PHENOTYPING METHODS AND QTL MAPPING FOR LATE LEAF SPOT RESISTANCE IN CULTIVATED PEANUT (*Arachis hypogaea* L.)

by

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(Under the Direction of Peggy Ozias-Akins)

ABSTRACT

Late leaf spot (LLS), caused by *Cercosporidium personatum,* is a major foliar disease of peanut. LLS resistance is quantitative in nature. To identify genomic regions that control disease resistance, quantitative trait locus (QTL) mapping is required. Obtaining reliable phenotyping data is vital for mapping. Therefore, a population, consisting of 78 recombinant inbred lines (RILs) segregating for LLS resistance, was used for quantitative trait locus (QTL) analysis. Data were collected using three different phenotyping methods and comparisons were made. A set of 447 simple sequence repeat (SSR) and 25 single nucleotide polymorphism (SNP) markers was used for parental screening. The population was genotyped using 141 polymorphic loci. A linkage map with 94 loci, distributed into 19 LGs, was constructed based on the marker segregation data obtained from the population. A total of 7 QTL were identified that explained 10.9-38.4% of the variation in the phenotyping data.

INDEX WORDS: Peanut, *Arachis hypogaea*, disease resistance, late leaf spot, *Cercosporidium personatum,* LLS, phenotyping, quantitative, molecular marker, QTL, mapping, host resistance, detached leaf

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DEDICATION

This thesis is dedicated to my family, my parents, Kashmir and Simarjit Gill, my brother, Kamaljeet Gill and my loving husband and best friend, Harwinder Sidhu.

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CHAPTER 1

INTRODUCTION

Cultivated peanut (*Arachis hypogaea*) is a recent allotetraploid legume (2n = 4x = 40) (AABB), and its A and B genomes are most likely derived from two wild diploid progenitors, *A. duranensis* and *A. ipaënsis,* respectively (Kochert *et al.,* 1996). It is a major legume crop and its kernels are primarily crushed for oil or used for direct consumption in different parts of the world (Tillman and Stalker, 2010). Peanut kernels are a rich source of oil, protein, and various minerals and vitamins (Savage and Keenan, 1994). Peanut plants also find use in animal consumption as the oil cake can be used as animal feed (Savage and Keenan, 1994) and the haulms make relatively palatable and protein-rich forage for cattle (Cook and Crosthwaite, 1994). Peanut grows mainly in tropical and sub-tropical regions of the world. World annual production of peanut is about 38.6 mt, with Asia, Africa and Americas accounting for 68%, 24%, and 8%, respectively. China is the world's largest producer of peanut and contributes to 42% of world production, followed by India at 18%. The USA contributes only about 4% to the total world production. However, the trends for yield are different. Average yield for peanut in the US is 3.7 t/ ha, more than twice the average yields in India and the world at 1.7 t/ ha and 1.8 t/ ha, respectively. On the other hand, average peanut yields in China are much higher than the world average and slightly lower than the US, at 3.4 t / ha (FAOSTAT, 2011). These variations in yields across countries may be attributed to the

level of farm mechanization, management of biotic and abiotic stresses, and investment in crop improvement and research efforts (Dwivedi *et al.*, 2007).

Among biotic stresses, early leaf spot, caused by *Cercospora arachidicola* S. Hori, and late leaf spot, caused by *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton, are the most destructive and economically important foliar diseases affecting peanut (*Arachis hypogaea* L.) throughout the world (Shokes and Culbreath, 1997). Yield losses due to these diseases may range from 10% to 80%, varying geographically, between seasons, and for availability of chemical control (McDonald *et al.*, 1985; Miller *et al.*, 1990; Shokes and Culbreath, 1997). Fungicide applications can reduce the yield losses due to diseases but they increase the economic burden on small-scale farmers in developing countries.

The two leaf spots occur wherever peanut is grown (Jackson and Bell, 1969; Porter *et al.*, 1984) and are often found together. Sometimes, one pathogen may be more predominant than the other, in a certain location or year, or a time of the year. This variation in occurrence of leaf spots is influenced by changing weather patterns, cultivar selection and other management inputs (Nutter *et al.*, 1995).

Symptoms of early and late leaf spots have been described by several authors (Jackson and Bell, 1969; Jenkins, 1938; McDonald *et al.*, 1985; Woodroof, 1933). Chemical control is historically the most prevalent method of peanut leaf spot management in the southeastern US and incurs about 20% of the total variable production costs (Tillman and Stalker, 2010). As mentioned in several previous studies, it is not a sustainable disease management strategy in many developing countries (Gibbons, 1980; Subrahmanyam *et al.*, 1982).

Host resistance offers great scope in this scenario and breeding for resistance to early and late leaf spots has been a major objective in peanut breeding programs since the early 1980s (Chiteka *et al.*, 1988; Fehr, 1987; Norden *et al.*, 1982). However, incorporation of late leaf spot (LLS) disease resistance in cultivars using conventional breeding methods has been challenging due to the polygenic and complex inheritance of the trait (Dwivedi *et al.*, 2002; Motagi, 2001; Nevill 1982; Sharief, *et al.*, 1978). Another limitation is that high levels of disease resistance have only been identified in wild *Arachis* spp. (Abdou *et al.*, 1974; Dwivedi *et al.*, 2002; Hassan and Beute, 1977; Jackson and Bell, 1969; Pande and Rao, 2001; Sharief *et al.*, 1978), that haven't yet been successfully utilized to develop commercially acceptable resistant cultivars.

With recent advances in the field of genetics and genomics, the potential for utilizing molecular markers to accelerate the development of improved peanut cultivars has increased significantly (Varshney *et al.*, 2007). It is difficult to develop a single or a few diagnostic markers, which can effectively discriminate between resistant and susceptible genotypes, for quantitative traits like disease resistance. Therefore, quantitative trait loci (QTL) or specific regions of the genome, which control disease resistance, need to be identified. Genotypic and phenotypic data of a mapping population, segregating for disease resistance, need to be analyzed to detect QTL through linkage mapping. Disease resistant cultivars could then be developed by introgressing the identified QTL into elite lines, with the help of tightly linked markers. One of the prerequisites of accurate QTL identification is reliable and highly informative phenotypic data. In addition to low genetic variation in cultivated peanut, lack of precise phenotyping is a major limitation for successful application of genomics in breeding

(Glaszmann *et al.*, 2010; Pandey *et al.*, 2011; Varshney *et al.*, 2006; Varshney *et al.*, 2007).

The Florida (1-10) scale (Chiteka *et al.*, 1988) is widely used to evaluate leaf spot resistance in peanut breeding programs (Gorbet, 2007; Gorbet and Tillman, 2008; Grichar *et al.*, 1998; Isleib *et al.*, 2003). Similar discrete disease scoring scales have been used in phenotyping mapping populations (Khedikar *et al.*, 2010; Sujay *et al.*, 2012). The Florida (1-10) scale provides comprehensive assessment of disease symptoms, including intensity of lesions, their location in canopy and total defoliation. Due to its discrete nature and narrow range (1-10), the Florida scale cannot capture the quantitative or continuous variability among genotypes of a segregating mapping population. The Florida scale is subjective in nature and requires training, which further questions the accuracy and reliability of this phenotyping method. Therefore, two other methods, a lateral stem assay and a detached leaf assay were separately used to phenotype the RIL population. Comparisons were made amongst the three methods to find the best combination of attributes that can be measured to obtain highly informative as well as reliable LLS phenotyping data. Both the alternate methods, lateral stem assay (Shokes *et al.*, 1987) and detached leaf assay (Melouk and Banks, 1978) have been used for LLS phenotyping for several years but comparison between these and the Florida 1-10 scale have not been reported for a mapping population study. Since automation in future disease phenotyping is desired (Dwivedi *et al.*, 2007), use of electronic imaging and disease analysis software, was included wherever possible to evaluate its feasibility and efficiency in disease phenotyping.

In contrast to the substantial phenotypic diversity that exists for various morphological, physiological, and agronomic traits in *Arachis hypogea*, low-levels of molecular marker variation have been observed in the germplasm of this species. This low-level genetic variation in cultivated peanut is mainly attributed to its evolution through a recent single polyploidization event. Another reason is the restricted gene flow between diploid relatives and tetraploid cultivated peanut. Moreover, years of conventional breeding and selection has led to a narrowed genetic base. Several researchers (Cuc *et al.*, 2008; Koppolu *et al.*, 2010; Singh *et al.*, 1998; Young *et al.*, 1996) have also discussed the lack of a suitable molecular marker system in peanut.

Various marker systems including, Restriction Fragment Length Polymorphisms (RFLPs) (Halward *et al.*, 1991; Kochert *et al.*, 1991), Random Amplification of Polymorphic DNA (RAPDs) (Garcia *et al.*, 1995; Raina *et al.*, 2001), Amplified Fragment Length Polymorphisms (AFLPs) (He and Prakash, 2001), and Simple Sequence Repeats (SSRs) (He, *et al.*, 2003) have been used in the past for detecting polymorphisms in cultivated peanut. Microsatellites or SSR markers are often preferred over other classes of molecular markers because they are multi-allelic, co-dominant, relatively abundant and provide extensive genome coverage (Gupta and Varshney, 2000). In peanut, SSRs are also the most informative and useful marker system for genetic applications (Gupta and Varshney, 2000, Pandey, *et al.*, 2012). More than 4000 SSR markers are now available in peanut (Gautami *et al.*, 2012; Pandey *et al.*, 2012) owing to the extensive efforts of various research groups across the globe (Pandey *et al.*, 2012). Therefore, SSRs were the markers of choice for this QTL mapping study. Depending on the in-house availability, SSR markers were selected from amongst several publications

(Gautami *et al.*, 2012; Guo *et al.*, 2012; Khedikar *et al.*, 2010; Qin *et al.*, 2012; Ravi *et al.*, 2011; Sujay *et al.*, 2012) and used for screening parents of the C1501 population.

Single nucleotide polymorphisms (SNPs) are another marker system with potential for higher polymorphism detection and high-throughput applications in cultivated peanut (Guo *et al.*, 2011; Pandey *et al.*, 2012; Paterson *et al.*, 2004). A small set of SNPs was used for genotyping the segregating RILs; therefore, high-throughput assay platforms such as BeadXpress and GoldenGate assays from Illumina Inc. (San Diego, CA) were not be economical. Instead, a more flexible and cost-effective option (Hiremath *et al.*, 2012), Competitive Allele Specific PCR (KASPar) assay from KBiosciences (Hertfordshire, UK) (www.kbioscience.co.uk) was used.

QTL mapping has now become routine in many species as a method to achieve higher genetic gains for quantitative traits in fewer generations than conventional breeding. It helps to identify marker-trait associations, that can be used in genotypic selection, and demarcate the genomic regions responsible for conferring favorable or unfavorable alleles for quantitative trait phenotypes. Owing to the low-level genetic variation and scarcity of polymorphic markers, QTL mapping for complex traits in cultivated peanut is still in its infancy. Various traits that have been mapped in peanut using QTL analysis include, abiotic stress (drought tolerance) (Gautami *et al.*, 2012; Ravi *et al.*, 2011), disease resistance (LLS, rust, and TSWV) (Khedikar *et al.*, 2010; Qin *et al.*, 2012; Sujay *et al.*, 2012), and nutritional quality (oleic and linoleic acid content) (Sarvamangala *et al.*, 2011). However, all these mapping studies have genetic maps based on SSRs alone. Due to their amenability to high- throughput genotyping and cost-effective approaches, SNPs have the potential to replace SSRs as markers of choice for

peanut genotyping in the near future (Guo *et al.*, 2011; Pandey *et al.*, 2012; Paterson *et al.*, 2004). Therefore, an attempt was made in this study to incorporate a small number of SNPs along with SSRs to construct a genetic map for QTL mapping.

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CHAPTER 2

LITERATURE REVIEW

Arachis Genetic Polymorphisms

In contrast to the substantial phenotypic diversity that exists for various morphological, physiological, and agronomic traits in *Arachis hypogea*, relatively low genetic diversity has been observed in this species. This low-level genetic variation in cultivated peanut is mainly attributed to its evolution through a recent single polyploidization event (Kochert *et al.*, 1996). Another reason is the restricted gene flow between diploid relatives and tetraploid cultivated peanut. Moreover, years of conventional breeding and selection has led to a narrowed genetic base. Several researchers (Cuc *et al.*, 2008; Koppolu *et al.*, 2010; Singh *et al.*, 1998; Young *et al.*, 1996) have also discussed the lack of a suitable molecular marker system in peanut.

Various marker systems including, Restriction Fragment Length Polymorphisms (RFLPs) (Halward *et al.*, 1991; Herselman *et al.*, 2004; Kochert *et al.*, 1991), Random Amplification of Polymorphic DNA (RAPDs) (Garcia *et al.*, 1995; Halward *et al.*, 1992; Raina *et al.*, 2001), Amplified Fragment Length Polymorphisms (AFLPs) (Herselman, *et al.*, 2004), Simple Sequence Repeats (SSRs) (He *et al.*, 2003; Pandey *et al.*, 2012a) and transposon markers (Shirasawa *et al.*, 2012) have been developed for detecting polymorphisms in *Arachis* spp. However, the inability of most molecular marker technologies (RFLPs, AFLPs, and RAPDs) to detect sufficient intraspecific

polymorphisms has impeded high-density molecular mapping in *Arachis hypogea* in the past (Halward *et al.*, 1991; Kochert *et al.*, 1991; Paterson *et al.*, 2004). SSR markers, first developed by Hopkins *et al.* (1999) in peanut, are often preferred over other classes of molecular markers because in addition to being multi-allelic and codominant, they are relatively abundant and provide extensive genome coverage (Gupta and Varshney, 2000). In peanut, SSRs are also the most informative and useful marker system available for genetic applications (Gupta and Varshney, 2000; Pandey *et al.*, 2012a). More than 4000 SSR markers are now available in peanut (Gautami *et al.*, 2012a; Pandey *et al.*, 2012a) owing to the extensive efforts of various research groups across the globe (Pandey *et al.*, 2012a). However, less than 38% of these have been found to be polymorphic among *A. hypogaea* lines (Pandey *et al.*, 2012a). These SSR markers are a useful tool to study the genetic variability in peanut germplasm of both cultivated and wild species. Large numbers of AFLPs and SSRs have served as a useful resource for plant diversity assessment, genetic studies, molecular mapping and gene discovery in peanut. Even though large numbers of markers are available for cultivated peanut, among a variety of marker systems, only a limited number of markers can be mapped in a given biparental mapping population due to polymorphism constraints (Holbrook *et al.*, 2011).

In spite of these hindrances, different molecular marker systems have played an important role in assessing plant diversity in peanut. The botanical varieties of *A. hypogea* were first distinguished on the molecular level by using AFLPs (He and Prakash, 2001). Herselman (2003) later distinguished closely related peanut cultivars using the same marker system. Halward *et al.* (1992) created the first linkage map in peanut by using RFLPs to analyze progenies of the cross *A. stenosperma* and *A.*

cardenasii (both A-genome species) and grouped 117 RFLP markers into 11 linkage groups (LGs). Burow *et al.* (1996) created another RFLP map, composed of 383 RFLP loci. Following this, linkage maps for diploid species of peanut were developed using various molecular marker systems, including AFLPs, RAPDs (Garcia *et al.*, 2005), and microsatellites (SSRs). Separate microsatellite maps were created for both A genome spp. (cross between *A. duranensis* and *A. stenosperma*) (Moretzsohn *et al.*, 2005) and B genome species (*A. ipaensis* and *A. magna* Krapov., W.C. Gregory, and C.E. Simpson) using the same set of SSR markers. Both A- and B- genome maps were reported to exhibit good synteny (Gobbi *et al.*, 2006).

Owing to the advances in technology and availability of molecular resources in recent years, moderate to high-density linkage mapping has been made possible in cultivated peanut (Holbrook *et al.*, 2011; Pandey *et al.*, 2012b). Varshney *et al.* (2009) developed the first SSR-based genetic linkage map for cultivated peanut. A total of 135 SSR loci were mapped on 22 LGs, covering 1270.5 cM of map distance. Since then, several genetic linkage maps have been constructed for different population backgrounds in cultivated peanut (Gautami *et al.*, 2012b; Hong *et al.*, 2010; Khedikar *et al.*, 2010; Ravi *et al.*, 2011; Sujay *et al.*, 2012). Before the advent of linkage mapping in peanut, information on very few marker-trait associations was available (Tillman and Stalker, 2010). Examples of such associations include, RAPD-linked corn rootworm and early leaf spot (ELS) resistance (Stalker and Mozingo, 2001), AFLP linked with resistance to aphid vector of groundnut rosette disease (GRD) (Herselman *et al.*, 2004), CAP (Cleaved Amplified Polymorphic Sequences) -linked nutritional quality (Lopez *et al.*, 2000) and SSR-linked sclerotinia blight resistance (Chenault and Maas, 2006). However,

availability of linkage maps encouraged numerous studies aimed at identifying marker-trait associations through quantitative trait loci (QTL) analysis. Various traits that have been mapped in peanut using QTL analysis include, abiotic stress (drought tolerance) (Gautami *et al.*, 2012b; Ravi *et al.*, 2011), disease resistance (foliar diseases and spotted wilt) (Khedikar *et al.*, 2010; Qin *et al.*, 2012; Sujay *et al.*, 2012) nutritional quality (oleic and linoleic acid content) (Sarvamangala *et al.*, 2011), and multiple agronomic traits (Shirasawa *et al.*, 2012). Improved marker-trait associations have paved the way for molecular breeding applications in peanut improvement (Janila *et al.*, 2013).

The most recent genetic linkage map for cultivated peanut consists of 1114 loci (SSRs and transposon markers) distributed on 21 linkage groups (LGs), covering a map distance of 2166.4 cM (Shirasawa *et al.*, 2013). While more markers are still required for developing ultra-dense genetic maps in cultivated peanut, consensus maps developed from diploid and tetraploid *Arachis* spp. serve as valuable resources for genetic and molecular breeding activities in peanut (Gautami *et al.*, 2012a; Shirasawa *et al.*, 2013).

Background of Cultivated Genotypes Selected for Study

'Gregory'

Gregory, a large seeded, virginia-type peanut cultivar, was released by the North Carolina Agricultural Research Service (NCARS) (Isleib *et al.*, 1999). Gregory has alternate branching pattern, and intermediate growth habit. Gregory is a selection from a cross between cultivars 'NC 7' (Wynne *et al.*, 1979) and 'NC 9' (Wynne *et al.*, 1986). At the time of release, Gregory had higher percentage of fancy, jumbo pods, extra-large kernels, and higher pod yield as compared to the then largest seeded virginia-type cultivar 'NC 7'. When evaluated for disease resistance, Gregory was found to be highly

susceptible to sclerotinia blight (*Sclerotinia minor* Jagger) just like 'NC 7'. Gregory was not recommended for planting in the fields that harbor *S. minor* because Gregory produces luxuriant canopy growth under high planting density or high soil moisture and fertility, and such luxuriant growth promotes the spread of the pathogen. Gregory was found to be susceptible to ELS (*Cercospora arachidicola* S. Hori). However, Gregory showed the lowest incidence of tomato spotted wilt virus (TSWV) among all the virginia-type cultivars released by then. Gregory performed well in North Carolina-Virginia peanut production area as well as the southeastern US production area that includes Georgia, Florida, and Alabama (Isleib *et al.*, 1999).

'Tifguard'

Tifguard is a runner-type peanut (*Arachis hypogaea* L. subsp. *hypogaea* var. *hypogaea*) cultivar released by the USDA-ARS and the Georgia Agricultural Experiment Stations (Holbrook *et al.*, 2008). Tifguard is a result of the efforts to bring resistance to root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood race 1) and spotted wilt caused by TSWV into a single peanut cultivar. To develop the breeding population, a cross between TSWV-resistant cultivar, 'C-99R' (Gorbet and Shokes, 2002), and the nematode-resistant cultivar, 'COAN' (Simpson and Starr, 2001), was made. Selection was performed until a breeding line, C724-19-15, was identified that had high resistance to both pathogens. Peanut fields in the peanut production region of the southeastern United States experienced biotic stress from both the peanut root- knot nematode and TSWV. The problem prevalent at that time was that the nematode resistant cultivars suffered yield reduction due to TSWV stress and the TSWV resistant cultivars due to nematode stress. Tifguard has root-knot nematode resistance similar to the nematode-

resistant check cultivars, 'COAN' and 'NemaTAM' (Simpson *et al.*, 2003), and higher resistance to TSWV than 'Georgia Green' (Branch, 1996) and 'C-99R', two cultivars with moderate levels of resistance to TSWV. Since Tifguard possesses a high level of resistance to both TSWV and *M. arenaria*, it had significantly higher yield than all other entries, when subjected to biotic pressure from both the pathogens. Therefore, this cultivar is valuable for peanut growers in the areas where both these pathogens are prevalent (Holbrook *et al.*, 2008).

