weighted– neighbour Joining with bootstrap analysis set at > 40 (Perrier *et al.*, 2003). A .DON file was used to extract identifiers for genotype name and manual input of colour codes according to traits.

Factorial analysis is based on the Principal Coordinate Analysis (PCoA). It considers the space of high dimension defined by the distances between units two by two. PCoA extracts a first axis (one dimension) such that,

$$\sum_{i,j} (d_{ij} - \delta_{ij})^2$$

is the minimum (where d_{ij} is the observed distance between i and j , δ_{ij} is the distance between the projections of i and j on this axis). Then it extracts a second axis, orthogonal to the previous one (independence condition) minimising the squared differences and so on. Solutions are given by eigenvectors and eigen values of the matrix W of scalar products between elements that is defined from the d_{ij} according to the Torgerson formula:

$$W_{ij} = \frac{-(d_{ij}^2 - d_{i.}^2 - d_{.j}^2 + d^2)}{2}$$

Factorial analysis was computed from the .DIS file as input file into an .AFT output file and viewed in axes 2/4 (Perrier *et al.*, 2003). A .DON file was used to extract identifiers for genotype name and manual input of colour codes according to traits.

CHAPTER FOUR

RESULTS

4.1 POLYMORPHIC SSRS

A total of 799 markers were screened across 16 genotypes to evaluate the most informative markers for QTL mapping and pre-/post-breeding applications. One hundred and seventy-nine markers gave scoring data availability less than 38 percent across the 16 genotypes and were omitted from the final dataset. Those that gave difficulty in scoring due to multiple loci were 36 in total. Monomorphic markers totalled 161 whilst a total of 376 (47.1 percent of total SSRs) polymorphic markers were carried forward to the final dataset for analysis. Polymorphic markers gave an average of 3.7 alleles per locus, giving a total of 1476 total alleles (Table 4.1).

TABLE 4.1: A summary account of the total number of markers that were analysed across 16 *Arachis* genotypes.

Description	Quantity
Total marker loci	817
Monomorphic Markers	161
Polymorphic marker loci	394
Excluded markers	262
Duplicated Markers (split heterozygosity)	18
Total markers	799

The summary statistics extracted from PowerMarker may be noted in (Appendix A) with emphasis on Allele number, Major Allele Frequency, Availability, Heterozygosity and Polymorphism Information Content (PIC).

Polymorphism Information Content (PIC) ranged from 0.06 to 0.86 for Ah-671 to Ah1TC4F12 respectively with a mean value of 0.47. PIC values above 0.5 were observed in 44 percent (174) of the markers that performed well.

Major Allele Frequency ranged from 0.18 to 0.47 (140 markers), 0.5 to 0.80 (180 markers) and 0.81 to 0.97 (74 markers).

Heterozygosity ranged from 0 to 0.38 in 77 percent of the markers analysed across the genotypes. The mean Heterozygosity was 0.20. Markers that were highly heterozygous were considered as having amplified two loci and so split into two alleles denoted with ($_1/2$) to the marker name. If both alleles were heterozygous and polymorphic, these were retained. If one half of the split allele was homozygous, it was discarded. Markers that resulted in two homozygous loci were not split.

Marker allele scores in GeneMapper were graded according to Figure 4.1 and the grade allocation per marker as per Appendix A. Table 4.2 gives information on the number of markers per grade.

The performance of the markers across the genotypes was evaluated with regards to availability per genotype (Table 4.3). The range of performance was 90.9 percent (ICGV-SM 95714) to 96.2 percent (55-437). The overall mean performance across genotypes was 94.2 percent. ELS resistant genotypes ICGV-SM 95714 and 7878 performed at 90.9 and 94.4 percent respectively.

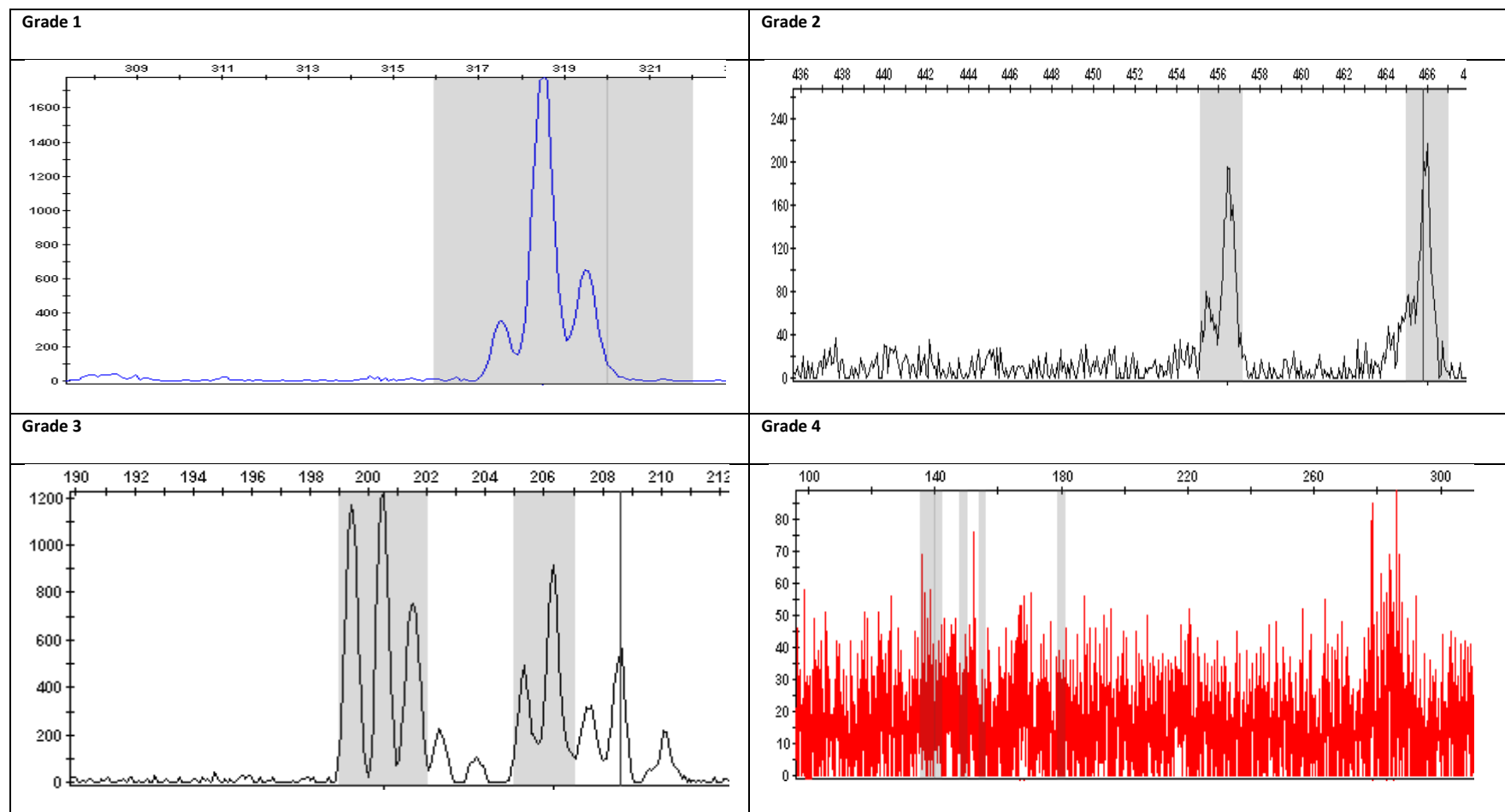


FIGURE 4.1 Allele fragments showing samples of the allele grading. Grade 1: Clear Single peak, 318base pairs; Grade 2: Heterozygous peaks, 456 and 466 base pairs; Grade 3: Multiple heterozygous peaks, main peaks 200 and 206bp; Grade 4, undefined peaks due to experimental and/ or mechanical error.

TABLE 4.2: Characteristics of markers allocated to each grade.

Grade	Description	Number of Markers	Number of Polymorphic Markers per Grade
1	Clear single peaks.	301	189
2	Clear peaks with multiple elevations.	76	68
3	Peaks not well defined, scored.	178	137
4	Difficult to score due to noise/ multiple loci binding/low availability.	262	NA
Total Number of Markers		817	394

TABLE 4.3: Marker performance per genotype across 788 Data Points (ELS resistant genotypes highlighted in grey).

Genotype	Missing Data	Percentage of Availability
55-437	30	96.2
ICGV 86124	34	95.7
ICGV-SM 90704	34	95.7
CHALIMBANA	36	95.4
CG7	38	95.2
MGV 5	38	95.2
ICGV-SM 99557	40	94.9
47_10	42	94.7
ICG 12991	44	94.4
ICG 7878	44	94.4
JL24	48	93.9
ICGV-SM 95342	52	93.4
ICGV 94114	54	93.1
PENDO	58	92.6
FLEUR_II	68	91.4
ICGV-SM 95714	72	90.9
MEAN	45.75	94.2

4.2 GENETIC RELATIONSHIP

4.2.1 Dissimilarity matrix

Dissimilarity was calculated (Table 4.5) and the values ranged from the most closely related 0.34 (MGV5: ICGV–SM 90704) to the most distance related 0.66 (Chalimbana: 47–10). The mean dissimilarity matrix value was 0.51. The most appropriate pair–wise combinations for ELS disease resistance and tolerance were highlighted in grey and bold font based on the highest pair wise combinations per row or column respective to ELS disease resistance conferring genotypes ICG 7878 and ICGV–SM 95714.

4.2.2 Tree diagram

The genetic distances of the genotypes were portrayed in a tree diagram (Figure 4.2). The genotypes were classified into three main clusters. Cluster I comprised of genotypes of Aflatoxin resistance: 55–437; High yielding and quality traits: FLEUR II; Farmer preferred: ICGV 86124, 47–10, JL 24 and Pendo). Sub–Cluster Ia comprised FLEUR II, ICGV 86124, JL 24 and Pendo whilst Sub–Cluster Ib comprised 47–10 and 55–437. Cluster II comprised of genotypes of Farmer preferred: ICGV–SM 99557; Rust resistance; ICGV 94114; GRD virus resistance: ICG 12991. Sub–Cluster IIa comprised ICGV–SM 99557 and ICGV 94114. Sub–Cluster IIb comprised ICG 12991.

Cluster III comprised of genotypes of traits for ELS resistance: ICG 7878; Rust resistance: ICGV–SM 95342; GRD aphid resistance: ICGV–SM 90704; High yielding and quality traits: CG7, Chalimbana and MGV5. Sub–Cluster IIIa comprised ICGV–SM 90704, Chalimbana, CG7, ICG 7878 and MGV5. Sub–Cluster IIIb comprised ICGV–SM 95342.

Cluster IV comprised of a single genotype for ELS resistance: ICGV–SM 95714.

Cluster II and III were more closely related (monophyletic) than to Cluster I (paraphyletic) whilst Cluster IV was independent of all other clusters. Factorial analysis was carried out and Eigen values less than one were obtained for each axis (Figure 4.3).

4.3 MARKER MAP LOCATIONS

A total of 139 (37 percent) polymorphic marker locations were ascertained with reference to Gautami *et al.* (2012) and Wang *et al.* (2012) genome maps (Table 4.5; Appendix A). The number of markers associated per Linkage Group (LG) across chromosomes ‘aa’ and ‘bb’ ranged from 0 (0.00 percent, b06) to 18 (12.59 percent, LG9, a04) of total marker map locations. Marker map location was distributed across all LGs with the exception of LG b06 of chromosome ‘bb’.

TABLE 4.4: The dissimilarity matrix of 16 *Arachis* sp. genotypes (The most appropriate ELS disease resistance pair wise comparisons > 0.54 have been highlighted in grey).

Genotype	ICG	ICG	55–	ICGV	ICGV–	ICGV	ICGV	ICGV–	ICGV–						
	7878	12991	437	86124	SM	94114	95342	SM	SM	47–10	CG7	Chalimbana	FLEUR–II	JL24	MGV 5
					90704			95714	99557						
ICG 12991	0.458														
55–437	0.508	0.407													
ICGV 86124	0.582	0.506	0.407												
ICGV–SM 90704	0.479	0.468	0.546	0.547											
ICGV 94114	0.538	0.458	0.441	0.496	0.542										
ICGV–SM 95342	0.507	0.572	0.552	0.520	0.550	0.544									
ICGV–SM 95714	0.567	0.532	0.491	0.514	0.519	0.543	0.548								
ICGV–SM 99557	0.532	0.452	0.442	0.488	0.499	0.427	0.549	0.499							
47–10	0.607	0.504	0.383	0.468	0.591	0.509	0.571	0.566	0.511						
CG7	0.513	0.479	0.579	0.551	0.404	0.499	0.537	0.522	0.446	0.611					
Chalimbana	0.409	0.483	0.577	0.594	0.400	0.566	0.534	0.512	0.527	0.662	0.439				
FLEUR–II	0.570	0.526	0.394	0.454	0.536	0.522	0.560	0.503	0.487	0.493	0.593	0.543			
JL24	0.597	0.532	0.419	0.412	0.563	0.542	0.567	0.567	0.525	0.419	0.615	0.580	0.425		
MGV 5	0.471	0.485	0.547	0.567	0.347	0.549	0.535	0.523	0.518	0.651	0.438	0.310	0.533	0.589	
PENDO	0.532	0.471	0.475	0.419	0.540	0.509	0.594	0.528	0.452	0.526	0.511	0.517	0.447	0.370	0.563

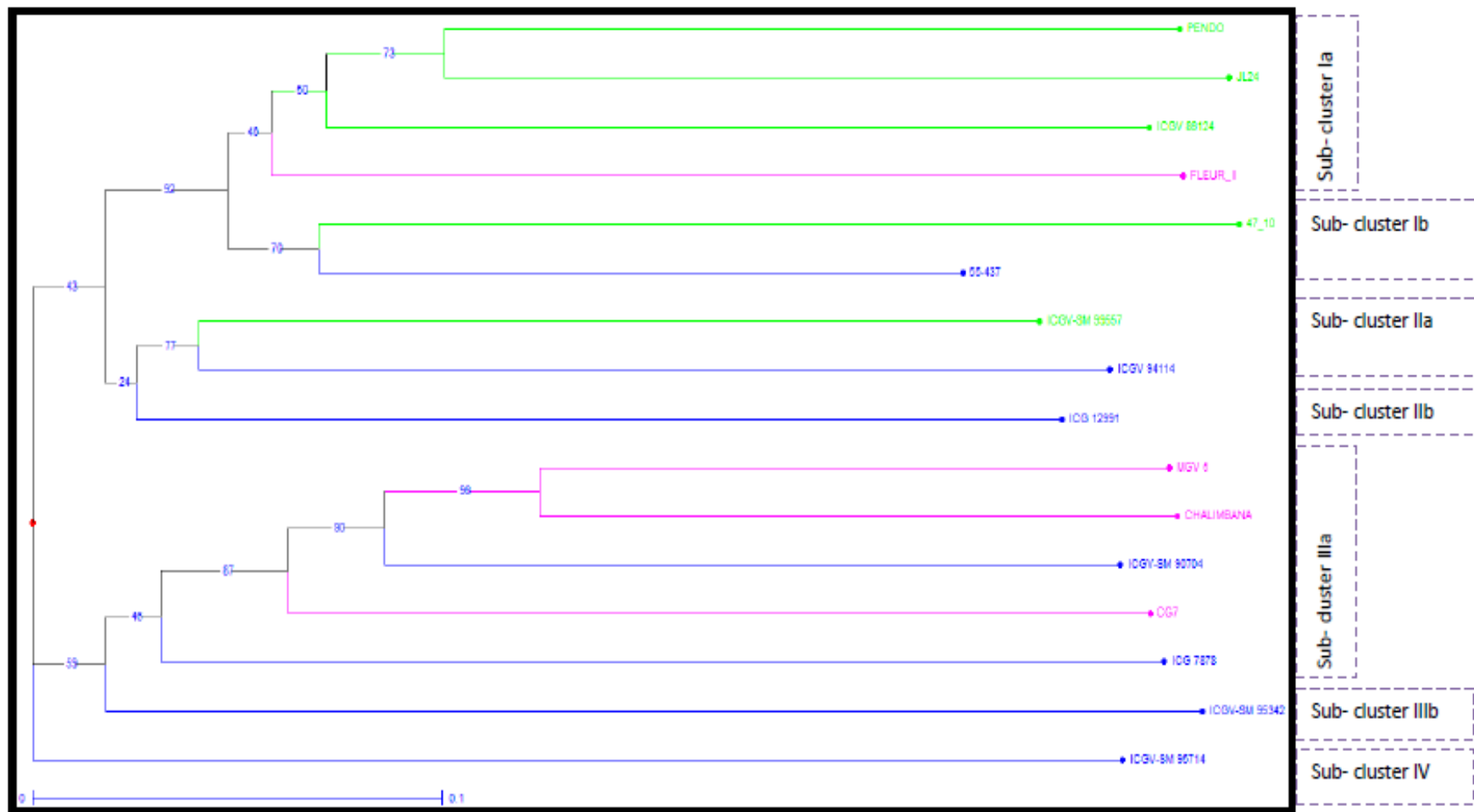


FIGURE 4.2: Tree diagram representing genetic relatedness of 16 *Arachis* genotypes with variable traits; resistance, yield and quality, and farmer preferred traits indicated in green, pink and blue respectively.

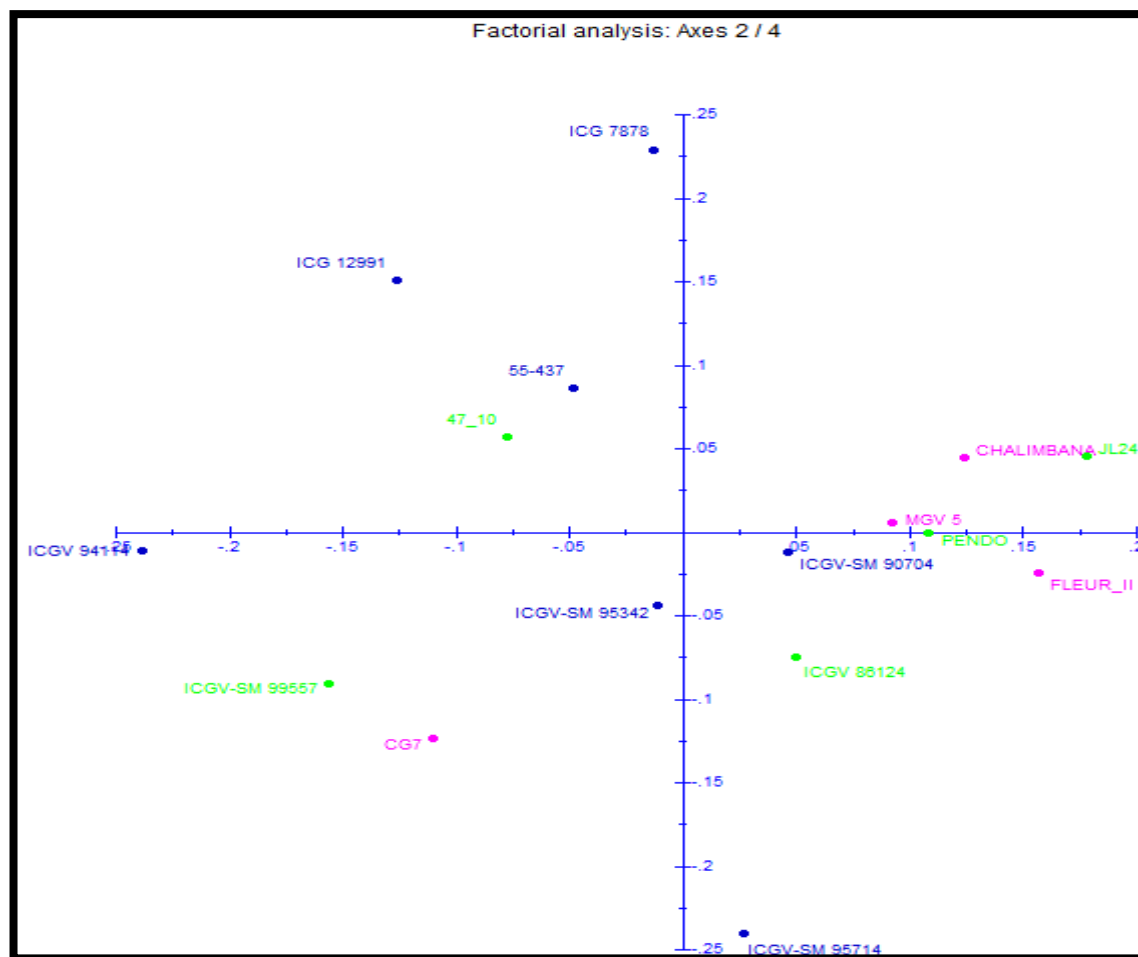


FIGURE 4.3: Factorial analysis of 16 *Arachis* sp. across axes 2/4 (Resistance Traits- Green; Yield and Quality Traits- Pink; Farmer preferred- Blue).

TABLE 4.5: The Location of 139 polymorphic SSRs per Linkage Group (LG) with reference to Gautami *et al.* (2012) and Wang *et al.* (2012) genome maps.

LG	MARKERS																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
a04 (LG9)	GM106 2	Ap40	GM89 0	GM224 6	TC11B04	GM172 0	IPAHM1 05	GM2589	GM191 9	GM131 1	IPAH M108	GM2 313	AHGS0 347	AHGS0 134	pPGSseq 18C5	GM2 480	Ah1TC 5A07	Ah2 TC7 G10
a09 (LG18)	GM245 0	GM8 49	GM23 59	GM129 1	GM1911	PM675	AHGS06 95	Ah1TC5D 06	Ah1TC 1D02	AHGS0 993	Ah– 671							
a06 (LG5,1 0)	IPAHM 659	GM1 489	GM14 90	GM233 7	IPAHM2 45	TC11A0 4	GM1573	IPAHM68 9	GM191 6	Ah2TC 7C06								
a03 (LG7)	GM171 7	GM2 402	GM22 15	GM252 8	GM2206	GM195 4	Ah1TC0 A01	pPGSseq 19G7	AHGS0 132									
a05 (LG19)	GM104 9	GA34	GM15 77	GM207 8	RN16F0 5	GM170 2	pPGSseq 10D4	Ah1TC6E 01	GA32									
b07 (LG2)	GM195 3	GM2 156	GM20 67	GM207 3	GA24	GM255 7	pPGPseq 5D5	pPGSseq 15C10										
a07 (LG4)	GM149 4	GM1 937	GM10 76	GM188 0	GM1986	GM192 2	GM1990											
a08 (LG12)	GM228 9	GM1 628	GM20 89	Ah1TC 3B04	Ah2TC7 A02	GM171 3	GM2571											
b03 (LG14)	GM185 4	GM1 618	GM19 96	GM238 8	GM2009	Ah2TC9 B12	GM2574											
b05 (LG21)	GM213 7	GM1 555	IPAH M136	GM184 3	Ah1TC5 D01	AHGS07 29												
b01 (LG6)	GM150 1	GM1 331	Ah3	GM260 7	pPGSseq 13A7	AHGS01 38												
b10 (LG5)	TC3E05	GM1 742	GM21 65	GM203 2	Ah1TC1 B02	Ah2TC1 1A02												
a10 (LG1)	GM253 1	GM1 788	GM24 11	GA161	GM799													

TABLE 4.5: The Location of polymorphic SSRs per Linkage Group (LG) with reference to Gautami *et al.* (2012) and Wang *et al.* (2012).