Leaf Spot Diseases of Peanut

Among biotic stresses, early leaf spot (ELS), caused by *Cercospora arachidicola* S. Hori, and late leaf spot (LLS), caused by *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton, are the most destructive and economically important foliar diseases affecting peanut throughout the world (Shokes and Culbreath, 1997). Yield losses due to these diseases may range from 10% to 80%, varying geographically, among seasons, and with availability of chemical control (McDonald *et al.*, 1985; Miller *et al.*, 1990; Shokes and Culbreath, 1997). Fungicide applications can reduce or limit the yield losses due to these diseases, but they represent a significant cost for producers, and may be cost-prohibitive for small-scale farmers in developing countries.

The two leaf spots occur wherever peanut is grown (Jackson and Bell, 1969; Porter *et al.*, 1984; Shokes and Culbreath, 1997) and are often found together. Sometimes, one pathogen may be more predominant than the other, in a certain location or year, or a time of the year. This variation in occurrence of leaf spots may be influenced by changing weather patterns, cultivar selection and other management inputs (Nutter *et al.*, 1995).

Leaf spots tend to reduce the vitality of affected plants by reducing photosynthetic area and promoting leaf abscission. A temperature range of 25 to 30°C, 95-100% relative humidity, and lack of crop rotation is conducive for conidial germination and infection by leaf spot pathogens (Alderman and Nutter, 1994; Butler *et al.*, 1994; Shew *et al.*, 1988; Sommartya and Beute, 1986; Wadia and Butler, 2007).

Several authors (Jackson and Bell, 1969; Jenkins, 1938; McDonald *et al.*, 1985; Woodroof, 1933) have described symptoms of early and late leaf spots. Host genotype and weather conditions can influence the disease symptoms of leaf spots. In general, the symptoms of ELS and LLS start as small necrotic flecks that later develop into light brown to black sub circular lesions on the leaflets. The symptoms extend beyond the leaflets and disease lesions may be observed on petioles, stems, stipules, and pegs (Shokes and Culbreath, 1997). Lesions of ELS are usually surrounded by a pale yellow halo. However, this is not considered a reliable diagnostic character because the halo may be influenced by the host genetics, nutritional status of host, or weather conditions, and LLS lesions may have a halo as well. In general, the lesions produced by *C. arachidicola* (CA) are circular to irregular in shape, brown in color and slightly raised on the adaxial leaflet surface, where most of sporulation occurs. They are light brown and usually smooth on the abaxial leaflet surface with fruiting structures, randomly distributed on adaxial surface. The lesions produced by *C. personatum* (CP) are circular in shape, dark brown to black and smooth on the adaxial leaflet surface, black and rough or granular on the abaxial leaflet surface, where most of the sporulation occurs. LLS lesions occur as circular rings of fruiting structures on the abaxial surface of leaflets. In both the leaf spots, as the severity of disease increases, the symptoms develop from

chlorosis, through necrosis, coalesced lesions to defoliation of the plant. Severe leaf spot attack may lead to total defoliation of the plant. Numerous pods may be lost from such defoliated plants while digging and inverting the plant. The amount of yield loss depends on how early the defoliation starts. Digging these defoliated plants before their expected maturity is usually recommended to avoid huge yield losses. However, digging early may result in poorer quality kernels.

Chemical control is historically the most prevalent method of peanut leaf spot management in the southeastern US and represents about 20% of the total variable production costs (Tillman and Stalker, 2010). However, it is not a sustainable disease management strategy in many developing countries (Gibbons, 1980; Subrahmanyam *et al.*, 1982).

Host plant resistance offers great scope in this scenario and breeding for resistance to early and late leaf spots has been a major objective in peanut breeding programs since the early 1980s (Chiteka *et al.*, 1988; Norden *et al.*, 1982). However, incorporation of leaf spot disease resistance in suitable cultivars using conventional breeding methods has been challenging due to the polygenic and complex inheritance of the trait (Dwivedi *et al.*, 2002; Nevill, 1982; Sharief *et al.*, 1978).

Nematodes in Peanut

A variety of plant parasitic nematodes can attack peanut. The principal nematode species of economic importance belong to genera *Meloidogyne* (root-knot nematode), *Pratylenchus* (root-lesion nematode) and *Criconemoides* (ring nematode) (Dickson and De Waele, 2005). Three major species of root-knot nematodes, including *M. arenaria, M. hapla,* and *M. javanica* attack peanut worldwide (Sasser, 1977), with *M. arenaria* and

M. javanica concentrated in warmer regions, and *M. hapla* in cooler regions. Among the *Meloidogyne* spp., *M. arenaria* occurs as the principal nematode parasite on peanut in Alabama, Florida, Georgia and Texas. It is also known to cause some peanut infestation in the northern peanut growing belt of the US, including North Carolina, South Carolina and Virginia. On the other hand, *M. javanica* is not as prominent as *M. arenaria* and has only been reported in Florida, Georgia and Texas (Cetintas *et al.*, 2003; Minton and McGill, 1969). *M. hapla* occurs more frequently in the northern regions of the US and sometimes on higher altitudes in tropical regions (Eisenback and Triantaphyllou, 1991). The general effects of all nematodes that attack peanut include reduction of feeder roots and root stunting. However, nematodes differ in their specific symptoms on roots and pods. To identify a nematode causal agent, the roots and pods should be carefully dug with a shovel for examination of nematode damage symptoms. The diagnosis should be verified by soil, root and pod assays (Rich and Kinloch, 2005; Rich and Tillman, 2009).

Root knot nematode

The most serious nematode pest of peanut is *Meloidogyne arenaria* race 1, the peanut root-knot nematode (RKN). The presence of galls or knots on roots and pods of peanut is the diagnostic character of RKN attack. These galls/knots appear as single or multiple wart-like growths on roots, pegs and pods and sometimes may even produce discoloration (Rich and Kinloch, 2005; Rich and Tillman, 2009). As RKN infection progresses, secondary infections occur due to diseases such as pod rot or white mold and may lead to eventual death of the plants. The foliar symptoms of RKN attack may include plant stunting, yellowing, and wilting. Foliar symptoms of root-knot disease may be expressed at any time during the growing season, but are most evident in a peanut crop

beginning about 100 days after planting and during or after periods of hot, dry weather. In a field, areas of RKN damaged peanut are usually round to oblong in shape, and rows of infected plants may never overlap, as would those of healthy plants. In areas of high nematode populations, plants may wilt and eventually die (Rich and Kinloch, 2005; Rich and Tillman, 2009).

Root lesion nematode

The root lesion nematode on peanut, *Pratylenchus brachyurus*, is not as serious a pest as *M.arenaria*, but it can drastically reduce yields and seriously disfigure the peanut hulls with unattractive brown lesions that lead to pod rotting. In a field with lesion nematode infection, oval spots of dull yellowing may be seen. As these symptoms are very similar to nutrient deficiencies, careful diagnosis is important. The symptoms on infected plants are initially seen as distinct light brown lesions on pods, which become less distinct and turn black in color as the disease progresses. High populations of lesion nematode may cause extensive root and pod rotting in peanut (Rich and Kinloch, 2005; Rich and Tillman, 2009).

Single Nucleotide Polymorphisms (SNPs) for Mapping in Diploids and Polyploids

Single nucleotide polymorphisms (SNPs), arising from single base changes in sequence, and small insertions and deletions (InDels) are the most abundant polymorphisms, both in genic and non-genic regions, across genomes (Bundock *et al.*, 2009; Kwok, 2001). SNPs are rapidly becoming the markers of choice owing to their abundance and slow mutation rate within genomes, amenability to high throughput genotyping, and cost and time-effectiveness (Close *et al.*, 2009; Gupta and Varshney, 2000; Han *et al.*, 2012). Although, SNPs are bi-allelic and have lower information

content than SSRs, their high frequency, even distribution in genome and better genotyping accuracy rate reinforces their preference over other marker systems (Allen *et al.*, 2011; Morin *et al.*, 2004; Rafalski, 2002). SNPs are versatile markers that have widespread applications in plants including, high-resolution genetic mapping, linkage disequilibrium (LD) based association mapping, characterization of genetic resources, phylogenetic analysis, and identification of marker-trait associations (Giancola *et al.*, 2006; Rafalski, 2002; Westermeier *et al.*, 2009). Therefore, extensive SNP discovery projects have been undertaken in several crop plants, such as maize (Ching *et al.*, 2002), soybean (Zhu *et al.*, 2003), wheat (Ablett *et al.*, 2006; Somers *et al.*, 2006), rice (McNally *et al.*, 2006; Shen *et al.*, 2004), barley (Rostoks *et al.*, 2005), and chickpea (Hiremath *et al.*, 2012), to name a few.

However, large-scale SNP detection in many cultivated crops has been hindered due to: (a) large genome-size, with much repetitive DNA (b) polyploidy (c) reduced diversity in elite germplasm due to recent evolutionary bottlenecks (d) unavailability of complete genome sequence (Close *et al.*, 2009; Ganal *et al.*, 2009).

Various methods for SNP discovery in plants are based on; screening of expressed sequence tag (EST) data for single feature polymorphisms (SFPs), amplicon resequencing, direct SNP mining or hybridization-based identification from sequenced genomes, and using advanced sequencing technologies (Ganal *et al.*, 2009; Kota *et al.*, 2008).

SNP genotyping in polyploid species poses many challenges. For autopolyploid species, SNP discovery based on comprehensive screening of EST collections or amplicon resequencing may be used for SNP genotyping (Ganal *et al.*, 2009). However,

SNP assays developed through direct mining from published ESTs may not be polymorphic for the population of interest. For the amplicon re-sequencing strategy, poor quality SNP detection may be achieved when low dosage alleles are subjected to conventional sequencing methods (Bundock *et al.*, 2009). Therefore, amplicon re-sequencing, using a genotyping platform that can distinguish different allele dosages and haplotype combinations in heterozygous genotypes is desired for autopolyploids (Han *et al.*, 2012). High-resolution melting (HRM) and next generation sequencing technology (454 pyrosequencing) has been used for SNP genotyping in alfalfa (Han *et al.*, 2012) and sugarcane (Bundock *et al.*, 2009), respectively.

In the context of allopolyploid species, SNP discovery is even more tedious as useful polymorphisms within a genome need to be distinguished from not useful polymorphisms that occur between the genomes. In general, both EST screening and amplicon re-sequencing can be used for SNP genotyping. However, SNP identification through EST screening will be cumbersome, as discrimination among EST sequences from various genomes and screening large collections of ESTs from different individuals will be required (Ganal *et al.*, 2009). Therefore, amplicon re-sequencing is the most preferred method for SNP genotyping in allopolyploids. Although, direct sequencing of polymerase chain reaction (PCR) products can be used when slight sequence variation exists between the constituent genomes (e.g., rapeseed), amplicon sequencing using genome-specific primers shall be employed for allopolyploids with diverse component genomes (e.g., bread wheat) (Ganal *et al.*, 2009).

Cultivated peanut is a recent allotetraploid (AABB), with a genetic bottleneck (Kochert *et al.*, 1996), and a large genome size of 2800 Mbp (Temsch and Greilhuber,

2000), for which the whole genome sequence is not yet available. Since the amount of genetic variation in the elite breeding material is relatively low, in crops such as peanut, tomato, and hexaploid wheat, initial SNP identification through amplicon re-sequencing, is performed across large sets of target germplasm. In peanut, two SNP discovery projects have identified polymorphisms in tetraploid and diploid peanut (Pandey *et al.*, 2012b). For tetraploid peanut, comparison of 17 genotypes, based on 454/FLX transcript sequences, yielded > 2000 SNPs and a 1536-SNP Illumina GoldenGate SNP array was developed at the University of Georgia (Peggy Ozias-Akins, pers. comm.). In another project at the University of California-Davis, a 768-SNP Illumina GoldenGate SNP array based on tentative orthologous genes (TOGs) between diploid genotypes was developed (Nagy *et al.*, 2012).

Single Nucleotide Polymorphism (SNP) Genotyping Platforms

In the last few years, there has been a lot of activity in the area of SNP genotyping technology development. The choice of method depends on the scale and the scientific question one is looking to address. No single genotyping method is ideally suited for all applications. Some of the areas in which improvements are being made include rapid assay development, reduced assay costs and realizing highly parallel genomic assays. According to Kwok (2001), an ideal genotyping method should be easy, quick and cost-effective to develop, produce reliable results even with low quality DNA samples, be flexible to accommodate low and high-throughput requirements, and amenable to automation while maintaining the accuracy of genotyping results. Genotyping typically involves the generation of allele-specific products for SNPs of interest followed by their detection for genotype determination. SNP genotyping methods are very diverse

(Syvänen, 2001). Each genotyping method broadly consists of two elements: allele discrimination method and allele detection method (Gut, 2004).

Allele discrimination strategies

These are a series of molecular biological, physical and chemical procedures for distinction of the alleles of a SNP (Gut, 2001). Four popular methods for allele discrimination include primer extension, hybridization, ligation, and enzymatic cleavage.

Primer extension

Primer extension is a stable and reliable way of distinguishing alleles of a SNP. In primer extension, a nucleotide is hybridized next to a SNP and enzyme specificity is then utilized to achieve allelic discrimination. Two main variations in this approach include, common primer extension (CPE) – use of a common primer to detect both alleles, and specific primer extension (SPE) – use of specific primers to detect each allele (Kim and Misra, 2007).

Common primer extension (CPE)

A common primer extension (CPE) reaction typically involves designing a primer that anneals to its 3'end adjacent to a SNP site and extension with nucleotides by DNA polymerase (Bannigan *et al.*, 2007). Fluorescence detection or mass spectroscopy can then be used to determine the identity of the extended base and reveal the SNP genotype. The simplicity in primer selection and assay design, and ability to detect multiple SNPs together make CPE one of the favorite commercial SNP genotyping methods. Examples of CPE based methods include the PinPoint assay (Haff and Smirnov, 1997; Ross *et al.*, 1998), MassEXTEND™ (Braun *et al.*, 1997; Cashman *et al.*, 2001), SPC-SBE (Kim *et al.*, 2002), and GOOD assay (Sauer and Gut, 2003). In these methods, SNP-specific

primer extension yields extension products of different masses. Since each extension product corresponds to one of the alleles of each SNP, mass analysis can then be used to detect the SNP genotype. CPE approaches that use fluorescence-based detection involve single base extension (SBE) of primer with fluorescently labeled ddNTPs. They have been implemented in different formats, including homogeneous reaction and detection (SNaPshot® approach (Applied Biosystems, CA) (Le Hellard *et al.*, 2002), detection on a solid phase SNPstream™ assay (Orchid Biosciences, NJ) (Bell *et al.*, 2002; Pastinen *et al.*, 1997), and solid phase–mediated reaction arrayed primer extension (APEX) (Pastinen *et al.*, 1997).

Specific primer extension (SPE)

Specific primer extension (SPE) approaches involve using two allele-specific primers that are identical except for a mismatch at their 3'end. Allelic discrimination is based on the principle that primer extension would occur only if the nucleotide at their 3' end binds with perfect complementarity to the SNP. Allele-specific PCR (AS-PCR) uses differently labeled allele specific primers and a common reverse primer. The basic principle of AS-PCR is that DNA amplification will occur only when the allele-specific primer binds at a SNP site with perfect complementarity. The SNP genotype of the PCR product can later be determined by fluorescence detection (Gibbs *et al.*, 1989). AS-PCR has been combined with real-time PCR and capillary array electrophoresis for SNP genotyping (Latorra *et al.*, 2003; McClay *et al.*, 2002; Medintz *et al.*, 2001). A variation of this approach is the allele-specific primer extension (ASPE), where the PCR product containing the polymorphic site serves as template, and the 3' end of the primer extension probe consists of the allelic base. Fluorescence analysis of extended product can be done

to determine the SNP genotype (Ugozzoli *et al.*, 1992). Tagged primers with labeled nucleotides are used for ASPE based Tag-It ™ approach (™Bioscience Corp., Canada) and the SNP genotype is determined using flow cytometry analysis (Bortolin *et al.*, 2004). Competitive Allele Specific PCR (KASPar) assay from KBiosciences (Hertfordshire, UK) is a PCR-based SNP fluorescent genotyping system that uses Fluorescence resonance energy transfer (FRET) for allele detection (http://www.kbioscience.co.uk/reagents/KASP_manual.pdf). Primer extension is a very robust allelic discrimination mechanism allowing specific genotyping of most SNPs at similar reaction conditions and hence suitable for high throughput SNP genotyping (Syvänen, 2001).

Hybridization

In hybridization approaches, the differential thermal stability of double-stranded DNA with perfectly matched or mismatched target-probe pairs is used as a basis to distinguish the different alleles of a SNP (Kim and Misra, 2007). With the allele specific oligonucleotide hybridization (ASO) approach, two allele-specific probes are designed, usually with the polymorphic base in a central position, such that they hybridize to the target sequence only when there is a perfect match (Kwok, 2001). Under optimized assay conditions, only the perfectly matched probe-target hybrids are stable, and hybrids with one-base mismatch are unstable (Sobrino *et al.*, 2005). The length and sequence of the probe, location of SNP in the probe, and the hybridization conditions together influence the thermal stability between an ASO probe and its SNP containing target sequence and hence the effectiveness of allele discrimination (Kim and Misra, 2007). However, the *a priori* prediction of optimal reaction conditions or ASO probe sequence to achieve

effective allele discrimination is very challenging because these parameters are different for different SNPs. Therefore, there is no single set of reaction conditions optimal for genotyping all SNPs. This makes the assay design for ASO probe based hybridization very difficult. A widely known approach used to overcome this problem is to carry out ASO hybridization reactions on microarrays (Syvänen, 2001). In GeneChip® array technology (Affymetrix, CA), probe array, consisting of 25-mer oligonucleotide allele-specific probes is formed and amplified SNP containing target sequences are cleaved, tagged, hybridized and fluorescently labeled to this probe array under stringent conditions. A computer algorithm to assign SNP genotypes interprets the complex fluorescence patterns. The ability to use multiple probes to analyze each SNP makes GeneChip® array technology (Affymetrix, CA) a high accuracy SNP genotyping platform (Matsuzaki *et al.*, 2004). Other approaches that use hybridization have been described below.

The TaqMan® genotyping assay (Applied Biosystems, CA) combines hybridization and 5' nuclease activity of polymerase coupled with fluorescence detection. Dynamic allele-specific hybridization (DASH™) (DynaMetrix, UK) is an allele discrimination approach that employs real-time denaturation of a probe-target duplex under homogeneous conditions for allele detection using fluorescence (Russom *et al.*, 2006).

Other approaches to increase the power of ASO hybridization include the use of peptide nucleic acid (PNA) analogues (Griffin *et al.*, 1997; Ross *et al.*, 1997) or locked nucleic acids (LNA) (Ørum *et al.*, 1999) that have very high affinities for complementary DNA. Due to their high affinity, shorter PNA and LNA probes can be used to enable

improved allele discrimination while analyzing a large number of samples (Syvänen, 2001). PNA probes are used for allele-specific hybridization with surface-immobilized DNA templates (Griffin *et al.*, 1997; Ross *et al.*, 1997). Hybridization is the simplest mechanism for genotyping as there are no enzymes involved in allele discrimination (Kwok, 2001). In order to take full advantage of new ASO probe formats for SNP genotyping, only high accuracy, high sensitivity and high throughput detection methods should be used (Sobrino *et al.*, 2005).

Ligation

The DNA ligase is known for its specificity in repairing nicks in DNA. This specific nature of ligase is employed in the ligation based allele discrimination approaches (Kim and Misra, 2007). When two adjacent oligonucleotides are annealed to a DNA template, they are ligated together only if the oligonucleotides perfectly match the template at the junction (Kwok, 2001). Three probes, one common and two allele specifics, are required for oligonucleotide ligation assay (OLA). The common probe binds to the target DNA immediately downstream of the SNP. One of the allele specific probes has at its 3' end the nucleotide complementary to one allele, while the other has it complementary to the alternative allele. These two allele specific probes compete to bind to the DNA target adjacent to the common probe, which generates a double stranded region containing a nick at the allele site. Only the allele specific probe with perfect complementarity to the target is ligated to the common probe by the DNA ligase (Sobrino *et al.*, 2005). The ligation products are analyzed by various means to reveal the nature of the base at the polymorphic site. One can infer the allele(s) present in the target DNA by determining whether ligation has occurred. Most ligation methods employ allele-specific

probes with their 3'ends at the SNP sites because ligases are more sensitive to mismatches at the 3'end.

OLA gave rise to another technique, Padlock (Nilsson *et al.*, 1994), which involves circularization of allele specific probes i.e. a linear oligonucleotide probe with its ends designed to mimic the allele-specific probe and common probe for ligation at the SNP site (Kim and Misra, 2007). The ligation of the Padlock probe can then be detected by using the rolling circle amplification (RCA), which is a high throughput genotyping technology (Faruqi *et al.*, 2001). Molecular inversion probe (MIP) technique (ParAllele Biosience, CA), involves the use of a modified Padlock probe and SBE at the SNP site prior to ligation (Hardenbol *et al.*, 2003). Combinatorial fluorescence energy transfer (CFET) tags, composed of fluorescent dyes that can transfer energy when in close proximity, have also been used with ligation for SNP genotyping (Tong *et al.*, 2001). Other technologies such as SNPlex™ (Applied Biosystems, CA) and Illumina genotyping system (Illumina Inc.) are also based on the specificity of ligases. In these approaches, SNP interrogation reactions are carried out directly on genomic DNA and ligation products are amplified simultaneously by universal PCR (Sobrino *et al.*, 2005).

The ligation approaches have the advantage of having the highest level of specificity and ease to optimize among all allelic discrimination mechanisms. On the other hand, the ligation mechanism has the slowest reaction and requires the largest number of modified probes (Kwok, 2001).

Enzymatic cleavage

Enzymatic cleavage for allele discrimination is based on sequence and structure specificity of certain classes of enzymes that can cleave DNA. The Invader assay (Third Wave™ Technology) is based on the specificity of recognition, and structure-specific cleavage, by a Flap endonuclease. In this assay, three probes, two allele-specific and one common probe/invader, are used such that the invader is complementary to the region 3' of the polymorphic site with a mismatch at the SNP site, and the allele-specific probes are complementary to the region 5' of the polymorphic site with an overhang at their 5' end of the three-dimensional structure formed when two overlapping oligonucleotides hybridize perfectly to a target DNA (Lyamichev *et al.*, 1999). On hybridization to a DNA template, a three dimensional structure is formed by the invader, and the allele-specific probe with perfect complementarity. This structure is recognized by the flap endonuclease, the overhang of the allele-specific probe at the SNP site is cleaved, and it is detected by fluorescence. If there is a mismatch, the structure formed will not be recognized by the Flap endonuclease and cleavage and fluorescence will not occur (Kim and Misra, 2007). In a modification of the original Invader® assay, the released overhang oligonucleotide may act as the invader probe in a secondary reaction to amplify fluorescent signal (Hall *et al.*, 2000). The Invader assay is a genotyping method that does not require PCR amplification but a large amount of target DNA is required. In order to increase the sensitivity, a PCR step may be performed before the invader reaction, and the assay is then known as PCR–Invader assay (Mein *et al.*, 2000). The DNA requirements for genotyping and the cost implications of using PCR templates can be

further reduced and the throughput of this platform increased by using large multiplex (100-plex) PCR reactions (Ohnishi *et al.*, 2001).

Allele detection methods

The products of allele discrimination have to be analyzed by various allele detection methods. Some of the common methods of allele-detection include mass-based detection, and fluorescence signal-based detection.

Mass-based detection

Matrix assisted laser desorption/ionization – time of flight mass spectrometry approach (MALDI-TOF MS)

MALDI-TOF MS is the most direct method of detection because it measures the molecular weight of the products formed in the allelic discrimination reaction. The resolution of MALDI-TOF MS is so high that it can easily distinguish between DNA molecules that differ by only one base (Berlin and Gut, 1999; Buetow *et al.*, 2001; Li *et al.*, 1999). The analysis is quick and even though each sample is analyzed serially, the throughput of this method is high. The DNA products are co-crystallized with matrix molecules and subjected to ionization using a laser beam. Resulting ions are accelerated by a voltage gradient, through a field-free region and separated by the time of flight of each ion that depends on the mass and charge of the ion. This is a very precise measurement of the molecular weight of the DNA products and specific software are used to convert this time of flight into exact mass. A new chip based genotyping approach using MALDI-TOF MS has been developed by Tang *et al.* (1999) which has increased the throughput and decreased the cost for this technique. The main limitation

of MALDI-TOF MS method is the need for extremely high purity of the analyte for it to work (Kwok, 2001).

Fluorescence signal-based detection

Fluorescence-based allele detection is widely used in genotyping because of its simple implementation and fast and sensitive detection ability. It has been used in most initial studies on SNP discovery and for direct sequencing (DS) using capillary array electrophoresis for the Human Genome Project. Fluorescently labeled ddNTPs used in a Sanger sequencing reaction generate a ladder of fluorescently tagged extension products (Sanger *et al.*, 1977), which are separated by electrophoresis and allele detection based on fluorescent signals is performed. This is a preferred method for SNP discovery due to its high throughput.

Fluorescence polarization (FP)

Fluorescence polarization (FP) involves the polarization of fluorescent dye by the plane-polarized light, which results in emission of polarized fluorescence. Under conditions of constant temperature and solvent viscosity, the degree of polarization is a measure of molecular motion, which is used to detect changes in molecular weight (Chen *et al.*, 1999). FP can be used as a detection method in virtually any SNP genotyping method in which the product of the allelic discrimination reaction is substantially larger or smaller than the starting fluorescent molecule e.g. primer extension (Chen *et al.*, 1999) or 5' nuclease reaction (Latif *et al.*, 2001). The need for smaller amounts of fluorescent dyes and cheaper probes makes it one of the favorite fluorescence based allele-detection methods.

Fluorescence resonance energy transfer (FRET)

Fluorescence resonance energy transfer (FRET) occurs when two fluorescent dyes are in close proximity to one another and the emission spectrum of one of the dyes (donor) overlaps the excitation spectrum of the other (acceptor) (Clegg, 1995). As a result, different fluorescence signals are observed depending on whether the dyes are in close proximity or are separated from one another (Kim and Misra, 2007). Because of the required proximity of the two dyes, FRET is a good detection method for a number of allelic discrimination mechanisms including primer extension, ligation, 5'nuclease, molecular beacon, and invasive cleavage reactions (Hall *et al.*, 2000; Kostrikis *et al.*, 1998; Livak, 1999; Tyagi *et al.*, 1998). The major drawback of this method is the cost of the labeled probes required in all the genotyping approaches with FRET detection. FRET has also been used in AS-PCR (Myakishev *et al.*, 2001), SBE reactions (Chen and Kwok, 1997) and KASPar (http://www.kbioscience.co.uk/reagents/KASP_manual.pdf) for SNP genotyping.

Quantitative Trait Loci (QTL) Mapping

The concept of QTL mapping was first demonstrated by Sax (Sax, 1923) in his experiment with beans, in which he studied the association between a complex polygenic trait and a simple monogenic trait. Not much progress could be achieved in this field until the 1980s, mainly due to the unavailability of adequate polymorphic markers (Vinod, 2011). The development of molecular marker technologies and statistical packages enabled the detection of variation at the molecular level and analysis of variation at the population level respectively. The acronym QTL (Quantitative Trait

Loci) is just another name for polygenes, and was coined by Gelderman (1975). QTL mapping involves the construction of linkage maps and performing QTL analysis to identify genomic regions associated with traits.

A suitable mapping population is a very important requirement for QTL mapping. The population should be generated from parental lines, which are polymorphic for one or more traits of interest. Other than this, a high-density linkage map, a suitable phenotypic screening method and an appropriate statistical package are vital for QTL mapping (Vinod, 2011). Depending on the experimental objectives, timeframe and available resources, a mapping population can be chosen among F_2, F_2 derived, RILs, BC (back cross), DH (double haploid) etc. In addition to these factors, the type of mapping population and genetic nature of the target trait govern the size of the mapping population. This mapping population is then genotyped using polymorphic markers and the genotypic data is analyzed by statistical packages such as MAPMAKER (Lander *et al.*, 1987) or JoinMap (Ooijen and Voorrips, 2002) to construct the linkage map of the polymorphic markers. Phenotypic evaluation of the mapping population needs to be done before subsequent QTL mapping (Vinod, 2011).

Three widely used statistical approaches for QTL detection include, single marker analysis (SMA), simple interval mapping (SIM), and composite interval mapping (CIM). SMA, also known as single point analysis or single factor analysis of variance (SF-ANOVA) is the simplest QTL detection method that does not require a complete linkage map and is compatible with all basic software programs. Limitations of this method include decreasing likelihood of a QTL to be detected as it gets farther from the marker and inability to distinguish if the markers are associated with one or more QTL (Vinod,

2011). Instead of analyzing single markers, SIM analyzes multiple points between the pair of adjacent linked marker loci. It is statistically more powerful than SMA. SIM is easily accessible through various statistical packages such as MapMaker/QTL (Lincoln *et al.*, 1993) and Qgene (Nelson, 1997). In CIM, interval mapping for single QTL is combined with multiple regression analysis on a marker linked with another QTL (Zeng, 1994). It is more powerful, precise and effective than SIM and SMA methods. CIM has been performed by researchers using various statistical packages such as WinQTLCart (Wang *et al.*, 2012), MapManager QTX (Manly *et al.*, 2001) and PLABQTL (Utz and Melchinger, 1996). Another latest approach in QTL mapping is multiple interval mapping (MIM), which extends the interval mapping to multiple QTL (Vinod, 2011).

QTL mapping has now become routine in many species as a method to achieve higher genetic gains for quantitative traits in fewer generations than conventional breeding. It helps to identify marker-trait associations, that can be used in genotypic selection, and demarcate the genomic regions responsible for conferring favorable or unfavorable alleles for quantitative trait phenotypes. Owing to the low-level genetic variation and scarcity of polymorphic markers, QTL mapping for complex traits in cultivated peanut is still in its infancy. Various traits that have been mapped in peanut using QTL analysis include, abiotic stress (drought tolerance)(Gautami *et al.*, 2012b; Ravi *et al.*, 2011), disease resistance (LLS, rust, and TSWV) (Khedikar *et al.*, 2010; Qin *et al.*, 2012; Sujay *et al.*, 2012), nutritional quality (oleic and linoleic acid content) (Sarvamangala *et al.*, 2011), and multiple agronomic traits (Shirasawa *et al.*, 2012).

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CHAPTER 3

COMPARISON OF LATE LEAF SPOT (LLS) PHENOTYPING METHODS IN A RIL POPULATION OF CULTIVATED PEANUT (*Arachis hypogaea* L.)[1]

[1] Gill, R., Cantonwine, E.G., Culbreath, A.K., Holbrook, C.C., and Ozias-Akins, P. To be submitted to *Plant Disease*

Abstract

Late leaf spot (LLS, caused by *Cercosporidium personatum,* is one of the most destructive, economically important, and widespread foliar diseases of peanut. Fungicide applications can reduce the yield losses due to diseases but they increase the economic burden on small-scale farmers in developing countries. Host plant resistance offers great scope in this scenario and breeding for resistance to leaf spots has been a major objective in peanut breeding programs since the early 1980s. Owing to the quantitative inheritance of this trait, conventional breeding for disease resistance is challenging. Molecular breeding through quantitative trait loci (QTL) analysis shows great potential in breeding for such traits. Reliable genotyping and phenotyping data are vital for conducting useful QTL studies. Therefore, three different phenotyping methods were used to characterize resistance to LLS in a recombinant inbred line (RIL) population developed from a cross between Gregory, a virginia type cultivar, and Tifguard, a runner type cultivar. Gregory has been previously characterized as susceptible and Tifguard is reported as moderately resistant to the disease. This biparental population consisted of 78 $F_{7:8}$ lines and was grown with the parents in three replications in a non-sprayed field trial. The leaf spot intensity was assessed four times during each season, in 2011 and 2012, using the traditional Florida (1 to 10 scale) and twice, in 2011, using a combination of lateral stem assay (LSA) and image analysis. Three lateral stems were randomly collected from each plot across the three replications in the field, and the leaves were then subjected to imaging by a flatbed scanner. The image analysis and manual phenotyping on these lateral stem samples yielded data on percent defoliation, percent lesion area, and number of lesions per unit area (infection frequency). In 2012, the RIL population was evaluated

in a detached leaf assay with artificial disease epiphytotics and data on LLS disease resistance components including, incubation period (IP), latent period (LP), days after inoculation (DAI) until lesion diameter (LD)=1mm (IP(1mm)), percent sporulation (SPp), sporulation index (SI), percent lesion area (LAp), lesion diameter (LD) and infection frequency (IF) were collected. Attributes measured using each method were tested for significance and correlations among and within the three methods were calculated. According to results from various statistical analyses, ease and efficiency of measurement, feasibility and objectivity, an order of priority for various phenotyping attributes was proposed.

Introduction

Cultivated peanut (*Arachis hypogaea* L.) is a major legume crop and its kernels are primarily crushed for oil or used for direct consumption in several different parts of the world (Tillman and Stalker, 2010). Peanut kernels are a rich source of oil, protein, and various minerals and vitamins (Savage and Keenan, 1994). After oil extraction, peanut cake can be used as animal feed (Savage and Keenan, 1994) and the haulms make relatively palatable and protein-rich forage for cattle (Cook and Crosthwaite, 1994). Peanuts are grown mainly in tropical and sub-tropical regions of the world. World annual production of peanut is about 38.6 mt, with Asia, Africa and the Americas accounting for 68%, 24%, and 8%, respectively. China is the world's largest producer of peanut and contributes 42% of the world production, followed by India at 18%. The US contributes only about 4% to the total world production. However, the trends for yield are different. Average yield for peanut in the US is 3.7 t/ ha, more than twice the average yields in India and the world at 1.7 t/ ha and 1.8 t/ ha, respectively. On the other hand,

average peanut yields in China are much higher than the world average and slightly lower than the US, at 3.4 t / ha (FAOSTAT, 2011). These variations in yields across countries may be attributed to the level of farm mechanization, management of biotic and abiotic stresses, and investment in crop improvement and research efforts (Dwivedi *et al.*, 2007).

Among biotic stresses, early leaf spot (ELS), caused by *Cercospora arachidicola* S. Hori, and late leaf spot (LLS), caused by *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton, are the most destructive and economically important foliar diseases affecting peanut throughout the world (Shokes and Culbreath, 1997). Yield losses due to these diseases may range from 10% to 80%, varying geographically, among seasons, and with availability of chemical control (McDonald *et al.*, 1985; Miller *et al.*, 1990; Shokes and Culbreath, 1997). Fungicide applications can reduce or limit yield losses due to these diseases, but they represent a significant cost for producers, and may be cost-prohibitive for small-scale farmers in developing countries.

The two leaf spots occur wherever peanut is grown (Jackson and Bell, 1969) and are often found together. Sometimes, one pathogen may be more predominant than the other, in a certain location or year, or a time of the year. This variation in occurrence of leaf spots may be influenced by changing weather patterns, cultivar selection and other management inputs (Nutter *et al.*, 1995).

Several authors (Jackson and Bell, 1969; Jenkins, 1938; McDonald *et al.*, 1985; Woodroof, 1933) have described symptoms of early and late leaf spots. Host genotype and weather conditions can influence the disease symptoms of leaf spots. In general the symptoms of early and late leaf spots start as small necrotic flecks that later develop into light brown to black sub-circular lesions on the leaflets. The symptoms extend beyond

the leaflets, and disease lesions may be observed on petioles, stems, stipules, and pegs. Lesions of early leaf spot are usually surrounded by a pale yellow halo. However, this is not considered a reliable diagnostic character because the halo may be influenced by the host genetics, nutritional status of host, or weather conditions, and late leaf spot lesions may have a halo as well. In general, the lesions produced by *C. arachidicola* (CA) are circular to irregular in shape, brown in color and slightly raised on the adaxial leaflet surface, where most sporulation occurs. They are light brown and usually smooth on the abaxial leaflet surface with fruiting structures, randomly distributed on the adaxial surface. The lesions produced by *C. personatum* (CP) are circular in shape, dark brown to black and smooth on the adaxial leaflet surface, black and rough or granular on the abaxial leaflet surface, where most of the sporulation occurs. LLS lesions occur as circular rings of fruiting structures on the abaxial surface of leaflets. In both the leaf spots, as the severity of disease increases, the symptoms develop from chlorosis, through necrosis, coalesced lesions to defoliation of the plant. Severe leaf spot attack may lead to total defoliation of the plant. Numerous pods may be lost from such defoliated plants while digging and inverting the plant. The amount of yield loss depends on how early the defoliation starts. Digging these defoliated plants before their expected maturity is usually recommended to avoid huge yield losses. However, digging early may result in poorer quality kernels.

Chemical control is historically the most prevalent method of peanut leaf spot management in the southeastern US and represents about 20% of the total variable production costs (Tillman and Stalker, 2010). As mentioned in several previous studies,

it is not a viable disease management strategy in many developing countries (Gibbons, 1980; Subrahmanyam *et al.*, 1982).

Host plant resistance offers great scope in this scenario and breeding for resistance to early and late leaf spots has been a major objective in peanut breeding programs since the early 1980s (Chiteka *et al.*, 1988a; Norden *et al.*, 1982). However, incorporation of LLS disease resistance in suitable cultivars using conventional breeding methods has been challenging due to the polygenic and complex inheritance of the trait (Dwivedi *et al.*, 2002; Nevill, 1982; Sharief *et al.*, 1978). Although high levels of disease resistance have been identified in wild *Arachis* spp. (Abdou *et al.*, 1974; Dwivedi *et al.*, 2002; Hassan and Beute, 1977; Jackson and Bell, 1969; Pande and Rao, 2001; Sharief *et al.*, 1978), harnessing these resistance sources for cultivar development is cumbersome. Owing to the additional challenges, such as linkage drag, associated with using unadapted germplasm, there has been limited success in utilizing these resistance sources for peanut cultivar development (Gowda *et al.*, 2002).

With recent advances in the field of genetics and genomics, the potential for utilizing molecular markers to accelerate the development of improved peanut cultivars has increased significantly (Varshney *et al.*, 2007). It is difficult to develop a single marker or a few diagnostic markers, which can effectively discriminate between resistant and susceptible genotypes, for quantitative traits like disease resistance. Therefore, quantitative trait loci (QTL) or specific regions of the genome, which control disease resistance, need to be identified. Genotypic and phenotypic data of a mapping population, segregating for disease resistance, need to be analyzed to detect QTL through linkage mapping. Disease resistant cultivars can be developed by introgressing the

identified QTL into elite lines, with the help of tightly linked markers. One of the prerequisites of accurate QTL identification is reliable and highly informative phenotypic data. In addition to low genetic variation in cultivated peanut, lack of precise phenotyping is a major limitation for successful application of genomics in breeding (Glaszmann *et al.*, 2010; Pandey *et al.*, 2011; Varshney *et al.*, 2006; Varshney *et al.*, 2007).

The Florida 1-10 scale (Chiteka *et al.*, 1988b) is widely used to evaluate leaf spot resistance in peanut breeding programs (Gorbet, 2007a; Gorbet, 2007b; Gorbet and Tillman, 2008; Grichar *et al.*, 1998; Isleib *et al.*, 2003). Similar discrete disease scoring scales have been used in phenotyping mapping populations (Khedikar *et al.*, 2010; Sujay *et al.*, 2012). The Florida 1-10 scale provides comprehensive assessment of disease symptoms, including intensity of lesions, their location in the canopy and total defoliation. It can be used quickly, and lends itself well to use in situations where large numbers of plots need to be evaluated, such as with mapping populations. It is discrete in nature. Due to its discrete nature and narrow range (1-10), the Florida scale cannot capture the quantitative or continuous variability among genotypes of a segregating mapping population. The Florida scale is subjective, and requires training, which further questions the accuracy and reliability of this phenotyping method. Therefore, two other methods, a lateral stem assay (LSA) and a detached leaf assay (DLA) were separately used to phenotype a recombinant inbred line (RIL) population. Comparisons were made among the three methods to find the best combination of attributes that can be measured to obtain highly informative as well as reliable LLS phenotyping data. Both the alternate methods, lateral stem assay (Shokes *et al.*, 1987) and detached leaf assay (Melouk and Banks, 1978) have been used for LLS phenotyping for several years, but comparison

between those and the Florida 1-10 scale have not been reported for a mapping population study. Since automation in future disease phenotyping is desired (Dwivedi *et al.*, 2007), use of electronic imaging and disease analysis software was included wherever possible to evaluate its feasibility and efficiency in disease phenotyping.

The objectives of this study were to (a) characterize the C1501 RIL population using the Florida (1-10) scale, lateral stem and detached leaf assays; and (b) statistically compare the three phenotyping methods and propose the best combination of attributes that constitute a reliable and informative phenotyping dataset for a mapping population.