LG	MARKERS																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
b02 (LG16)	GM2196	Ah26	GA166	Ah1TC4F12														
b04 (LG13)	GM2584	GM1445	GM2033	AHGS0230														
b08 (LG4)	GM1961	IPAHM123	IPAHM606	GM1798														
(LG3)	GM2063	AHGS0369	AHGS0798	AHGS0278														
(LG17)	GM1821	pPGPseq2F5	GM1985															
(LG20)	AHGS0147	Ah2TC9H08	AHGS0151															
(LG11)	AHGS0357	pPGPseq1B9	GM1598															
b09	GM1483	Lec1																
(LG15)	GA166	Ah1TC4F12																
a02	RI1F06																	

CHAPTER FIVE

DISCUSSION

5.1 SSR MARKER PROPERTIES AND PERFORMANCE

The methods adopted in this study were effective and economical considering the financial challenges faced associated with molecular biology research in Africa (Varshney *et al.*, 2009 b, Schuelke, 2000). Markers of low availability were excluded from the final data set and assumed unreliable to ensure only those markers that performed well across the genotypes of interest were considered for further analysis. Favourable PIC values were obtained for distinction of genotypes. Forty-five percent of the selected markers gave a Polymorphism Information Content (PIC) value greater than or equal to 0.5 and an average PIC value of 0.47. These values were high in comparison to recent findings by Pandey *et al.* (2012 a) in which only 15.7 percent of markers gave PIC values greater than 0.5. Comparable findings were detected by other groundnuts (Cuc *et al.*, 2008; Mace *et al.*, 2006) with variations occurring due to methodology and number of samples/markers. The polymorphic performance was very high in comparison to other studies in groundnuts which ranged from 3 to 33 percent with PIC values (Song *et al.*, 2010; He *et al.*, 2003, Moretzsohn *et al.*, 2004) and so this selection of markers was highly informative. The Major Allele Frequency (MAF) was inversely proportional to the PIC value hence the lower the occurrence of the most common allele the greater the PIC. Markers with MAF that ranged from 0.5 to 0.8 (180 polymorphic markers) give approximately equal information in information pertaining to linkage in linkage disequilibrium studies (useful in QTL mapping) whilst those of values greater than 0.8 (74 polymorphic markers) are

limited in information to that respect (Goddard *et al.*, 2000). The number of alleles per marker ranged from 2 to 11 with a mean value of 3.74 with the most and least polymorphic giving values of 2 and 10 respectively. A higher number of alleles ranging 2 to 14 were reported (Pandey *et al.*, 2012 a; He *et al.*, 2003) and a lower number of alleles ranging 2 to 8 (Cuc *et al.*, 2008) by previous studies. The most polymorphic markers of PIC value greater than 0.70 (Hildebrand *et al.*, 1992) had allele values ranging from 5 to 11. Thus, the number of alleles was directly proportional to the PIC (Tantasawat *et al.*, 2011). The most informative SSR markers Ah2TC7H11, Ah1TC3E02, Ah1TC4F12, GNB70, Ah2TC11H06, AHGS0798, pPGPseq3B5, Ah2TC9H09, Lec1, Ah1TC2G05, AHGS0965, GA161, TC04G02, Ah1TC3B04, TC11A04, TC3E05, TC05A06 and GNB18 gave a high number of alleles ranging from 8 to 11 and PIC values 0.78 to 0.86 hence being significant in distinguishing all the genotypes for use in MAS and other diversity studies.

The PIC value range and mean allele value were in agreement with recent studies by Pandey *et al.* (2012 a) and lower than that detected by He *et al.* (2003). Marker GM2009 gave a PIC value of 0.67 and has been shown to be closely associated with the major QTL for Late Leaf Spot (LLS) (Sujay *et al.*, 2011). The genetic similarity of LLS and ELS disease resistance mechanisms (McDonald *et al.*, 1985) further clues in the significance of this marker for QTL analysis with regards to ELS. Markers IPAHM108_1/2 and IPAHM123_2 gave PIC of 0.69/0.72 and 0.73 across 5 and 6 alleles. These values were similar to a previous study by Cuc *et al.* (2008) with variation in number of alleles. Other polymorphic IPAHMx markers varied with those investigated by Cuc *et al.* (2008). Variation was with regards to low polymorphism (IPAHM659_2/105/136/177) and high polymorphism (IPAHM689)

whilst allele numbers were fairly consistent in comparison. This variation in marker characteristics may be attributed to the inherent genotypic constitution of the cultivars used. There was no common genotype used in this study and that of Cuc *et al.* (2008). Other markers that gave high PIC in this study and that of Varshney *et al.* (2009 c) were Ah1TC1E01, Ah1TC4F12 and Ah1TC6E01 with PIC values in the range of 0.60 to 0.90. The presence of similar marker characteristics further highlights the usefulness of the markers in the present study across cultivated *Arachis* sp. The polymorphic markers in this study may also be useful across other legume species in comparative genomics studies as was ascertained of polymorphic soybean derived EST–SSRs in the *Arachis* genome (Hong *et al.*, 2010 b). The overall performance of markers gave a high average 94.2 percent across the 16 genotypes.

5.2 DISSIMILARITY MATRIX PAIR WISE COMPARISONS ACROSS 16 ARACHIS sp. GENOTYPES

The dissimilarity matrix (scale 0 to 1) highlighted the most distantly related genotypes. The values obtained are directly proportional to the genetic distance of genotypes under consideration (Perrier *et al.*, 2003) and are expressed as a proportion of the total polymorphic markers respective to each pair–wise comparison. Hence, this aided in determining the most appropriate parental genotypes for breeding programmes. The values obtained were high in comparison to those of previous studies (Tshilenge–Lukanda *et al.*, 2012; Koppolu *et al.*, 2010). Highlighted in the results were possible pair–wise combinations for introgression of ELS disease resistance and/or tolerance introgression. The pairs were selected with respect to the highest values per row/column for genotypes ICG 7878 and ICGV–SM 95714. ICG 7878 gave the highest pair wise comparisons with farmer preferred genotypes 47–10,

JL 24, ICGV 86124 (dissimilarity values: 0.607, 0.597, 0.582 respectively) and high yielding and quality trait genotype FLEUR II (dissimilarity value: 0.57). Farmer preferred genotypes 47–10 and JL 24 in addition to favourable dissimilarity values with ICG 7878, also paired well with ELS resistant genotype ICGV–SM 95714 (dissimilarity values: 0.566 and 0.567 respectively). ICGV–SM 95714 was also favourably highlighted in pair wise combination with rust resistant genotypes ICGV 94114 and ICGV–SM 95342 (dissimilarity values 0.543 and 0.548 respectively).

With regards to considering disease resistance across all the genotypes, appreciable values were noted to allow for introgression of more than one resistance trait into a single genotype. ICG 12991 is predominantly cultivated for its GRD *Aphis* sp. resistance but has otherwise been shown to exhibit ELS resistance (Deom *et al.*, 2006). It may therefore confer multiple disease resistances in breeding crosses with Rust resistant ICGV–SM 95432 (0.572) and ELS resistant ICGV–SM 95714 (0.5320).

In addition to JL24 being a farmer preferred variety, a study by Tshilenge–Lukanda *et al.* (2012) showed that it exhibits tolerance to both ELS and LLS and may be further improved by crossing with other resistant genotypes i.e., ICG 7878 (ELS resistant), ICG 12991, ICGV–SM 90704, ICGV–SM 95432, ICGV–SM 95714 (ELS resistant) (dissimilarity values 0.597, 0.532, 0.563, 0.567 and 0.567 respectively). Other genotypes may also be considered for pair wise introgression of traits and disease resistance, consider Rust resistant genotype ICGV–SM 95432 gave the following values: 0.552 with 55437 (*Aspergillus flavus* resistant), 0.572/ 0.550 with ICG 12991/ICGV–SM 90704 (GRD resistant).

Sixty-three percent of the dissimilarity values gave a range of 0.50 to 0.66 thus, accounting for approximately 237 polymorphic markers significantly differentiating each of these genotypes of variable traits of yield, quality and disease resistance. Nineteen percent of the values were associated with crosses for introgression of ELS resistance. The high number of markers used in this study therefore enhances the potential for introgression of multiple disease resistance and yield and quality into farmer preferred and commercial genotypes.

5.3 GENETIC CLUSTERING

Genotypes were distinguished into three large clusters and one independent cluster (single genotype: ICGV–SM 95714). The majority of farmer preferred genotypes namely; 47–10, ICGV 86124, 47–10, JL 24 and Pendo were clustered together in Cluster I with ICGV 86124, 47–10, JL 24 and Pendo sub-clustered and so more closely related. This may be attributed to groundnuts exhibiting a level of out crossing (Smartt, 1960) ranging from 0 to 8 percent (Reddy *et al.*, 1993; Knauff *et al.*, 1992). The overall genetic relatedness of the genotypes may further be attributed to the exchange of seed among small holder farmers and proximity of planting of farmer preferred genotypes within a common geographic location. Thus, long term effects of farmer preferences and selection may have influenced the genetic composition of seed for this study.

Genotypes for ELS resistance notably ICG 7878 and ICGV–SM 95714 were notably distanced from the majority of the genotypes hence more relevant in trait introgression into the other 14 genotypes.

ICGV–SM 95714 showed the highest missing data at 90.9 percent availability (Table 4.3). This may have contributed to independent clustering. The Eigen values

obtained further raised the significance for the incorporation of populations for increased accuracy in relationship analysis. However, tree analysis added further weight to the genetic diversity demonstrated by dissimilarity analysis values.

5.4 SSR MARKER DISTRIBUTION

In this study 537 (68 percent of total marker loci) were scored and graded 1 to 3 of which 394 (73 percent scored) were polymorphic and can be carried forward for QTL mapping. The locality of a total of 139 (37 percent) polymorphic markers was ascertained with reference to Guatami *et al.* (2012) and Wang *et al.* (2012) genome maps (Table 4.5, Appendix A). The number of markers associated per Linkage Group (LG) was evenly spread across the LGs. These values ranged from 0 (0.00 percent, b06) to 18 (12.59 percent, LG9, a04). Previous studies have resulted in the construction of genetic maps from SSR marker numbers as low as 144 (Varshney *et al.*, 2009 a), 175 (Hong *et al.*, 2010 b), 181/188 (Sujay *et al.*, 2011), 324 (Qin *et al.*, 2011) on Recombinant Inbred Line (RIL) populations and marker numbers as high as 895–tetraploid genome (Guatami *et al.*, 2012) and 1724–diploid genome (Nagy *et al.*, 2012). The use of 16 genotypes of variable traits allowed for a repertoire not only beneficial for future QTL mapping and MAS restricted to ELS resistance but other quality traits and disease resistances.

CHAPTER SIX

CONCLUSIONS

In this study, 376 highly informative SSR markers were identified from 799 that were screened. The genetic distinction of the 16 groundnut cultivars analysed was figuratively demonstrated. The genotypes were notably distinguished into four main clusters with ICG 7878 and ICGV–SM 95714 notably distinct from the majority of the genotypes. A dissimilarity ‘toolkit’ was constructed that indicates the most suitable parents for mapping populations and marker assisted introgression to aid breeders in groundnut improvement. In addition, a subset of 139 SSR markers, that were polymorphic across the genotypes analysed and that are evenly spread across the groundnut genome for future ELS resistance QTL mapping, was identified.

CHAPTER SEVEN

RECOMMENDATIONS

This study allowed for the initial identification of the most informative SSR markers in African *Arachis* sp. germplasm. These markers may further be integrated into a Quantitative Loci Trait Linkage study so as to identify the most critical markers linked to resistance to ELS disease resistance and other traits of interest. This would, hasten breeding studies and provide the farmer with ideal genotypes for cultivation within a reduced time frame as opposed to the conventional studies that require a minimum of six years for the provision of an ideal genotype to the farmer.

The dissimilarity matrices obtained in this study are a significant guideline for the most appropriate parents in backcross breeding for integration of resistance genes from the ELS resistant donor genotypes (ICGV 95714 and ICG 7878) into local susceptible genotypes preferred by the small holder and commercial farmer.

The polymorphic markers in this study may be carried forward to other studies for future genetic improvement studies for resistance, tolerance and other traits.

The methodology is not restricted to peanut germplasm and so similar methodology with the exception of genotypes and markers may be used in future studies of local nutritional crops. Aspects of optimisation of PCR and DNA extraction would still be necessary.

In the event that populations are integrated into a study such as this, it would be exceedingly useful in phylogenetic studies.

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APPENDIX A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
1	Ah1TC3G05	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGATCCCAAGTCT CCAGAGGA, AACACAAGGAGGCAGAGGA	N.A
2	Ah1TC4E08	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCTCATCCACCCT CCTGACT, GATGCGTGAGTGGTCATACG	N.A
3	Ah–272	1	No	No	N.A	G	1.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACGATCAACCCTCA TGGCAACAAC, GCGGAAAGGAGCTTGTGAGTTT	N.A
4	Ah2AC1B01	1	No	No	N.A	G	0.81	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCCCCATCACACA CTCTTTT, GGACAGCGTTAGGGATTTATGA	N.A
5	Ah2AC1C11	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCTCCCACACCAA ACTTAAAAGC, CCCACTCCTATAAATACCCCTCTT	N.A
6	Ah2AC1G11	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCCCTTTTCTTTCA AGGCTCCTA, TTCGTAAATCAGAGGTGGTGAG	N.A
7	Ah2AC2A06	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACATCATCTCGATCC ATCCTTCTG, CTCCTTCTTCTCGCGTATTGT	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
8	Ah2AC2D04	1	No	No	N.A	G	1.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACCTCCAACAATTAC AAGCACCTG, GCTTCTCTGTTCTTCCTATTTTC	N.A
9	Ah2AC2H11	1	No	No	N.A	G	0.88	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCCTTTACTTGTG CAGTTGTGC, AAAACGCCATGTGGTGGAT	N.A
10	Ah–330	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGCCTCAGCAAG TCAAGTCACA, GATTTATGTAGCCGACCCACC	N.A
11	Ah–394	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCGACAACAATC ATCCTCAACAG, AAGAAGTAGTGCGGAGGTTGG	N.A
12	Ah–408	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCATGGACATAGC AGCACAACA, GAGGGTGGTGGCGTTTTAGA	N.A
13	Ah–428	1	No	No	N.A	G	0.81	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGGGATTATCA TGGAGGGACA, TGTTGGATTGTTGTCTTCGGT	N.A
14	Ah–462	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGGCTTCCTGGTC CTTCATTGTC, CAACCCCACTTCACAAACCAG	N.A
15	Ah–521	1	No	No	N.A	G	1.00	N.A	0.52	N.A	CACGACGTTGTAAAACGACATTCTGAGGCTG CTTCCCAAC, CGCTGAGAAAGGGAGTCACAAA	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
16	Ah-522	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGTCAATGCCGAA CCTCAACGTA, TTCACCATCATCTCCAACGCTT	N.A
17	Ah-558	1	No	No	N.A	G	1.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACTGTGACACCATC AATCAAAGGG, CAAAACCCAAATCATCACCACC	N.A
18	Ah-594	1	No	No	N.A	G	1.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACTGACCAAATCAT CCCATCTTGC, CAGCACATCTCCAACCTTCTCCA	N.A
19	Ah-607	1	No	No	N.A	G	0.81	N.A	0.00	N.A	CACGACGTTGTAAAACGACGGGCAGAGGATT ATTGAGTCCC, GTCCAAACACCACCAACAAACA	N.A
20	Ah-649	1	No	No	N.A	G	1.00	N.A	0.42	N.A	CACGACGTTGTAAAACGACGGAATGCCAAA TCCATCCTTC, GTTGTTGCGGTGTGAAAACGGTC	N.A
21	Ah-681	1	No	No	N.A	G	1.00	N.A	0.37	N.A	CACGACGTTGTAAAACGACATCAAGGGTGGA GGGATAGTGC, TG GTTGGCTTGGTAGTTGGTGT	N.A
22	Ah-715	1	No	No	N.A	G	0.94	N.A	0.38	N.A	CACGACGTTGTAAAACGACGCAGAGAGAAC GGTGGAATCA, CGGTAAGTCTGGCCTTGGTTCT	N.A
23	Ah-727	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGTTGAACTTGA ATCTGGGCTG, AAGCAACACAACAACCACCACT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
24	Ah-735	1	No	No	N.A	G	1.00	N.A	0.37	N.A	CACGACGTTGTAAAACGACTATCCGTCTCAA GGCCAAAACA, AAGCCCTCATCCATTATCCA	N.A
25	Ah-736	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACACCTTGTTTTG GTTGTGGTGA, GGAGGGATAATGCACAAGGGAG	N.A
26	Ah-742	1	No	No	N.A	G	0.88	N.A	0.37	N.A	CACGACGTTGTAAAACGACATGGACCAGAGA TCCTCCATGC, TGGAGCCATTCACTCATCA	N.A
27	AHGS0202	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCAATTCGTTTATC ACCCGCT, TTGCAACGACGAGTCTATCG	N.A
28	AHGS0338	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCCTAATTCAGCC CACGAAG, AGCACATTTGGGGTATTTGG	N.A
29	AHGS0461	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCACTGTGTTGCC CATGTAGG, AGGACATTCAATCTGCAGGC	N.A
30	GA151	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACATCAATGCACAT GGGACAAA, GGGCTGAGGTAAAGGTAAGCA	N.A
31	GA175	1	No	No	N.A	G	0.56	N.A	0.00	N.A	CACGACGTTGTAAAACGACCATCCAACACTTC CAAATACCA, GGTAAGCATGAAGAACGCAAA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
32	GA194	1	No	No	N.A	G	0.50	N.A	0.37	N.A	CACGACGTTGTAAAACGACTTGGGGTTTGA GAGTGAGAA, CCTTGACCTTCCTTGCCTTA	N.A
33	GA37	1	No	No	N.A	G	0.69	N.A	0.00	N.A	CACGACGTTGTAAAACGACCCTCACAAGGC TAAAGAGGAA, TATACCATCCCTGCCACAGAA	N.A
34	GA4	1	No	No	N.A	G	0.94	N.A	0.00	N.A	CACGACGTTGTAAAACGACCTCACCAAGGAA TGTTGAGA, CGCCAAAATGCATATAAGAGC	N.A
35	GA47	1	No	No	N.A	G	0.88	N.A	0.00	N.A	CACGACGTTGTAAAACGACTAGGTGCCTTTG CTTCTCTTG, CCATCTCTTGCTTTGTCATGG	N.A
36	GA54	1	No	No	N.A	G	0.88	N.A	0.00	N.A	CACGACGTTGTAAAACGACGCCATCAGGAGA AGAACTTGA, TGCTGAGAGAGAGCAATGAG	N.A
37	GA58	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACAAATTCACATTCA TCTAACCATTTCA, GAGTGCTAGAGGCAGCAACTTT	N.A
38	GA68	1	No	No	N.A	G	1.00	N.A	0.52	N.A	CACGACGTTGTAAAACGACGAAGACTATTGC ACCCTCCAA, CGGCACTGAATAGAGCAATGT	N.A
39	GM1047	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCCCAAATCTCA ATTCCTAGA, TCCAAAGGGTCAAATTTCTCA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
40	GM1099	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGAGCAGATTGTT AGGTTTTACCC, ATTGCTGATGCTGCTGTTGTA	N.A
41	GM1122	1	No	No	N.A	EST	0.88	N.A	0.00	N.A	CACGACGTTGTAAAACGACACGACTAATAAC CCCGTCAGC, GGTGCAGCAAATGTAGAGGAC	N.A
42	GM1127	1	No	No	N.A	EST	0.94	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGCTAAGAGGTT ACTTGGTTTGC, CAAATGGAAATGACTGCTCCT	N.A
43	GM1184	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGCAAGCATCTTC TTCCTCTCC, CCTTCACTTCCACCATCATCT	N.A
44	GM1527	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACAACCACCATC ACCACTACC, GCAACGACGATTATGAACATTT	N.A
45	GM1655	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGTTTCAGCCGCC TAGAGAAT, GCAGGTAACCTCCCCGTAAA	N.A
46	GM1670	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCGCAACTTCTGT GAGGAACA, TCATTGTTCCAAAACATGC	N.A
47	GM1694	1	No	No	N.A	EST	1.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACGCAAGAGAGAG AAACACCACA, CACGGATATTTGGAATTGACG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
48	GM1724	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCGGCTTGATTGA TAGGGAAA, TGTCTTCCTCATCCCACTCT	N.A
49	GM1760	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGAAGAGCCATG TCAGATCG, AGGGCCCCAACAAAGATAAGT	N.A
50	GM1808	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCTCCGTCACTTCC GTTCAAT, CACAGAAGCAGGCAACAAAA	N.A
51	GM1839	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGAATCTGAGAGT GAAACAGAGCA, GAATTTGGGAAGACGAGGTTG	N.A
52	GM1840	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCGGCAACAACCTT TCCTCTTTT, GCCTCGATATCCTGAAAGCTC	N.A
53	GM1878	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCAGTGGTTCAG TGCATCAAG, GTCCCTTGGTCATCTTCGATT	N.A
54	GM1899	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGGCTCCGATTCTTCT ACGTTTCT, GGAGCTCCGATGAGAAGCTAA	N.A
55	GM1959	1	No	No	N.A	EST	0.94	N.A	0.38	N.A	CACGACGTTGTAAAACGACGTGTTCTCAGCC ATCTTTTCG, GTGAAGGTGTTGTGAATGCAG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
56	GM2120	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCCACTGCCACCT CTATCATC, TCCACCCACATAGACAGAAGC	N.A
57	GM2251	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACAGGCTGAGGAA GAAGGGTAGA, GAGAATCAGGTTTCAGATTCAGGA	N.A
58	GM2444	1	No	No	N.A	EST	0.94	N.A	0.38	N.A	CACGACGTTGTAAAACGACCCCTGTTACACAC AAGCCATT, TGAGCAAGTGTTAGCCATGAA	N.A
59	GM2445	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGCTCAGATTTTA TGGGCAAC, CAGACCACTTTGCCTCTTCTG	N.A
60	GM2515	1	No	No	N.A	EST	0.94	N.A	0.37	N.A	CACGACGTTGTAAAACGACTCGGTTCACTC AACCCCTA, CGGGTCTGGTATCTTGTTCA	N.A
61	GM2605	1	No	No	N.A	EST	1.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACACTGCTGCCATG GTTGAGTTA, TTTCGCACTTTCTCAGTTTCC	N.A
62	GM2606	1	No	No	N.A	EST	0.88	N.A	0.00	N.A	CACGACGTTGTAAAACGACAGAAACACGCGT AGTGGAATG, TGCCGTTTACTCTCACTCACA	N.A
63	GM917	1	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGTATTGGTGGAG GGTTATTGG, CTAGGGTTTGGCTTCACATCA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
64	GNB1	1	No	No	N.A	G	0.81	N.A	0.38	(TA)44	GCTTTTATTCCTTCATTGGGC, CGAAGGATAACAAAGTGTACG	N.A
65	GNB106	1	No	No	N.A	G	0.94	N.A	0.00	(TTAT)7	TGCATATTCACCGTTGATGTT, CCAATTATGAGACTACAGTTATACCAA	N.A
66	GNB107	1	No	No	N.A	G	1.00	N.A	0.42	(GA)33	ATGGCACATGAACAGCAAAA, TCCTCTTGCAAGCAAAATCC	N.A
67	GNB11	1	No	No	N.A	G	0.56	N.A	0.00	(AT)7	TTTCAATTGTGCTCTCCTCTG, TTTGTGTAATCAACGTAACCATCA	N.A
68	GNB113	1	No	No	N.A	G	1.00	N.A	0.38	(GAA)6	CAGCAGCCCTTTTCATCTTT, GGAGGAGGAAGCACAGTGTC	N.A
69	GNB114	1	No	No	N.A	G	1.00	N.A	0.00	(GGT)5	ACGTAACCTTCGTGCCTCCAC, TTCTCCACTTTATGCCACCC	N.A
70	GNB118	1	No	No	N.A	G	1.00	N.A	0.00	(TTTA)5	GCACCAAATAAATGGTGGAAG, TTACCATTGGCCACAACATC	N.A
71	GNB119	1	No	No	N.A	G	1.00	N.A	0.00	(TA)6	CCAATTGTGAACAGAACAAATGA, GGTTATTAAATGCATTCTATCTCGTG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
72	GNB120	1	No	No	N.A	G	0.75	N.A	0.00	(TCA)5	ATTGATTGCCGTTTAGCTGG, CAATATGGTGGTGGTGCAGA	N.A
73	GNB121	1	No	No	N.A	G	0.94	N.A	0.37	(TAA)5	AACCATTTTGC GTGTGTGAA, TCTTAGAATCGGCCCTTGAA	N.A
74	GNB129	1	No	No	N.A	G	1.00	N.A	0.00	(GT)7	GATTTGAGCCCACTTACCCA, TTCATCAACCAAAGTTCCCA	N.A
75	GNB131	1	No	No	N.A	G	1.00	N.A	0.00	(CA)44	CCCCCTTCTTAGACCACAA, GATCTGCTACAATCTTCTCAAGACA	N.A
76	GNB134	1	No	No	N.A	G	1.00	N.A	0.37	(AT)6	GCAATTCCACCCAGAAGGTA, CGGTATCTTCGCAACCAGAT	N.A
77	GNB135	1	No	No	N.A	G	0.81	N.A	0.00	(GT)20	AGTTTGGGATTATATTTTGGGT, ACTCGACCTGCGAAGCTAAG	N.A
78	GNB141	1	No	No	N.A	G	0.94	N.A	0.00	(AT)21	TCGGTTATACAGCCAATCCC, AATGGTACCCCACTGAGC	N.A
79	GNB142	1	No	No	N.A	G	1.00	N.A	0.37	(ATA)5	TGCTGAACTTGAAAAGAAGTCTG, TGGATCCTGGAGGAATTGAG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
80	GNB145	1	No	No	N.A	G	1.00	N.A	0.00	(AC)9gaaacactcaccacg gagagggagagagcac(GA) 16	CCCAAACCCACACTCTCCTA, AAAGGGTTTTTGGCATCTCC	N.A
81	GNB153	1	No	No	N.A	G	1.00	N.A	0.00	(TC)6	GATGATTTTGTGCACACGG, CACACAAAGGAGCGAGATCA	N.A
82	GNB158	1	No	No	N.A	G	0.94	N.A	0.37	(AAT)5	CCCACGGAGATTGTTGGTAT, TTGACAATGGTGATGCCCTA	N.A
83	GNB175	1	No	No	N.A	G	1.00	N.A	0.00	(TA)7	TGGAACAACGGATAGAGTTGG, AAGCAACTCCAATAGTAAAGTTAGAAA	N.A
84	GNB183	1	No	No	N.A	G	0.63	N.A	0.00	(AGA)6	TAAAATATTGCTCGCCCTCG, CCTACCTCGCCCTCTCTTT	N.A
85	GNB20	1	No	No	N.A	G	0.63	N.A	0.00	(AGA)5	GCAATGCTTTTACCACACCA, ACATAACCGAACCCTACCCC	N.A
86	GNB21	1	No	No	N.A	G	0.44	N.A	0.00	(CT)8	GATGACTGGGCGTAACCTCGT, CGCCACCCCTATTCTAACAA	N.A
87	GNB24	1	No	No	N.A	G	1.00	N.A	0.00	(TA)7	TGCGGCTAGTTCCTATGAT, TTGTTACGCAAAGCTAACG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
88	GNB28	1	No	No	N.A	G	1.00	N.A	0.38	(TG)7	TAATGCTGTCGTCAAAACGG, GCTCACACTCATCAGAAGTTCG	N.A
89	GNB30	1	No	No	N.A	G	0.94	N.A	0.00	(AT)9	GGCCTAAACTTTACGGGTCG, TCGCGAAGTCGTATCATTCA	N.A
90	GNB32	1	No	No	N.A	G	0.81	N.A	0.00	(ACA)16	GGGCTTATGGTTCGAATGAA, TAATTCATGTAGCCGACCCC	N.A
91	GNB39	1	No	No	N.A	G	1.00	N.A	0.10	(TA)11	TTGTTCAATGCTAGAACTCAACC, CGAGAATAAACTCCTAAAATTCTCAA	N.A
92	GNB42	1	No	No	N.A	G	1.00	N.A	0.37	(AG)6	TCAGCTTCAACAAGAATGCC, AACAATCCTCCTTGATAATTG	N.A
93	GNB48	1	No	No	N.A	G	0.56	N.A	0.00	(TTC)5	GGCATTGGAATGAAAGAATG, TCCAATCGAACTCCAAAACC	N.A
94	GNB52	1	No	No	N.A	G	0.94	N.A	0.00	(AT)6	CTCCAAATTGGGATGGAGAG, ACTCATAGCAGTCGGCCTTG	N.A
95	GNB53	1	No	No	N.A	G	1.00	N.A	0.37	(TGA)5	GCCCACTCGTTCTAAAAGGG, TTGCTTGCTAGCTTCTGTG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
96	GNB64	1	No	No	N.A	G	1.00	N.A	0.00	(AAAG)5	GCTTGGGAAGTCACCAAAAA, TCACTGGAAGCTCTTTAGTCCA	N.A
97	GNB69	1	No	No	N.A	G	0.50	N.A	0.38	(CTT)5	TCTTGACTTTCCCATGGAGG, TTCAGGCAACCATTCAACAA	N.A
98	GNB71	1	No	No	N.A	G	1.00	N.A	0.00	(AGA)5	ATCCAAACACAAGGGTGAGG, TTTTATTTCCTTTAATTTTCG	N.A
99	GNB72	1	No	No	N.A	G	1.00	N.A	0.00	(TTC)5	TTGGGTTAGGGAGCTTTGTG, CCAATTGGATGAAGAACGAAA	N.A
100	GNB77	1	No	No	N.A	G	0.81	N.A	0.00	(AG)6	AGACCCTTTTCGGTCCAGAT, TCATGGTGTTGCTTGATTT	N.A
101	GNB82	1	No	No	N.A	G	1.00	N.A	0.00	(CAT)6	ATCAAAACCCAAAACCTCCC, AAGCATCAAGGTTTCCGATG	N.A
102	GNB96	1	No	No	N.A	G	0.56	N.A	0.00	(AT)6	AGATTGCGGAACATTGGTTG, GGCTTTATTTTGGGGCATT	N.A
103	PM650	1	No	No	N.A	N.A	1.00	N.A	0.00	N.A	CACGACGTTGTAAACGACAACCTCTCAATG CCCCTTCT, GTGGTGAGGGGAAGCAGATA	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
104	pPGPseq2B9	1	No	No	N.A	G	1.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACGCAACATGCTCT GAATTTTGAC, TGTGCAACCCAATTCAATAACTT	N.A
105	pPGPseq8H1	1	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTTGTGACAACCTT CCCCTTC, CATCCGCTTTCTCTCACCAT	N.A
106	RM15B5	1	No	No	N.A	N.A	0.56	N.A	0.16	N.A	CACGACGTTGTAAAACGACGCTGGTTGTTGG TTTAAGA, GGTCAAGTGAATTGGACAG	N.A
107	RM2H10	1	No	No	N.A	N.A	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACAAAGTGGGAGC ACAAGAAACAT, TAAAGCATTGGGAGAGAACCAT	N.A
108	RM3H10	1	No	No	N.A	N.A	1.00	N.A	0.40	N.A	CACGACGTTGTAAAACGACATCTATCACTTGT CCCCTTTCG, GGTTTCATGTTGTGCGCTCTAT	N.A
109	RN25B1	1	No	No	N.A	N.A	0.81	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCGTTTCGCATAT TCGTCTTC, GAAGAAGCTGGGGTATGAGATG	N.A
110	TC2A02	1	No	No	N.A	N.A	1.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACCTCCCTTGTGGG TATGTGGT, GGCTCCCATTCATTCTCAAA	N.A
111	GM1858	1	No	No	N.A	EST	1.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACAATTTGGCGTTG AGATTGTTG, CGTTTTGTTGTTGAAGTCTTCCT	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
112	AC2C05_2	1	Yes	Yes	3	N.A	1.00	0.44	0.56	N.A	N.A, N.A	N.A
113	Ah193	1	Yes	No	4	G	1.00	0.50	0.60	N.A	CACGACGTTGTAAAACGACCTTGCTGAAGGC AACTCCTACG, TCGGTTTGTCTCTTTGGTCACTC	a01
114	Ah1TC0A01_2	1	Yes	Yes	6	G	1.00	0.31	0.76	N.A	N.A, N.A	a03 (LG7)
115	Ah1TC1D12	1	Yes	No	2	G	1.00	0.88	0.19	N.A	CACGACGTTGTAAAACGACCCCTTTCATTCTC CCTTTCC, TTCTCCTGCACTAGGTTTCCA	N.A
116	Ah1TC2C07	1	Yes	No	2	G	0.94	0.97	0.06	N.A	CACGACGTTGTAAAACGACCACCACTCCC AAGGTTTT, TCAAGAACGGCTCCAGAGTT	N.A
117	Ah1TC2C11_1	1	Yes	Yes	2	G	0.94	0.88	0.19	N.A	N.A, N.A	N.A
118	Ah1TC2D06	1	Yes	No	7	G	0.88	0.36	0.78	N.A	CACGACGTTGTAAAACGACAGGGGGAGTCA AAGGAAAGA, TCACGATCCCTTCTCCTTCA	N.A
119	Ah1TC2D08	1	Yes	No	6	G	0.81	0.31	0.76	N.A	CACGACGTTGTAAAACGACATGTGGGGAGGT CGGTAAC, TCACAGGTTTTGTGTGCTCG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
120	Ah1TC4D02	1	Yes	No	4	G	1.00	0.56	0.55	N.A	CACGACGTTGTAAAACGACAAGTTGTTCCCG TTGCACTC, AAAACACCATAAGGTGAATCAAA	N.A
121	Ah1TC4F01	1	Yes	No	2	G	1.00	0.81	0.26	N.A	CACGACGTTGTAAAACGACGAACAACCGGGA GCAATTTA, CGTCCAGTTCCTATAGAACCTATCA	N.A
122	Ah1TC4F02	1	Yes	No	3	G	0.94	0.60	0.48	N.A	CACGACGTTGTAAAACGACGCACTGCACCCC AATCTCTA, GATGGGTGGTTGGTGTCTC	N.A
123	Ah1TC4G06	1	Yes	No	2	G	0.88	0.93	0.12	N.A	CACGACGTTGTAAAACGACATTTACATTCCC TAGCCCC, CATCGACTGACTTGAAAAATGG	N.A
124	Ah1TC4G10	1	Yes	No	5	G	0.94	0.40	0.68	N.A	CACGACGTTGTAAAACGACTTCGGTCATGTTT GTCCAGA, CTCGAGTGCTCACCTTCAT	N.A
125	Ah1TC4H02	1	Yes	No	5	G	1.00	0.41	0.69	N.A	CACGACGTTGTAAAACGACACCGCAAACCTCA TCCATCTC, GATAGCGTCAGAGGCAGAGG	N.A
126	Ah1TC5A07	1	Yes	No	2	G	0.44	0.86	0.21	N.A	CACGACGTTGTAAAACGACGTTTGGTTCTCCC TCCTCCT, AGCCTCTTCATCCCCTCAT	a04 (LG9)
127	Ah1TC5D01	1	Yes	No	3	G	0.94	0.60	0.41	N.A	CACGACGTTGTAAAACGACCATTGACCACTC ACATCCGT, GATGGGAGTGTGTATTCGGC	b05 (LG21)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
128	Ah1TC6E01	1	Yes	No	5	G	1.00	0.34	0.68	N.A	CACGACGTTGTAAAACGACCTCCCTCGCTTCC TCTTTCT, ACGCATTAAACCACACACCAA	a05 (LG19)
129	Ah1TC6G09	1	Yes	No	2	G	0.81	0.77	0.29	N.A	CACGACGTTGTAAAACGACGGAGGTTGCATG CATCATAGT, TCATTGAACGTATTTGAAAGCTC	N.A
130	Ah26	1	Yes	No	5	G	1.00	0.34	0.71	N.A	CACGACGTTGTAAAACGACGAAAATGATGCC ATAAAGCGTA, AGTGTAACACCCCGTTAGCC	b02 (LG16)
131	Ah2AC2A04_1	1	Yes	Yes	2	G	0.94	0.88	0.19	N.A	N.A, N.A	N.A
132	Ah2AC2B05	1	Yes	No	4	G	1.00	0.47	0.50	N.A	CACGACGTTGTAAAACGACGATCCAATCAGC TATCAACTAT, CCAAGTTCTTACCCATTTCT	N.A
133	Ah2AC2F08_2	1	Yes	Yes	2	G	1.00	0.81	0.26	N.A	N.A, N.A	N.A
134	Ah2TC11A02_2	1	Yes	Yes	2	G	1.00	0.75	0.30	N.A	N.A, N.A	b10 (LG5)
135	Ah2TC11B11	1	Yes	No	2	G	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACAGAGTAGGCAAC ATCCTTGGA, ACATTAACAGCCACACCCTCTT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
136	Ah2TC11C04	1	Yes	No	4	G	1.00	0.66	0.46	N.A	CACGACGTTGTAAAACGACTTTTGACCTAACC CTATTCC, CTCTCTCTTCTCCCTCACTC	N.A
137	Ah2TC11E04	1	Yes	No	4	G	1.00	0.59	0.52	N.A	CACGACGTTGTAAAACGACACGACACCCTGA AATCAAGTTT, CCGAAGGCACCAAAAAGTAT	N.A
138	Ah2TC11H06	1	Yes	No	10	G	1.00	0.25	0.83	N.A	CACGACGTTGTAAAACGACCCATGTGAGGTA TCAGTAAAGAAAGG, CCACCAACAACATTGGATGAAT	N.A
139	Ah2TC7C06	1	Yes	No	3	G	0.94	0.80	0.31	N.A	CACGACGTTGTAAAACGACGGCAGGGGAAT AAAACACTAACT, TTTTCCTTCCTTCTCCTTTGTC	a06 (LG5,1 0)
140	Ah2TC7D03	1	Yes	No	6	G	0.94	0.50	0.59	N.A	CACGACGTTGTAAAACGACCCCTTTATTCACT AAGAAACATGC, CAATTTTCGGCGATCAGAG	N.A
141	Ah2TC7H09	1	Yes	No	2	G	0.94	0.80	0.27	N.A	CACGACGTTGTAAAACGACAACCTTTATGCCA GTCCCCTCTT, GGATGATGACAAGGGTGATTTC	N.A
142	Ah2TC9B08	1	Yes	No	5	G	1.00	0.47	0.64	N.A	CACGACGTTGTAAAACGACGGTTGGGTTGAG AACAAGG, ACCCTCACCCTAACTCCATTA	N.A
143	Ah2TC9F04	1	Yes	No	5	G	1.00	0.78	0.36	N.A	CACGACGTTGTAAAACGACCCTAAACAACGA CAAACACTCA, AAGCACAACACAGAACCCTAAA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
144	Ah2TC9F10	1	Yes	No	5	G	0.75	0.33	0.73	N.A	CACGACGTTGTAAAACGACATCACAATCACA GCTCCAACAA, GGCAAGTCTAATCTCCTTTCCA	N.A
145	Ah2TC9H08	1	Yes	No	2	G	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACGCCAAAGGGGA CCATAAAC, TCCATCTTCCATCTCATCCAC	(LG20)
146	Ah–460	1	Yes	No	2	G	1.00	0.56	0.37	N.A	CACGACGTTGTAAAACGACTGGAGAGATAAT GCACAAGGCA, TG GTTGGCTTGGTAGTTGGTGT	N.A
147	Ah–638	1	Yes	No	3	G	1.00	0.75	0.35	N.A	CACGACGTTGTAAAACGACTCCCTTTTACTA GCCCGCAAC, TTTCCGTCAGGTTCTGCTCTTG	N.A
148	Ah–655	1	Yes	No	2	G	0.88	0.64	0.35	N.A	CACGACGTTGTAAAACGACATCAAAATCAAG GGTGGAGGGA, ACAGCGGCTCTAAGCTCATCAA	N.A
149	Ah–671	1	Yes	No	2	G	1.00	0.97	0.06	N.A	CACGACGTTGTAAAACGACAGAAAGAGCAC GGGACATTACC, ATGAATGAGTGGTCATACGCGA	N.A
150	AHGS0091	1	Yes	No	5	G	1.00	0.28	0.74	N.A	CACGACGTTGTAAAACGACGCCCTCCACCAG TTCTATGA, AGCAGAAGCACTCCCTGAAC	N.A
151	AHGS0134	1	Yes	No	6	G	1.00	0.34	0.74	N.A	CACGACGTTGTAAAACGACGAATCTGCTGTG GACCGTCT, GCAGATGGAGAAGCCATGA	a04 (LG9)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
152	AHGS0151	1	Yes	No	7	G	1.00	0.31	0.76	N.A	CACGACGTTGTAAAACGACAGCAAATGAAGG TGAGGGTG, GCCCAAAAATGCTAACGAAG	(LG20)
153	AHGS0230	1	Yes	No	5	G	1.00	0.31	0.71	N.A	CACGACGTTGTAAAACGACGGAAGCATCACC CTTCAAAT, GATGTTAGCATTTGATGAAAGTCA	b04 (LG13)
154	AHGS0278	1	Yes	No	2	G	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACGGAACCTCCAGTG ACAGGCTC, ACCTGCCGTTGTTTCTCATC	(LG3)
155	AHGS0347	1	Yes	No	4	G	1.00	0.34	0.62	N.A	CACGACGTTGTAAAACGACAACCAAGTATCCC TTCCGCTT, AGTTTGTTGCCAGTTTCGCT	a04 (LG9)
156	AHGS0369	1	Yes	No	4	G	1.00	0.81	0.31	N.A	CACGACGTTGTAAAACGACACGATTCGACGG AGAGAGAG, TTCAAACACAGAACCCCTCC	(LG3)
157	AHGS0372	1	Yes	No	5	G	1.00	0.44	0.62	N.A	CACGACGTTGTAAAACGACTTTGAGTTACTTT GCGCGTG, GCAGAAGCCTACCTAAGCCA	N.A
158	AHGS0482	1	Yes	No	4	G	0.94	0.53	0.49	N.A	CACGACGTTGTAAAACGACCACTGCCTCCTGC AAATGTA, CTTGTGGCCAATATGTCCCT	N.A
159	AHGS0535	1	Yes	No	5	G	1.00	0.53	0.58	N.A	CACGACGTTGTAAAACGACCTCGCAAGAGT CCTTTGAC, TGTAGCAGAAGCACGACCAC	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
160	AHGS0590	1	Yes	No	4	G	1.00	0.38	0.68	N.A	CACGACGTTGTAAAACGACAAGCCCTTCTCCC TCACTTC, ATAGTGGACCTCAACCACGG	N.A
161	AHGS0651	1	Yes	No	2	G	1.00	0.75	0.30	N.A	CACGACGTTGTAAAACGACTTAACTGAGCGA GTGTGCGT, TTAGCCCTGTAGCAGAAGCC	N.A
162	AHGS0695	1	Yes	No	2	G	1.00	0.69	0.34	N.A	CACGACGTTGTAAAACGACTGGTGTGTTGGG AAATGAGA, ACAGCAGAATCTCGGGCTAA	a09 (LG18)
163	AHGS0926	1	Yes	No	3	G	1.00	0.50	0.46	N.A	CACGACGTTGTAAAACGACCCAATGCTTGGT TCTCCATT, AGTGGTTGGCATTGTAAGGG	N.A
164	AHGS0929	1	Yes	No	2	G	1.00	0.69	0.34	N.A	CACGACGTTGTAAAACGACTCCCCTTTCTAGT TTTGCAATC, GCATTTTGTCAAACACTTGGA	N.A
165	AHGS0965	1	Yes	No	9	G	1.00	0.25	0.84	N.A	CACGACGTTGTAAAACGACAACATTCCAGCA GGCAATTC, CGTTGCCAGGAATTGATTTT	N.A
166	Ap40	1	Yes	No	4	G	0.69	0.36	0.68	N.A	CACGACGTTGTAAAACGACCTGTTTGATCGCC GCTATG, GTCAAGTGCTTCCTCCGATG	a04 (LG9)
167	GA145	1	Yes	No	3	G	0.94	0.50	0.52	N.A	CACGACGTTGTAAAACGACTCTCACTTCCATC GTTGCTCT, AAGCGTGGCGTTACAAGTATG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
168	GA166	1	Yes	No	7	G	0.94	0.27	0.80	N.A	CACGACGTTGTAAAACGACTGGTCGCAGATA GTATTCTCCT, TGGAATTTGAATCGCACTCTT	(LG15)
169	GA168	1	Yes	No	3	G	1.00	0.50	0.46	N.A	CACGACGTTGTAAAACGACCTTGAATGGTTCT ACGCTTCG, GATTATCTTCGTTCAATTCATCA	N.A
170	GA199	1	Yes	No	2	G	1.00	0.63	0.36	N.A	CACGACGTTGTAAAACGACTGCTTTGTTTATG TACCTCTTGA, CAATCTGTCCCTCAGGCTTTA	N.A
171	GA24	1	Yes	No	4	G	0.75	0.42	0.62	N.A	CACGACGTTGTAAAACGACAACGAAATATTT TGAGAAAGGAT, AGCATTAGCAACTCTAAGCTCAT	b07 LG2)
172	GA32	1	Yes	No	3	G	0.56	0.50	0.55	N.A	CACGACGTTGTAAAACGACCAGCAATTCAGC AAACTAATGAA, TCCTCCACGTCCTTTTATTT	a05 (LG19)
173	GA34	1	Yes	No	3	G	0.75	0.79	0.32	N.A	CACGACGTTGTAAAACGACTTCAATCATTCA CGTGTCAATC, AGTGCTAGGAGCCAGCAATTT	a05 (LG19)
174	GA48	1	Yes	No	2	G	0.69	0.68	0.34	N.A	CACGACGTTGTAAAACGACAATGGCACTGTG AAGGACAAC, TTCCGGTTATGGAGCATCTAC	N.A
175	GA57	1	Yes	No	2	G	0.94	0.50	0.38	N.A	CACGACGTTGTAAAACGACATCGTCCTCGCA GGTTCTTA, CTTGATTTGGTAATGGGCTGA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
176	GA71_1	1	Yes	Yes	2	G	0.81	0.85	0.23	N.A	N.A, N.A	N.A
177	GA73_2	1	Yes	Yes	2	G	0.75	0.75	0.30	N.A	N.A, N.A	N.A
178	GM1049	1	Yes	No	4	EST	1.00	0.41	0.63	N.A	CACGACGTTGTAAAACGACTTTTAATGCACG AAGCCAATC, CACACCCCGTCATAGGTTCTA	a05 (LG19)
179	GM1062	1	Yes	No	2	EST	1.00	0.88	0.19	N.A	CACGACGTTGTAAAACGACAAGAAAAGGAG GCATGAGAGG, TTCAATTGGGCTTCATCATC	a04 (LG9)
180	GM1114	1	Yes	No	3	EST	1.00	0.44	0.56	N.A	CACGACGTTGTAAAACGACTCAAGCCCCAAA GAAGTATCA, ATCCAGTCCAAATCCGTGAC	N.A
181	GM1128	1	Yes	No	2	EST	0.94	0.97	0.06	N.A	CACGACGTTGTAAAACGACTGTGGTAAATGC ATGGATGG, TGATTCCATGGCCAAAATTAG	N.A
182	GM1241	1	Yes	No	2	EST	1.00	0.56	0.37	N.A	CACGACGTTGTAAAACGACATGAAGAGCGG AAGATGGTG, CTCCTCCCTTCATTCAAAC	N.A
183	GM1254	1	Yes	No	5	EST	0.94	0.50	0.61	N.A	CACGACGTTGTAAAACGACCAAGGACTACA TTTACCACTGC, TGAAGTTGATGGTTTGGTTGG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
184	GM1291_2	1	Yes	Yes	2	EST	1.00	0.88	0.19	N.A	N.A, N.A	a09 (LG18)
185	GM1445	1	Yes	No	5	EST	1.00	0.41	0.66	N.A	CACGACGTTGTAAAACGACGTTGGCAGAGTG GAAGAACTG, CGCTTTTATAATAGGCCGAGGT	b04 (LG13)
186	GM1462	1	Yes	No	2	EST	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACCTTGCAATTAATA GCTTCACACA, TCCATAAGTCGATCAAGGAAAA	N.A
187	GM1483	1	Yes	No	2	EST	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACGCTGTTACATGG GCATCATTT, TCATCAGAGACCCAAGATCCA	b09
188	GM1489	1	Yes	No	7	EST	0.94	0.23	0.81	N.A	CACGACGTTGTAAAACGACGGAAGATGTGGT TGCAAATTC, CTCCCAGCTATCAACTTCACG	a06 (LG5,1 0)
189	GM1490	1	Yes	No	2	EST	1.00	0.84	0.23	N.A	CACGACGTTGTAAAACGACAACTCAAGGGC CAGCATATC, CAAGCCCCTTTGTGTGTGTAT	a06 (LG5,1 0)
190	GM1494_2	1	Yes	Yes	2	EST	1.00	0.56	0.37	N.A	N.A, N.A	a07 (LG4)
191	GM1495	1	Yes	No	2	EST	1.00	0.63	0.36	N.A	CACGACGTTGTAAAACGACGCACCAAACCT TACAATTCA, CAAGAACACACTGCTTCCTTTG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
192	GM1573	1	Yes	No	2	EST	1.00	0.88	0.19	N.A	CACGACGTTGTAAAACGACGAGACCGGAGA CGGAGAGTAT, ACGCCCATAGATTAACCCAGT	a06 (LG5,1 0)
193	GM1596	1	Yes	No	5	EST	1.00	0.44	0.59	N.A	CACGACGTTGTAAAACGACTTGGGTCAAGGT ATGAACAAG, ATGAAGGAAGCGTCAAAATCA	N.A
194	GM1616	1	Yes	No	3	EST	1.00	0.53	0.54	N.A	CACGACGTTGTAAAACGACGCGTAACTCTTTC GGATCTCTT, CCCGTATTTTGAGCAGAACA	N.A
195	GM1628	1	Yes	No	5	EST	1.00	0.41	0.68	N.A	CACGACGTTGTAAAACGACAAACGTGCTCTA GAAACATACAAAA, CAACACATGCAATGCAACAA	a08 (LG12)
196	GM1713	1	Yes	No	2	EST	1.00	0.78	0.28	N.A	CACGACGTTGTAAAACGACTCTGCATGAACT GGACCATC, CACACACACAACACTCAACACA	a08 (LG12)
197	GM1717	1	Yes	No	2	EST	0.94	0.57	0.37	N.A	CACGACGTTGTAAAACGACTGCGAGTGTGTT GATTCCTT, GTGTCGCTTTGCCTTTAACA	a03 (LG7)
198	GM1730	1	Yes	No	3	EST	1.00	0.44	0.57	N.A	CACGACGTTGTAAAACGACCCCCACACCCAC AACATAAT, ATTGTGGCCTTCACAAGAGG	N.A
199	GM1742	1	Yes	No	3	EST	1.00	0.56	0.51	N.A	CACGACGTTGTAAAACGACGCTTGTTGCAA TCATCACA, ACCTCCAACAGGAACATTGC	b10 (LG5)