Materials and Methods

The C1501 population was composed of 78 RILs derived from the cross Gregory x Tifguard. Gregory is a high yielding virginia-type peanut cultivar but is susceptible to early leaf spot (Isleib *et al.*, 1999), whereas Tifguard is a result of the efforts to combine resistance to root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood race 1) and tomato spotted wilt tospovirus (TSWV), the causal agent of tomato spotted wilt in a single peanut cultivar (Holbrook *et al.*, 2008) and is moderately resistant to the early leaf spot (Li *et al*., 2012). Single seed descent (SSD) was used to advance the population from F_2 to $F_{4,}$ followed by advancing the material from $F_{4:5}$ to $F_{7:8}$ by harvesting individual plants and increasing seed in winter nurseries over the next two years. The plant material was advanced from $F_{7:8}$ to $F_{7:10}$ by bulk harvesting plots from 2010-2012. Field phenotyping was done on $F_{7:9}$ and $F_{7:10}$ generations in 2011 and 2012 respectively, whereas the growth chamber study was conducted on the $F_{7:9}$ generation.

The C1501 RIL population was evaluated for late leaf spot resistance at the University of Georgia Coastal Plain Experiment Station, Gibbs Farm in Tifton for two

consecutive years. Planting dates were May 25, 2011 and May 22, 2012. Experimental design for the field trials in both years was a randomized block design with three replications. Planting was done in 2.79 m^2 rectangular plots that consisted of two rows (1.52 m each). Forty seeds were planted in each of these plots, such that there were 20 seeds at uniform distance per row. Plots were irrigated and sprayed to control insects throughout the season as and when required. Low intensity spotted wilt (caused by TSWV) was observed in some plots, but white mold (caused by *Sclerotium rolfsii* Sacc.) was controlled by a single spray of Folicur, a.i. tebuconazole (Folicur 3.6F, Bayer Crop Protection, Research Triangle Park, NC) and prothioconazole (Proline, Bayer Crop Protection), in June 2011 and June 2012 respectively. The leaf spot epidemics occurred naturally in both years. Although ELS and irregular leaf spot (Cantonwine *et al.*, 2010) were observed early in the season, the LLS epidemic soon took over and *C. personatum* was the predominant pathogen at the time of harvest during both years.

Florida 1-10 scale rating

Late leaf spot severity per plot was evaluated four times in each year, using the Florida 1-10 scale rating system, where 1 = no leaf spot; 2 = very few lesions on the leaves and none on the upper canopy; 3 = few lesions on the leaves and very few on the upper canopy; 4 = some lesions with more on the upper canopy and 5% defoliation; 5 = lesions noticeable even on upper canopy and 20% defoliation; 6 = lesions numerous and very evident on upper canopy and 50% defoliation; 7 = lesions numerous on upper canopy and 75% defoliation; 8 = upper canopy covered with lesions and 90% defoliation; 9 = very few leaves remaining and those covered with lesions and 98% defoliation; and 10 = plants completely defoliated and dead (Chiteka *et al.*, 1988a). Ratings were taken at

82, 110, 130 and 141 days after planting (DAP) in 2011 and 111, 121, 132, and 141 DAP in 2012. To compare disease progress, area under the disease progress curve (AUDPC) value was calculated for each plot from the disease ratings each year (Shaner and Finney, 1977). The disease progress values were standardized (stAUDPC) by dividing AUDPC values by the number of days between the first and the last rating of each season, to account for differences in the duration of LLS epidemics (Singh *et al.,* 2011; Woodward *et al.*, 2008; Woodward *et al.,* 2010).

Lateral stem assay

Lateral stem assay (LSA) was used to assess the late leaf spot epidemic more objectively by separately measuring the attributes, defoliation and disease severity, that constitute the Florida 1-10 scale rating. Three lateral branches were randomly collected from each plot, twice during the 2011 season, first at 118-120 DAP and then at 132-134 DAP. The sample collection from three replications was staggered over three days such that the samples from the first, second and third replications were collected on the first, second, and third day, respectively. The lateral stems were brought to the laboratory where the number of nodes (n) and total number of leaflets present (p) were counted. The potential number of leaflets was calculated by n*4, and number of missing leaflets by subtracting the actual number of leaflets present (p) from the potential number of leaflets (n*4). Percent defoliation (DEF) was calculated from these data (Aquino *et al.*, 1995). All the leaflets were manually removed from the rachis and covered with a clear plastic sheet with sporulation side (abaxial side) up next to a blue background. The leaflets were scanned at 300 dpi using a flatbed scanner (Canon CanoScan LiDE 210) and stored as .tiff or .png files. Leaflet images were processed using ASSESS 2.0 image

analysis software (American Phytopathological Society, St. Paul, MN) to give the percent lesion area (LAp) (Erickson *et al.*, 2003) and infection frequency (i.e. number of lesions per cm^2) (Cantonwine *et al.*, 2008).

Detached leaf assay

The C1501 RIL population was evaluated for the components of resistance to LLS in a growth chamber study. The $F_{7:9}$ generation plant material was raised in the greenhouse. Four to five seeds for each line were sown in 15-cm pots filled with potting mix and sterilized field soil in 1:1 ratio. When the plants were 9 weeks old, a DLA was set up in the growth chamber by carefully excising the first or second fully expanded leaf at the base of the petiole from a randomly selected plant in each pot. The experimental design was a randomized complete block with three replications and a control. The control consisted of uninoculated detached leaf samples of the whole population. The study was carried out in March-April 2012 over a period of 30 days. All plants of one line, CB25A died in the greenhouse, so it was not included in the study. In addition, an alternate source of seed for Gregory (certified seed from 2007, designated "Gregory-Certified"), the susceptible parent, was evaluated in this study. Leaf sample collection for the first, second and third replications was scheduled on three consecutive days, respectively. Therefore, each of the three replications had individual initiation, inoculation and harvest dates during the experiment. After being detached from the plant, the cut ends of leaves were dipped in a gel-based formulation of indole butyric acid (IBA) (CLONEX Rooting Gel), and placed individually in 16 ml test tubes containing autoclaved water. The tubes with detached leaves were arranged randomly in test tube racks, placed in a transparent plastic tub with clear plexiglass on top, and put in a growth

chamber. The conditions in the growth chamber were set at 22-24°C, 95% -100% RH, and a 10/14 h light/dark photoperiod to provide an optimal environment for CP germination and infection (Alderman and Nutter Jr, 1994; Butler *et al.*, 1994; Shew *et al.*, 1988; Sommartya and Beute, 1986; Wadia and Butler, 2007). Two inches of standing water was maintained in the tubs to achieve very high humidity. Water was added to test tubes and tubs as needed (Cantonwine *et al.*, 2008). Detached leaves were misted with autoclaved water three times over the first 24 h to help them recover from excision shock and water stress. After this period, leaf samples were observed for any signs of wilting. None of the samples had to be removed because of wilting, and inoculations were administered following this step.

Peanut leaves with sporulating LLS lesions from natural infections were collected from the University of Georgia Coastal Plain Experiment Station Rigdon Farm, Tifton, GA, in 2011. Sporulating lesions were cut out, and stored in test tubes at 4° C for 5 to 8 months. These served as the inoculum source for the detached leaf experiment. For inoculation, conidial suspensions were prepared by stirring these leaf discs, bearing CP conidia, in 0.005% Tween 20 to suspend the conidia, and standardized to a concentration of 1.0×10^4 conidia ml^{-1} using a hemacytometer as described by Cantonwine *et al.* (2008).

Since the inoculations of three replications were performed over three separate days, fresh conidial suspensions were prepared each day. Inoculation was achieved by individually spraying each leaf sample for 1 second using an atomizing sprayer held at 30 cm from the leaf. A sterile solution of 0.005% Tween 20 was used on the control samples (Cantonwine *et al.*, 2008).

The leaf samples were observed daily for the development of initial late leaf spot lesions for the first 11 days after inoculation (DAI) in each replication and then on every second day until harvest (29 DAI). During this period, the DAI for the appearance of symptoms and sporulation were recorded for the first three lesions on each leaf sample for estimating incubation period (IP) and latent period (LP), respectively. Days after inoculation until the first three lesions reached 1 mm in diameter were recorded as another component that represented an interaction of incubation period with lesion diameter (designated as "IP (1mm)") (E.G. Cantonwine pers. comm.). The final values of IP, LP and IP (1mm) for each sample were recorded as mean values for the three lesions. LP estimations were made at two different magnifications (2X and 16X) and recorded as LP (2X) and LP (16X), respectively. The leaves were removed from test tubes at 29DAI and percent sporulation (SPp) and sporulation index (SI) were measured. Numbers of sporulating and non-sporulating lesions were counted to calculate percent lesions with sporulation on each leaflet and the mean of four leaflets was recorded as the SPp for that sample. A 0-4 scale, where 0= few or no stromata; 1= few stromata with little sporulation; 2= stromata over more than half of the lesion, with moderate sporulation; 3= stromata over most of the lesion, moderate to high sporulation; 4= stromata over entire lesion with heavy sporulation (Chiteka *et al.*, 1988a; Subrahmanyam *et al.*, 1982; Subrahmanyam *et al.*, 1985), was used to score sporulation on three oldest lesions in each leaf sample and the mean was recorded as SI for that leaf sample. Leaflets were removed from the rachis and prepared for imaging as described under the LSA method described above. Leaflet images were processed using ASSESS 2.0 image analysis software to give the LAp (Erickson *et al.*, 2003), infection frequency (i.e.

number of lesions per cm^2) (Cantonwine *et al.*, 2008), and mean lesion diameter of the five largest lesions for each leaf sample. IF was calculated by measuring the number of lesions and total leaf area (cm^2) separately by using 'count' and 'leaf area' options, respectively in ASSESS 2.0. To calculate mean lesion diameter (LD), the five largest lesions were delineated using 'freehand' option and 'length' and 'width at centroid' were checked under 'feature extraction options'. Since most lesions were not perfectly round, the software generated a dataset with different 'long axis' and 'short axis' values for each lesion. The diameter of each lesion was recorded as the mean of 'long axis' and 'short axis' and the mean of five lesions was recorded as mean lesion diameter for that leaf sample. Alternatively, the area of each lesion could be measured using the software and diameter calculated from the formula area= $\pi d^2/4$. Equivalent diameter values were obtained using either method. However, the former was used in this study.

Day 29 was chosen as the cutoff date for the experiment so that defoliation of the leaf samples would not occur, and biased evaluation of percent sporulation, number of lesions etc. could be prevented since when a lesion sporulates, it continues to sporulate until the leaf abscises. In that case, it would be difficult to assess if the lesion sporulation increased, decreased or stopped entirely after defoliation (Ricker *et al.*, 1985).

Statistical analyses

Analysis of variance (ANOVA) was performed on field disease severity ratings, and components of resistance to late leaf spot measured during lateral stem and detached leaf assays, to test the significance of differences between genotypes. Data were appropriately transformed, as needed, to meet the assumptions of ANOVA (Table 3.1). For the attributes that were significantly (at $P <= 0.05$) affected by genotype, differences

among genotype levels were determined by using Duncan's multiple range tests. Duncan's mean separation was performed on transformed means, but the untransformed or actual means were reported in the tables. Correlation coefficients (*r*) (Falconer, 1981) between different attributes of late leaf spot, measured using the Florida 1-10 scale, LSA and DLA, were calculated. Correlation coefficients were also calculated among various attributes of disease measured within each method. Correlation analyses were performed on untransformed data for all variables. Significance level for all correlation coefficients was $P \leq 0.05$.

Results

Gregory showed higher disease susceptibility than Tifguard for all the attributes measured using the three different methods to phenotype LLS. The RILs in the C1501 population also showed segregation for disease resistance (Table 3.2).

Analysis of variance

Florida 1-10 scale

Genotypic differences were significant ($P \leq 0.05$) for each of the four Florida scale disease severity ratings, and disease progress values (AUDPC and stAUDPC) from data pooled across years (2011-2012). For field disease severity, year (Y) x genotype (G) interaction was significant only for the first Florida scale rating (Table 3.3). A possible explanation for this observation may be that the first Florida scale rating was taken very early in the season in 2011, 82 DAP, as opposed to 111 DAP in 2012, and LLS epidemic had not yet masked the effect of irregular leaf spot (ILS), which is known to appear on peanut and closely mimic fungal leaf spot early in the season (Cantonwine *et al.*, 2010). This may have contributed to the inaccurate early season disease ratings and thus

inconsistency in disease severity for genotypes over years. Since proliferation of ILS decreases as the growth season progresses (Cantonwine *et al.*, 2010), the confounding effect was not observed in LLS ratings taken later in the season. LLS epidemic in 2012 was more severe than 2011 (Table 3.4), consistent with higher rainfall and relative humidity in 2012 that enhances CP conidial germination and infection (Alderman and Nutter, 1994; Butler *et al.*, 1994; Shew *et al.*, 1988; Sommartya and Beute, 1986; Wadia and Butler, 2007).

The field disease severity data were also analyzed separately for years (2011 and 2012). Genotypic differences were significant ($P \leq 0.05$) for each of the four Florida scale disease severity ratings, and disease progress values (AUDPC and stAUDPC) in 2011 and 2012, except for the third Florida scale rating in 2011 (FSRIII) (Table 3.5). Untransformed means with Duncan grouping were reported for field disease severity ratings and disease progress values, which were significantly ($P \leq 0.05$) affected by genotype in 2011 (Tables 3.6-3.10) and 2012 (Tables 3.11-3.16) field trials.

Lateral stem assay

Percent lesion area (LAp), and infection frequency (IF) measured during LSAII (132-134 DAP) showed significant ($P \leq 0.05$) differences among genotypes in the C1501 RIL population but the same attributes measured during LSAI (118-120 DAP) lacked ability to significantly distinguish among genotypes. DEF did not significantly differ among genotypes in either LSAI or LSAII (Table 3.17). Untransformed means with Duncan grouping were reported for LAp, and IF measured during LSAII, to further distinguish among genotypes (Tables 3.18-3.19).

Detached leaf assay

Among components of resistance to LLS measured during DLA, LAp, IP, SI, SPp, IP (1mm), LP (under 2X and 16X magnification), and LD showed significant ($P \leq 0.05$) differences among genotypes. However, no significant ($P \leq 0.05$) differences among genotypes were observed for IF. Untransformed means with Duncan grouping are being reported for all components of resistance to LLS measured during DLA, except IF (Tables 3.20-3.27).

Correlations

Florida 1-10 scale

Among the four Florida scale disease severity ratings (FSRI-FSIV) taken in 2011, each rating showed significant correlation with the corresponding rating taken in 2012. Disease progress values for both years were also significantly correlated. Although all the above mentioned associations were significant, the coefficients were low ($r < 0.5$). Correlation coefficients calculated between first Florida scale rating in 2011 and 4 ratings in 2012 showed negative values (Table 3.27). This may be due to inaccuracies in the first Florida scale rating of 2011 as it was taken very early in the season (82 DAP) and is suspected to be confounded by presence of ILS during that time.

Lateral stem assay

Attributes of resistance to LLS, LAp, IF and DEF, measured during LSAI and LSAII had significant (P=0.05) correlation (r) with AUDPC, the fourth Florida scale rating, and another FSR closest in time to each of the lateral stem assays (FSRII for LSAI, FSRIII for LSAII) in 2011. LAp had the highest correlation coefficients with the corresponding disease severity ratings and AUDPC values for both lateral stem assays,

followed by DEF and IF, except between IF in LSAII and FSRIV (Table 3.29). These attributes of resistance to LLS had significant coefficients of correlation across data collected in LSAI and LSAII. Among the three attributes, LAp had the highest correlation coefficient (r = 0.58) across the two lateral stem assays, followed by DEF (r = 0.45) and IF (r = 0.32) (Table 3.30) as well as within each assay separately (Table 3.31). In general, correlation coefficients among attributes measured in LSAII (r = 0.69-0.95) were stronger than LSAI (r = 0.3-0.45) (Table 3.32).

Detached leaf assay

IP, LP, and IP (1mm), were positively associated with each other and negatively associated with remaining components of LLS resistance. Results from correlation coefficient calculations, between components of resistance and field disease severity data (2011-2012) (Table 3.32), and among the components (Table 3.33) are summarized below:

Percent diseased leaf area

Significant ($P \leq 0.05$) correlations were found between LAp and final disease severity (FSRIV) and disease progress values (AUDPC) for 2012. Among other components measured in the DLA, LAp showed lowest and highest associations with IP (r = 0.49) and IF (r = 0.82), respectively.

Infection frequency

There was a significant ($P \leq 0.05$) but weak correlation between IF and AUDPC for 2011. Among other components, IF had lowest and highest correlation coefficients with SI (r = 0.29) and LAp (r = 0.82), respectively.

Incubation period

Correlation coefficients between IP, FSRIV, and AUDPC for 2012 were significant. Among other components, IP had lowest and highest correlation coefficients with SPp (r = 0.35) and IP (1mm) (r = 0.74), respectively.

Latent period

Latent period was recorded under two magnifications, 16X and 2X, LP (16X) and LP (2X), respectively. Latent period (2X) showed significant associations with field disease severity and progress values for both years (2011 and 2012). Correlations of LP (16X) were significant for field disease scores (FSRIV) of both years but disease progress values (AUDPC) of 2012 only. Concerning associations among components, both LP (16X) and LP (2X) had lowest correlation with IF (r = 0.41 and 0.42, respectively) and highest with each other (r = 0.83).

Sporulation index and percent sporulation

Both SI and SPp had significant correlation coefficients with field disease severity and AUDPC values from both years (2011 and 2012). Among correlations with other components, both SI and SPp had lowest correlation with IF, (r = 0.29 and 0.31, respectively) and highest with each other (r = 0.80).

Lesion diameter

Correlations between lesion diameter (LD) and field disease severity and progress values were significant only for 2012. The association between LD and other resistance components ranged from, r= 0.30 to 0.70, lowest with IF and highest with LP (16X).

DAI until lesion reaches 1mm diameter

Significant associations of IP (1mm) were found for both years with field disease severity but only for 2012 with AUDPC values. Among other components, IP (1mm) had lowest and highest correlation coefficients with IF (r = 0.41) and IP (r = 0.75), respectively.

Lateral stem and detached leaf assays

Since only LAp and IF measured during LSAII showed significant differences among genotypes (Table 3.34), only the correlation coefficients between these attributes and components of resistance measured during DLA were discussed. Among the components of resistance, LP (16X), LP (2X), SI, and SPp showed significant correlations with both LAp and IF measured during LSAII.

Discussion

C. personatum was the predominant pathogen at the time of harvest during both years. The Florida 1-10 scale is a suitable reference method for characterizing field LLS resistance in peanut. Absence of year (Y) x genotype (G) interactions for field disease ratings, except for the anomalous FSRI of 2011, suggests there is reasonable consistency across years in scoring genotypes using this method. This consistency in phenotyping is corroborated by significant correlations observed between corresponding field ratings during two years. However, care should be taken to schedule the ratings such that phenotyping is not confounded by factors like irregular leaf spot (ILS). Use of any field evaluation method requires considerable space and an ample supply of seed. In this study, 40 seeds were used for each replicate. It may be difficult to maintain conditions that are conducive for disease development in some locations and years. In this study,

irrigation was used to help facilitate epidemic development. With the dry hot conditions that were prevalent during the 2011 season, it is likely that disease development would not have been sufficient to allow comparison of the genotypes had irrigation not been available.

In addition, inter-plot interference can also play an important role. In this study, there were no obvious aberrations in disease severity among replicates of the more resistant lines that could be attributed to their proximity to more susceptible lines. However, if a certain genotype escapes/tolerates disease *via* reduced inoculum production and not resistance to infection, susceptibility of such lines may be overestimated when they are planted adjacent to genotypes that allow more inoculum production. Therefore, multiple disease evaluations during the season and over years are needed to reduce the risk of erroneous phenotyping.

To address the concerns of reliable and detailed phenotyping of LLS in a mapping population, two other methods, lateral stem assay and detached leaf assay were also tested. Lateral stem assays were done twice during the 2011 field season. However, significant differences among genotypes could not be detected from the data collected in the first lateral stem assay. Among the attributes of disease resistance measured during the second lateral stem assay, percent disease area and IF were able to show significant differences among genotypes. Therefore, considering the time, labor and logistic challenges associated with carrying out a LSA, it may not be feasible for testing large populations. Alternatively, only percent disease incidence could be recorded by counting the number of leaves that show LLS disease symptoms (Cantonwine *et al.*, 2008) or the disease assessment task could be confined to measuring LAp using ASSESS (Singh *et*

al., 2011). However, it is important to note that large plots of few genotypes were evaluated using these methods in the reported studies. The feasibility and success of these assays may not be repeatable in a large mapping population. For example, Singh *et al.* (2011) used a preset length of lateral stem samples, which makes the assay more uniform and feasible. However, a predefined stem length may not help in the choice of a representative sample from all plots of a mapping population, which is often segregating for morphological characters, such as internodal distance, plant habit etc., in addition to disease resistance. From the results of the present study, it was evident that DEF measured by counting the missing leaflets on lateral stems could not account for differences among RILs. This may be attributed to the unreliable method of calculating DEF, which erroneously assumes that each missing leaflet can be accounted for by the disease. It neglects to take into account the defoliation that may occur due to natural senescence in lower canopy regions or other physiological or environmental factors (Shokes *et al.*, 1987). Therefore, it is best to use LSA to measure disease incidence, and/or LAp as indices for LLS resistance in a mapping population.