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
200	GM1798	1	Yes	No	3	EST	1.00	0.50	0.46	N.A	CACGACGTTGTAAAACGACGCCACATGGGAG TTTCAGTT, TGCAACATTTGCTGGGTTTA	b08 (LG4)
201	GM1813	1	Yes	No	3	EST	1.00	0.91	0.17	N.A	CACGACGTTGTAAAACGACTAATGGAAGGCC CTTGAGC, CGGAGCCATTAAACTCCAAA	N.A
202	GM1836	1	Yes	No	2	EST	1.00	0.81	0.26	N.A	CACGACGTTGTAAAACGACCGCCTCTCGTTAC CATCACTA, AACCCAACAAATCCACATTCA	N.A
203	GM1854	1	Yes	No	3	EST	1.00	0.69	0.40	N.A	CACGACGTTGTAAAACGACCCCAACCCTTTC TTTCTCTT, TGGTGGTGTTTTGTTGTTGTT	b03 (LG14)
204	GM1911	1	Yes	No	2	EST	1.00	0.75	0.30	N.A	CACGACGTTGTAAAACGACCAGCTTTCTTTCA ATTCATCCA, CACTTCGTGTTCTTCCTGCTC	a09 (LG18)
205	GM1916	1	Yes	No	2	EST	1.00	0.69	0.34	N.A	CACGACGTTGTAAAACGACATCGACAAGAAT TGGGGTGA, TCTTCCACTCTTCGAAACAA	a06 (LG5,1 0)
206	GM1922	1	Yes	No	3	EST	1.00	0.59	0.46	N.A	CACGACGTTGTAAAACGACGGAGAGTCGGT GAGAGGAGAG, CGCTCGTTTCTCTTTTCATT	a07 (LG4)
207	GM1937_2	1	Yes	Yes	2	EST	1.00	0.69	0.34	N.A	N.A, N.A	a07 (LG4)

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
208	GM1940	1	Yes	No	2	EST	0.94	0.73	0.31	N.A	CACGACGTTGTAAAACGACCAACTAAGGTTG AAGGTTGAAGG, TTTCTTGTGTGAGCTGTGCTG	N.A
209	GM1953	1	Yes	No	3	EST	1.00	0.44	0.58	N.A	CACGACGTTGTAAAACGACTCCACAGTCGAG AGTCCAG, GCCGTCCCAACATTTTATTC	b07 (LG2)
210	GM1954	1	Yes	No	7	EST	1.00	0.34	0.76	N.A	CACGACGTTGTAAAACGACGAGGAGTGTGA GGTTCTGACG, TGGTTCATTGCATTTGCATAC	a03 (LG7)
211	GM1961	1	Yes	No	5	EST	1.00	0.47	0.62	N.A	CACGACGTTGTAAAACGACTGTATTCTCCCTG AAATGACGA, CTTCTTCTCCATCCTCCCTA	b08 (LG4)
212	GM1990	1	Yes	No	6	EST	0.94	0.30	0.75	N.A	CACGACGTTGTAAAACGACTCCTTCCACAAT AACAATGAA, GAGGAGAAAACATGGCCTAAAA	a07 (LG4)
213	GM1996	1	Yes	No	7	EST	0.88	0.39	0.73	N.A	CACGACGTTGTAAAACGACCATCCCATCATTT TCCCTCTT, TACAGTGAAGGTGGGATCCTG	b03 (LG14)
214	GM2009	1	Yes	No	6	EST	0.94	0.43	0.68	N.A	CACGACGTTGTAAAACGACCAAACGCATACA CCCCATAAC, TTTGGTTCTCGTTTGTGTTTT	b03 (LG14)
215	GM2033	1	Yes	No	3	EST	0.88	0.86	0.24	N.A	CACGACGTTGTAAAACGACAAGGTGAAACCA GGAAGCAAT, CCATGACACAAATCCATTGTC	b04 (LG13)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
216	GM2063	1	Yes	No	3	EST	1.00	0.56	0.45	N.A	CACGACGTTGTAAAACGACAAAGTGGTGATT CCCAGTTCC, GAGCAGCGTTGTTGTGGTTAG	(LG3)
217	GM2080	1	Yes	No	2	EST	1.00	0.56	0.37	N.A	CACGACGTTGTAAAACGACCGTCGATTTCGTC GGAGT, GCACAACAAGCATTCTCAGTC	N.A
218	GM2093	1	Yes	No	3	EST	1.00	0.59	0.46	N.A	CACGACGTTGTAAAACGACAATCATGCACAA GATCGAGGA, AGCAGTAATGGCGGAGAAGAT	N.A
219	GM2137	1	Yes	No	4	EST	1.00	0.59	0.54	N.A	CACGACGTTGTAAAACGACCAAAGTCCCATG AATGCTCTC, GTATGAACCGAGCCAAATCCT	b05 (LG21)
220	GM2182_1	1	Yes	Yes	2	EST	0.94	0.67	0.35	N.A	N.A, N.A	N.A
221	GM2280	1	Yes	No	3	EST	1.00	0.81	0.29	N.A	CACGACGTTGTAAAACGACATCTCAATCCAAC CCTTCCAT, TCATCGGTGATCTTACCTCCA	N.A
222	GM2284	1	Yes	No	4	EST	0.94	0.40	0.62	N.A	CACGACGTTGTAAAACGACACACCCCAAATA GCTTCGTCT, TCCACAACACCAACCTTCTTC	N.A
223	GM2388	1	Yes	No	2	EST	1.00	0.50	0.38	N.A	CACGACGTTGTAAAACGACGTGAGGAGCATC TTGTGTGGT, GAACTTCTTGCAAGGGAACAA	b03 (LG14)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
224	GM2402_1	1	Yes	Yes	4	EST	1.00	0.46	0.64	N.A	N.A, N.A	a03 (LG7)
225	GM2402_2	1	Yes	Yes	2	EST	1.00	0.66	0.35	N.A	N.A, N.A	a03 (LG7)
226	GM2411	1	Yes	No	4	EST	1.00	0.75	0.39	N.A	CACGACGTTGTAAAACGACGAACTAATCGC GTGATGGAA, TTTCGCCAAGATTGAAGACAT	a10 (LG1)
227	GM2450	1	Yes	No	3	EST	1.00	0.84	0.26	N.A	CACGACGTTGTAAAACGACAGCAGCAGCAAG AAGATGAAC, ACGCTGCAGAGACGATTTTC	a09 (LG18)
228	GM2499	1	Yes	No	2	EST	0.94	0.80	0.27	N.A	CACGACGTTGTAAAACGACTTCCCTTACCCT TAGAAACAA, TGTTTCATGAAAGGCAAATCCT	N.A
229	GM2528_1	1	Yes	Yes	2	EST	1.00	0.94	0.11	N.A	N.A, N.A	a03 (LG7)
230	GM2531	1	Yes	No	3	EST	1.00	0.47	0.55	N.A	CACGACGTTGTAAAACGACCAAGGATGTCCC AGATGATGT, GGACTCAATTTGTCGACCCTA	a10 (LG1)
231	GM2557	1	Yes	No	4	EST	0.94	0.50	0.53	N.A	CACGACGTTGTAAAACGACGGTAGTATCGCA AGCTCAACG, TACCGTAATGCCAGGGAGAAT	b07 (LG2)

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
232	GM2571	1	Yes	No	2	EST	1.00	0.59	0.37	N.A	CACGACGTTGTAAAACGACGATCGTTTTCACA ACCAAAGC, AGCGATCATGTAACACCGTTC	a08 (LG12)
233	GM2574	1	Yes	No	2	EST	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACCACCACCGCCAA TCTACC, AACTTGTGCACCGGGTTG	b03 (LG14)
234	GM2589	1	Yes	No	5	EST	1.00	0.53	0.61	N.A	CACGACGTTGTAAAACGACTTTTCAGCTACCCT TCTTCCAAA, CAAAAGTGAACCCCGTAAGAA	a04 (LG9)
235	GM2607	1	Yes	No	2	EST	1.00	0.91	0.16	N.A	CACGACGTTGTAAAACGACAGAAACACGCGT AGTGAATG, CCGTGCCGTTTACTCTCACT	b01 (LG6)
236	GM939_1	1	Yes	Yes	3	EST	1.00	0.56	0.45	N.A	N.A, N.A	N.A
237	GM939_2	1	Yes	Yes	2	EST	1.00	0.93	0.12	N.A	N.A, N.A	N.A
238	GM986	1	Yes	No	6	EST	0.94	0.30	0.75	N.A	CACGACGTTGTAAAACGACAGCCAAAATGGG CTATACGAT, TTTCTGTGGCTCTTGCTT	N.A
239	GM988	1	Yes	No	4	EST	1.00	0.44	0.64	N.A	CACGACGTTGTAAAACGACGCCACTTCTCAA GGAATGACA, GGAGGAGGACCATCATCAAAT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
240	GNB104	1	Yes	No	3	G	1.00	0.72	0.40	(TA)7	CGGAAATATTGCTGGATGCT, CCACAATGCATTTATGGGAT	N.A
241	GNB105	1	Yes	No	2	G	1.00	0.88	0.19	(AGA)6	TAAAAGATTGCTCATCCCCG, AACCATTTCCCTCCCATT	N.A
242	GNB112	1	Yes	No	2	G	1.00	0.94	0.11	(AT)23	CAATGTCTGGGTGACAACCTT, TTATCCGACCGTGAGTAATGC	N.A
243	GNB117	1	Yes	No	2	G	0.50	0.94	0.11	(AG)6	AGAAGTCCTTAAATCCCTCTCC, CGCGATAAGGAAAAATCCAA	N.A
244	GNB122	1	Yes	No	2	G	1.00	0.69	0.34	(CT)6	CATGAATGGTCCATAAGCCA, GACTGGATACCGGGATACTCA	N.A
245	GNB127	1	Yes	No	3	G	1.00	0.53	0.48	(TG)8tc(TA)22	GGATGTAATATTGGCATGTTACTTGT, AGTGTCACGCTCCGGTTAT	N.A
246	GNB136	1	Yes	No	5	G	1.00	0.47	0.65	(GA)16	GATTTAATGGGGGATGAGGG, TCACCCACACCCTTTTGATT	N.A
247	GNB14	1	Yes	No	2	G	1.00	0.81	0.26	(AT)10	AACTGGGATGTTGAACAAAGG, CTCCCCTTCTTGACGATGA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
248	GNB144	1	Yes	No	2	G	1.00	0.59	0.37	(TA)6	TAACGGAGGAGCCAAAAGTG, CACCATTCCATCACCTCAAA	N.A
249	GNB156	1	Yes	No	3	G	1.00	0.78	0.33	(CTT)6	CCACTTTTCAAATTACGCGA, CACAGCATCAGAACCTGCAT	N.A
250	GNB161	1	Yes	No	2	G	0.94	0.57	0.37	(TA)9	CGACAACTTTAAACTCTCCCC, CATCCACGCAAAACAATTTTA	N.A
251	GNB174	1	Yes	No	2	G	0.94	0.93	0.12	(TA)7	GAAGTTTTTGGGGGATTTCGT, CCACCTTGGTCGAGATGTTT	N.A
252	GNB180	1	Yes	No	5	G	0.88	0.46	0.58	(AAC)7	CAAAATTGACGAGAGCAGCA, ACGAGCTGTTTCACTTTCGC	N.A
253	GNB29_1	1	Yes	Yes	2	G	1.00	0.81	0.26	N.A	N.A, N.A	N.A
254	GNB40	1	Yes	No	4	G	1.00	0.44	0.55	(GAAAG)5	GGCGAGAGAATAATGTGAGGA, AACAAGGTCGCATCTGCTCT	N.A
255	GNB41	1	Yes	No	3	G	0.88	0.71	0.40	(TAGA)11	TTGATGTGGCATCATTAGCAA, TGCCAATTCAGCTTTCACTTT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
256	GNB43_2	1	Yes	Yes	3	G	1.00	0.81	0.29	N.A	N.A, N.A	N.A
257	GNB51	1	Yes	No	3	G	0.94	0.70	0.39	(TTA)10	AAGCTTATCATGTGCCCCAA, GGAAAAGGTAATGCCACCAA	N.A
258	GNB54	1	Yes	No	3	G	1.00	0.66	0.45	(ATA)7	ACCGGCTGATTTTGAAGTTG, TTTACGTGATTGATTTTATGCTAAC	N.A
259	GNB68	1	Yes	No	2	G	0.50	0.56	0.37	(TTA)19	CACCCTTCTCTCAAACCT, CTGGAGTGTCTACCCCCA	N.A
260	GNB8	1	Yes	No	3	G	1.00	0.50	0.46	(CT)16	ACGTGGCCCCAAATAAGTCT, CACACCCATTTCACTCA	N.A
261	GNB85	1	Yes	No	2	G	1.00	0.94	0.11	(AAT)6	TGAGTGTAATTCTAGAGCCAAAA, AAAGGAATAAAAATAAAGTCGGC	N.A
262	GNB87	1	Yes	No	2	G	0.88	0.57	0.37	(AT)11	GCCTGTAGCACTGCAAACAA, GGAATAGGGGCAAGAATGGT	N.A
263	GNB88	1	Yes	No	2	G	0.81	0.92	0.13	(TAA)5	CCACGTGAGAGCAAAAGAACT, CGGGACGATTTTGATTTTGA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
264	GNB90	1	Yes	No	2	G	1.00	0.97	0.06	(TA)6	CTCCACAAGCTGTGGCTAGA, CTTCCACACGTCGTAAAGCA	N.A
265	GNB98	1	Yes	No	3	G	0.44	0.64	0.46	(TA)7(GA)8	GAGAAGGAGACAGAGTGTAAAGGG, TTCATTCATTCACCGACTCATC	N.A
266	IPAHM105	1	Yes	No	3	G	0.94	0.73	0.37	N.A	CACGACGTTGTAAAACGACCAGAGTTTGGGA ATTGATGCT, GCCAGATCTGAGCAAGAACC	a04 (LG9)
267	IPAHM108_1	1	Yes	Yes	5	G	1.00	0.40	0.69	N.A	N.A, N.A	a04 (LG9)
268	IPAHM108_2	1	Yes	Yes	5	G	1.00	0.31	0.73	N.A	N.A, N.A	a04 (LG9)
269	IPAHM297	1	Yes	No	3	G	0.94	0.73	0.37	N.A	CACGACGTTGTAAAACGACAATGCCAAGCTA GATAGAGTAGTGA, CAGCGAATAGAGCAGAAGGA	N.A
270	IPAHM606	1	Yes	No	2	G	1.00	0.63	0.36	N.A	CACGACGTTGTAAAACGACCCTAACTCAGCCT GCGAAAC, CAGAGGTGTTTGGAGAACTAGGA	b08 (LG4)
271	IPAHM659_2	1	Yes	Yes	3	G	0.94	0.67	0.44	N.A	N.A, N.A	a06 (LG5,1 0)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
272	PM741	1	Yes	No	2	N.A	1.00	0.75	0.30	N.A	CACGACGTTGTAAAACGACAGGATCAAGAAA AGGCAGCA, GGCGACGCAATTGAGACTAT	N.A
273	pPGPseq1B9_1	1	Yes	Yes	3	G	1.00	0.56	0.45	N.A	No Sequence, No Sequence	(LG11)
274	pPGPseq1B9_2	1	Yes	Yes	2	G	1.00	0.63	0.36	N.A	N.A, N.A	(LG11)
275	pPGPseq2C11_2	1	Yes	Yes	5	G	1.00	0.44	0.68	N.A	N.A, N.A	N.A
276	pPGPseq2D12B	1	Yes	No	7	G	1.00	0.41	0.72	N.A	CACGACGTTGTAAAACGACAAGCTGAACGAA CTCAAGGC, TGCAATGGGTACAATGCTAGA	N.A
277	pPGPseq2F5	1	Yes	No	4	G	1.00	0.69	0.45	N.A	CACGACGTTGTAAAACGACTGACCAAAGTGA TGAAGGGA, AAGTTGTTGTACATCTGTCATCG	(LG17)
278	pPGPseq3B5_2	1	Yes	Yes	9	G	1.00	0.19	0.86	N.A	N.A, N.A	N.A
279	pPGPseq3E10	1	Yes	No	3	G	1.00	0.59	0.48	N.A	CACGACGTTGTAAAACGACTCCCAAAAATAA CAAACATGGA, ACGCTTTGAGACTCGTCGTT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
280	pPGPseq5D5	1	Yes	No	5	G	1.00	0.34	0.72	N.A	CACGACGTTGTAAAACGACAAAAGAAAGACC TTCCCCGA, GCAGGTAATCTGCCGTGATT	b07 LG2)
281	pPGPseq7G2	1	Yes	No	5	G	1.00	0.41	0.61	N.A	CACGACGTTGTAAAACGACACTCCCGATGCA CTTGAAAT, AACCTCTGTGCACTGTCCCT	N.A
282	pPGSseq13A10	1	Yes	No	2	G	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACAACTCGCTTGTA CCGGCTAA, AGGAATAATAACAATACCAACAGCA	N.A
283	pPGSseq13A7	1	Yes	No	3	G	1.00	0.66	0.41	N.A	CACGACGTTGTAAAACGACAATCCGACGCAA TGATAAAAA, TCCCCTTATTGTTCCAGCAG	b01 (LG6)
284	pPGSseq16C6	1	Yes	No	5	G	1.00	0.44	0.64	N.A	CACGACGTTGTAAAACGACTTGCTACTAAGC CGAAAATGAAG, CTTGAAATTAACACATATGCACACA	N.A
285	pPGSseq17E1	1	Yes	No	5	G	1.00	0.47	0.64	N.A	CACGACGTTGTAAAACGACTTCGTTGACGTG AGCGTTAC, TTAGGATTGTTCCAAGGCCA	N.A
286	pPGSseq18C5	1	Yes	No	5	G	1.00	0.31	0.67	N.A	CACGACGTTGTAAAACGACGGACGCCGGAT GCTATTTA, ACATGAGTCCCTTTCCCTT	a04 (LG9)
287	pPGSseq18G1_2	1	Yes	Yes	3	G	1.00	0.56	0.45	N.A	N.A, N.A	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
288	RM14G2	1	Yes	No	3	N.A	1.00	0.66	0.39	N.A	CACGACGTTGTAAAACGACGCTTTACCAGAC ACAGCAATTC, CTAGCAATAACAGCATGAACCG	N.A
289	RM1G8_2	1	Yes	Yes	4	N.A	1.00	0.56	0.56	N.A	N.A, N.A	N.A
290	RM8E8	1	Yes	No	5	N.A	1.00	0.38	0.68	N.A	CACGACGTTGTAAAACGACGAACGCGTCAAA GAACATAACA, TTAACAACAGCAACAGCTTCGT	N.A
291	RN26A8	1	Yes	No	2	N.A	1.00	0.75	0.30	N.A	CACGACGTTGTAAAACGACAGGATTGGGTCA AGTCATTTTC, GGTAACAGGAAAGCGTGCAT	N.A
292	TC03A12	1	Yes	No	3	N.A	1.00	0.47	0.55	N.A	CACGACGTTGTAAAACGACGCCCATATCAAG CTCCAAAA, TAGCCAGCGAAGGACTCAAT	N.A
293	TC03E02	1	Yes	No	4	N.A	1.00	0.47	0.62	N.A	CACGACGTTGTAAAACGACTGAAAGATAGGT TTCGGTGGA, CAAACCGAAGGAGGAACTTG	N.A
294	TC04H02	1	Yes	No	4	N.A	1.00	0.41	0.63	N.A	CACGACGTTGTAAAACGACACCGCAAACCTCA TCCATCTC, GATAGCGTCAGAGGCAGAGG	N.A
295	TC06E01_1	1	Yes	Yes	4	N.A	1.00	0.44	0.53	N.A	N.A, N.A	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
296	TC06E01_2	1	Yes	Yes	4	N.A	0.81	0.38	0.64	N.A	N.A, N.A	N.A
297	TC07A02	1	Yes	No	6	N.A	1.00	0.28	0.76	N.A	CACGACGTTGTAAAACGACCGAAAACGACAC TATGAAACTGC, CCTTGGCTTACACGACTTCCT	N.A
298	TC11A04_1	1	Yes	No	3	N.A	1.00	0.56	0.45	N.A	N.A, N.A	a06 (LG5,1 0)
299	TC11A04_2	1	Yes	No	8	N.A	1.00	0.25	0.83	N.A	N.A, N.A	a06 (LG5,1 0)
300	TC11B04_1	1	Yes	No	4	N.A	1.00	0.72	0.43	N.A	N.A, N.A	a04 (LG9)
301	TC11B04_2	1	Yes	No	7	N.A	1.00	0.29	0.79	N.A	N.A, N.A	a04 (LG9)
302	Ah1TC2B05	2	No	No	N.A	G	1.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACGGCGGGAAGAG CTTAAGAAG, TTGCAATGGATCTCACCAAA	N.A
303	Ah2AC1D11	2	No	No	N.A	G	1.00	N.A	0.37	N.A	CACGACGTTGTAAAACGACTGCAGAGAGGTT GGATGGAGTA, CCCGCTAATCCCCGAAGT	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
304	GM1542	2	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGTTCTCTGATGGA CTCGGAGA, CAATACGCATAGATGCAAACAG	N.A
305	GNB103	2	No	No	N.A	G	1.00	N.A	0.00	(TTC)5	CCTTGCCGAATACACATCT, CCCATGACTCGGAGGTAGAA	N.A
306	GNB162	2	No	No	N.A	G	1.00	N.A	0.19	(TTA)7	TGGCTTTTCTGCTTGCTTT, TGAAGGCTTGAACAGTGCAT	N.A
307	GNB164	2	No	No	N.A	G	1.00	N.A	0.00	(TCA)6(TCT)5tttttttctt attcatcttttgttttctgttct atcttttcaagtttcttctgttt tactctttaataagaataaa	TCTCCTCCTTATCTTCTGCTGC, TGCAAGTTCATCCTCTTCCAA	N.A
308	GNB188	2	No	No	N.A	G	1.00	N.A	0.37	(TA)14	ATCAATGCACATGGGACAAA, AATGGGGCTGAGGTAAAGGT	N.A
309	GNB4	2	No	No	N.A	G	0.94	N.A	0.00	(TA)7	GGGGCTAAGGTAAGGGTGAG, TGGGTAGCAAGGCATGATCT	N.A
310	Ah1TC1A02_1	2	Yes	Yes	4	G	1.00	0.47	0.54	N.A	N.A, N.A	N.A
311	Ah1TC1A02_2	2	Yes	Yes	3	G	1.00	0.88	0.21	N.A	N.A, N.A	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
312	Ah1TC1D01	2	Yes	No	3	G	0.94	0.70	0.39	N.A	CACGACGTTGTAAAACGACTGCCAATCTCCTC TTCAACC, TCAGGCAAGGGTTCCTACTG	N.A
313	Ah1TC1D02_1	2	Yes	Yes	3	G	1.00	0.63	0.43	N.A	N.A, N.A	a09 (LG18)
314	Ah1TC1D02_2	2	Yes	Yes	4	G	1.00	0.63	0.48	N.A	N.A, N.A	a09 (LG18)
315	Ah1TC1E01	2	Yes	No	8	G	1.00	0.41	0.75	N.A	CACGACGTTGTAAAACGACCAGCAAAGAGTC GTCAGTCG, GAAAGTTCACTTGAGCAAATTCA	N.A
316	Ah1TC2B01	2	Yes	No	2	G	1.00	0.50	0.38	N.A	CACGACGTTGTAAAACGACTTGCAGAAAAGG CAGAGACA, GAAAGAAGCTAAGAAGGACCCATA	N.A
317	Ah1TC2G05	2	Yes	No	9	G	1.00	0.25	0.84	N.A	CACGACGTTGTAAAACGACGCCGAGCTAGTT TGATTTGG, TTGGATTGAATGGAGGAATG	N.A
318	Ah1TC3B04	2	Yes	No	9	G	1.00	0.25	0.81	N.A	CACGACGTTGTAAAACGACGAAGAAGAAGTC ACTGCGGC, AAGCTAGTTTCTGATTAAAGCACCA	a08 (LG12)
319	Ah1TC3E02	2	Yes	No	11	G	1.00	0.34	0.80	N.A	CACGACGTTGTAAAACGACTGAAAGATAGGT TTCGGTGGA, CAAACCGAAGGAGGAACCTG	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
320	Ah1TC3E05	2	Yes	No	7	G	1.00	0.31	0.78	N.A	CACGACGTTGTAAAACGACCACCACTTGAGT TGGTGAGG, CTTCTTCTCTCCCGCAATG	N.A
321	Ah1TC3H01	2	Yes	No	3	G	1.00	0.69	0.40	N.A	CACGACGTTGTAAAACGACCTAGGATGGGTG ACCTCCTG, TAACACTATCCGCGAAGCCT	N.A
322	Ah1TC5D06	2	Yes	No	5	G	0.81	0.58	0.58	N.A	CACGACGTTGTAAAACGACGAAATTTTAGTTT TCAGCACAGCA, TTTTCCCCTCTAAATTTTCTCG	a09 (LG18)
323	Ah2TC7G10_1	2	Yes	Yes	2	G	1.00	0.94	0.11	N.A	N.A, N.A	a04 (LG9)
324	Ah2TC7G10_2	2	Yes	Yes	6	G	1.00	0.44	0.65	N.A	N.A, N.A	a04 (LG9)
325	Ah2TC7H11	2	Yes	No	11	G	0.75	0.29	0.82	N.A	CACGACGTTGTAAAACGACAGGTTGGAAC TA TGGCTGATTG, CCAGTTTAGCATGTGTGGTTCA	N.A
326	Ah2TC9H09	2	Yes	No	9	G	0.88	0.18	0.86	N.A	CACGACGTTGTAAAACGACTTAGCGACAAAG GATGGTGAG, TAGGGACGAAAATAGGGACTGA	N.A
327	Ah3	2	Yes	No	6	G	1.00	0.31	0.75	N.A	CACGACGTTGTAAAACGACTCGGAGAACAAG CACACATC, TTGCGCTCTTTCTCACACTC	b01 (LG6)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
328	Ah-723	2	Yes	No	2	G	0.94	0.77	0.29	N.A	CACGACGTTGTAAAACGACTACTCCTCAGAC ATGGTGGCGA, TCACGTCACACCACTCTCCCTT	N.A
329	GA12	2	Yes	No	2	G	0.75	0.71	0.33	N.A	CACGACGTTGTAAAACGACAAACGAGAAGC GTTGAGTTAGC, CGTTGAAGAAGAAGCTCGAAA	N.A
330	GA141	2	Yes	No	2	G	1.00	0.81	0.26	N.A	CACGACGTTGTAAAACGACAACATCTCTCTCC CACCCAAT, CAGGGATCTCTTGATTGTCAG	N.A
331	GA161	2	Yes	No	9	G	0.50	0.25	0.84	N.A	CACGACGTTGTAAAACGACTGAGGCCGTCTT GTTTAGAGA, CCTCTTCCATCACCGTTCATA	a10 (LG1)
332	GM1331	2	Yes	No	3	EST	1.00	0.59	0.44	N.A	CACGACGTTGTAAAACGACTCAAGCAAAGT GTCAGAGAA, AAGGAACCGGAAACCATACAC	b01 (LG6)
333	GM1457	2	Yes	No	2	EST	1.00	0.50	0.38	N.A	CACGACGTTGTAAAACGACTGCTCCATAGATT TCCCAAAA, TCCACTAGGGCCTGTTACTGA	N.A
334	GM1918	2	Yes	No	3	EST	1.00	0.78	0.31	N.A	CACGACGTTGTAAAACGACCTACCGCTTCTCC TCCTCCT, GGGATCCTCTCTGCCATCTAC	N.A
335	GM1971_1	2	Yes	Yes	3	EST	1.00	0.50	0.55	N.A	N.A, N.A	a01