Among the nine components of resistance to LLS measured during DLA, all but IF, showed significant differences among genotypes. All components, except IF, were also significantly associated with field disease severity and progress values in 2012. SI, SPp and LP (2X) were significantly correlated with the field disease data from two years. LP (16X) and IP (1mm) were significantly associated with field disease scores from both years but AUDPC values for one year. IF was least correlated with the field data, and only showed significant but weak correlation with AUDPC values from 2011. All components measured during DLA were significantly correlated to each other. Six

components had their weakest correlation coefficients with IF but percent disease area had its highest correlation with IF. It was interesting to observe that the new component, IP (1mm), showed high (r > 0.68-0.74) correlation coefficients with both IP and LD. Evaluation of large populations for components of disease resistance can be time and labor intensive. LAp had high (r > 0.5) correlation coefficients with all the other components measured during DLA. The number of components to be recorded may be reduced by omitting the ones that provide nearly redundant information as another component. Both LP (2X) and LP (16X) had similar values for correlation coefficients with the other components. Since, recording latent period under 2X magnification is less cumbersome and significant for all the field disease severity and progress values for both years, use of that magnification is much more efficient than determining LP at 16X. SI and SPp also showed nearly similar correlation coefficients with the other components. Choice between SI and SPp would depend on the need for objectivity versus labor and time limitations. While SI measurement requires less time, SPp is more objective and quantitative. SPp, SI, LP (2X) and LP (16X) also showed significant correlations with the attributes measured in second lateral stem assay.

Percent defoliation and days until defoliation have been used as components of resistance in the past (Melouk and Banks, 1978; Ricker *et al.*, 1985; Subrahmanyam *et al.*, 1982). However, in the present study, evaluations were terminated before defoliation occurred, to prevent biased evaluations of SPp and IF (Ricker *et al.*, 1985).

Although each of the components recorded with different phenotyping methods conveys relevant information about the disease, it is not possible to measure all components in large populations. According to results from various statistical analyses,

ease and efficiency of measurement, feasibility and objectivity, the following order of priority is proposed: SPp or SI, LP (2X) or LP (16X), IP (1mm), LAp, LD, IP, and IF for DLA; LAp, IF, and DEF for LSA.

Among the three methods of phenotyping, both the Florida 1-10 scale and detached leaf assay were mostly able to differentiate among RILs segregating for LLS resistance. While the Florida 1-10 scale is easier to use in large populations and provides sufficient information on disease severity and defoliation, detached leaf assay is tedious to carry out but it provides exhaustive information on components of disease resistance and helps to dissect the basis of resistance for each segregating line in the population. For example, it was observed that in a few lines (CB75A, CB62B, CB52B, CB49A), LLS lesions appeared quite early in the experiment (low IP), but these lesions exhibited delayed sporulation (high LP). Therefore, the difference between LP and IP for these lines is high. A possible explanation for such observations could be that resistance to LLS in these lines is achieved *via* arrested sporulation, such that the lines were susceptible to CP infection but have an underlying mechanism to prevent secondary inoculum production. It is difficult to detect such subtleties in field scoring methods as they are based on the overall disease response of a genotype and do not provide any information on how different components contribute to produce that response.

The present study provides detailed comparisons of available leaf spot phenotyping methods in peanut. To the best of our knowledge, this is the first report of integrating electronic imaging and disease analysis in a peanut mapping population. In addition, two new components of disease resistance including, IP (1mm) and LP-IP are being proposed. As already discussed, choice of a phenotyping method would vary

depending upon objectives of the study, number of genotypes to be evaluated, and availability of resources (skill, labor, time). The components to be evaluated can be chosen based on the proposed priority order above.

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Table 3.1 List of C1501 RIL population late leaf spot (LLS) phenotyping components and their corresponding transformation functions.

Method/Assay	Rating/Component	Transformation function
Florida (1-10)scale 2011	FSRI[a]	*ln* (x)
	FSRII[b]	*ln*(x)
	FSRIII[c]	none
	FSRIV[d]	*ln*(x)
	AUDPC[e]	*ln*(x)
Florida (1-10)scale 2012	FSRI[f]	*ln*(x)
	FSRII[g]	*ln*(x)
	FSRIII[h]	*ln*(x)
	FSRIV[i]	none
	AUDPC[j]	*ln*(x)
Lateral stem assay LSAI	Diseased leaf area (%)	*ln*(x)
	Infection frequency	*ln*(x)
	Defoliation (%)	*sq. root*(x)
Lateral stem assay LSAII	Diseased leaf area (%)	*ln*(x)
	Infection frequency	*sq. root*(x)
	Defoliation (%)	*ln*(x)
Detached leaf assay	Diseased leaf area (%)	*sq. root*(x)
	Infection frequency	*sq. root*(x)
	Incubation period	*ln*(x)
	Latent period (2X)	*ln*(x)
	Latent period (16X)	*ln*(x)
	Sporulation index	none
	Sporulation (%)	[(*arcsine* (*sq. root* x)]^2
	Lesion diameter (mm)	none
	DAI until lesion diameter=1mm	*ln*(x)

Table 3.2 Late leaf spot phenotyping data on Gregory, Tifguard, and the C1501 RIL population.

Phenotyping Method	Trait†	Year	Mean				Variation in RILs	σ^2
			Gregory (old)	Gregory*	Tifguard	RILs		
Florida (1-10) scale	FSRI (82 DAP)	2011	1.08	.	1.25	1.29	1.00-3.17	0.10
	FSRII (110 DAP)	2011	1.67	.	1.33	2.31	1.33-3.83	2.31
	FSRIII (130 DAP)	2011	4.58	.	2.67	4.78	3.50-6.83	0.43
	FSRIV (141 DAP)	2011	5.67	.	4.25	6.17	4.75-8.33	0.91
	stAUDPC	2011	2.61	.	1.89	3.01	2.24-4.17	0.21
	FSRI (111 DAP)	2012	2.67	3.58	1.58	2.66	1.67-4.50	0.28
	FSRII (121 DAP)	2012	4.33	5.75	3.50	4.42	3.00-6.17	0.44
	FSRIII (132 DAP)	2012	5.58	7.50	4.17	5.56	4.00-8.83	0.59
	FSRIV (141 DAP)	2012	8.58	9.67	6.33	8.17	5.50-9.92	1.00
	stAUDPC	2012	5.11	6.56	3.83	5.07	3.51-6.94	0.47
Lateral Stem Assay I	Lesion area (%)(LAp)	2011	0.40	.	0.26	1.17	0.23-5.07	0.77
	Infection frequency (IF)(number of lesions/cm2)	2011	0.18	.	0.16	0.58	0.12-6.22	0.55
	Defoliation (%) (DEF)	2011	24.07	.	17.68	18.47	5.01-38.29	32.00
Lateral Stem Assay II	LAp	2011	2.10	.	0.76	3.03	0.72-9.48	3.27
	IF	2011	0.92	.	0.33	1.36	0.32-4.26	0.66
	DEF	2011	34.05	.	31.66	41.00	22.38-71.34	144.58
Detached Leaf Assay	Sporulation index (SI)	2012	3.22	3.67	1.11	2.98	0.89-4.00	0.76
	IF	2012	3.66	2.62	1.65	2.49	0.91-4.34	0.52
	Incubation period (IP)(days)	2012	9.11	10.67	12.67	10.25	8.44-12.33	0.85

Latent period (LP2X)(days)	2012	20.44	17.56	25.67	19.47	14.00-25.50	6.40
Latent period (LP16X)(days)	2012	15.56	15.33	20.33	17.33	14.22-23.00	4.25
LP (2X)-IP	2012	11.33	7.72	15.22	9.21	1.89-14.89	4.87
IP (1mm)	2012	14.22	14.11	20.22	15.74	12.55-20.77	2.93
Sporulation (%)	2012	91.38	97.31	42.90	79.87	19.91-100	335.13
Lesion diameter (LD) (mm)	2012	0.24	0.27	0.20	0.24	0.10-0.29	0.00
LAp	2012	10.17	9.65	2.74	7.05	2.10-12.90	9.13

†First Florida scale rating (FSRI) was not included for both years (2011 and 2012) because disease scoring early in the season tends to be confounded by presence of irregular leaf spot (ILS)

*alternate seed source for Gregory ("Gregory-new" for field phenotyping data and "Gregory-certified" for growth chamber phenotyping data).

Table 3.3 Effect of peanut genotype, year and their two-way interaction, on late leaf spot disease progress and disease severity in the C1501 RIL population, 2011-2012.

Source	FSRI [a*]	FSRII [b†]	FSRIII [c]	FSRIV [d†]	AUDPC [e†]	stAUDPC [f†]
Genotype (G)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Year (Y)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
(Y)*(G)	<.0001	0.7638	0.6263	0.5361	0.8212	0.8208

[a] *p* – values for first Florida scale ratings (FSRI), taken at 82 days after planting (DAP) and 111 DAP in 2011 and 2012 respectively. From analysis of untransformed data, ANOVA conditions could not be satisfied.
[b] *p* – values for second Florida scale ratings (FSRII), taken at 110 DAP and 121 DAP in 2011 and 2012 respectively.
[c] *p* – values for third Florida scale ratings (FSRIII), taken at 130 DAP and 132 DAP in 2011 and 2012 respectively. From analysis of untransformed data, ANOVA conditions satisfied.
[d] *p* – values for fourth Florida scale ratings (FSRIV), taken at 141 DAP and 141 DAP in 2011 and 2012 respectively.
[e] *p* – values for area under disease progress curve (AUDPC) in 2011 and 2012.
[f] *p* – values for standardized (stAUDPC),calculated by dividing AUDPC values by the number of days from the first observed symptoms till harvest to account for differences in the duration of late leaf spot epidemics.
† From analysis of log transformed data.

Table 3.4 Comparison of the effect of year on late leaf spot disease progress and disease severity in the C1501 RIL population, 2011-2012.

	Duncan grouping [i]					
Year	FSRI [c]	FSRII [d]	FSRIII [e]	FSRIV [f]	AUDPC [g]	stAUDPC [h]
2011	1.29 a	2.28 a	4.74 a	6.13 a	177 b	2.99 a
2012	2.65 b	4.42 b	5.57 b	8.16 b	152 a	5.07 b

[c] Untransformed means for first Florida scale ratings (FSRI), taken at 82 days after planting (DAP) and 111 DAP in 2011 and 2012 respectively.
[d] Untransformed means for second Florida scale ratings (FSRII), taken at 110 DAP and 121 DAP in 2011 and 2012 respectively.
[e] Untransformed means for third Florida scale ratings (FSRIII), taken at 130 DAP and 132 DAP in 2011 and 2012 respectively.
[f] Untransformed means for fourth Florida scale ratings (FSRIV), taken at 141 DAP and 141 DAP in 2011 and 2012 respectively.
[g] Untransformed means for area under disease progress curve (AUDPC) in 2011 and 2012.
[h] Untransformed means for standardized (stAUDPC),calculated by dividing AUDPC values by the number of days from the first observed symptoms till harvest to account for differences in the duration of late leaf spot epidemics.
[i] Means within a column with different letters differ at P=0.05 as determined by analyses of variance (ANOVA) and Duncan's multiple range test procedures on log transformed data in FSRII, FSRIV, AUDPC, stAUDPC and untransformed data in FSRI and FSRIII. While FSRIII did not need transformation, appropriate transformation for FSRI could not be found to satisfy ANOVA conditions.

Table 3.5 Analysis of variance for field disease severity readings on Florida 1-10 scale, taken on the C1501 RIL population during 2011 and 2012 growing seasons.

	2011[a]	2012[b]
FSRI	<.0001 [d]	<.0001 [c]
FSRII	0.0079 [c]	<.0001 [c]
FSRIII	0.1311 [e]	<.0001 [c]
FSRIV	<.0001 [c]	<.0001 [d]
AUDPC	0.0204 [c]	<.0001 [c]
stAUDPC	0.0206 [c]	<.0001 [c]

[a] *p* – values for Florida scale ratings taken at 82 DAP (FSRI), 110 DAP (FSRII), 130 DAP (FSRIII), 141 DAP (FSRIV), area under disease progress values (AUDPC) and disease progress values standardized over duration of epidemic (stAUDPC) in 2011.
[b] *p* – values for Florida scale ratings taken at 111 DAP (FSRI), 121 DAP (FSRII), 132 DAP (FSRIII), 141 DAP (FSRIV), area under disease progress values (AUDPC) and disease progress values standardized over duration of epidemic (stAUDPC) in 2012.
[c] From analysis of log transformed data.
[d] From analysis of untransformed data, ANOVA conditions could not be satisfied.
[e] From analysis of untransformed data, ANOVA conditions satisfied.

Table 3.6 Mean FSRI values and Duncan grouping for the RIL C1501 population, 2011 field trial.

Genotype	FSRI [a]	Duncan grouping [b]					
CB01B	1.12		I			J	
CB02B	1.42	F	I	E	G	J	H
CB03B	1.03					J	
CB04A	1.25		I		G	J	H
CB05A	1.67	F	C	E	B	D	
CB06B	1.37	F	I	E	G	J	H
CB07B	1.12		I			J	
CB08A	1.08		I			J	
CB09A	1.58	F	C	E	G	D	H
CB10B	1.12		I			J	
CB11A	1.03					J	
CB12B	1					J	
CB13B	1.5	F	I	E	G	D	H
CB14B	1.2		I			J	H
CB15A	1.28	F	I		G	J	H
CB16B	1.67	F	C	E	B	D	
CB17A	1.58	F	C	E	G	D	H
CB18B	1.15		I			J	
CB19B	1.42	F	I	E	G	J	H
CB20A	1.12		I			J	
CB21B	1.33	F	I		G	J	H
CB22B	1.17		I			J	
CB23A	1.03					J	
CB24B	1.37	F	I	E	G	J	H
CB25B	1.28	F	I		G	J	H
CB26A	1.17		I			J	
CB27A	1.42	F	I	E	G	J	H
CB28A	1.03					J	
CB29B	1.25		I		G	J	H
CB30B	1.17		I			J	
CB31B	1.25		I		G	J	H
CB32A	1.42	F	I	E	G	J	H
CB33A	2				B		
CB34B	1					J	
CB35A	1					J	
CB36A	1.03					J	
CB37A	1.17		I			J	

CB38B	1.17		I			J	
CB39A	1.17		I			J	
CB40A	1.28	F	I		G	J	H
CB41A	1					J	
CB42A	1.08		I			J	
CB43B	1.33	F	I		G	J	H
CB44B	1					J	
CB45A	1.08		I			J	
CB46B	1.2		I			J	H
CB47A	1.25		I		G	J	H
CB48B	1.2		I			J	H
CB49A	1.92		C		B		
CB50B	1.62	F	C	E	G	D	
CB51A	1.17		I			J	
CB52B	1.37	F	I	E	G	J	H
CB53B	1.17		I			J	
CB54B	1.75		C	E	B	D	
CB55A	1.15		I			J	
CB56A	1					J	
CB57B	3.17				A		
CB58B	1.75		C	E	B	D	
CB59B	1.03					J	
CB60A	1.12		I			J	
CB61B	1.25		I		G	J	H
CB62B	1.58	F	C	E	G	D	H
CB63A	1.08		I			J	
CB64B	1.28	F	I		G	J	H
CB65B	1.12		I			J	
CB66A	1.2		I			J	H
CB67A	1.5	F	I	E	G	D	H
CB68A	1.33	F	I		G	J	H
CB69A	1.17		I			J	
CB70B	1.08		I			J	
CB71A	1.37	F	I	E	G	J	H
CB72B	1.83		C		B	D	
CB73A	1.2		I			J	H
CB74A	1.25		I		G	J	H
CB75A	1.83		C		B	D	
CB76B	1.08		I			J	
CB77A	1					J	
CB78A	1.03					J	

G-OLD	1.08	I		J		
TIFGU	1.25	I	G	J		H

[a] Means from untransformed data for first Florida scale rating, 82 days after planting, 2011.

[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on untransformed data.

Table 3.7 Mean FSRII values and Duncan grouping for the RIL C1501 population, 2011 field trial.

Genotype	FSRII	Duncan grouping
CB01B	2.5	E B D A G C F
CB02B	2	E B D A G C F
CB03B	2.08	E B D A G C F
CB04A	3.75	B A
CB05A	3.17	B D A C
CB06B	1.67	E B D G C F
CB07B	2.58	E B D A G C F
CB08A	2.67	E B D A G C F
CB09A	2.58	E B D A G C F
CB10B	1.83	E B D A G C F
CB11A	1.42	G F
CB12B	2.67	E B D A G C F
CB13B	2	E B D A G C F
CB14B	3.08	B D A C
CB15A	3.33	B A
CB16B	3.25	B D A C
CB17A	1.83	E B D A G C F
CB18B	2	E B D A G C F
CB19B	1.55	E D G F
CB20A	2.25	E B D A G C F
CB21B	2	E B D A G C F
CB22B	2.08	E B D A G C F
CB23A	3.33	B A C
CB24B	2.17	E B D A G C F
CB25B	3.58	A
CB26A	2.25	E B D A G C F
CB27A	2.33	E B D A G C F
CB28A	2.38	E B D A G C F
CB29B	2	E B D A G C F
CB30B	1.88	E B D A G C F
CB31B	3.83	A
CB32A	1.75	E B D G C F
CB33A	2	E B D A G C F
CB34B	2.75	E B D A G C F
CB35A	1.47	G F

CB36A	1.83	E	B	D	A	G	C	F
CB37A	1.5	E				G		F
CB38B	2.33	E	B	D	A	G	C	F
CB39A	1.83	E	B	D	A	G	C	F
CB40A	2.92	E	B	D	A		C	F
CB41A	2.75	E	B	D	A		C	F
CB42A	2.08	E	B	D	A	G	C	F
CB43B	2.08	E	B	D	A	G	C	F
CB44B	2.25	E	B	D	A	G	C	F
CB45A	3	E	B	D	A		C	
CB46B	2	E	B	D	A	G	C	F
CB47A	2	E	B	D	A	G	C	F
CB48B	2.38	E	B	D	A	G	C	F
CB49A	1.58	E		D		G	C	F
CB50B	1.83	E	B	D	A	G	C	F
CB51A	2.67	E	B	D	A	G	C	F
CB52B	2.58	E	B	D	A	G	C	F
CB53B	3.5		B		A		C	
CB54B	1.92	E	B	D	A	G	C	F
CB55A	3.5		B		A			
CB56A	1.92	E	B	D	A	G	C	F
CB57B	2.5	E	B	D	A	G	C	F
CB58B	1.58	E		D		G	C	F
CB59B	2.67	E	B	D	A	G	C	F
CB60A	3.42		B		A			
CB61B	2.67	E	B	D	A	G	C	F
CB62B	1.97	E	B	D	A	G	C	F
CB63A	1.75	E	B	D		G	C	F
CB64B	1.92	E	B	D	A	G	C	F
CB65B	2.42	E	B	D	A	G	C	F
CB66A	2.58	E	B	D	A	G	C	F
CB67A	2.08	E	B	D	A	G	C	F
CB68A	2.92	E	B	D	A	G	C	F
CB69A	1.33					G		
CB70B	2.5	E	B	D	A	G	C	F
CB71A	2.17	E	B	D	A	G	C	F
CB72B	1.83	E	B	D	A	G	C	F
CB73A	1.92	E	B	D	A	G	C	F
CB74A	2.5	E	B	D	A	G	C	F

CB75A	2.08	E	B	D	A	G	C	F
CB76B	1.92	E	B	D	A	G	C	F
CB77A	1.33					G		
CB78A	1.58	E		D		G		F
G-OLD	1.67	E	B	D		G	C	F
TIFGU	1.33					G		

[a] Means from untransformed data for second Florida scale rating, 110 days after planting, 2011. [b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.8 Mean FSRIV values and Duncan grouping for the RIL C1501 population, 2011 field trial.