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
336	GM1971_2	2	Yes	Yes	3	EST	1.00	0.50	0.51	N.A	N.A, N.A	a01
337	GM1986_1	2	Yes	Yes	3	EST	0.94	0.50	0.55	N.A	N.A, N.A	a07 (LG4)
338	GM1986_2	2	Yes	Yes	4	EST	0.94	0.37	0.64	N.A	N.A, N.A	a07 (LG4)
339	GM2056	2	Yes	No	3	EST	1.00	0.81	0.29	N.A	CACGACGTTGTAAAACGACGCTGAAACACCA CCAACAACCT, GAAGCGTCAATGATGAAGGTC	N.A
340	GM2081	2	Yes	No	2	EST	0.94	0.70	0.33	N.A	CACGACGTTGTAAAACGACCTGAAATCTCGA TGCGAATG, ACAAGATCAATGGTGCCGTAG	N.A
341	GM2156	2	Yes	No	3	EST	0.94	0.53	0.54	N.A	CACGACGTTGTAAAACGACCGTGCTCTTGTTTC TCTTCTCTG, TGGAGGAATGAAGGAAGGAAT	b07 LG2)
342	GM2177	2	Yes	No	2	EST	0.94	0.80	0.27	N.A	CACGACGTTGTAAAACGACGTCAGAAAGTGCT TTCCCATG, GTGTGTCACTCTTTCGCCATT	N.A
343	GM2206_1	2	Yes	Yes	2	EST	1.00	0.69	0.34	N.A	N.A, N.A	a03 (LG7)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
344	GM2206_2	2	Yes	Yes	2	EST	1.00	0.80	0.27	N.A	N.A, N.A	a03 (LG7)
345	GM2584	2	Yes	No	3	EST	1.00	0.81	0.30	N.A	CACGACGTTGTAAAACGACACATTTTCAGCAC CCTTCCTT, ACTCGTCAATGTTCCCTTGTG	b04 (LG13)
346	GNB10	2	Yes	No	7	G	0.88	0.36	0.76	(TA)27	CACTATAGTTATCGGAGCCACTG, AAAACAGCGTTTTTAGTTTCAACA	N.A
347	GNB108	2	Yes	No	4	G	0.94	0.50	0.58	(AAAAG)5	AACTTTGGGACATTGGTTGG, TACTTTGGGCATTTTGTCC	N.A
348	GNB111	2	Yes	No	2	G	1.00	0.88	0.19	(TA)6	GTATTTTGCCCTTTGGGTT, TGCAACACTCCGATCGATAA	N.A
349	GNB12_1	2	Yes	Yes	2	G	1.00	0.88	0.19	N.A	N.A, N.A	N.A
350	GNB15	2	Yes	No	3	G	0.44	0.71	0.41	(TA)12	GCATACATTCAAGTGGCCCTA, CGTGATTAGGCATGAAAGTGA	N.A
351	GNB150	2	Yes	No	2	G	0.94	0.93	0.12	(TA)6	GACTGGTTATCTTCGAGCC, AAAAGCGCAACACTCCGAT	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
352	GNB18	2	Yes	No	8	G	0.88	0.32	0.79	(AT)27	AAAAATTCTTCCTAGTTTACCCCC, CTCAACACAACAAGTGGAGCA	N.A
353	GNB189	2	Yes	No	2	G	1.00	0.81	0.26	(TATTT)5tttttcgccac(T)10	TGGCTTTTCTGCTTGCTTT, TTGATGTGGTGGCAATAATT	N.A
354	GNB44	2	Yes	No	3	G	1.00	0.56	0.41	(TGG)5	AGTTGGACACGTAACCTCCCG, CGTCGAGTGCATAGTGATCG	N.A
355	GNB45	2	Yes	No	5	G	0.63	0.50	0.62	(TC)19	CCACCACAACAGTGGTTCAG, AAGCATAACGCGAAGGAGAA	N.A
356	GNB55	2	Yes	No	2	G	1.00	0.53	0.37	(AG)7	TTTTGACCCAGATTTAGCC, AAGAGGAGTCCTAATCCTAATCCT	N.A
357	GNB70	2	Yes	No	10	G	1.00	0.25	0.86	(AT)30	CTGCAGCTGCGGTAAC TAGA, GGTAAGGTGCTCCTGCAAAG	N.A
358	GNB79	2	Yes	No	2	G	0.94	0.70	0.33	(TA)27	CAAGGTTGTAACGGGGCTAA, GAGGAATTTTGGTAGCTAAGCAT	N.A
359	GNB9	2	Yes	No	2	G	0.94	0.93	0.12	(TA)6	TTCCAACACTTTTGCAATTTCA, GTAACCACACCATTGGGCTC	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
360	IPAHM123_1	2	Yes	Yes	4	G	1.00	0.69	0.44	N.A	N.A, N.A	b08 (LG4)
361	IPAHM123_2	2	Yes	Yes	6	G	1.00	0.38	0.73	N.A	N.A, N.A	b08 (LG4)
362	IPAHM136	2	Yes	No	2	G	0.88	0.86	0.21	N.A	CACGACGTTGTAAAACGACCCCTTTCTCCAC TACTACCA, TTCTCCTAGGGACTCCGATG	b05 (LG21)
363	IPAHM245	2	Yes	No	5	G	0.94	0.47	0.54	N.A	CACGACGTTGTAAAACGACCCAAGGACCTA GTGACCAA, GGACCCTTAGCACATTCCAA	a06 (LG5,1 0)
364	Lec1	2	Yes	No	9	N.A	1.00	0.19	0.86	N.A	CACGACGTTGTAAAACGACCAAGCATCAACA ACAACGA, GTCCGACCACATACAAGAGTT	b09
365	pPGPseq2G4	2	Yes	No	7	G	1.00	0.28	0.78	N.A	CACGACGTTGTAAAACGACTTCTTGTTTCCTT TGGCTTC, TGCTCAAGTGCCTTATTGGTG	N.A
366	pPGSseq10D4_2	2	Yes	Yes	5	G	1.00	0.63	0.52	N.A	N.A, N.A	a05 (LG19)
367	pPGSseq18A5	2	Yes	No	7	G	1.00	0.50	0.66	N.A	CACGACGTTGTAAAACGACTGATTCGATTTC TCATGCACA, GAGGATTCTTGAGCCTCGAC	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
368	pPGSseq19D9	2	Yes	No	5	G	1.00	0.56	0.59	N.A	CACGACGTTGTAAAACGACTGTTGCCCACTGT TCTAATCA, TCAAATGGCATAGTCTCCCC	N.A
369	pPGSseq9G5	2	Yes	No	3	G	0.88	0.50	0.46	N.A	CACGACGTTGTAAAACGACCAAATTGTGCAG CCAAGAGA, CATATGCCCAGGAAGAGGAA	N.A
370	RM19A10	2	Yes	No	4	N.A	1.00	0.38	0.62	N.A	CACGACGTTGTAAAACGACCTTCGCACTCAA AAGTTTGTTTT, TGAACCTTGCTTCAGAACCTTGA	N.A
371	RM6D9	2	Yes	No	2	N.A	1.00	0.63	0.36	N.A	CACGACGTTGTAAAACGACAGTCTCTCCCCAC AAAACCTCA, TTGATAACATCCAGACAAACCG	N.A
372	RN16F05	2	Yes	No	5	N.A	0.94	0.50	0.60	N.A	CACGACGTTGTAAAACGACCTACCTCCTTGC CGTCCT, CCGATTCTCCTTCTCTTCG	a05 (LG19)
373	TC04G02	2	Yes	No	9	N.A	1.00	0.25	0.81	N.A	CACGACGTTGTAAAACGACGATCCAACCTGTG AATTGGGC, CACACCAGCAACAAGGAATC	N.A
374	TC05A06	2	Yes	No	8	N.A	1.00	0.31	0.80	N.A	CACGACGTTGTAAAACGACTCGGTTTGGGAG ACACTCTT, TTGTAAGCAGACGCCACATC	N.A
375	TC05D06_2	2	Yes	Yes	6	N.A	1.00	0.38	0.73	N.A	N.A, N.A	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
376	TC06H03	2	Yes	No	6	N.A	1.00	0.25	0.76	N.A	CACGACGTTGTAAAACGACTCACAATCAGAG CTCCAACAA, CAGGTTACCAGGAACGAGT	N.A
377	TC3E05	2	Yes	No	8	N.A	1.00	0.31	0.80	N.A	CACGACGTTGTAAAACGACCACCACTTGAGT TGGTGAGG, CTTCTTCTCTCCCGCAATG	b10 (LG5)
378	Ah1TC1G04	3	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGCTGTGAGAGA AATGGCAG, GCGCATTCTTCGATTAAAGG	N.A
379	Ah1TC3G01	3	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGACGGTAATCGT GCCCTAAA, TGCAGTAGTGGCAGCAGAAC	N.A
380	Ah1TC5H11	3	No	No	N.A	G	0.94	N.A	0.00	N.A	CACGACGTTGTAAAACGACAAAAATCGGACC ATGATTGG, TCTTCTCCTCCCTCACTCCA	N.A
381	Ah2TC9C08	3	No	No	N.A	G	0.69	N.A	0.00	N.A	CACGACGTTGTAAAACGACACTTTTGGGGCA GGATGAG, GCCTCTATTGCTGAGATTATTGC	N.A
382	Ah–545	3	No	No	N.A	G	0.75	N.A	0.00	N.A	CACGACGTTGTAAAACGACTTGGTGAGGTGC TCTGTAAGGC, CAGAGGGAGGCAGAGACAACAA	N.A
383	Ah–556	3	No	No	N.A	G	0.88	N.A	0.00	N.A	CACGACGTTGTAAAACGACACCTTCTCAAG GCCAAAACAG, TGCTCTCATCTCGCTTCTCCAT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
384	Ah-657	3	No	No	N.A	G	0.81	N.A	0.00	N.A	CACGACGTTGTAAAACGACAATCACACCTTTC CCTTCTCGC, TGC GTTGGGTATGTTTCTGCTT	N.A
385	AHGS0124	3	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACAGGGAGAAACG GGAAGGTTA, GCAACGCTGCATTTTATTCA	N.A
386	AHGS0194	3	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGGATGGGACAG GTTGAGAGA, GATATCAATTGGCACGCTTTT	N.A
387	AHGS0393	3	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCCCAACATCCAC ATCAATCA, ACCCCTTTCTTTCAAGGCTC	N.A
388	GA131	3	No	No	N.A	G	0.63	N.A	0.37	N.A	CACGACGTTGTAAAACGACATGTCCTTGCCTT GTTTCGTT, TAGTTGGCGGTATGGCTTAGA	N.A
389	GA195	3	No	No	N.A	G	0.94	N.A	0.37	N.A	CACGACGTTGTAAAACGACAAACATGCTCCT GCCTCTCT, TCAGAGTACAATCATGCAATCAAA	N.A
390	GA27	3	No	No	N.A	G	0.88	N.A	0.00	N.A	CACGACGTTGTAAAACGACCATCCAAAGCCA AAGTTCACA, GCTTAGCTTGCTTTGATTAGGG	N.A
391	GA43	3	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGGCCAGAACAAA TCG GTTATT, TTGTTCAAGATTTAGTCACTCAACATA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
392	GA53	3	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCTATGTTCCCGG TGTACGTT, TAATGCCTCACTTGGAGTTGG	N.A
393	GM1021	3	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCAAAGCACCCAT GGCTAGATA, ATTTGAGCTACACCGAGAGCA	N.A
394	GM1171	3	No	No	N.A	EST	0.94	N.A	0.00	N.A	CACGACGTTGTAAAACGACCGATTAGGACCT CACCAACAC, TGTCTTCAGCCTCATCATTCC	N.A
395	GM1309	3	No	No	N.A	EST	0.81	N.A	0.00	N.A	CACGACGTTGTAAAACGACCTCCTCCTCTTCC ACCTGTTT, GCACGACTTGACAGCTTC	N.A
396	GM1521	3	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACACAACAACCGAG TCAGCATCT, ACTCCTCGATGTGTTTGTGG	N.A
397	GM1549	3	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTTCCAAGAATCA ACATCACCA, AAGGAAGAAGCAGAGCAATCC	N.A
398	GM1726	3	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGAGTGTTGTTGG CATATATAATAGTGA, GGAGGTGGTGATCTTCCTCA	N.A
399	GM1864	3	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACCAACACACCCAG TCACTCTCTC, TCCTTTCTGATGTTCTGTGTGTG	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
400	GM2007	3	No	No	N.A	EST	0.88	N.A	0.00	N.A	CACGACGTTGTAAAACGACGGCCTCTATTC CTTCCTTT, TTGGGATTCTTGATCAGATG	N.A
401	GM2058	3	No	No	N.A	EST	0.94	N.A	0.00	N.A	CACGACGTTGTAAAACGACATCATCATATCCC ACCGTTCA, ATGCTCCTCGCTTTGTCCT	N.A
402	GM2637	3	No	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACATGCTCTCAGTTC TTGCCTGA, AAGGAGCCAGCTAGCTACATAGT	N.A
403	GNB101	3	No	No	N.A	G	0.50	N.A	0.00	(AG)31	GAACCTGATAGGCGTGGA,AA, AACCACCACCCCTCTTCTCT	N.A
404	GNB109	3	No	No	N.A	G	0.56	N.A	0.00	(AT)6	TTTTTGATGAATATCTTTTGGTG, CGATTATTTGCACCCAATTTT	N.A
405	GNB123	3	No	No	N.A	G	0.81	N.A	0.00	(TTC)5	TTGGTCTTGGCACTGTCTTG, CTTGCCGGTTAGTTTGTGGT	N.A
406	GNB128	3	No	No	N.A	G	0.56	N.A	0.00	(TCT)5	TCCTATAGCCGAACCCCTCT, CTCGGAAGTGGAAGCTTTTG	N.A
407	GNB166	3	No	No	N.A	G	1.00	N.A	0.10	(AAAG)5	TGTGCAAGCTAAGTGTGGGA, TCATTTGGGATGCTTTCATTC	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
408	GNB191	3	No	No	N.A	G	0.81	N.A	0.00	(AAT)21	TGAAGCCATCCACAGTTTGT, GCTCAGACAGGAGCATGA	N.A
409	GNB35	3	No	No	N.A	G	1.00	N.A	0.00	(TA)37	CATCTTCGCAACCGAATTTT, TGTAAGTGGAAAGCGCAACA	N.A
410	GNB62	3	No	No	N.A	G	1.00	N.A	0.37	(TTAT)5	TATTGACTTGGCATTGGGGT, AAGTGTGGGGAAAAGTGTGG	N.A
411	GNB80	3	No	No	N.A	G	1.00	N.A	0.00	(AC)7	TTGTAATGGGGCCAAGGTAA, TGTTAGCCACTTTGAGCCTTT	N.A
412	GNB83	3	No	No	N.A	G	1.00	N.A	0.00	(TAAA)5	AGGGTAAGAACAGGGGCAGT, TCGAAATTCTTCCCTCTCTTC	N.A
413	GNB94	3	No	No	N.A	G	0.75	N.A	0.00	(TATG)5	CGCGTCTGGAATTCCTAAG, AAATTTTGAAGTCTGACCTGCG	N.A
414	pPGSseq13E9_1	3	No	Yes	N.A	G	0.94	N.A	0.00	N.A	N.A, N.A	N.A
415	pPGSseq14H6	3	No	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAACGACGCAACTAGGGTG TATGCCGT, CAACCCTATACACCGAGGGA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
416	pPGSseq19D6	3	No	No	N.A	G	0.63	N.A	0.00	N.A	CACGACGTTGTAAAACGACTTTGTTATGCTCA CACCCCA, AAAAATGAAGCAATATTTTGTGTAG	N.A
417	pPGSseq19H3	3	No	No	N.A	G	1.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACTGGCAGGCAGTA AACATCAG, TTGAGGACGTGATGAACTGG	N.A
418	RM11H6_2	3	No	No	N.A	N.A	0.81	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCAAGGGTCCAC TAATAAGACCA, TGCAACTGATAAGGAAGCTGAA	N.A
419	RM3H9_2	3	No	No	N.A	N.A	0.69	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGCATCCACATG GCTCTTC, TTGTTTGGTCTGTTTCTCCCAT	N.A
420	Ah1TC1B02	3	Yes	No	3	G	0.94	0.50	0.49	N.A	CACGACGTTGTAAAACGACAACATGCATGCA AATGGAAA, GCCAAAGTCACTTGTTTGCTT	b10 (LG5)
421	Ah1TC1E06	3	Yes	No	4	G	0.88	0.39	0.63	N.A	CACGACGTTGTAAAACGACACCGTTACGAAC GCTTTGTC, TCCCTCTCATACGACACCCT	N.A
422	Ah1TC1H04	3	Yes	No	2	G	0.94	0.93	0.12	N.A	CACGACGTTGTAAAACGACCATTACTTCTCTAG GTTTGTTTTCCA, ATGGCGTGACAACGGAAC	N.A
423	Ah1TC4F12	3	Yes	No	10	G	1.00	0.19	0.86	N.A	CACGACGTTGTAAAACGACGATCTTTCCGCCA TTTTCTC, GGTGAATGACAGATGCTCCA	(LG15)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
424	Ah1TC4G02	3	Yes	No	4	G	1.00	0.31	0.69	N.A	CACGACGTTGTAAAACGACGATCCAAGTGTG AATTGGGC, CACACCAGCAACAAGGAATC	N.A
425	Ah2AC2A09_2	3	Yes	Yes	3	G	0.94	0.70	0.37	N.A	N.A, N.A	N.A
426	Ah2TC7A02	3	Yes	No	4	G	0.81	0.38	0.64	N.A	CACGACGTTGTAAAACGACCGAAAACGACAC TATGAACTGC, CCTTGGCTTACACGACTTCCT	a08 (LG12)
427	Ah2TC9B12	3	Yes	No	3	G	0.88	0.93	0.13	N.A	CACGACGTTGTAAAACGACGGCTGGGCTATG TTGATGT, TGCAGTACCTAAACCACCACTAC	b03 (LG14)
428	Ah–614	3	Yes	No	2	G	1.00	0.59	0.37	N.A	CACGACGTTGTAAAACGACGCCAACTATCAA CTCCCTCGCT, GAGTGAATGGCGAGAACTGGAA	N.A
429	AHGS0132	3	Yes	No	4	G	1.00	0.50	0.60	N.A	CACGACGTTGTAAAACGACCAAATGTACCTTC GGCGATT, TTACGAACACCCCTTTCTG	a03 (LG7)
430	AHGS0138_1	3	Yes	Yes	3	G	1.00	0.50	0.46	N.A	N.A, N.A	b01 (LG6)
431	AHGS0138_2	3	Yes	Yes	2	G	1.00	0.88	0.19	N.A	N.A, N.A	b01 (LG6)