Genotype	FSRIV [a]	Duncan grouping [b]
CB01B	7	E B D A G C F
CB02B	6.33	E B D H A G C F
CB03B	5.33	E I D H G F
CB04A	7.92	B A C
CB05A	7.33	E B D A C
CB06B	6	E B I D H A G C F
CB07B	6.25	E B I D H A G C F
CB08A	7.25	E B D A C F
CB09A	7.92	B A
CB10B	5.83	E B I D H A G C F
CB11A	4.92	I H G F
CB12B	6.58	E B D H A G C F
CB13B	5.67	E B I D H A G C F
CB14B	7.58	B D A C
CB15A	8.33	A
CB16B	6.75	E B D H A G C F
CB17A	5.25	E I D H G F
CB18B	5.67	E B I D H G C F
CB19B	4.75	I H
CB20A	6.08	E B I D H A G C F
CB21B	4.83	I H G
CB22B	6.17	E B I D H A G C F
CB23A	8.33	A
CB24B	6.08	E B I D H A G C F
CB25B	7.33	E B D A C
CB26A	5.42	E I D H G F
CB27A	5.92	E B I D H A G C F
CB28A	5.58	E I D H G C F
CB29B	5.58	E I D H G F
CB30B	5.42	E I D H G F
CB31B	7.67	B D A C
CB32A	5.83	E B I D H A G C F
CB33A	5	E I H G F
CB34B	6.92	E B D H A G C F
CB35A	4.83	I H G
CB36A	6.25	E B I D H A G C F

CB37A	4.75				I	H		G		
CB38B	6.42		E		B	D	H	A	G	C F
CB39A	5.83		E		B	I	D	H	A	G C F
CB40A	7.17		E		B	D		A		C F
CB41A	7.92				B			A		
CB42A	6.08		E		B	I	D	H	A	G C F
CB43B	6.33		E		B	D	H	A	G	C F
CB44B	6.5		E		B	D	H	A	G	C F
CB45A	7.08		E		B	D		A	G	C F
CB46B	5.5		E		B	I	D	H		G C F
CB47A	5.25		E			I	D	H		G F
CB48B	5.92		E		B	I	D	H	A	G C F
CB49A	5		E			I	H		G	F
CB50B	5.08		E			I	H		G	F
CB51A	6		E		B	I	D	H	A	G C F
CB52B	5.83		E		B	I	D	H	A	G C F
CB53B	8.08				B			A		C
CB54B	5.67		E		B	I	D	H		G C F
CB55A	7.67				B	D		A		C
CB56A	6.58		E		B	D	H	A	G	C F
CB57B	4.92					I	H		G	F
CB58B	5.33		E			I	D	H		G F
CB59B	6.92		E		B	D	H	A	G	C F
CB60A	7.33		E		B	D		A		C
CB61B	6.58		E		B	D	H	A	G	C F
CB62B	5.08		E			I	H		G	F
CB63A	5.25		E			I	D	H		G F
CB64B	6.08		E		B	I	D	H	A	G C F
CB65B	6.17		E		B	I	D	H	A	G C F
CB66A	6.83		E		B	D	H	A	G	C F
CB67A	5.42		E		B	I	D	H		G C F
CB68A	7.17		E		B	D		A		C F
CB69A	5.42		E			I	D	H		G F
CB70B	6.75		E		B	D	H	A	G	C F
CB71A	6.25		E		B	I	D	H	A	G C F
CB72B	6.08		E		B	I	D	H	A	G C F
CB73A	5.83		E		B	I	D	H	A	G C F
CB74A	6.25		E		B	I	D	H	A	G C F
CB75A	4.75					I	H			

CB76B	5.92	E	B	I	D	H	A	G	C	F
CB77A	4.83			I		H		G		F
CB78A	5.25	E		I	D	H		G		F
G-OLD	5.67	E	B	I	D	H	A	G	C	F
TIFGU	4.25			I						

[a] Means from untransformed data for fourth Florida scale rating,141 days after planting, 2011.

[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.9 Mean AUDPC values and Duncan grouping for the RIL C1501 population, 2011 field trial.

Genotype	AUDPC [a]	Duncan grouping [b]
CB01B	187.33	E B D A G C F
CB02B	172.33	E B D H A G C F
CB03B	161.67	E B D H A G C F
CB04A	231.67	B D A C
CB05A	223	B A C
CB06B	163.67	E B D H A G C F
CB07B	191.33	E B D A G C F
CB08A	195.33	E B D A G C F
CB09A	213.33	E B D A C F
CB10B	159.67	E B D H A G C F
CB11A	131.67	H G
CB12B	188	E B D A G C F
CB13B	158.67	E B D H A G C F
CB14B	221	B A C
CB15A	221.67	B A C
CB16B	226.33	B A C
CB17A	156	E B D H A G C F
CB18B	159	E B D H A G C F
CB19B	134.33	H G
CB20A	169.33	E B D H A G C F
CB21B	144.67	E D H G C F
CB22B	161.33	E B D H A G C F
CB23A	223.33	B A C
CB24B	174.33	E B D H A G C F
CB25B	229	B A
CB26A	166.67	E B D H A G C F
CB27A	182.33	E B D A G C F
CB28A	165	E B D H A G C F
CB29B	169	E B D H A G C F
CB30B	155.67	E B D H A G C F
CB31B	236.33	B A
CB32A	158.67	E B D H A G C F
CB33A	167	E B D H A G C F
CB34B	188	E B D A G C F
CB35A	139	E D H G F

CB36A	164	E	B	D	H	A	G	C	F
CB37A	137.33	E			H		G		F
CB38B	173.67	E	B	D	H	A	G	C	F
CB39A	157.33	E	B	D	H	A	G	C	F
CB40A	215	E	B	D		A		C	F
CB41A	205.67	E	B	D		A	G	C	F
CB42A	169	E	B	D	H	A	G	C	F
CB43B	176	E	B	D	H	A	G	C	F
CB44B	180.33	E	B	D		A	G	C	F
CB45A	208.67	E	B	D		A	G	C	F
CB46B	162.67	E	B	D	H	A	G	C	F
CB47A	163.67	E	B	D	H	A	G	C	F
CB48B	174	E	B	D	H	A	G	C	F
CB49A	153.67	E	B	D	H	A	G	C	F
CB50B	157.33	E	B	D	H	A	G	C	F
CB51A	184.67	E	B	D		A	G	C	F
CB52B	183	E	B	D		A	G	C	F
CB53B	246.33					A			
CB54B	175.67	E	B	D	H	A	G	C	F
CB55A	229.67		B			A		C	
CB56A	166	E	B	D	H	A	G	C	F
CB57B	193.33	E	B	D		A	G	C	F
CB58B	150.33	E	B	D	H	A	G	C	F
CB59B	195.33	E	B	D		A	G	C	F
CB60A	217.67	E	B	D		A		C	
CB61B	181	E	B	D		A	G	C	F
CB62B	151	E	B	D	H		G	C	F
CB63A	141	E		D	H		G	C	F
CB64B	155.67	E	B	D	H	A	G	C	F
CB65B	173.67	E	B	D	H	A	G	C	F
CB66A	187.33	E	B	D		A	G	C	F
CB67A	162	E	B	D	H	A	G	C	F
CB68A	201.33	E	B	D		A	G	C	F
CB69A	138.33				H		G		F
CB70B	187	E	B	D		A	G	C	F
CB71A	180.33	E	B	D		A	G	C	F
CB72B	181	E	B	D		A	G	C	F
CB73A	167.33	E	B	D	H	A	G	C	F
CB74A	188.33	E	B	D		A	G	C	F

CB75A	161.67	E	B	D	H	A	G	C	F
CB76B	162.67	E	B	D	H	A	G	C	F
CB77A	135.67				H		G		F
CB78A	149.33	E	B	D	H	A	G	C	F
G-OLD	154	E	B	D	H	A	G	C	F
TIFGU	111.67				H				

[a] Means from untransformed data for area under disease progress curve (AUDPC) values, 2011. [b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.10 Mean stAUDPC values and Duncan grouping for the RIL C1501 population, 2011 field trial.

Genotype	stAUDPC [a]	Duncan grouping [b]
CB01B	3.18	E B D A C F
CB02B	2.92	E B D A G C F
CB03B	2.74	E B D A G C F
CB04A	3.93	B A C
CB05A	3.78	B A C
CB06B	2.77	E B D A G C F
CB07B	3.24	E B D A C F
CB08A	3.3	E B D A C F
CB09A	3.62	E B D A C
CB10B	2.7	E B D A G C F
CB11A	2.24	G F
CB12B	3.19	E B D A C F
CB13B	2.69	E B D A G C F
CB14B	3.75	B A C
CB15A	3.76	B A C
CB16B	3.83	B A C
CB17A	2.65	E B D A G C F
CB18B	2.7	E B D A G C F
CB19B	2.28	G F
CB20A	2.87	E B D A G C F
CB21B	2.45	E D G C F
CB22B	2.73	E B D A G C F
CB23A	3.79	B A C
CB24B	2.96	E B D A G C F
CB25B	3.88	B A
CB26A	2.82	E B D A G C F
CB27A	3.09	E B D A C F
CB28A	2.8	E B D A G C F
CB29B	2.86	E B D A G C F
CB30B	2.63	E B D A G C F
CB31B	4.01	B A
CB32A	2.69	E B D A G C F
CB33A	2.83	E B D A G C F
CB34B	3.18	E B D A C F
CB35A	2.35	E D G F

CB36A	2.78		E	B	D	A	G	C	F
CB37A	2.33		E		D		G		F
CB38B	2.94		E	B	D	A	G	C	F
CB39A	2.67		E	B	D	A	G	C	F
CB40A	3.64		E	B	D	A		C	
CB41A	3.49		E	B	D	A		C	F
CB42A	2.86		E	B	D	A	G	C	F
CB43B	2.98		E	B	D	A	G	C	F
CB44B	3.05		E	B	D	A		C	F
CB45A	3.54		E	B	D	A		C	F
CB46B	2.76		E	B	D	A	G	C	F
CB47A	2.77		E	B	D	A	G	C	F
CB48B	2.95		E	B	D	A	G	C	F
CB49A	2.6		E	B	D	A	G	C	F
CB50B	2.66		E	B	D	A	G	C	F
CB51A	3.13		E	B	D	A		C	F
CB52B	3.11		E	B	D	A		C	F
CB53B	4.17					A			
CB54B	2.97		E	B	D	A	G	C	F
CB55A	3.89			B		A		C	
CB56A	2.81		E	B	D	A	G	C	F
CB57B	3.27		E	B	D	A		C	F
CB58B	2.55		E	B	D	A	G	C	F
CB59B	3.31		E	B	D	A		C	F
CB60A	3.69			B	D	A		C	
CB61B	3.07		E	B	D	A		C	F
CB62B	2.56		E	B	D		G	C	F
CB63A	2.39		E		D		G	C	F
CB64B	2.64		E	B	D	A	G	C	F
CB65B	2.94		E	B	D	A	G	C	F
CB66A	3.17		E	B	D	A		C	F
CB67A	2.74		E	B	D	A	G	C	F
CB68A	3.41		E	B	D	A		C	F
CB69A	2.34		E				G		F
CB70B	3.17		E	B	D	A		C	F
CB71A	3.06		E	B	D	A		C	F
CB72B	3.06		E	B	D	A		C	F
CB73A	2.84		E	B	D	A	G	C	F
CB74A	3.19		E	B	D	A		C	F

CB75A	2.74	E	B D A G C F
CB76B	2.75	E	B D A G C F
CB77A	2.3	E	G F
CB78A	2.53	E	B D A G C F
G-OLD	2.61	E	B D A G C F
TIFGU	1.89		G

[a] Means from untransformed data for standardized area under disease progress curve (stAUDPC) values, calculated by dividing AUDPC values by the number of days from the first observed symptoms till harvest , the duration of late leaf spot epidemic in 2011.
[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.11 Mean FSRI values and Duncan grouping for the RIL C1501 population, 2012 field trial.

Genotype	FSRI [a]	Duncan grouping [b]												
CB01B	3	K	E	J	B	I	D	H	A	G	C		F	
CB02B	2.17	K		J	N	I		H	L	G		O		M
CB03B	3.17		E		B		D	H	A	G	C		F	
CB04A	4.5								A					
CB05A	2.83	K	E	J	B	I	D	H	L	G	C		F	M
CB06B	2.42	K	E	J	N	I	D	H	L	G		O	F	M
CB07B	2.75	K	E	J	B	I	D	H	L	G	C		F	M
CB08A	3.25		E		B		D	H	A	G	C		F	
CB09A	1.92				N							O		M
CB10B	2.58	K	E	J		I	D	H	L	G	C		F	M
CB11A	2.42	K	E	J	N	I	D	H	L	G		O	F	M
CB12B	2.58	K	E	J		I	D	H	L	G	C		F	M
CB13B	2.67	K	E	J	B	I	D	H	L	G	C		F	M
CB14B	3.33		E		B		D		A		C		F	
CB15A	3.5				B		D		A		C			
CB16B	2.75	K	E	J	B	I	D	H	L	G	C		F	M
CB17A	1.92				N				L			O		M
CB18B	2.75	K	E	J	B	I	D	H	L	G	C		F	M
CB19B	2.08	K		J	N				L			O		M
CB20A	2.58	K	E	J		I	D	H	L	G	C		F	M
CB21B	2.17	K		J	N	I		H	L			O		M
CB22B	3	K	E	J	B	I	D	H	A	G	C		F	
CB23A	2.75	K	E	J	B	I	D	H	L	G	C		F	M
CB24B	2.5	K	E	J		I	D	H	L	G	C		F	M
CB25B	3.25		E		B		D		A	G	C		F	
CB26A	3.08		E	J	B	I	D	H	A	G	C		F	
CB27A	2	K			N				L			O		M
CB28A	3.25		E		B		D		A	G	C		F	
CB29B	2.42	K	E	J	N	I	D	H	L	G	C	O	F	M
CB30B	2.5	K	E	J	N	I	D	H	L	G	C		F	M
CB31B	3.58				B				A		C			
CB32A	2.08	K		J	N				L			O		M
CB33A	2.67	K	E	J	B	I	D	H	L	G	C		F	M
CB34B	3.17		E		B	I	D	H	A	G	C		F	
CB35A	2.08	K		J	N				L			O		M

CB36A	3.25			E		B		D	H	A	G	C		F		
CB37A	1.83					N							O		M	
CB38B	3.5					B		D		A		C				
CB39A	2.33	K	E	J	N	I	D	H	L	G	C	O	F	M		
CB40A	2.92	K	E	J	B	I	D	H	L	G	C		F			
CB41A	3.92				B				A							
CB42A	2.75	K	E	J	B	I	D	H	L	G	C		F	M		
CB43B	3		E	J	B	I	D	H	A	G	C		F			
CB44B	3.08		E	J	B	I	D	H	A	G	C		F			
CB45A	2.33	K	E	J	N	I	D	H	L	G		O	F	M		
CB46B	2.5	K	E	J	N	I	D	H	L	G	C		F	M		
CB47A	2.25	K	E	J	N	I		H	L	G		O	F	M		
CB48B	2.67	K	E	J	B	I	D	H	L	G	C		F	M		
CB49A	2.08	K		J	N	I			L			O		M		
CB50B	2.08	K		J	N	I			L			O		M		
CB51A	3.33		E		B		D		A		C		F			
CB52B	2.08	K		J	N	I			L			O		M		
CB53B	2.75	K	E	J	B	I	D	H	L	G	C		F	M		
CB54B	2.08	K		J	N	I			L			O		M		
CB55A	3.17		E		B	I	D	H	A	G	C		F			
CB56A	3.33		E		B		D		A		C					
CB57B	2.08	K		J	N				L			O		M		
CB58B	1.92				N							O		M		
CB59B	2.92	K	E	J	B	I	D	H	L	G	C		F			
CB60A	3.17		E		B	I	D	H	A	G	C		F			
CB61B	2.42	K	E	J	N	I	D	H	L	G	C	O	F	M		
CB62B	2.58	K	E	J		I	D	H	L	G	C		F	M		
CB63A	1.67				N							O				
CB64B	2.25	K		J	N	I		H	L	G		O	F	M		
CB65B	2.58	K	E	J		I	D	H	L	G	C		F	M		
CB66A	3.08	K	E	J	B	I	D	H		G	C		F			
CB67A	2.5	K	E	J	N	I	D	H	L	G	C		F	M		
CB68A	2.75	K	E	J	B	I	D	H	L	G	C		F	M		
CB69A	2.25	K	E	J	N	I		H	L	G		O	F	M		
CB70B	3.17		E		B	I	D	H	A	G	C		F			
CB71A	2.33	K	E	J	N	I		H	L	G		O	F	M		
CB72B	1.92				N							O		M		
CB73A	2.67	K	E	J	B	I	D	H	L	G	C		F	M		
CB74A	2.42	K	E	J	N	I	D	H	L	G	C		F	M		

CB75A	2.25	K	E	J	N	I		H	L	G		O	F	M
CB76B	3		E	J	B	I	D	H	A	G	C		F	
CB77A	2.42	K	E	J	N	I	D	H	L	G	C		F	M
CB78A	2.33	K	E	J	N	I	D	H	L	G		O	F	M
G-NEW	3.58				B		D		A		C			
G-OLD	2.67	K	E	J		I	D	H	L	G	C		F	M
TIFGU	1.58											O		

[a] Means from untransformed data for first Florida scale rating, 111 days after planting, 2012. [b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.12 Mean FSRII values and Duncan grouping for the RIL C1501 population, 2012 field trial.

Genotype	FSRII [a]	Duncan grouping [b]
CB01B	4.75	K E J B I D H P Q O G N C M F L
CB02B	3.67	W S T V R Q U
CB03B	5.08	E B D H A G C F
CB04A	6.17	A
CB05A	4.92	K E J B I D H A G C F L
CB06B	4.83	K E J B I D H A O G N C M F L
CB07B	4.42	K E J S I T D R H P Q O G N U M F L
CB08A	5	E B I D H A G C F
CB09A	3.75	W S T V R P Q O U
CB10B	4.17	K J S I T V R H P Q O G N U M F L
CB11A	4.25	K J S I T V R H P Q O G N U M F L
CB12B	4.5	K E J B I D R H P Q O G N C M F L
CB13B	3.92	K J S I T V R P Q O N U M L
CB14B	5.58	B D A C
CB15A	5.5	E B D A C
CB16B	3.83	W S T V R P Q O N U
CB17A	3.67	W S T V R Q U
CB18B	4.5	K E J S I T D R H P Q O G N C M F L
CB19B	3.33	W V
CB20A	4	K J S I T V R H P Q O N U M L
CB21B	4.08	K J S I T V R H P Q O G N U M L
CB22B	4.67	K E J B I D H P Q O G N C M F L
CB23A	4.67	K E J B I D H P Q O G N C M F L
CB24B	3.92	K J S T V R P Q O N U M L
CB25B	5.08	E B D H A G C F
CB26A	4.83	K E J B I D H A G C M F L
CB27A	3.67	W S T V R Q U
CB28A	5.5	E B D A C
CB29B	3.75	W S T V R P Q U
CB30B	4.08	K J S I T V R H P Q O G N U M F L
CB31B	5.58	B D A C
CB32A	3.58	W S T V R U
CB33A	4.08	K J S I T V R H P Q O G N U M L
CB34B	5	E B I D H A G C F
CB35A	3.75	W S T V R P Q O N U

CB36A	5		E	J	B	I		D		H		A		G		C		F			
CB37A	3.42		W					V								U					
CB38B	5		E		B	I		D		H		A		G		C		F			
CB39A	3.92	K		J	S	I	T	V	R		P	Q	O		N	U	M		L		
CB40A	5		E		B	I		D		H		A		G		C		F			
CB41A	5.75				B							A									
CB42A	4.5	K	E	J	B	I		D	R	H	P	Q	O	G	N	C	M	F	L		
CB43B	4.83	K	E	J	B	I		D		H		A		G		C	M	F	L		
CB44B	5.5		E		B			D				A				C					
CB45A	4.17	K		J	S	I	T	V	R	H	P	Q	O	G	N	U	M	F	L		
CB46B	3.83		W		S		T	V	R		P	Q	O		N	U	M				
CB47A	3.92	K		J	S	I	T	V	R		P	Q	O		N	U	M		L		
CB48B	4.75	K	E	J	B	I		D		H	P	A	O	G	N	C	M	F	L		
CB49A	3.58		W		S		T	V	R							U					
CB50B	3.83				S		T	V	R		P	Q	O		N	U	M		L		
CB51A	5.08		E		B			D		H		A		G		C		F			
CB52B	3.83	K			S		T	V	R		P	Q	O		N	U	M		L		
CB53B	5.25		E		B			D				A				C		F			
CB54B	3.83				S		T	V	R		P	Q	O		N	U	M		L		
CB55A	5.17		E		B			D				A		G		C		F			
CB56A	5		E		B	I		D		H		A		G		C		F			
CB57B	4	K		J	S	I	T	V	R	H	P	Q	O		N	U	M		L		
CB58B	3.5		W				T	V								U					
CB59B	5.08		E		B			D		H		A		G		C		F			
CB60A	4.83	K	E	J	B	I		D		H		A		G	N	C	M	F	L		
CB61B	4.5	K	E	J	B	I		D	R	H	P	Q	O	G	N	C	M	F	L		
CB62B	3.92	K		J	S	I	T	V	R		P	Q	O		N	U	M		L		
CB63A	3		W																		
CB64B	3.83				S		T	V	R		P	Q	O		N	U	M		L		
CB65B	4.33	K	E	J	S	I	T		R	H	P	Q	O	G	N	U	M	F	L		
CB66A	4.92	K	E	J	B	I		D		H		A		G		C		F	L		
CB67A	4.17	K		J	S	I	T	V	R	H	P	Q	O	G	N	U	M	F	L		
CB68A	4.67	K	E	J	B	I		D		H	P	Q	O	G	N	C	M	F	L		
CB69A	3.42		W					V								U					
CB70B	4.92	K	E	J	B	I		D		H		A		G		C		F			
CB71A	4.25	K		J	S	I	T	V	R	H	P	Q	O	G	N	U	M	F	L		
CB72B	3.92	K		J	S	I	T	V	R		P	Q	O		N	U	M		L		
CB73A	4.75	K	E	J	B	I		D		H	P	A	O	G	N	C	M	F	L		
CB74A	4.25	K		J	S	I	T	V	R	H	P	Q	O	G	N	U	M	F	L		

CB75A	3.92	K			J	S	I	T	V	R			P	Q	O			N	U	M		L
CB76B	4.75	K	E		J	B	I		D			H	P	A	O	G	N	C	M	F	L
CB77A	3.83	K				S		T	V	R			P	Q	O			N	U	M		L
CB78A	4.5	K	E		J	S	I		D	R	H	P	Q	O	G	N	C	M	F	L		
G-NEW	5.75					B							A				C					
G-OLD	4.33	K	E		J	S	I	T		R	H	P	Q	O	G	N	U	M	F	L		
TIFGU	3.5		W			S		T	V								U					

[a] Means from untransformed data for second Florida scale rating, 121 days after planting, 2012. [b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.13 Mean FSRIII values and Duncan grouping for the RIL C1501 population, 2012 field trial.

Genotype	FSRIII [a]	Duncan grouping [b]														
CB01B	6.08		F	L	K	J	E	I	C	H		D		G	
CB02B	5.25	N		L	K	J	S	I	M	H	Q	R	P	G	O
CB03B	5.92	N	F	L	K	J	E	I	M	H		D		G	O
CB04A	7.67				B				A						
CB05A	6.33		F		B		E	I	C	H		D		G	
CB06B	5.83	N	F	L	K	J	E	I	M	H		D	P	G	O
CB07B	5.17	N		L	K	J	S	I	M		Q	R	P		O
CB08A	6.42		F		B		E		C			D		G	
CB09A	4.75		T		U		S		V		Q	R	P		
CB10B	5.08	N	T	L	K		S		M		Q	R	P		O
CB11A	5.5	N	F	L	K	J	E	I	M	H	Q	R	P	G	O
CB12B	5.67	N	F	L	K	J	E	I	M	H	Q	D	P	G	O
CB13B	4.83		T		U		S		V		Q	R	P		O
CB14B	7				B				C			D			
CB15A	8.83								A						
CB16B	5.33	N		L	K	J	S	I	M	H	Q	R	P	G	O
CB17A	4.75		T		U		S		V		Q	R	P		
CB18B	5.58	N	F	L	K	J	E	I	M	H	Q		P	G	O
CB19B	4.92	N	T		U		S		M		Q	R	P		O
CB20A	5.58	N	F	L	K	J	E	I	M	H	Q		P	G	O
CB21B	5.08	N	T	L	K		S		M		Q	R	P		O
CB22B	5.5	N	F	L	K	J	E	I	M	H	Q	R	P	G	O
CB23A	5.58	N	F	L	K	J	E	I	M	H	Q		P	G	O
CB24B	5.42	N	F	L	K	J		I	M	H	Q	R	P	G	O
CB25B	5.75	N	F	L	K	J	E	I	M	H		D	P	G	O
CB26A	6.08		F	L	K	J	E	I	C	H		D		G	
CB27A	4.33		T		U		S		V						
CB28A	6		F	L	K	J	E	I	M	H		D		G	
CB29B	4.92	N	T		U		S				Q	R	P		O
CB30B	5.25	N		L	K	J	S	I	M	H	Q	R	P	G	O
CB31B	6.67		F		B		E		C			D			
CB32A	4.67		T		U		S		V		Q	R			
CB33A	4.92	N	T		U		S		V		Q	R	P		O
CB34B	5.83	N	F	L	K	J	E	I	M	H		D		G	O
CB35A	4.83		T		U		S		V		Q	R	P		O

CB36A	6.33		F		B		E	I	C	H		D		G		
CB37A	4								V							
CB38B	6.08		F	L	K	J	E	I	C	H		D		G		
CB39A	4.92	N	T		U		S		M		Q	R	P		O	
CB40A	6	N	F	L	K	J	E	I	M	H		D		G		
CB41A	6.67				B		E		C			D				
CB42A	5.75	N	F	L	K	J	E	I	M	H		D	P	G	O	
CB43B	6		F	L	K	J	E	I	M	H		D		G		
CB44B	6.58		F		B		E		C			D				
CB45A	5.17	N		L	K	J	S	I	M	H	Q	R	P		O	
CB46B	5	N	T	L			S		M		Q	R	P		O	
CB47A	5.17	N		L	K	J	S	I	M	H	Q	R	P		O	
CB48B	5.83	N	F	L	K	J	E	I	M	H		D	P	G	O	
CB49A	4.33		T		U		S		V							
CB50B	5	N	T	L			S		M		Q	R	P		O	
CB51A	6.25		F		B	J	E	I	C	H		D		G		
CB52B	5.17	N		L	K	J	S	I	M		Q	R	P		O	
CB53B	6.42		F		B		E		C			D		G		
CB54B	4.83		T		U		S		V		Q	R	P		O	
CB55A	6.25		F		B	J	E	I	C	H		D		G		
CB56A	6.17		F		K	J	E	I	C	H		D		G		
CB57B	5.08	N	T	L	K	J	S		M		Q	R	P		O	
CB58B	5.08	N	T	L	K	J	S		M		Q	R	P		O	
CB59B	6.35		F		B		E		C	H		D		G		
CB60A	6	N	F	L	K	J	E	I	M	H		D		G		
CB61B	5.75	N	F	L	K	J	E	I	M	H		D	P	G	O	
CB62B	4.92	N	T		U		S		M		Q	R	P		O	
CB63A	4.08				U				V							
CB64B	5.08	N	T	L	K		S		M		Q	R	P		O	
CB65B	5.5	N	F	L	K	J	E	I	M	H	Q	R	P	G	O	
CB66A	6	N	F	L	K	J	E	I	M	H		D		G		
CB67A	4.92	N	T		U		S		M		Q	R	P		O	
CB68A	5.67	N	F	L	K	J	E	I	M	H	Q	D	P	G	O	
CB69A	4.5		T		U		S		V			R				
CB70B	5.67	N	F	L	K	J	E	I	M	H	Q	D	P	G	O	
CB71A	5.75	N	F	L	K	J	E	I	M	H		D	P	G	O	
CB72B	5.25	N		L	K	J	S	I	M	H	Q	R	P	G	O	
CB73A	5.75	N	F	L	K	J	E	I	M	H		D	P	G	O	
CB74A	5.67	N	F	L	K	J	E	I	M	H	Q	D	P	G	O	

CB75A	5.33	N		L	K	J		I	M	H	Q	R	P	G	O
CB76B	5.75	N	F	L	K	J	E	I	M	H		D	P	G	O
CB77A	5	N	T	L			S		M		Q	R	P		O
CB78A	5.58	N	F	L	K	J	E	I	M	H	Q		P	G	O
G-NEW	7.5				B				C						
G-OLD	5.58	N	F	L	K	J	E	I	M	H	Q		P	G	O
TIFGU	4.17		T		U				V						

[a] Means from untransformed data for third Florida scale rating, 130 days after planting, 2012.

[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.14 Mean FSRIV values and Duncan grouping for the RIL C1501 population, 2012 field trial.

Genotype	FSRIV [a]	Duncan grouping [b]													
CB01B	8.75		E		B	I	D	H	A	G	C		F		
CB02B	8.67		E	J	B	I	D	H	A	G	C		F		
CB03B	8.67		E	J	B	I	D	H	A	G	C		F		
CB04A	9		E		B		D	H	A	G	C		F		
CB05A	8.75		E		B	I	D	H	A	G	C		F		
CB06B	8.58	K	E	J	B	I	D	H	A	G	C		F		
CB07B	8	K	E	J	O	I	D	H	M	G		N	F	L	
CB08A	9.25		E		B		D		A		C		F		
CB09A	8.33	K	E	J	B	I	D	H	M	G	C		F	L	
CB10B	6.83		R		O		Q		M		P	N	S		
CB11A	7.75	K		J	O	I		H	M	G	P	N	F	L	
CB12B	8.83		E		B	I	D	H	A	G	C		F		
CB13B	7.42	K	R	J	O	I	Q		M		P	N		L	
CB14B	9.42		E		B		D		A		C				
CB15A	9.92								A						
CB16B	7.58	K		J	O	I	Q	H	M	G	P	N		L	
CB17A	7.67	K		J	O	I		H	M	G	P	N		L	
CB18B	8.33	K	E	J	B	I	D	H	M	G	C		F	L	
CB19B	6.5		R		O		Q				P		S		
CB20A	7.92	K	E	J	O	I		H	M	G		N	F	L	
CB21B	8	K	E	J	O	I	D	H	M	G		N	F	L	
CB22B	8.08	K	E	J		I	D	H	M	G	C	N	F	L	
CB23A	8.5	K	E	J	B	I	D	H	A	G	C		F	L	
CB24B	8.5	K	E	J	B	I	D	H	A	G	C		F	L	
CB25B	9.08		E		B		D		A	G	C		F		
CB26A	9.5				B		D		A		C				
CB27A	6.5		R		O		Q				P		S		
CB28A	9.25		E		B		D		A		C		F		
CB29B	8.17	K	E	J	B	I	D	H	M	G	C	N	F	L	
CB30B	7.42	K	R	J	O	I	Q		M		P	N		L	
CB31B	9.08		E		B		D		A	G	C		F		
CB32A	7.92	K	E	J	O	I		H	M	G		N	F	L	
CB33A	7.5	K	R	J	O	I	Q	H	M		P	N		L	
CB34B	8.58	K	E	J	B	I	D	H	A	G	C		F		
CB35A	7.67	K		J	O	I		H	M	G	P	N		L	

CB36A	9.5				B		D		A		C				
CB37A	6.08		R				Q						S		
CB38B	9		E		B		D	H	A	G	C		F		
CB39A	7.67	K		J	O	I		H	M	G	P	N		L	
CB40A	8.75		E		B	I	D	H	A	G	C		F		
CB41A	9.5				B		D		A		C				
CB42A	9.33		E		B		D		A		C				
CB43B	9		E		B		D	H	A	G	C		F		
CB44B	9.08		E		B		D		A	G	C		F		
CB45A	7.5	K	R	J	O	I	Q	H	M		P	N		L	
CB46B	7.58	K		J	O	I	Q	H	M	G	P	N		L	
CB47A	7.17	K	R	J	O		Q		M		P	N		L	
CB48B	8.92		E		B	I	D	H	A	G	C		F		
CB49A	6.5		R		O		Q				P		S		
CB50B	7.17	K	R	J	O		Q		M		P	N		L	
CB51A	9.08		E		B		D		A	G	C		F		
CB52B	7		R		O		Q		M		P	N		L	
CB53B	9.67				B				A						
CB54B	6.67		R		O		Q				P	N	S		
CB55A	8.92		E		B	I	D	H	A	G	C		F		
CB56A	9.58				B				A		C				
CB57B	7.67	K		J	O	I		H	M	G	P	N		L	
CB58B	6.83		R		O		Q		M		P	N	S		
CB59B	9.67				B				A						
CB60A	9.25		E		B		D		A		C		F		
CB61B	9		E		B		D	H	A	G	C		F		
CB62B	6.83		R		O		Q		M		P	N	S		
CB63A	5.5												S		
CB64B	8	K	E	J	O	I	D	H	M	G		N	F	L	
CB65B	8.17	K	E	J	B	I	D	H	M	G	C	N	F	L	
CB66A	9		E		B		D	H	A	G	C		F		
CB67A	7.17	K	R	J	O		Q		M		P	N		L	
CB68A	8.83		E		B	I	D	H	A	G	C		F		
CB69A	7.08	K	R		O		Q		M		P	N		L	
CB70B	7.92	K	E	J	O	I		H	M	G		N	F	L	
CB71A	8.08	K	E	J		I	D	H	M	G	C	N	F	L	
CB72B	7.67	K		J	O	I		H	M	G	P	N		L	
CB73A	8.08	K	E	J		I	D	H	M	G	C	N	F	L	
CB74A	8.25	K	E	J	B	I	D	H	M	G	C		F	L	

CB75A	6			R									S		
CB76B	8.25	K	E	J	B	I	D	H	M	G	C		F	L	
CB77A	6.92			R		O		Q		M		P	N	S	
CB78A	9.25			E		B		D		A		C		F	
G-NEW	9.67					B				A					
G-OLD	8.58	K	E	J	B	I	D	H	A	G	C		F		
TIFGU	6.33			R				Q				P		S	

[a] Means from untransformed data for fourth Florida scale rating, 141 days after planting, 2012.

[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on untransformed data.

Table 3.15 Mean AUDPC values and Duncan grouping for the RIL C1501 population, 2012 field trial.

Genotype	AUDPC [a]	Duncan grouping [b]
CB01B	165	L E K M J D H I G C F N
CB02B	141	L T K M J S I R Q P O N
CB03B	167.67	L E K B J D H I G C F
CB04A	204.67	B A
CB05A	168.67	L E K B J D H I G C F
CB06B	160	L E K M J D H I G P O F N
CB07B	148	L T K M J S H I R G Q P O N
CB08A	174.67	E B D H A G C F
CB09A	134	T S U R Q P O V
CB10B	138.67	L T K M S U R Q P O N
CB11A	146.67	L T K M J S H I R Q P O N
CB12B	156.33	L E K M J D H I R G Q P O F N
CB13B	136.33	T M S U R Q P O V N
CB14B	187.67	E B D A C
CB15A	208	A
CB16B	141.33	L T K M J S U R Q P O N
CB17A	130	T S U R Q P V
CB18B	154	L K M J H I R G Q P O F N
CB19B	124	T W S U V
CB20A	146.33	L T K M J S H I R Q P O N
CB21B	140.67	L T K M J S U R Q P O N
CB22B	155.33	L E K M J H I R G Q P O F N
CB23A	157	L E K M J D H I R G Q P O F N
CB24B	146	L T K M J S H I R Q P O N
CB25B	168	E K B J D H I G C F
CB26A	169.67	E B J D H I G C F
CB27A	121	T W U V
CB28A	176	E B D H A G C F
CB29B	137.33	T S U R Q P O V N
CB30B	141.33	L T K M J S R Q P O N
CB31B	184	E B D A C F
CB32A	130.33	T S U R Q V
CB33A	139.33	L T M S U R Q P O N
CB34B	165.67	L E K M J D H I G C F N
CB35A	132.67	T S U R Q P O V

CB36A	174.67		E		B		D	H	A		G		C		F			
CB37A	112.33				W										V			
CB38B	171.33		E		B		D	H	I		G		C		F			
CB39A	136.33		T		M		S		U	R		Q	P	O	V	N		
CB40A	166.33	L	E	K	M	J	D	H	I		G		C		F	N		
CB41A	189.33				B		D		A				C					
CB42A	160.33	L	E	K	M	J	D	H	I		G		C	O	F	N		
CB43B	166.33	L	E	K	M	J	D	H	I		G		C		F			
CB44B	180		E		B		D		A		G		C		F			
CB45A	141	L	T	K	M	J	S		I	R		Q	P	O		N		
CB46B	136.67		T		M		S		U	R		Q	P	O		N		
CB47A	136		T				S		U	R		Q	P	O	V	N		
CB48B	161.67	L	E	K	M	J	D	H	I		G		C	O	F	N		
CB49A	120.67		T		W				U						V			
CB50B	133		T				S		U	R		Q	P	O	V			
CB51A	173.33		E		B		D	H	A		G		C		F			
CB52B	134		T				S		U	R		Q	P	O	V			
CB53B	177		E		B		D	H	A		G		C		F			
CB54B	129		T				S		U	R		Q			V			
CB55A	173		E		B		D	H	A		G		C		F			
CB56A	174		E		B		D	H	A		G		C		F			
CB57B	137.67	L	T		M		S		U	R		Q	P	O		N		
CB58B	128		T				S		U	R					V			
CB59B	175		E		B		D	H	A		G		C		F			
CB60A	168.33	L	E	K	B	J	D	H	I		G		C		F			
CB61B	157.33	L	E	K	M	J	D	H	I		G	Q	P	O	F	N		
CB62B	133.67		T				S		U	R		Q	P	O	V			
CB63A	105.67				W													
CB64B	138.67	L	T	K	M		S		U	R		Q	P	O		N		
CB65B	150.33	L		K	M	J	S	H	I	R	G	Q	P	O	F	N		
CB66A	168	L	E	K	B	J	D	H	I		G		C		F			
CB67A	137.67	L	T		M		S		U	R		Q	P	O		N		
CB68A	159	L	E	K	M	J	D	H	I		G			O	F	N		
CB69A	124		T		W		S		U						V			
CB70B	159.67	L	E	K	M	J	D	H	I		G		C	O	F	N		
CB71A	150	L		K	M	J	S	H	I	R	G	Q	P	O	F	N		
CB72B	137.67	L	T		M		S		U	R		Q	P	O		N		
CB73A	157.33	L	E	K	M	J	D	H	I		G	Q	P	O	F	N		
CB74A	150.67	L		K	M	J	S	H	I	R	G	Q	P	O	F	N		

CB75A	132.67			T				S		U	R		Q	P	O	V			
CB76B	159.67		L	E	K	M	J	D	H	I		G		C	O	F		N	
CB77A	133.33			T				S		U	R		Q	P	O	V			
CB78A	156.67		L	E	K	M	J	D	H	I		G	Q	P	O	F		N	
G-NEW	196.67					B				A				C					
G-OLD	153.33		L	E	K	M	J		H	I	R	G	Q	P	O	F		N	
TIFGU	114.67					W				U						V			

[a] Means from untransformed data for area under disease progress curve (AUDPC) values, 2012. [b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.16 Mean stAUDPC values and Duncan grouping for the RIL C1501 population, 2012 field trial.