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
432	AHGS0147	3	Yes	No	7	G	0.94	0.30	0.77	N.A	CACGACGTTGTAAAACGACTAACAGCCGGAT CAAACCTCC, ACCACCCTGCAATCACTT	(LG20)
433	AHGS0231	3	Yes	No	4	G	0.88	0.43	0.60	N.A	CACGACGTTGTAAAACGACAAGGAAGCATTG ACCTCGTG, AGCGGAAAAGGACAAGTGAA	N.A
434	AHGS0266	3	Yes	No	5	G	1.00	0.31	0.74	N.A	CACGACGTTGTAAAACGACAGAGCTCGAAGA GGGAGCTT, TGATCTCTCTGCTTGGAACA	N.A
435	AHGS0357	3	Yes	No	6	G	1.00	0.31	0.72	N.A	CACGACGTTGTAAAACGACCCTTTCCTTCCTT CATTCCC, AACATCTGCCAAATGCAACA	(LG11)
436	AHGS0365	3	Yes	No	6	G	1.00	0.41	0.73	N.A	CACGACGTTGTAAAACGACTTTGCTCCCTTGA TTGCTTT, GAAGGTGTAGGGTTTGGGGT	N.A
437	AHGS0502	3	Yes	No	5	G	0.94	0.47	0.60	N.A	CACGACGTTGTAAAACGACATGTCACATTTTC GATTGCG, GAGACCTTTGCGATTTTGCT	N.A
438	AHGS0626	3	Yes	No	2	G	0.88	0.75	0.30	N.A	CACGACGTTGTAAAACGACATTGCCTCACTGC GAAATCT, ACCCAAGTGAGTGATGGGAA	N.A
439	AHGS0729	3	Yes	No	8	G	0.94	0.40	0.76	N.A	CACGACGTTGTAAAACGACTGGTTGTTCTAAC CCTTCGG, TCACTATCCCATCCCTGCTC	b05 (LG21)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
440	AHGS0798	3	Yes	No	10	G	0.94	0.37	0.78	N.A	CACGACGTTGTAAAACGACCGTAGTTGGTGG TAGCCGAT, GAACCGTTAACCTCTTCCC	(LG3)
441	AHGS0909	3	Yes	No	2	G	1.00	0.59	0.37	N.A	CACGACGTTGTAAAACGACTCTTCGACGAGG TTGGATTC, GCAAGTCCACCATTTCTCT	N.A
442	AHGS0993	3	Yes	No	4	G	1.00	0.31	0.69	N.A	CACGACGTTGTAAAACGACTGGAGAACTGT GGGAAACC, TCACATATGCATTGACCTTTCA	a09 (LG18)
443	AHGS0996	3	Yes	No	2	G	1.00	0.50	0.38	N.A	CACGACGTTGTAAAACGACCCAAGTTTAGGC ATTACGTGC, CACTCCACACCTTCACTGC	N.A
444	GA138	3	Yes	No	5	G	1.00	0.31	0.73	N.A	CACGACGTTGTAAAACGACTCCAGTGAGGGT TAATAGCTCAA, AGAGTGAAGGTAGAATGGTGGAA	N.A
445	GA147	3	Yes	No	3	G	0.69	0.55	0.48	N.A	CACGACGTTGTAAAACGACCTCAGCATAGCA ACACACACG, GGCTTCTCCTTCCCAGTCT	N.A
446	GA169	3	Yes	No	2	G	0.94	0.67	0.35	N.A	CACGACGTTGTAAAACGACCTCCTTAACAATA AATCGAGTGATGA, CAACCCATTTGCCACCTCTAT	N.A
447	GA172	3	Yes	No	2	G	0.81	0.69	0.34	N.A	CACGACGTTGTAAAACGACCATACCGACAGA TTTTGAACTCG, GTTACTGTTGCTGCCACCATT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
448	GA196	3	Yes	No	4	G	0.81	0.46	0.55	N.A	CACGACGTTGTAAAACGACGTCTGAGGACAT GACAGAACCA, CGTACCACTCTCCACCATGC	N.A
449	GA197	3	Yes	No	2	G	0.94	0.73	0.31	N.A	CACGACGTTGTAAAACGACTGGAAGAAAATG GAAGGAACA, GGGGATTCCAATCATTACAT	N.A
450	GA198	3	Yes	No	2	G	0.44	0.71	0.32	N.A	CACGACGTTGTAAAACGACACTGGCATTATG GTAGTAGGATAACA, CATTGCGCTGCACAACCTAC	N.A
451	GA29	3	Yes	No	3	G	0.88	0.57	0.45	N.A	CACGACGTTGTAAAACGACCTTTTCCCAGATG CATTTTGA, CCCAAATTGAGATTGACCAAA	N.A
452	GA33	3	Yes	No	2	G	0.56	0.83	0.24	N.A	CACGACGTTGTAAAACGACCAAGTGATAGCA CGCTGTTTG, TTAAGTCCCATGCCTGTCTTG	N.A
453	GA59	3	Yes	No	4	G	0.69	0.41	0.58	N.A	CACGACGTTGTAAAACGACCCCTCTGCTCCAT ATTCATCA, GGTAGTGGTGGTGCATGTTTT	N.A
454	GA60	3	Yes	No	2	G	0.88	0.96	0.07	N.A	CACGACGTTGTAAAACGACGAGGAAGAGCA AGCCAAGAAT, CTCGCACCAATTACACAAACC	N.A
455	GM1073_2	3	Yes	Yes	3	EST	1.00	0.69	0.43	N.A	N.A, N.A	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
456	GM1076	3	Yes	No	3	EST	1.00	0.50	0.46	N.A	CACGACGTTGTAAAACGACAACAGACCAAAG GGTGTGTGA, TGCAGATTCTCACTTCCTAAGC	a07 (LG4)
457	GM1125	3	Yes	No	2	EST	1.00	0.50	0.38	N.A	CACGACGTTGTAAAACGACCCAAAGAGGAGT TGGAATGTG, TGAGGCCAAAGAGTGTGTTTC	N.A
458	GM1311_2	3	Yes	Yes	3	EST	1.00	0.56	0.45	N.A	N.A, N.A	a04 (LG9)
459	GM1358	3	Yes	No	3	EST	0.75	0.54	0.50	N.A	CACGACGTTGTAAAACGACTCTCAATGGTGA AGCAGTTCC, GTCAACCGTGTGTCTGTCTCA	N.A
460	GM1389	3	Yes	No	4	EST	0.63	0.40	0.60	N.A	CACGACGTTGTAAAACGACGAGAGTGAGAG TGTGGTGAGTGA, CCGAGTGAGTTGAGTGATTCTG	N.A
461	GM1482	3	Yes	No	2	EST	1.00	0.78	0.28	N.A	CACGACGTTGTAAAACGACTTGAAGCGGTGT TCATCAAAT, GGAGATGCTGGAGAAGAAAGG	N.A
462	GM1487	3	Yes	No	2	EST	1.00	0.75	0.30	N.A	CACGACGTTGTAAAACGACCCTGTTGTGGTG GTTCAATTC, CATTGGACCAGAAGGTTGGTA	N.A
463	GM1501	3	Yes	No	5	EST	1.00	0.56	0.59	N.A	CACGACGTTGTAAAACGACTCTGCAGTGTGT GTGTGATGA, TAAGAACCAAATTGCGACCA	b01 (LG6)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
464	GM1543	3	Yes	No	5	EST	1.00	0.69	0.47	N.A	CACGACGTTGTAAAACGACGACGGAAGA ACACAGGAAA, AAGCCTGATGATCTTGAAAAGC	N.A
465	GM1551	3	Yes	No	3	EST	1.00	0.38	0.59	N.A	CACGACGTTGTAAAACGACTCACTGTCTCGGT GTTTCCT, TCAGCCTTCTCTACCGTAAA	N.A
466	GM1555	3	Yes	No	6	EST	0.94	0.27	0.78	N.A	CACGACGTTGTAAAACGACCGTAGACGTGAA CCACTACCAA, CGCCTAGTGTCTCAGAAAACG	b05 (LG21)
467	GM1562	3	Yes	No	4	EST	1.00	0.34	0.62	N.A	CACGACGTTGTAAAACGACTCTTCTAATCTTC GGTCAACAAT, TCATCAGATCTCCAAAGCAC	N.A
468	GM1572	3	Yes	No	2	EST	1.00	0.56	0.37	N.A	CACGACGTTGTAAAACGACGCGTGGTTCTTC TGGACATT, TACATCCACTTCCAACCGAAG	N.A
469	GM1577	3	Yes	No	6	EST	1.00	0.50	0.66	N.A	CACGACGTTGTAAAACGACGCGGTGTTGAAG TTGAAGAAG, TAACGCATTAACCACACACCA	a05 (LG19)
470	GM1585	3	Yes	No	7	EST	1.00	0.34	0.77	N.A	CACGACGTTGTAAAACGACAGAAAGGGCAT GATGAAACTG, TAACCGCCGCTAAATCAAAAT	N.A
471	GM1591	3	Yes	No	6	EST	1.00	0.31	0.77	N.A	CACGACGTTGTAAAACGACTCATCACATTTGA TTGCTTG TG, TGCCTTAATAAGCTGGCCTTT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
472	GM1598	3	Yes	No	5	EST	1.00	0.41	0.65	N.A	CACGACGTTGTAAAACGACGGCCAGGCAAGT AAAGGTAAT, TGGTCGGTTAGGCAAAGTCTA	(LG11)
473	GM1618	3	Yes	No	2	EST	1.00	0.69	0.34	N.A	CACGACGTTGTAAAACGACCTGAATCCATTTT GGTCCTCA, CAAGTTGCTAAGCACCACGAG	b03 (LG14)
474	GM1638	3	Yes	No	2	EST	0.88	0.71	0.32	N.A	CACGACGTTGTAAAACGACCCACATGTTTCAC TGGCTTTT, GCCTCATTACATTATTTGATGCAC	N.A
475	GM1652	3	Yes	No	3	EST	0.94	0.67	0.44	N.A	CACGACGTTGTAAAACGACGCTGGTGAATTT CTGCCATT, CATCACCCGGCATAATAAGG	N.A
476	GM1661_1	3	Yes	Yes	5	EST	1.00	0.60	0.51	N.A	N.A, N.A	a01
477	GM1702	3	Yes	No	2	EST	0.81	0.92	0.13	N.A	CACGACGTTGTAAAACGACGATTGGGAAGCA GCAAGAAG, CAAGGATCTCAGCAGCACAG	a05 (LG19)
478	GM1720_1	3	Yes	Yes	2	EST	1.00	0.94	0.11	N.A	N.A, N.A	a04 (LG9)
479	GM1720_2	3	Yes	Yes	2	EST	1.00	0.88	0.19	N.A	N.A, N.A	a04 (LG9)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
480	GM1788	3	Yes	No	3	EST	0.88	0.79	0.33	N.A	CACGACGTTGTAAAACGACGTTCTACCGTG ATCCCTGA, CCAAGTTAGAGGGCTGCAAG	a10 (LG1)
481	GM1821_1	3	Yes	Yes	2	EST	1.00	0.94	0.11	N.A	N.A, N.A	(LG17)
482	GM1821_2	3	Yes	Yes	2	EST	1.00	0.67	0.35	N.A	N.A, N.A	(LG17)
483	GM1843	3	Yes	No	2	EST	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACCCACACACACA CACACACTA, AGGTGATGCTGCGTTTCTTT	b05 (LG21)
484	GM1880	3	Yes	No	7	EST	1.00	0.31	0.79	N.A	CACGACGTTGTAAAACGACCCAAAATGGAGG GTCCTTAGT, GAACTCCTCAAGCAAAGATCTGA	a07 (LG4)
485	GM1919	3	Yes	No	3	EST	1.00	0.91	0.17	N.A	CACGACGTTGTAAAACGACTGGAAGGGGTCA GAAGAAGTT, CCGTGTCTCTCTTTCTCTCC	a04 (LG9)
486	GM1985	3	Yes	No	3	EST	0.81	0.54	0.47	N.A	CACGACGTTGTAAAACGACTGGCATGTTCTCT GTCATAGG, TGTTGAAGATTTTGTGTTGTTG	(LG17)
487	GM2017	3	Yes	No	4	EST	1.00	0.44	0.64	N.A	CACGACGTTGTAAAACGACTTGCAGAATACC ATGTGTTGC, GTGGGTGAGAGCTCAAGTGAC	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
488	GM2032	3	Yes	No	4	EST	0.94	0.40	0.62	N.A	CACGACGTTGTAAAACGACGCCGATGATGTA CGTTTCTTC, GAGACGGCATGTCAAAGAAT	b10 (LG5)
489	GM2060	3	Yes	No	3	EST	0.94	0.73	0.37	N.A	CACGACGTTGTAAAACGACCACGGCAGTTCT AGTGGACTT, AGGACGATCTCTTCTGGAACC	N.A
490	GM2067	3	Yes	No	3	EST	0.63	0.50	0.49	N.A	CACGACGTTGTAAAACGACTCGCCAAGAAGA ACAAAACAC, CTGGTCGAAGAAGGGTTCTCT	b07 LG2)
491	GM2073	3	Yes	No	6	EST	0.81	0.27	0.77	N.A	CACGACGTTGTAAAACGACGAGATTGTTGAT CGGATTGGA, AGCAGCCTTCAATGGATTCTC	b07 LG2)
492	GM2076	3	Yes	No	2	EST	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACCCACCATTGCTAT GATGAACC, TGATGGATGATGAAGATGTGC	N.A
493	GM2078	3	Yes	No	4	EST	0.94	0.40	0.64	N.A	CACGACGTTGTAAAACGACTCATGATGCAAT GATAATAGGC, CTGGTCCATTGGGGACTCT	a05 (LG19)
494	GM2083	3	Yes	No	4	EST	0.69	0.45	0.58	N.A	CACGACGTTGTAAAACGACTCACCGATCATC ATCATCAA, ATTGGGGTTGTTTCCATTCTC	N.A
495	GM2089	3	Yes	No	6	EST	0.88	0.36	0.74	N.A	CACGACGTTGTAAAACGACATCGCGCAGTTA AAGAAGTGA, ATCTGAGTCCGAGCAGTTCA	a08 (LG12)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
496	GM2105	3	Yes	No	3	EST	0.94	0.63	0.45	N.A	CACGACGTTGTAAAACGACGAAGGTGAGAG ACAACAGCTTG, CGAGTGCCATTGGTAAAGAAG	N.A
497	GM2106	3	Yes	No	6	EST	1.00	0.38	0.73	N.A	CACGACGTTGTAAAACGACTTTGACATCATTG TTGATTGTTG, GATGAGGCCATTAAGGAGTGA	N.A
498	GM2165	3	Yes	No	3	EST	0.75	0.67	0.42	N.A	CACGACGTTGTAAAACGACCTACGCGCATCG CATAATATC, GTGAGATGGGGTTGGAGATTT	b10 (LG5)
499	GM2196	3	Yes	No	3	EST	0.88	0.64	0.46	N.A	CACGACGTTGTAAAACGACCCTTGCTTTTCGG CTTCTATT, GAGCTTTGGCTTTTGTGTGTG	b02 (LG16)
500	GM2215	3	Yes	No	4	EST	0.88	0.29	0.68	N.A	CACGACGTTGTAAAACGACGAAATCGGAGTC GGAGAGGT, TCCCCTTCTTTCTTCGTTCTT	a03 (LG7)
501	GM2240	3	Yes	No	7	EST	1.00	0.41	0.71	N.A	CACGACGTTGTAAAACGACAACCTGTTGTGG TTTGATTGG, ACTTGAAAGCCCTCCAGAAAA	N.A
502	GM2246	3	Yes	No	4	EST	0.94	0.47	0.59	N.A	CACGACGTTGTAAAACGACGCAATTTATGTG CACCTTTT, CGCTTGACACCAATGAAGTCT	a04 (LG9)
503	GM2263	3	Yes	No	3	EST	0.81	0.65	0.43	N.A	CACGACGTTGTAAAACGACGTGGCCGATTTC TTGATTCTC, AATGGTTCTGGGGCAAGTAAT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
504	GM2289_2	3	Yes	Yes	3	EST	0.94	0.67	0.44	N.A	N.A, N.A	a08 (LG12)
505	GM2303	3	Yes	No	4	EST	0.88	0.71	0.43	N.A	CACGACGTTGTAAAACGACTATGGAATCCAC AGCATCCA, AATGGAAGGGGCTTCTGAGTA	N.A
506	GM2313	3	Yes	No	4	EST	0.94	0.57	0.57	N.A	CACGACGTTGTAAAACGACGATGCTGCTAAA TCGAGATGC, GTTGTTTTGTTTCACGCCAGT	a04 (LG9)
507	GM2322_2	3	Yes	Yes	4	EST	1.00	0.63	0.51	N.A	N.A, N.A	N.A
508	GM2333	3	Yes	No	4	EST	0.81	0.42	0.65	N.A	CACGACGTTGTAAAACGACAATCTGGTCGGA TCTTGCTTT, GGAAGAAAGGAATGGCTTTTG	N.A
509	GM2337	3	Yes	No	3	EST	1.00	0.75	0.35	N.A	CACGACGTTGTAAAACGACTGTGGAAATGAA GGGACATTG, TGAGAGTGAGTGCGAGTGAGA	a06 (LG5,1 0)
510	GM2349	3	Yes	No	3	EST	1.00	0.50	0.46	N.A	CACGACGTTGTAAAACGACAACACCAATCAT TTCCCCAAC, AAAACCACCAACCACACAGTC	N.A
511	GM2359	3	Yes	No	5	EST	1.00	0.72	0.41	N.A	CACGACGTTGTAAAACGACAAATCCCCAATTC AAACCACT, GGGACCGAGTATTCGACTCAT	a09 (LG18)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
512	GM2369	3	Yes	No	5	EST	0.94	0.37	0.70	N.A	CACGACGTTGTAAAACGACAGGTTCTCTGT GGGTGATTG, CCAAACCTCCAGCTTCCTTCTT	N.A
513	GM2381	3	Yes	No	4	EST	1.00	0.31	0.70	N.A	CACGACGTTGTAAAACGACGTGTGCAAAGGG AGTGTGTG, CTTCTGCTGCTACTGCTGCTT	N.A
514	GM2480	3	Yes	No	3	EST	1.00	0.44	0.57	N.A	CACGACGTTGTAAAACGACAGCGCCTTCTTTT GTCTCTCT, GATGAAGAGGTGAGGGTGGA	a04 (LG9)
515	GM2558	3	Yes	No	2	EST	1.00	0.66	0.35	N.A	CACGACGTTGTAAAACGACGTGTGTGTGTGT GTGGGAGAG, CTGGTCTCATGGTACCGACAC	N.A
516	GM799_1	3	Yes	Yes	2	EST	0.94	0.64	0.35	N.A	N.A, N.A	a10 (LG1)
517	GM799_2	3	Yes	Yes	2	EST	0.94	0.93	0.12	N.A	N.A, N.A	a10 (LG1)
518	GM849	3	Yes	No	2	EST	0.94	0.93	0.12	N.A	CACGACGTTGTAAAACGACCGCCAAAAGAAG AAGAGAAGC, ATCCCCACCAACAATACATGA	a09 (LG18)
519	GM890	3	Yes	No	5	EST	1.00	0.34	0.72	N.A	CACGACGTTGTAAAACGACCATGGCTTCCCAT TCTTCTTA, GGCACATAGTTGCAAGGCATA	a04 (LG9)

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
520	GM908	3	Yes	No	2	EST	1.00	0.75	0.30	N.A	CACGACGTTGTAAAACGACGAAACGACAGT ACACGAATCA, TCACTCTTAACAAAACCAACTCG	N.A
521	GM919	3	Yes	No	2	EST	1.00	0.56	0.37	N.A	CACGACGTTGTAAAACGACTTTTCGTTAGGA GGAGCCACT, TGATCATAAAAGTGACCCTAAA	N.A
522	GM951	3	Yes	No	6	EST	1.00	0.38	0.75	N.A	CACGACGTTGTAAAACGACCCACCACCACCA TTCAATAAC, TTGCAGACATGTGTGGAGAAC	N.A
523	GM963	3	Yes	No	2	EST	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACCGTTTCACTCTGC TCAGCTTC, CGTGTTTTCTCAACACCACAA	N.A
524	GM980	3	Yes	No	2	EST	1.00	0.63	0.36	N.A	CACGACGTTGTAAAACGACGAAGAGGGAGA GGAAGGTGAG, GCCACAAGAATACCCAGATT	N.A
525	GM982	3	Yes	No	3	EST	0.94	0.53	0.46	N.A	CACGACGTTGTAAAACGACCTCAGGTCAGGT CATCAAGGA, GCAAGTTCAGAGCCATTCAAA	N.A
526	GNB100	3	Yes	No	4	G	0.81	0.38	0.63	(TA) ¹⁹	TTCCTAGAGTGTTTTGGCTTACTG, AAAAACCTAATTATTCGACTTAAAGCA	N.A
527	GNB151_1	3	Yes	Yes	2	G	1.00	0.56	0.37	N.A	N.A, N.A	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
528	GNB159	3	Yes	No	3	G	0.81	0.62	0.48	(GT)8c(TG)24	CGAATCGGAACACCGTACTT, TTTAAATGTTGGCCAGG	N.A
529	GNB178	3	Yes	No	5	G	0.50	0.38	0.71	(TA)24	GGCATGGTTGGTTAGTCACA, TTACTGAGCATGCCCCTTT	N.A
530	GNB190	3	Yes	No	2	G	1.00	0.84	0.23	(TTG)5	TCGTTTGATTCATGTAGCCG, TCGATCAAATTGTGTATTTTGT	N.A
531	GNB192	3	Yes	No	2	G	0.50	0.88	0.19	(TA)19	AAGGCAAAATGGTAGGATGC, CCACGTTTACCATTGCTTG	N.A
532	GNB38	3	Yes	No	4	G	0.94	0.43	0.60	(GA)11ggat(GGAG)6	TCCAGGGTCACTGTTCTTCC, CGTTGGTTTCATCAAAGGCT	N.A
533	GNB5	3	Yes	No	2	G	1.00	0.75	0.30	(CTTT)5	GCCTGTCTTTCTTTCGGTG, CCTTCAGCAAACAATCAGCA	N.A
534	GNB81	3	Yes	No	2	G	0.94	0.87	0.20	(AAT)5	TGCGAATCTGACTTGATTGC, AAAGGCTTGCTATTGCCAAA	N.A
535	GNB99	3	Yes	No	3	G	0.88	0.79	0.33	(AAT)11(TAT)27	TGGACATTTTAGTCTCCAAAAACA, TCTTAACGCAAACCACTTGAA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
536	IPAHM177	3	Yes	No	2	G	0.88	0.71	0.32	N.A	CACGACGTTGTAAAACGACTCAGCGGAGAAG AAAATAAGG, GAGGTGTTTGGAGAACTAGGATTT	a01
537	IPAHM689	3	Yes	No	7	G	1.00	0.38	0.74	N.A	CACGACGTTGTAAAACGACGATGACAATAGC GACGAGCA, GTAAGCCTGCAGCAACAACA	a06 (LG5,1 0)
538	PM675	3	Yes	No	6	N.A	0.69	0.27	0.78	N.A	CACGACGTTGTAAAACGACAATACCCTTCCCC AATCACC, TGCTTCTGCTCGATGTTCTG	a09 (LG18)
539	PM702	3	Yes	No	5	N.A	1.00	0.31	0.70	N.A	CACGACGTTGTAAAACGACCTCACCTTCGCAA ATCACCT, ACCCCCTCTCACTCTCCATT	N.A
540	PM727	3	Yes	No	2	N.A	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACCGAGGATCTCGA AGGGATGT, CAATAACCAGCAAGCAGCAA	N.A
541	pPGSseq11G3_2	3	Yes	Yes	5	G	0.94	0.40	0.70	N.A	N.A, N.A	N.A
542	pPGSseq15C10	3	Yes	No	4	G	1.00	0.81	0.31	N.A	CACGACGTTGTAAAACGACATTCCCATGTCGT CAAGACC, GCGACGGTATTGGCTTTTAG	b07 LG2)
543	pPGSseq16G8	3	Yes	No	7	G	1.00	0.56	0.62	N.A	CACGACGTTGTAAAACGACCTCAAAAAGCGC TTAGCCAC, CTGCCTACTGCCTACTGCCT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
544	pPGSseq17E3	3	Yes	No	4	G	1.00	0.59	0.47	N.A	CACGACGTTGTAAAACGACTTTCCTTTCAACC CTTCGTG, AATGAGACCAGCCAAAATGC	N.A
545	pPGSseq18E7	3	Yes	No	5	G	0.81	0.46	0.66	N.A	CACGACGTTGTAAAACGACAACGTGCGTGGA AAGAGTTC, TGAGAGTGGTTTTGTTGGTG	N.A
546	pPGSseq19B1	3	Yes	No	3	G	1.00	0.50	0.46	N.A	CACGACGTTGTAAAACGACTTGGTGATGGTG TTGGAGAA, TTAACCAGGCCAAAAGTGG	N.A
547	pPGSseq19B12	3	Yes	No	2	G	1.00	0.81	0.26	N.A	CACGACGTTGTAAAACGACCGTCCTTGTC GCTGTATTG, CCAATGTGCAACAAAACAAGA	N.A
548	pPGSseq19G7	3	Yes	No	5	G	1.00	0.31	0.71	N.A	CACGACGTTGTAAAACGACATTCAATTCCTCT CTCCCC, TCAATCAATCAATCGCAGGA	a03 (LG7)
549	pPGSseq9H8_2	3	Yes	Yes	5	G	1.00	0.31	0.72	N.A	N.A, N.A	N.A
550	RI1F06	3	Yes	No	3	N.A	0.94	0.67	0.43	N.A	CACGACGTTGTAAAACGACTGTCTCTCTCCT TTCCTTGCT, CCTTTGCTTCTTGCTTCC	a02
551	RM15C11	3	Yes	No	4	N.A	0.88	0.57	0.52	N.A	CACGACGTTGTAAAACGACGGACTGAACATC CGGCAC, GGACCAAATGACTGCTCTCTCT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
552	RM17E9	3	Yes	No	3	N.A	0.50	0.75	0.35	N.A	CACGACGTTGTAAAACGACACCACTCTCTTTG CGCTCTC, TGCCTCTGTGAAGAACTTGTA	N.A
553	TC02D06	3	Yes	No	5	N.A	0.63	0.40	0.70	N.A	CACGACGTTGTAAAACGACAGGGGGAGTCA AAGGAAAGA, TCACGATCCCTTCTCCTTCA	N.A
554	TC04D09	3	Yes	No	2	N.A	1.00	0.94	0.11	N.A	CACGACGTTGTAAAACGACTTGTGCTCTGCTC TTGGTTG, CTTGCTGGAGGAAACACACA	N.A
555	TC04F12	3	Yes	No	6	N.A	1.00	0.28	0.78	N.A	CACGACGTTGTAAAACGACGATCTTTCCGCCA TTTTCTC, GGTGAATGACAGATGCTCCA	N.A
556	GNB137	4	N.A	No	N.A	G	1.00	N.A	0.68	(AGA)7	CCCTCGAGCAAGAAAGAAAA, CCTCTTCCCAATTACCCCAT	N.A
557	GNB139	4	N.A	No	N.A	G	1.00	N.A	0.37	(TA)12	TGCCTTTTGCTAGATAAGTCCTG, AAATGTAGCCTTAGATCTCGTTTTT	N.A
558	GNB154	4	N.A	No	N.A	G	1.00	N.A	0.42	(AT)19	AGCACGGACACAACAAGAAA, GACTAGCCGGCCTAACTCC	N.A
559	GNB17	4	N.A	No	N.A	G	0.94	N.A	0.55	(GT)7	CCAAGCGTTCATACTGGTCA, GCGTGACTGCAGGTATGCT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
560	GNB173	4	N.A	No	N.A	G	1.00	N.A	0.10	(GAA)5	GCGTGCGTTAGAAGGTAGGA, TACCCCACTCACCCATTCTT	N.A
561	GNB56	4	N.A	No	N.A	G	1.00	N.A	0.37	(AGC)5	CGTTATTGGGTTTGTGTAGCA, CCAACACAAGGTGATGATTCC	N.A
562	GNB63	4	N.A	No	N.A	G	1.00	N.A	0.45	(TAT)8	GAGTTGGAGGACGTGGACAT, TCCTCGTGTGCAAACTCTTTT	N.A
563	GNB74	4	N.A	No	N.A	G	0.94	N.A	0.59	(AT)9	TGGATATCATGTAAGAATTCAACGA, TCAAACACTATAAATCCTTTTGGTT	N.A
564	RN13H12	4	N.A	No	N.A	N.A	0.75	N.A	0.55	N.A	CACGACGTTGTAAAACGACAAAGATAGGTTC GAAAGCACCA, ATATCCTACTGCCCCACTTGAG	N.A
565	Ah–524	4	N.A	No	N.A	G	1.00	N.A	0.50	N.A	CACGACGTTGTAAAACGACATTTTGGGTGGG GAAACCAAC, GTTGCCTGATTGATGGTTGAGG	N.A
566	GA14	4	N.A	No	N.A	G	0.94	N.A	0.38	N.A	CACGACGTTGTAAAACGACTGAAGTCACCAA GCATTCTCC, TGATCCAGAACCTGAAAGGAC	N.A
567	GA165	4	N.A	No	N.A	G	1.00	N.A	0.35	N.A	CACGACGTTGTAAAACGACATTTGTGCCCTAC CACCTTCT, TCCCTCCTAGAGGTTGACTTGA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
568	GA42	4	N.A	No	N.A	G	0.88	N.A	0.38	N.A	CACGACGTTGTAAAACGACTTGTGCGAAGGG TAAGATAGAAA, TCCTGTGCTTGAATCTGGAAT	N.A
569	GNB138	4	N.A	No	N.A	G	0.88	N.A	0.84	(TA)18	CAGCCCGATGAAGGAATAAA, CGACCTAAACTCCGTAGGCA	N.A
570	GNB140	4	N.A	No	N.A	G	1.00	N.A	0.00	(CTT)5	TCTTGACTTTCCCATGGAGG, GGATCGATTTTAGCAACCA	N.A
571	GNB148	4	N.A	No	N.A	G	1.00	N.A	0.58	(AT)29	CTTTAACTCCCCGGTTCTC, TTGCACAGTCCCTCTGTACG	N.A
572	GNB149	4	N.A	No	N.A	G	0.56	N.A	0.10	(AT)6	TGCCTAATGATACACGTGCC, AAAATGGCGATTCAAACCAC	N.A
573	GNB165	4	N.A	No	N.A	G	0.81	N.A	0.52	(AG)6	TCTGCACTTTACTTTCCCGC, CCTTTAAGTGAATCCAAATCC	N.A
574	GNB172	4	N.A	No	N.A	G	1.00	N.A	0.56	(GT)6	GCCGCTAACAGCACGTAAAT, TGAACCCAAACACTCATAACACA	N.A
575	GNB2	4	N.A	No	N.A	G	0.88	N.A	0.30	(TC)17acacgcgcg(CA)1 2cgcacgcacgcacg(CA)7	CTTGCTGTGGTGGATTTCTT, GCCTCCAATTTGCGAATCTA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
576	GNB22	4	N.A	No	N.A	G	0.94	N.A	0.10	(AG)6	CGCGATCAGCAGAATTAACA, TTGAACCTGGAAATTGGAGAA	N.A
577	GNB26	4	N.A	No	N.A	G	1.00	N.A	0.37	(CTT)8	CTCCGTGCTTCCTCTTCTTC, CAGAGAGAAAAACACGTAAATGAA	N.A
578	GNB27	4	N.A	No	N.A	G	1.00	N.A	0.69	(CAT)5	CGGTCTTTAGTGCGGTTCTC, TCTACGCTTTGGAGCCATCT	N.A
579	GNB33	4	N.A	No	N.A	G	1.00	N.A	0.54	(ATT)5	TTGGATGTTGGCTTTGTGAA, GGAAAAGGGCACTCTTGTTG	N.A
580	GNB49	4	N.A	No	N.A	G	1.00	N.A	0.49	(TA)6	CAAAATCCAGTGAGGGTTCA, TTTGAAGTGGTTGAATGCCA	N.A
581	GNB50	4	N.A	No	N.A	G	0.94	N.A	0.53	(AT)7	CAATGCAGAGTGTCAACAACAAA, GCTGCTTGTTACATGAGCCA	N.A
582	GNB59	4	N.A	No	N.A	G	0.44	N.A	0.37	(TA)7	CCAGAGGCCAGAAGATTGAG, GGCGTGACTCTCTGTACGCT	N.A
583	GNB66	4	N.A	No	N.A	G	1.00	N.A	0.53	(TA)7	TTACTTGGGGCATTTTGTCC, TGAAAATCTTGGGAATTGGG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
584	GNB7	4	N.A	No	N.A	G	0.81	N.A	0.00	(CTT)5	GTGCACCAGACACCAACATC, TGTTTCATCCCCTTACCCTA	N.A
585	GNB76	4	N.A	No	N.A	G	0.81	N.A	0.63	(AT)19	GGGAACGAATGAAGTAGGCA, GCATGGGTTTCAAGGTCTGT	N.A
586	GNB78	4	N.A	No	N.A	G	1.00	N.A	0.38	(TA)6	TTGTTTCTTGATGCAACCCA, TCATTTGAGACAGCCTCTGC	N.A
587	GNB95	4	N.A	No	N.A	G	0.94	N.A	0.42	(ATTA)5	TTCAATTAATAGCATAACTGATGTGA, GCTAACACCCGCTTCTCTTG	N.A
588	RM17C6	4	N.A	No	N.A	N.A	0.75	N.A	0.68	N.A	CACGACGTTGTAAAACGACGCTTATGGGACA CAGGAGACTT, AAACCACAACCACCAACACATA	N.A
589	RM18F5	4	N.A	No	N.A	N.A	0.94	N.A	0.42	N.A	CACGACGTTGTAAAACGACCAGCATTACAGT GTTTCTGTGTAAGA, TTTGCCCGGTCTGTAGTTATT	N.A
590	RN24H2	4	N.A	No	N.A	N.A	0.94	N.A	0.62	N.A	CACGACGTTGTAAAACGACATGCTGAGTGAG TTGATGCTTG, AAGGCAGGGAATGAAACAGAC	N.A
591	RN27A10	4	N.A	No	N.A	N.A	1.00	N.A	0.62	N.A	CACGACGTTGTAAAACGACACAGCCAAAAGC TCCTCCTAAC, GGACATTGGACCTAACTGATGAA	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
592	Ah1TC4A02	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACATTCAAATCGGA ATGGCAAG, GAGCAAAGGGCGAATCTATG	N.A
593	Ah–208	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACAGGGAAACCAAC AAGACCAAGG, AGATGGTGGGTTGAGATTGGGT	N.A
594	Ah–229	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGCAAACATCTTC CTTCCCAACA, ATTGACGTAAGCTGCCAAGAGG	N.A
595	Ah–264	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCGCACGGAGCGT CTTTTCTAT, CACCTTGAGGAGGGTTAATG	N.A
596	Ah–275	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCATGCAGGCAAC AAGATGATGA, CTTTGGAAATCCGCACGTTATG	N.A
597	Ah–296	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACAGTTCGCGTTG ATGAGTGTTG, CATTACCTTACCACTGGCACC	N.A
598	Ah2AC2B03	4	N.A	No	N.A	G	0.19	N.A	0.54	N.A	CACGACGTTGTAAAACGACCTCGCTATACTA GGTTTTGGGTGT, TGGTTTGCCTTTCTAGCCATTA	N.A
599	Ah2AC2C02	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTTCGACGGAAG ATCGTATTT, GGGTGCTATAATGGCTGAAC	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
600	Ah2AC2C08	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTTCAGTGTGGT GTCTCTCGAC, CCTCTAACTTTCCGGGTTCTT	N.A
601	Ah2AC3C07	4	N.A	No	N.A	G	0.00	N.A	0.75	N.A	CACGACGTTGTAAAACGACGGGGGTTTAGGA GCAAGATT, CAAGGTGAGAACAAAGGCAAAG	N.A
602	Ah2AC3D07	4	N.A	No	N.A	G	0.19	N.A	0.59	N.A	CACGACGTTGTAAAACGACTAGCTTCGATAA CCAGGGAGAC, CCCTAACACTCGTTCATTCTC	N.A
603	Ah2TC11F12	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTGAGTCTGTGGA AGAATAAGAGAAG, TGAGTCTATCGCCGCCTAC	N.A
604	Ah2TC7E04	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGAAGGACCCCAT CTATTCAA, TCCGATTCTCTCTCTCTCTC	N.A
605	Ah–321	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCGACGATGAGAA TGAAACACGC, GATGGCTGAATCACTCCAACCC	N.A
606	Ah–325	4	N.A	No	N.A	G	0.38	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGAGGTTGCTCT TCTCCTTCTC, GAGCACCACTGACAACACAAGG	N.A
607	Ah–331	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCCATCCCTCAGA ACTGGAACCT, CTGATCTCGGCGAAGGAAAAGT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
608	Ah–345	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGTTTGATTTCATG TAGCCGACC, TCCCTCCGCTAGGGTTTAACTG	N.A
609	Ah–390	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTACGACAAACAA TGTCCTCCG, CCAGCCTCAAGAACCACCTTTT	N.A
610	Ah–437	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTGGGTTGGTAGA GGACGTGTTG, AAGATTTGGGTGGAGAGAGTGGA	N.A
611	Ah–448	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACAACCTGGCTATTT CCTGGATGC, GCAAAGGCAAATGATGGAACAG	N.A
612	Ah–492	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGAACCTATAACAA CCGTCCGCCC, GCCTTTGTCCCCTTTATCCACA	N.A
613	Ah–530	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTCCATTGATGATC CCTCCTGAA, CAGCCACAACCACCACCATATC	N.A
614	Ah–569	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGCGTTTTAGAGC CCCATTGAG, CATGGACATAGCAGCACAACAAC	N.A
615	Ah–573	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTCTCTGTGACCA ACCACATCCA, AACACATGCAATCCCAACAACC	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
616	Ah–590	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCAAAACGCAATG CAAGGTACG, ACGAACGTGCAGCAAGAAGAAG	N.A
617	Ah–636	4	N.A	No	N.A	G	0.00	N.A	0.45	N.A	CACGACGTTGTAAAACGACAAGAAGAGGCTT GGTTGGGATG, CAAGGGTGGAGAGATAATGCACA	N.A
618	Ah–659	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCCGGTGTTCCTTT TGTTGTTGC, GGACGAAAATACCCTTCCCCTT	N.A
619	Ah–692	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTCCACGGAGAT TGTTGTTTTG, GCACCTCCTCCTTGAACACCTT	N.A
620	Ah–700	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCACCACCTCCTTC ACCAACACT, TGGCAGAAGAAAAGAACGCAAG	N.A
621	Ah–745	4	N.A	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGTTGTTCTGCTC CTGCTTTTG, ATTCGGACCAAAATGTCCCTTC	N.A
622	AHGS0599	4	N.A	No	N.A	G	0.38	N.A	0.45	N.A	CACGACGTTGTAAAACGACAGATTGGTGGTG ACAGAGGC, GCCATCGATGTAACCCCTCAC	N.A
623	GA1	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGCGTGAAATGAG TGTTGTGAG, CATAGCCACCATAGACACCAAA	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
624	GA101	4	N.A	No	N.A	G	0.31	N.A	0.38	N.A	CACGACGTTGTAAAACGACTGAAATGATGCA ACCACACAT, AAGGGAAAGTAAAACCATGCAA	N.A
625	GA102	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACAGAGTCTCGTGC TAATAACGTGT, TGGATAACCCATTTTCTATGTTG	N.A
626	GA108	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTGGATGCTGTAA GGAATGGAC, TTATCGAGCTTGCCCTCAGAAA	N.A
627	GA110	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGGAGAACCAGTG ACGTGACATA, GGATTAATTCTGATACCATGAAAGG	N.A
628	GA119	4	N.A	No	N.A	G	0.13	N.A	0.00	N.A	CACGACGTTGTAAAACGACCGATGCTCTCTTC CTTCTCT, CCGCTACTCCCTAACTCAAGC	N.A
629	GA120	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTAACCCCTAGCC GCCATACC, GTTGATGGCTTCACGGTGAG	N.A
630	GA122	4	N.A	No	N.A	G	0.25	N.A	0.00	N.A	CACGACGTTGTAAAACGACCCCATCTCAAGT GTAAAAGTGCT, CCTCCCCACCCTTAAACAATA	N.A
631	GA124	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGCGGAAACAGA AACAAATCAA, GCCGTTACATACCAGAGGAAA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
632	GA127	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGGCGCAGAAGTT ACGAAGAGT, TTTCATTCAATAACGTACATCCAT	N.A
633	GA133	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGGTGTTATGTAT AGCCACCAG, AAATAGTATGGACCAGAAATAATAAG	N.A
634	GA135	4	N.A	No	N.A	G	0.25	N.A	0.00	N.A	CACGACGTTGTAAAACGACCAGAAGCTGAAG TTGAACCAGA, CACCTGTTTCGTCGTTTGTT	N.A
635	GA140	4	N.A	No	N.A	G	0.00	N.A	0.38	N.A	CACGACGTTGTAAAACGACTTAGGCTGGTGG AAAGTGATG, CAAATAAAACAATGAATTGATAATCG	N.A
636	GA146	4	N.A	No	N.A	G	0.06	N.A	0.00	N.A	CACGACGTTGTAAAACGACGGCTTAACTCC GCAAGCTAC, TCACACGAACTAACGCACAA	N.A
637	GA150	4	N.A	No	N.A	G	0.31	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCCACAATGTGT AGTTCAAGCA, TGGTGACCATTTCAACTCTTG	N.A
638	GA155	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACATCGTGCAGAGG AATAGCAGA, GCGTAGTTAATTTGCCAGGTG	N.A
639	GA156	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTACTCCCTCTGC TGCTTCCT, TAGGGTTTCGTTGAGGAGGTT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
640	GA160	4	N.A	No	N.A	G	0.13	N.A	0.30	N.A	CACGACGTTGTAAAACGACTCTTTATCCCGAT GAATGAAA, CTCCCACAAACACAAACACAC	N.A
641	GA163	4	N.A	No	N.A	G	0.06	N.A	0.00	N.A	CACGACGTTGTAAAACGACATGTATAGTGGC GGATCCAAT, TTTTGAAGTATTCTCTTTTCAACA	N.A
642	GA167	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCCTTAGATGGCA ACATTGGAA, ACCTAAACCTAACACCCACAC	N.A
643	GA174	4	N.A	No	N.A	G	0.00	N.A	0.30	N.A	CACGACGTTGTAAAACGACAGCTTTCTGATT TGCCACTT, GCCAAACAAGTTTAGAACAATCC	N.A
644	GA177	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGATAGGGCCCAT AACATGTCC, TGAGGGTTGAGCTGAAGAGAA	N.A
645	GA19	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACACCAACGATGCT GCTGATAAC, ATCCTCAACCCATGCTTTCTT	N.A
646	GA2	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGTGTCCTGTGGT GTCCAAATTA, TGCATACCGGAGAAATTCAAG	N.A
647	GA20	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACACCCATAACA CACCACAAGT, ACCGAAGCACAGAGAAGACAA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
648	GA21	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCAGGATGAACAG GCACAGAAT, ATGAACAATTGCGATTTGGAC	N.A
649	GA217	4	N.A	No	N.A	G	0.13	N.A	0.38	N.A	CACGACGTTGTAAAACGACACAGACAGCGCA GATTAGAGG, AAGGAACATTAAACCTGGATCG	N.A
650	GA26	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCCTCACTTCTTTT TGCATGGT, TGGAAAGGAAATGATTTGGTG	N.A
651	GA28	4	N.A	No	N.A	G	0.00	N.A	0.55	N.A	CACGACGTTGTAAAACGACAGATGGTGGTGT AGGAGTTGTGT, TGGCCGTTGGATATTTATTTG	N.A
652	GA30	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCAAGAGGGACG GATAATAGCA, GACGCAAGGAAATGAGCATAC	N.A
653	GA35	4	N.A	No	N.A	G	0.25	N.A	0.00	N.A	CACGACGTTGTAAAACGACCAAGTTTGCAG TGATTTTGTG, AAATTTTCAGGTAAATCATTCTT	N.A
654	GA44	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTGACTTTAATTTT GAGCTTCTATAA, TTTTCTGTCCATAATTATATCGTATTT	N.A
655	GA45	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACAGCACATTGGCG AGATCACTA, AGCAAACCTGGAGAGAAGCACA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
656	GA49	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACACGTTTCCCAAT AAGACCAC, TGGACACCTTATCGGCTTATC	N.A
657	GA5	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACAACTTGGACGT TGGCTTTGT, TTGATCCAGAACCTGAAAGGA	N.A
658	GA50	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCGAAATCGCATA TATCCTCGA, TAACAATAGCCACGGACTCGA	N.A
659	GA51	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACAGGAAGTGGCTT CAAGGGTAT, CTGTCTCCTGAAAGTTGGGTTT	N.A
660	GA56	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTTTGGTCTCTCTC CTCCATCA, TTTCCAGCATCAATAGCAACC	N.A
661	GA6	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCCTCTTCTTGATT TCGTGCTG, GAAACATACATTCTCTTGCATCA	N.A
662	GA61	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTACCTTCGGCAT GGTGATT, TCGAGCCCTCTTTGACTCT	N.A
663	GA62	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTCCAAAATACAC ATTCTCATGG, CTACACGCAAAAAGCTCAGAA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
664	GA64	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGAGTGGTCTTTT GGTCTGTGC, TGTTTGGACTTTGCATGTTTTTC	N.A
665	GA65	4	N.A	No	N.A	G	0.06	N.A	0.00	N.A	CACGACGTTGTAAAACGACTTGCATGTTAGTC CCACCACT, CCGCACTCGTTTGGAAATTA	N.A
666	GA72	4	N.A	No	N.A	G	0.19	N.A	0.35	N.A	CACGACGTTGTAAAACGACACTTTTGGTGGCT TTCCTTCAT, TCTCTGTGCCCTCTTTCTTCA	N.A
667	GA79	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCGCAATGAGAAA TTCAACAGC, TGATAATTCTTCCGCGATTGT	N.A
668	GA8	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTGAAGGGAGGA AGAGACGAAT, CCTCTGTGCGACATGACTCTAA	N.A
669	GA80	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTGAAAGTAACTC GTTTACAGTTTGAAG, TCACTAAACATGTGGGTAAGTAAGAAA	N.A
670	GA81	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACAAGGAACGGCGT AATAGCATC, TATCTAACCTAGCCGCCATT	N.A
671	GA84	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTTGCAGAACACT CCAACATCC, TTTAGGTGGTGGCGGTGAAT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
672	GA85	4	N.A	No	N.A	G	0.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGCATATTGCATG GTAACGAAG, GGGGATGTGTATTAATTTACTTGA	N.A
673	GA87	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTTCACCTCCAAA ATCAACCA, ACCGCTGACATTGTATTGTTC	N.A
674	GA88	4	N.A	No	N.A	G	0.00	N.A	0.30	N.A	CACGACGTTGTAAAACGACATTTGAACGGCA GAGAGCATA, TCATTCCTCCCTCACATCTT	N.A
675	GA91	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTGAACTTTGGGA GTGTATATGACC, CCCACACCCCTAAATTATGT	N.A
676	GA96	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACACCAAGTCAAAT GTTGCCCTA, CGTTCTTCTCTGTTCTTCA	N.A
677	GA99	4	N.A	No	N.A	G	0.31	N.A	0.47	N.A	CACGACGTTGTAAAACGACAAAATGCCCTA TCCCTTCTT, TGATTTTCTGAGTTTGGCAGTG	N.A
678	GM1453	4	N.A	No	N.A	EST	1.00	N.A	0.66	N.A	CACGACGTTGTAAAACGACCTCACACACACT GCCAACACT, AGCTTAGTCGGAATCGGAGAC	N.A
679	GM1992	4	N.A	No	N.A	EST	1.00	N.A	0.74	N.A	CACGACGTTGTAAAACGACTGATGCTTGGTC AATGTATGAG, TTTCTCTGCTGCGTCATTTT	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
680	GM2008	4	N.A	No	N.A	EST	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTACAATCACCATC ACCATTC, CCTTCGCCGACAACACTACTA	N.A
681	GM2428	4	N.A	No	N.A	EST	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTCGTTTAGCGGT CTCTTCAA, CCCCATTTCTAACCCTTTTCA	N.A
682	GM2553	4	N.A	No	N.A	EST	1.00	N.A	0.80	N.A	CACGACGTTGTAAAACGACTCCCTTCAGTTCC GTTGAATA, GCCCCTTCCTCTTTTGTATG	N.A
683	GNB102	4	N.A	No	N.A	G	0.00	N.A	1.00	(TAAA)5	TTCTTGCCTTGCTCCTGCT, CGAAAATCACTAACCCTTTTCA	N.A
684	GNB110	4	N.A	No	N.A	G	0.00	N.A	1.00	(TA)8	TTAATTTCATACGCTTGTTAGAAAGA, TAAAGCTGTTGGGGATTGG	N.A
685	GNB115	4	N.A	No	N.A	G	0.00	N.A	1.00	(GAG)12	AATCACAATGGGCTGAGAGG, TCTTTTGCAGACATGGCAAG	N.A
686	GNB116	4	N.A	No	N.A	G	0.00	N.A	1.00	(AT)9	GCATGGGCATCTCAAAAAGT, TTGAATGCATATATGGTAAGTTTGA	N.A
687	GNB124	4	N.A	No	N.A	G	0.00	N.A	1.00	(ATT)5	TTGAATATTTGGATCATCTTCAATG, CGAATTCGTGAAATGTGGTG	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
688	GNB125	4	N.A	No	N.A	G	0.00	N.A	0.38	(TTAT)6	TTTAGCCGCTTAGGCCTGTA, AAGCAAAAGCTTGCGAAAAA	N.A
689	GNB126	4	N.A	No	N.A	G	0.00	N.A	1.00	(TC)10tt(TC)10	AAAAGGAACATTGAACCTGGAA, GGTTTCGCAATCAGCAATTT	N.A
690	GNB13	4	N.A	No	N.A	G	0.00	N.A	1.00	(TG)6	TTCCTGTAGAGCCTGAGGGA, AAAGTCCCAAATGCATGCTC	N.A
691	GNB130	4	N.A	No	N.A	G	0.00	N.A	0.55	(AAT)7	TAAGGGTCGATCCACAGAG, AAGGAACACAGCACCTCCAT	N.A
692	GNB132	4	N.A	No	N.A	G	0.00	N.A	1.00	(ACA)6	TTGTGCAAGTGAAACCACAAG, TGCCTTGCAATTTGTTGAAT	N.A
693	GNB133	4	N.A	No	N.A	G	0.00	N.A	1.00	(TCA)6tctgtactgtactt attggtcacatgcatatgcc tatgctgccact(ACC)6	AAAGCAAGCATCAACAACCA, TCGTTGAAACTGGGCTCTTC	N.A
694	GNB143	4	N.A	No	N.A	G	0.00	N.A	1.00	(TC)6	CCACCCAATCAGCCAAATTA, GAAAAGAGAAGAAGGAAGAAGAAGG	N.A
695	GNB146	4	N.A	No	N.A	G	0.00	N.A	1.00	(AGA)9	CGGAGCTACCAACTCGTCA, TGGTTGCAGTGATGAAGGTC	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
696	GNB147	4	N.A	No	N.A	G	0.00	N.A	1.00	(AGA)8	CGCCCTAAATGGTTATGGAA, CCCATCACCTCTTCACCACT	N.A
697	GNB152	4	N.A	No	N.A	G	0.00	N.A	1.00	(AGA)5	ATATTGCTCACCTCCAGCA, AATGGCCGAACAACTCCCT	N.A
698	GNB155	4	N.A	No	N.A	G	0.00	N.A	1.00	(TA)6	AGAACGCGAGATTACGCAAC, GTCTGGGCGTCAGATTAGGA	N.A
699	GNB157	4	N.A	No	N.A	G	0.00	N.A	1.00	(AG)6	CGCGATCTGCAGAATTACAA, TCCTACATCCTAACAAAGAGGAGA	N.A
700	GNB16	4	N.A	No	N.A	G	0.13	N.A	0.38	(AAT)8ag(TAA)14	ATGTGTGGGGCTAACCAAAA, CCAACCCGACAGATTAAGGA	N.A
701	GNB160	4	N.A	No	N.A	G	0.00	N.A	0.00	(AAG)10	GCCTGAACGACAAATCACAA, GCCCAATTATAGGCCCATTT	N.A
702	GNB163	4	N.A	No	N.A	G	0.00	N.A	1.00	(ATA)23	CAAACCATAATCCTCGTGGG, CTTTTGCAATAGAATTGTTTATGATTT	N.A
703	GNB167	4	N.A	No	N.A	G	0.00	N.A	1.00	(TTA)13	GGCAAATTTTTACCTAGCG, TCAATACTTTCGTTGCATGGAT	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
704	GNB168	4	N.A	No	N.A	G	0.00	N.A	1.00	(AT)26	TTCTGTTGTCATTTATGTGTGTGA, CCGTAGAACTTCTCTTGCGG	N.A
705	GNB169	4	N.A	No	N.A	G	0.31	N.A	0.00	(TA)9	GAACCAGTGGCAGGTTTCAT, GATGTGAGCCCATCCATGTT	N.A
706	GNB170	4	N.A	No	N.A	G	0.00	N.A	1.00	(TTC)5ttt(TTC)5ttttctt tttcttctccaaattcgcgcac gcaggttcttctcccttgatt c(CCT)6cttccaattgttgcac	TGTTTTCTTCTCTTCTCATTCTCC, TTGGTGTTATTGACGATGATGA	N.A
707	GNB171	4	N.A	No	N.A	G	0.00	N.A	1.00	(CT)6	ACGTGTCCTTTTTTCGTGCTC, CATTTTTCCCCATTTCTT	N.A
708	GNB176	4	N.A	No	N.A	G	0.00	N.A	1.00	(AT)12	CCTCCGGTTCTCATTCCATA, CTCAACCTACGAAGCCAAGG	N.A
709	GNB177	4	N.A	No	N.A	G	0.00	N.A	1.00	(GA)10	AGATGATTCGCCTCCCTTTT, TTATGAAAAATTCGCGACCC	N.A
710	GNB179	4	N.A	No	N.A	G	0.00	N.A	1.00	(AT)10	TCATGGTTTGGTTCGACTGA, TCGTTTCCTCATATGCTAATGC	N.A
711	GNB181	4	N.A	No	N.A	G	0.00	N.A	1.00	(AT)11	TGGAACCAATAATGAAACTAGGC, TGAGAAGCTTGTGGACTTGG	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
712	GNB182	4	N.A	No	N.A	G	0.00	N.A	1.00	(AG)68	AATATAGCTAGGCGGGGCAT, CACCCGCCCTAAATTGTAT	N.A
713	GNB184	4	N.A	No	N.A	G	0.00	N.A	1.00	(AT)6	TCAAATGCCAAATGGCTACA, TCGACGAGTGGTAATGTGGT	N.A
714	GNB185	4	N.A	No	N.A	G	0.00	N.A	1.00	(TC)7	GGCCAGAATTTTCATCTTGGA, CTGAGAACCCTATGCCTTGC	N.A
715	GNB186	4	N.A	No	N.A	G	0.00	N.A	1.00	(TG)6	GCCTTGGCATCAATTATCGT, GTGTTCACACCAAGCCAA	N.A
716	GNB187	4	N.A	No	N.A	G	0.00	N.A	1.00	(GA)26	AGGATTTGGGAGCTTGAGGT, TTCAAAGATGGTCCCCACAT	N.A
717	GNB19	4	N.A	No	N.A	G	0.00	N.A	1.00	(TA)6	TCCAAGTGAATAAATTTGGATTAGG, AATGCGATACGTGAATTTCTG	N.A
718	GNB23	4	N.A	No	N.A	G	0.00	N.A	1.00	(TTA)13	GGGGTCGATACGTCCACTAA, CATGCATCCATGAGACTTCC	N.A
719	GNB25	4	N.A	No	N.A	G	0.31	N.A	0.27	(AT)6	GGACCAGAGGGACAAAGACA, GGGGCCTCTATAGGTTGAGC	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
720	GNB3	4	N.A	No	N.A	G	1.00	N.A	0.38	(ATC)5	CGCTCTCCGGTGATACAAAC, CCCTCAAAACACCAAAATGG	N.A
721	GNB31	4	N.A	No	N.A	G	0.00	N.A	1.00	(GA)10	TGAAAGTGAGGTAGGTGGGG, GCATGCCTAAAAGAAGCAGG	N.A
722	GNB34	4	N.A	No	N.A	G	0.00	N.A	1.00	(AGA)6	TAAATATTGCTCGCCCTCG, CCTACCTCGCCCTCTCTCTT	N.A
723	GNB36	4	N.A	No	N.A	G	0.13	N.A	0.00	(AC)6	TTCATTCTAAGTTGCGTGCG, CGCAAGTTTGACAACAATGG	N.A
724	GNB37	4	N.A	No	N.A	G	0.00	N.A	0.00	(AT)10	TTGTGTATGTGTAGTGAGACGACG, AAAAAGTTGGCTCCTTAATATTACTC	N.A
725	GNB46	4	N.A	No	N.A	G	0.00	N.A	1.00	(AGA)5	CCCTCGAGCAAGAAAGAAAA, TCCCACATCACACTTTCCAA	N.A
726	GNB47	4	N.A	No	N.A	G	0.25	N.A	0.00	(AC)7	GCCAAATGGGCTAAACAGAC, GCTCGACACTGCAATCACAT	N.A
727	GNB57	4	N.A	No	N.A	G	0.00	N.A	1.00	(AAAT)6	TGTTTTCTCCTTCGTTTCGCT, ACCCACCACTTCCAGTTCAG	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
728	GNB58	4	N.A	No	N.A	G	0.00	N.A	1.00	(TA)32	TCTGCTTTATACCACACTTTTCC, ATTTCCCAAATCCAGGGTC	N.A
729	GNB6	4	N.A	No	N.A	G	0.25	N.A	0.19	(AG)6	ATAGGTGAGGGGTTTTGGG, AGGATTGGCAGAAAGAGCAA	N.A
730	GNB60	4	N.A	No	N.A	G	0.00	N.A	1.00	(AAG)5	AGCATTGAGCACGTATGCAG, GGCATCTCATCTTTCAGGGA	N.A
731	GNB61	4	N.A	No	N.A	G	0.00	N.A	1.00	(TC)7	GATCTAATGGCGGACCTGAA, TGGAAGAGCAGAAGGAAGGA	N.A
732	GNB65	4	N.A	No	N.A	G	0.00	N.A	1.00	(TTA)23	TTTTTCTGGACCAAGCCTCT, TGAATTCGGGCTACTGATCC	N.A
733	GNB67	4	N.A	No	N.A	G	0.00	N.A	1.00	(GC)6	CCAGTTGGGTTGGAAAGCTA, GGCCACATGTCTCCTAAAA	N.A
734	GNB73	4	N.A	No	N.A	G	0.00	N.A	1.00	(AG)9	GAAGGTGAAGGGGAAGAACC, TAACTCTCTCCGCCACCT	N.A
735	GNB75	4	N.A	No	N.A	G	0.00	N.A	1.00	(TG)9	GCTCTTGAAGCGAAGTGGTT, CCAAGCGACAAGCTCCTAAC	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
736	GNB84	4	N.A	No	N.A	G	0.00	N.A	1.00	(TA)6	TATTTGTCCGTTTTGGAGGC, TCTTTTGTGGGGTCCTTG	N.A
737	GNB86	4	N.A	No	N.A	G	0.00	N.A	1.00	(AT)12	ATTCGAGCATCATCACTCCC, ATATACCCAAAGAGGAAATTCTACC	N.A
738	GNB89	4	N.A	No	N.A	G	0.00	N.A	1.00	(TA)7	TTTTTAATGATGGCAGATTGAGA, TTTTTGATAATTTATTCTTCGCA	N.A
739	GNB91	4	N.A	No	N.A	G	0.00	N.A	1.00	(TTCT)5	CAGCCCATGAGGAGCTAAAC, ACCCCTTCCCAGAATACCAG	N.A
740	GNB92	4	N.A	No	N.A	G	0.00	N.A	1.00	(AGC)5	TTCAAATAACTTGGTGGGCA, GTCCTTGTTCCAAACCCTCA	N.A
741	GNB93	4	N.A	No	N.A	G	0.19	N.A	0.38	(TTC)5ttattctctctcaaat gcacattctaagttattactca tcacaacaaggc(AAT)5	AATGCTAACTTTCGCACACAA, TCCATAGAGGATGAGGATTGC	N.A
742	GNB97	4	N.A	No	N.A	G	0.00	N.A	1.00	(ATT)5	CTTGAATTTTCACCGTTCTTAAA, AATCTTGGTTTGGTGCAAGC	N.A
743	IPAHM295	4	N.A	No	N.A	G	0.00	N.A	0.00	N.A	N.A, N.A	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
744	PM692	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGAACGCGTGATG AAGGAAAT, GTCGTCCCTCTTTGTGTCGT	N.A
745	RIIF06	4	N.A	No	N.A	N.A	0.00	N.A	0.00	N.A	N.A, N.A	N.A
746	RM11A4	4	N.A	No	N.A	N.A	0.13	N.A	0.55	N.A	CACGACGTTGTAAAACGACGAGACCTTATGG TGGATCTTGG, AACGCTCCGTACAAGAGAAGAG	N.A
747	RM13A9	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACAAACCCACCTTTC CACTATATCAC, GGGTACATGGGAGAAATTGAAG	N.A
748	RM13B10	4	N.A	No	N.A	N.A	0.00	N.A	0.00	N.A	N.A, N.A	N.A
749	RM14B10	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACATCGAATCCCAA CTCACTCACT, TAAAATCACCCACCAAAAGCTC	N.A
750	RM14H12	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTCCTGAGATCCG AAAGCTACTC, AAGCAATGGCACGGTAGATACT	N.A
751	RM15C2	4	N.A	No	N.A	N.A	0.06	N.A	0.38	N.A	CACGACGTTGTAAAACGACAAAATGCGCTCC CTTCAC, TGTACGGCAATTCTCTGGAGT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
752	RM15H8	4	N.A	No	N.A	N.A	0.06	N.A	0.00	N.A	CACGACGTTGTAAAACGACATCTAACAA AACGACACCG, CAGGGGTGTAAGTGCA GTGTA	N.A
753	RM16B3	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCACGACGTA AATCCTTATT, TGTGAAGCTGCTCATACTC	N.A
754	RM16E1	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACATTGCTCTGT GTGTGTGTGT, ACCGGAACATTAAACAGTGACCT	N.A
755	RM16H10	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCCTTCTACAC ACATCCAATTAAAA, GACGTAGCGGCTTACTCTTGTT	N.A
756	RM18A10	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCAAGAGGGATT AAAGAACTCG, ATGGCTTTACTATGGAGACGGA	N.A
757	RM5G8	4	N.A	No	N.A	N.A	0.19	N.A	0.51	N.A	CACGACGTTGTAAAACGACATAGTCCATG AGCCCCATGT, TTACAACCGAATCTGCAAAGAC	N.A
758	RM6E8	4	N.A	No	N.A	N.A	0.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACATTTTCATTGT GGGTGCTAC, GGCTGAGGAGATAAGGGAGAAT	N.A
759	RM8G12	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTAATCCTCCC ATCTCTCT, AATCCCCGTTTCAATTTT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
760	RN13F12	4	N.A	No	N.A	N.A	0.38	N.A	0.35	N.A	CACGACGTTGTAAAACGACCTTGGGAGAGAA GCAATTCAGT, GCTCAAGCTACACAATCATGGA	N.A
761	RN14A4	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGACAAGAAGGC AAATCCAACC, GTCAAACAAAAGAGCCAAGGAA	N.A
762	RN23A7	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTGGATCTATCATC TTTTAATCCTCA, ACCCATACATTCAAACTCAAAA	N.A
763	RN23E11	4	N.A	No	N.A	N.A	0.31	N.A	0.27	N.A	CACGACGTTGTAAAACGACGTCAACCCCAAA CACAATCC, GAGAACCTGAGCGTATTCTCTGA	N.A
764	RN23F10	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACATGGCTAAAGAT GTTGAAGTTG, GTGCAGTAAACAAGGATGAAAA	N.A
765	RN23F12	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTTGGGAGAGAA GCAATTCAGT, GCCATGCAATAGTAGTCACCAA	N.A
766	RN24F3	4	N.A	No	N.A	N.A	0.25	N.A	0.16	N.A	CACGACGTTGTAAAACGACCCCTTCCGTTTTTC CATTATTCT, TGGTCAGTTTCTTCTTCCCAT	N.A
767	RN25D11	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGGGCACCCAAGA TGTGGA, GGCGACTTCGAGATAGTGGTCT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
768	RN25E8	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGCAACAAACGAA TCAGCAAT, CCAGAATCATCCATCCAATCTA	N.A
769	RN25H6	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGGGATCTATTGC ATTCCACAAA, TACTCTCTGGCCTCTGCCTCTA	N.A
770	RN26G9	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTTCACACAAGT GCTTCAACTG, TGACTTTCATATGCACCTCTCA	N.A
771	RN27H12	4	N.A	No	N.A	N.A	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGAGATGGAAGAT TATGGTACTCCAAG, AATCCTTTGCCGGGATCA	N.A
772	TC011A04	4	N.A	No	N.A	N.A	0.00	N.A	0.00	N.A	N.A, N.A	N.A
773	TC011B04	4	N.A	No	N.A	N.A	0.00	N.A	0.00	N.A	N.A, N.A	N.A
774	Ah1TC1A01	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTCAACGCGACAC AAGAAGTC, GTCGGTAAATCCGACGAAAA	N.A
775	Ah1TC2A11	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCAGCAGATTGAC GGGTTAGC, CAGCAAAGAGTCGTCAGTCG	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
776	Ah1TC2C03	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACAGAGCTGAGTGC TTGGTTCA, CAGCCTAGAGCCGAATTCAC	N.A
777	Ah1TC2C12	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGGAGTTTTGAGT TTTGAGTTTTGA, CCCGCTATTCCCCAAAAT	N.A
778	Ah1TC2E05	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGAATTTATAAGG CGTGGCGA, CCATCCCTTCTTCCTTCACA	N.A
779	Ah1TC3A10	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGCATGGGGTAAA TCTTCAA, ATGTGCCTATCAGGGGTTTG	N.A
780	Ah1TC3B05	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGGAGAAAACGC ATTGGAAT, TTTGTCCCGTTGGGAATAGT	N.A
781	Ah1TC3H02	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTCTCCGCCATCC ATGTAAT, ATGGTGAGCTCGACGCTAGT	N.A
782	Ah1TC3H07	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCAATGGGAGGCA AATCAAGT, GCCAAATGGTTCCTTCTCAA	N.A
783	Ah1TC4B01	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGAGGCTCCAACA TTGCTCTAA, CTAGTAGGTTCTTGTGAGAGAGAGC	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
784	Ah1TC4E09	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTTTCTCCCCCT TGAACCT, GATCAAGTGAAAATGTTAGTATAAG	N.A
785	Ah1TC4F04	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTCCCCACTACCTC GACTCTC, GGTATTTGGGGAAGGGTGT	N.A
786	Ah1TC4H07	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTCCGTTGCTCT TCTGAAC, GATCAAGCACTTCAGACAATGG	N.A
787	Ah1TC5A06	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTCGGTTTGGGAG AACTCTT, TTGTAAGCAGACGCCACATC	N.A
788	Ah1TC5C05	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTCATCTTCACTTC CCCTTTCA, CCCAACCAAATCCAACACTT	N.A
789	Ah2AC1E11	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCTTTGTCCATCTC TGCAACTCC, GCGCTCGAAGCTCTATGAATAA	N.A
790	Ah2AC2C12	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTATCGAGCCGAA TATGAAT, GCAGGATTTTGTAAATTGAGAG	N.A
791	Ah2AC3C02	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTCTAACGCACAC AAATCGAA, CTTGTACCTGCGCCATTCT	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
792	Ah2AC3F05	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGAGATTCTGTATA TGCCCTTA, GATCATAGATACAAGATAATCAAAGT	N.A
793	Ah2TC11C06	4	N.A	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCCAACAAACCC TCTCTCTCT, GAACAAGGAAGCGAAAAGAA	N.A
794	Ah2TC9E08	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGAAACAGCCGCG AGAGAA, CCCTAACCTCTCTTCATTGTGC	N.A
795	AHGS0108	4	N.A	No	N.A	G	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGGTGAGGGAAA GAATCCACA, ACAAGGGTGACTTTGTTGGC	N.A
796	GM1259	4	N.A	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGATTTGGTTATT GGGGAACA, CCCCTCCTGTACATACTCAA	N.A
797	GM1418	4	N.A	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACTGGACCTCCACT CACAAACT, CAATCCCAACCCATAAAA	N.A
798	GM1503	4	N.A	No	N.A	EST	0.94	N.A	0.00	N.A	CACGACGTTGTAAAACGACTCCGATGCTTATC ACAAAAGG, ATTGCATTGACAGAAGGAGGA	N.A
799	GM1539	4	N.A	No	N.A	EST	1.00	N.A	0.00	N.A	CACGACGTTGTAAAACGACGCTCTTGTTAATT CGATTCCA, CCTCATCAGAGTCAGGCAAGA	N.A

APPENDIX A: Characteristics of SSR Markers screened across 16 *Arachis* sp.: Grade (Gr.), Polymorphism (Poly.), Heterozygosity (Het.), Number of Alleles (All. #), EST/Genomic (G) SSR, Availability (Avail.), Major Allele Frequency (MAF), PIC, Repeat motif, Primer sequences and Marker Map Locations (MML)

No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
800	GM1855	4	N.A	No	N.A	EST	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTGGTACTCTTCGT TTGCCACT, GAATTGGAATGAGCTCTGGTG	N.A
801	GM1901	4	N.A	No	N.A	EST	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGAAACACCGATA TTTTCGATACA, TGACGAGCAAGTCATGTATGTG	N.A
802	GM2027	4	N.A	No	N.A	EST	0.69	N.A	0.00	N.A	CACGACGTTGTAAAACGACCAATAAAATTG GTTGGGGTCT, CCATGGCGGTATGAACAATAA	N.A
803	GM2084	4	N.A	No	N.A	EST	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCGCAGAAATGAA CCGAAATTA, GGATGCATTCTTCTCTCTCT	N.A
804	GM2259	4	N.A	No	N.A	EST	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGGAAGCACCGAT AGTTCCTCT, TTCTGCTGCTTTCATCATCA	N.A
805	GM2291	4	N.A	No	N.A	EST	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGCTTCCAACCAA ATTCAATCC, CGTCATTGAATCCTTGTGTC	N.A
806	GM2565	4	N.A	No	N.A	EST	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACGGATGATAGTGA CGGGGAAGT, GCCATATCCGGCAGAGTAAAT	N.A
807	IPAHM165	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACCAACACGTTTCGC TTCCAGAT, TCACTCTCATTTCCGCCATT	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
808	IPAHM524	4	N.A	No	N.A	G	0.88	N.A	0.00	N.A	CACGACGTTGTAAAACGACGCCATGGATAAG AACCTGAAA, CAGTAAGCTGAGCTGGCAGA	N.A
809	IPAHM569	4	N.A	No	N.A	G	0.81	N.A	0.00	N.A	CACGACGTTGTAAAACGACATTGGTGGATAT CGGACTCG, CCTATCCTTCAAGGCTTCTCG	N.A
810	pPGPseq2E6	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACTACAGCATTGCC TTCTGGTG, CCTGGGCTGGGGTATTATTT	N.A
811	pPGPseq3B8	4	N.A	No	N.A	G	0.06	N.A	0.00	N.A	CACGACGTTGTAAAACGACGGAGAAAGATCA AACGAGAACA, TTCGAATATCTGACATTTGCTTTT	N.A
812	pPGSseq18G9	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACATATCAGCGCCA ATGACTCC, TCGCTCCTGGCACCTATATC	N.A
813	pPGSseq19A5	4	N.A	No	N.A	G	0.00	N.A	1.00	N.A	CACGACGTTGTAAAACGACATTCGTCTCCTTC TTTTGGC, TTTTGCTTCCAAATGGCTTC	N.A
814	IPAHM138	4	N.A	No	N.A	G	0.00	N.A	0.00	N.A	N.A, N.A	N.A
815	RN13B10	4	N.A	No	N.A	N.A	0.56	N.A	0.57	N.A	CACGACGTTGTAAAACGACATAATCGTCTTGT AATCTTTGG, TTGAGACTATATACGAATGTTCA	N.A

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No	Marker	Gr.	Poly.	Het.	All. #	EST/ G SSR	Avail.	MAF	PIC	Repeat motif	forward, reverse primer sequence (5'– 3')	MML
816	GM10732	4	N.A	No	N.A	EST	0.00	N.A	0.00	N.A	N.A, N.A	N.A
817	gi-4925	4	No	No	N.A	N.A	0.38	N.A	0.00	N.A	CACGACGTTGTAAAACGACAACTTGTATGT GTAGCAGACC, GGTGAAAAATCGGAAAGA	N.A