Genotype	stAUDPC [a]	Duncan grouping [b]															
CB01B	5.5	L	E	K	O	J	D	H		I		G		C	N	F	M
CB02B	4.69	L	U	K	O	J	T		S	I	R		P	Q	N		M
CB03B	5.58	L	E	K	B	J	D	H		I		G		C		F	
CB04A	6.81				B					A							
CB05A	5.62	L	E	K	B	J	D	H		I		G		C		F	
CB06B	5.33	L	E	K	O	J	D	H		I		G	P	Q	N	F	M
CB07B	4.93	L	U	K	O	J	T	H	S	I	R	G	P	Q	N		M
CB08A	5.82		E		B		D	H		A		G		C		F	
CB09A	4.47		U				T		S	V	R		P	Q		W	
CB10B	4.61	L	U	K	O		T		S	V	R		P	Q	N		M
CB11A	4.89	L	U	K	O	J	T	H	S	I	R		P	Q	N		M
CB12B	5.22	L	E	K	O	J	D	H		I	R	G	P	Q	N	F	M
CB13B	4.54		U		O		T		S	V	R		P	Q		W	
CB14B	6.26		E		B		D			A				C			
CB15A	6.94									A							
CB16B	4.72	L	U	K	O	J	T		S	V	R		P	Q	N		M
CB17A	4.34		U				T		S	V	R			Q		W	
CB18B	5.14	L		K	O	J		H	S	I	R	G	P	Q	N	F	M
CB19B	4.13		U		X		T			V						W	
CB20A	4.88	L	U	K	O	J	T	H	S	I	R		P	Q	N		M
CB21B	4.68	L	U	K	O	J	T		S	V	R		P	Q	N		M
CB22B	5.18	L	E	K	O	J		H	S	I	R	G	P	Q	N	F	M
CB23A	5.23	L	E	K	O	J	D	H	S	I	R	G	P	Q	N	F	M
CB24B	4.87	L	U	K	O	J	T	H	S	I	R		P	Q	N		M
CB25B	5.6		E	K	B	J	D	H		I		G		C		F	
CB26A	5.66		E		B	J	D	H		I		G		C		F	
CB27A	4.04		U		X					V						W	
CB28A	5.85		E		B		D	H		A		G		C		F	
CB29B	4.58		U		O		T		S	V	R		P	Q	N	W	
CB30B	4.71	L	U	K	O	J	T		S		R		P	Q	N		M
CB31B	6.14		E		B		D			A				C		F	
CB32A	4.34		U				T		S	V	R					W	
CB33A	4.64	L	U		O		T		S	V	R		P	Q	N		M
CB34B	5.51	L	E	K	O	J	D	H		I		G		C	N	F	M
CB35A	4.42		U				T		S	V	R		P	Q		W	
CB36A	5.83		E		B		D	H		A		G		C		F	
CB37A	3.75				X											W	

CB38B	5.71		E		B		D	H		I		G		C		F		
CB39A	4.55		U		O		T		S	V	R		P	Q	N	W	M	
CB40A	5.55	L	E	K	O	J	D	H		I		G		C	N	F	M	
CB41A	6.31				B		D			A				C				
CB42A	5.35	L	E	K	O	J	D	H		I		G	P	C	N	F	M	
CB43B	5.54	L	E	K	B	J	D	H		I		G		C	N	F	M	
CB44B	6		E		B		D			A		G		C		F		
CB45A	4.69	L	U	K	O	J	T		S	I	R		P	Q	N		M	
CB46B	4.56		U		O		T		S	V	R		P	Q	N		M	
CB47A	4.54		U		O		T		S	V	R		P	Q	N	W		
CB48B	5.39	L	E	K	O	J	D	H		I		G	P	C	N	F	M	
CB49A	4.02		U		X					V						W		
CB50B	4.43		U				T		S	V	R		P	Q		W		
CB51A	5.78		E		B		D	H		A		G		C		F		
CB52B	4.46		U				T		S	V	R		P	Q		W		
CB53B	5.88		E		B		D	H		A		G		C		F		
CB54B	4.3		U				T		S	V	R					W		
CB55A	5.76		E		B		D	H		A		G		C		F		
CB56A	5.8		E		B		D	H		A		G		C		F		
CB57B	4.59	L	U		O		T		S	V	R		P	Q	N		M	
CB58B	4.26		U				T		S	V						W		
CB59B	5.83		E		B		D	H		A		G		C		F		
CB60A	5.61	L	E	K	B	J	D	H		I		G		C		F		
CB61B	5.24	L	E	K	O	J	D	H		I	R	G	P	Q	N	F	M	
CB62B	4.47		U				T		S	V	R		P	Q		W		
CB63A	3.51				X													
CB64B	4.61	L	U	K	O		T		S	V	R		P	Q	N		M	
CB65B	5.01	L		K	O	J	T	H	S	I	R	G	P	Q	N		M	
CB66A	5.58	L	E	K	B	J	D	H		I		G		C		F	M	
CB67A	4.59	L	U		O		T		S	V	R		P	Q	N		M	
CB68A	5.31	L	E	K	O	J	D	H		I		G	P		N	F	M	
CB69A	4.13		U		X		T			V						W		
CB70B	5.33	L	E	K	O	J	D	H		I		G	P	C	N	F	M	
CB71A	5.01	L		K	O	J	T	H	S	I	R	G	P	Q	N	F	M	
CB72B	4.59	L	U		O		T		S	V	R		P	Q	N		M	
CB73A	5.24	L	E	K	O	J	D	H		I	R	G	P	Q	N	F	M	
CB74A	5.02	L		K	O	J	T	H	S	I	R	G	P	Q	N		M	
CB75A	4.42		U				T		S	V	R		P	Q		W		
CB76B	5.32	L	E	K	O	J	D	H		I		G	P	C	N	F	M	
CB77A	4.45		U				T		S	V	R		P	Q		W		
CB78A	5.21	L	E	K	O	J	D	H		I	R	G	P	Q	N	F	M	

G-NEW	6.56					B						A					C		
G-OLD	5.11		L		K	O	J		H	S	I	R	G	P	Q	N	F		M
TIFGU	3.83					X					V						W		

[a] Means from untransformed data for standardized area under disease progress curve (stAUDPC) values, calculated by dividing AUDPC values by the number of days from the first observed symptoms till harvest , the duration of late leaf spot epidemic in 2012.
[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.17 Effect of peanut genotype on components of resistance to late leaf spot measured during detached leaf and lateral stem assays in the RIL C1501 population.

Source	DLA [a]	LSAI [b]	LSAII [c]
Diseased leaf area (%) [d]	<.0001 [m]	0.5781 [m]	0.0085 [m]
Infection frequency [e]	0.0578 [m]	0.6918 [m]	0.0016 [m]
Defoliation (%)	.	0.9908 [m]	0.0969 [m]
Incubation period [f]	0.0118 [m]	.	.
Sporulation index [g]	<.0001 [n]	.	.
Sporulation (%) [h]	<.0001 [m]	.	.
DAI until lesion diameter=1mm [i]	<.0001 [m]	.	.
Latent period (16x) [j]	<.0001 [m]	.	.
Latent period (2x) [k]	<.0001 [m]	.	.
Lesion diameter [l]	<.0001 [o]	.	.

[a,b,c] *p* – values for components of resistance to late leaf spot (LLS) measured during detached leaf assay (March-April 2012) in growth chamber, first lateral stem assay (118-120 DAP) and second lateral stem assay (132-134 DAP) in field 2011, respectively.
[d] *p* – values for percent leaf area affected by LLS, 29 days after inoculation (DAI) in DLA, 118-120 DAP in LSAI and 132-134 DAP in LSAII.
[e] *p* – values for number of lesions per unit leaf area (cm^2), 29 DAI in DLA, 118-120 DAP in LSAI and 132-134 DAP in LSAII.
[f] *p* – values for mean DAI until the first three lesions appear.
[g] *p* – values for mean degree of sporulation (0-4 scale) on three oldest lesions.
[h] *p* – values for mean percentage of sporulating lesions on four leaflets, 29 DAI.
[i] *p* – values for mean DAI until the first three lesions reach 1mm in diameter.
[j,k] *p* – values for mean DAI until sporulation is observed on the first three lesions, under 2x and 16x magnifica0tions respectively.
[l] *p* – values for mean diameter of five largest lesions, 29 DAI.
[m] From analysis of log transformed data.
[n] From analysis of untransformed data, ANOVA conditions could not be satisfied.
[o] From analysis of untransformed data, ANOVA conditions satisfied.

Table 3.18 Duncan grouping for percent leaf area affected by late leaf spot lesions in the RIL C1501 population, measured during second lateral stem assay.

Genotype	Diseased leaf area (%) [a]	Duncan grouping [b]									
CB01B	5.97	E		B		D		A		C	
CB02B	2.54	E	J	B	I	D	H	A	G	C	F
CB03B	3.57	E	J	B	I	D	H	A	G	C	F
CB04A	6.66	E		B		D		A		C	F
CB05A	2.77	E	J	B	I	D	H	A	G	C	F
CB06B	2.54	E	J	B	I	D	H	A	G	C	F
CB07B	3.59	E	J	B	I	D	H	A	G	C	F
CB08A	5.05	E		B	I	D	H	A	G	C	F
CB09A	4.14	E		B		D	H	A	G	C	F
CB10B	1.32	E	J		I		H		G		F
CB11A	1.24		J		I		H		G		
CB12B	3.14	E	J	B	I	D	H	A	G	C	F
CB13B	2.41	E	J	B	I	D	H	A	G	C	F
CB14B	8.00			B				A		C	
CB15A	6.80			B		D		A		C	
CB16B	5.11	E		B		D		A	G	C	F
CB17A	1.33	E	J		I		H		G		F
CB18B	1.73	E	J	B	I	D	H		G	C	F
CB19B	1.50	E	J		I	D	H		G		F
CB20A	3.47	E		B	I	D	H	A	G	C	F
CB21B	1.38	E	J		I		H		G		F
CB22B	2.33	E	J	B	I	D	H		G	C	F
CB23A	3.73	E		B		D	H	A	G	C	F
CB24B	2.66	E	J	B	I	D	H	A	G	C	F
CB25B	6.75	E		B		D		A		C	F
CB26A	2.31	E	J	B	I	D	H	A	G	C	F
CB27A	1.93	E	J		I		H		G		F
CB28A	3.59	E	J	B	I	D	H	A	G	C	F
CB29B	1.97	E	J		I	D	H		G		F
CB30B	2.85	E	J	B	I	D	H	A	G	C	F
CB31B	4.53	E		B		D		A	G	C	F
CB32A	1.92	E	J	B	I	D	H	A	G	C	F
CB33A	1.31	E	J		I		H		G		F
CB34B	4.05	E	J	B	I	D	H	A	G	C	F
CB35A	1.93	E	J	B	I	D	H		G	C	F
CB36A	3.35	E	J	B	I	D	H	A	G	C	F
CB37A	1.50	E	J		I	D	H		G	C	F

CB38B	2.32	E	J	B	I	D	H	A	G	C	F
CB39A	3.05	E	J	B	I	D	H	A	G	C	F
CB40A	3.59	E	J	B	I	D	H	A	G	C	F
CB41A	8.87							A			
CB42A	1.97	E	J	B	I	D	H		G	C	F
CB43B	3.02	E	J	B	I	D	H	A	G	C	F
CB44B	3.42	E	J	B	I	D	H	A	G	C	F
CB45A	4.93	E		B		D	H	A	G	C	F
CB46B	1.68	E	J		I		H		G		F
CB47A	1.42		J		I		H		G		
CB48B	2.62	E	J	B	I	D	H	A	G	C	F
CB49A	1.23		J		I		H		G		
CB50B	0.91		J		I		H				
CB51A	3.44	E	J	B	I	D	H	A	G	C	F
CB52B	2.07	E	J	B	I	D	H		G	C	F
CB53B	2.37	E	J	B	I	D	H	A	G	C	F
CB54B	2.83	E	J	B	I	D	H	A	G	C	F
CB55A	9.49			B				A			
CB56A	4.23	E	J	B	I	D	H	A	G	C	F
CB57B	1.14		J		I		H		G		F
CB58B	1.65	E	J	B	I	D	H		G	C	F
CB59B	3.54	E	J	B	I	D	H	A	G	C	F
CB60A	4.15	E		B	I	D	H	A	G	C	F
CB61B	2.88	E	J	B	I	D	H	A	G	C	F
CB62B	1.28		J		I		H		G		F
CB63A	1.15		J		I		H		G		
CB64B	1.25	E	J		I		H		G		F
CB65B	2.22	E	J	B	I	D	H	A	G	C	F
CB66A	4.84	E		B		D		A	G	C	F
CB67A	2.81	E	J	B	I	D	H	A	G	C	F
CB68A	3.73	E	J	B	I	D	H	A	G	C	F
CB69A	2.25	E	J	B	I	D	H	A	G	C	F
CB70B	3.66	E		B		D	H	A	G	C	F
CB71A	2.23	E	J	B	I	D	H	A	G	C	F
CB72B	1.66	E	J	B	I	D	H		G	C	F
CB73A	2.56	E	J	B	I	D	H	A	G	C	F
CB74A	3.56	E	J	B	I	D	H	A	G	C	F
CB75A	1.16		J		I		H		G		
CB76B	2.09	E	J	B	I	D	H	A	G	C	F
CB77A	0.72		J		I						
CB78A	1.33	E	J		I		H		G		F
G-OLD	2.10	E	J	B	I	D	H	A	G	C	F
TIFGU	0.76		J								

[a]Means for percent leaf area affected by late leaf spot, 118-120 days after planting
[b]Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.19 Duncan grouping for infection frequency in the RIL C1501 population, measured during second lateral stem assay.

Genotype	Infection frequency [a]	Duncan grouping [b]								
CB01B	2.51	E	B		D		A	G	C	F
CB02B	1.16	E	B	I	D	H		G	C	F
CB03B	1.60	E	B	I	D	H	A	G	C	F
CB04A	3.26		B		D		A		C	
CB05A	1.50	E	B	I	D	H	A	G	C	F
CB06B	1.27	E	B	I	D	H		G	C	F
CB07B	1.72	E	B	I	D	H	A	G	C	F
CB08A	3.33	E	B		D		A		C	F
CB09A	2.04	E	B	I	D	H	A	G	C	F
CB10B	0.59			I		H		G		
CB11A	0.62			I		H		G		
CB12B	1.71	E	B	I	D	H	A	G	C	F
CB13B	1.07	E		I	D	H		G		F
CB14B	3.76		B				A		C	
CB15A	2.69	E	B		D		A		C	
CB16B	2.10	E	B		D	H	A	G	C	F
CB17A	0.70	E		I		H		G		F
CB18B	0.74	E		I		H		G		F
CB19B	0.71	E		I		H		G		F
CB20A	1.49	E	B	I	D	H	A	G	C	F
CB21B	0.60			I		H		G		
CB22B	1.06	E		I	D	H		G		F
CB23A	1.77	E	B	I	D	H	A	G	C	F
CB24B	1.34	E	B	I	D	H		G	C	F
CB25B	2.73	E	B		D		A	G	C	F
CB26A	0.87	E		I		H		G		F
CB27A	0.86	E		I		H		G		F
CB28A	1.59	E	B	I	D	H		G	C	F
CB29B	0.77	E		I		H		G		F
CB30B	1.36	E	B	I	D	H		G	C	F
CB31B	2.14	E	B		D	H	A	G	C	F
CB32A	0.84	E		I		H		G		F
CB33A	0.71	E		I		H		G		F
CB34B	1.65	E	B	I	D	H	A	G	C	F
CB35A	0.84	E		I		H		G		F
CB36A	1.31	E		I	D	H		G	C	F
CB37A	0.71	E		I		H		G		F
CB38B	0.86	E		I	D	H		G		F
CB39A	1.30	E	B	I	D	H		G	C	F

CB40A	1.35	E	B	I	D	H	A	G	C	F
CB41A	3.59		B				A			
CB42A	0.85	E		I		H		G		F
CB43B	1.77	E	B	I	D	H	A	G	C	F
CB44B	1.25	E	B	I	D	H		G	C	F
CB45A	2.21	E	B		D	H	A	G	C	F
CB46B	0.64			I		H		G		
CB47A	0.76			I		H		G		F
CB48B	1.12	E		I	D	H		G		F
CB49A	0.55			I		H				
CB50B	0.47			I		H				
CB51A	1.56	E	B	I	D	H	A	G	C	F
CB52B	0.98	E		I	D	H		G		F
CB53B	1.01	E		I	D	H		G		F
CB54B	1.24	E		I	D	H		G		F
CB55A	4.26						A			
CB56A	1.49	E	B	I	D	H		G	C	F
CB57B	0.52			I		H				
CB58B	0.65			I		H		G		F
CB59B	1.51	E	B	I	D	H		G	C	F
CB60A	1.48	E	B	I	D	H	A	G	C	F
CB61B	1.14	E	B	I	D	H		G	C	F
CB62B	0.74	E		I		H		G		F
CB63A	0.59			I		H				
CB64B	0.62			I		H		G		F
CB65B	1.00	E		I	D	H		G		F
CB66A	2.22	E	B		D	H	A	G	C	F
CB67A	1.20	E	B	I	D	H		G	C	F
CB68A	1.62	E	B	I	D	H	A	G	C	F
CB69A	1.02	E		I	D	H		G		F
CB70B	1.89	E	B	I	D	H	A	G	C	F
CB71A	1.08	E		I	D	H		G		F
CB72B	0.86	E		I	D	H		G		F
CB73A	1.13	E	B	I	D	H		G	C	F
CB74A	1.37	E	B	I	D	H		G	C	F
CB75A	0.44			I		H				
CB76B	0.94	E		I	D	H		G		F
CB77A	0.32			I						
CB78A	0.71	E		I		H		G		F
G-OLD	0.92	E		I	D	H		G		F
TIFGU	0.33			I						

[a]Means from untransformed data for number of lesions per unit leaf area (cm^2), 118-120 days after planting.

[b]Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.20 Duncan grouping for incubation period in the RIL C1501 population, measured during detached leaf assay.

Genotype	Incubation period [a]	Duncan grouping [b]										
CB01B	10.00	K	E	J	B	I	D	H	A	G	C	F
CB02B	10.33	K	E	J	B	I	D	H	A	G	C	F
CB03B	12.33				B				A			
CB04A	9.00	K		J		I		H				
CB05A	11.89		E		B		D		A		C	
CB06B	9.89	K	E	J	B	I	D	H	A	G	C	F
CB07B	10.56	K	E	J	B	I	D	H	A	G	C	F
CB08A	9.11	K		J		I		H		G		F
CB09A	10.44	K	E	J	B	I	D	H	A	G	C	F
CB10B	11.66		E		B		D	H	A	G	C	F
CB11A	9.45	K	E	J		I	D	H		G	C	F
CB12B	8.44	K										
CB13B	11.11		E	J	B	I	D	H	A	G	C	F
CB14B	9.67	K	E	J	B	I	D	H		G	C	F
CB15A	10.33	K	E	J	B	I	D	H	A	G	C	F
CB16B	9.11	K		J		I		H		G		F
CB17A	12.11		E		B		D		A		C	F
CB18B	10.34	K	E	J	B	I	D	H	A	G	C	F
CB19B	12.11				B		D		A		C	
CB20A	10.11	K	E	J	B	I	D	H	A	G	C	F
CB21B	10.44	K	E	J	B	I	D	H	A	G	C	F
CB22B	9.56	K	E	J	B	I	D	H		G	C	F
CB23A	10.67	K	E	J	B	I	D	H	A	G	C	F
CB24B	8.78	K		J								
CB26A	10.33	K	E	J	B	I	D	H	A	G	C	F
CB27A	11.00		E	J	B	I	D	H	A	G	C	F
CB28A	10.33	K	E	J	B	I	D	H	A	G	C	F
CB29B	10.78	K	E	J	B	I	D	H	A	G	C	F
CB30B	11.00		E	J	B	I	D	H	A	G	C	F
CB31B	12.00		E		B		D		A		C	F
CB32A	11.34		E	J	B	I	D	H	A	G	C	F
CB33A	9.67	K	E	J	B	I	D	H		G	C	F
CB34B	9.67	K	E	J	B	I	D	H		G	C	F
CB35A	11.00	K	E	J	B	I	D	H	A	G	C	F
CB36A	9.22	K	E	J		I		H		G		F
CB37A	9.44	K	E	J		I	D	H		G	C	F
CB38B	10.00	K	E	J	B	I	D	H	A	G	C	F
CB39A	9.11	K		J		I		H		G		F
CB40A	9.44	K	E	J		I	D	H		G	C	F

CB41A	9.22	K	E	J		I		H		G		F
CB42A	9.33	K	E	J		I	D	H		G	C	F
CB43B	9.89	K	E	J	B	I	D	H	A	G	C	F
CB44B	10.11	K	E	J	B	I	D	H	A	G	C	F
CB45A	10.00	K	E	J	B	I	D	H	A	G	C	F
CB46B	10.67	K	E	J	B	I	D	H	A	G	C	F
CB47A	9.33	K	E	J		I	D	H		G	C	F
CB48B	10.11	K	E	J	B	I	D	H	A	G	C	F
CB49A	12.33				B				A		C	
CB50B	10.11	K	E	J	B	I	D	H	A	G	C	F
CB51A	9.33	K	E	J		I	D	H		G	C	F
CB52B	11.78		E		B	I	D	H	A	G	C	F
CB53B	10.56	K	E	J	B	I	D	H	A	G	C	F
CB54B	10.89	K	E	J	B	I	D	H	A	G	C	F
CB55A	9.78	K	E	J	B	I	D	H		G	C	F
CB56A	9.11	K		J		I		H		G		F
CB57B	9.89	K	E	J	B	I	D	H	A	G	C	F
CB58B	10.78	K	E	J	B	I	D	H	A	G	C	F
CB59B	10.33	K	E	J	B	I	D	H	A	G	C	F
CB60A	10.22	K	E	J	B	I	D	H	A	G	C	F
CB61B	9.45	K	E	J		I	D	H		G	C	F
CB62B	10.78	K	E	J	B	I	D	H	A	G	C	F
CB63A	10.33	K	E	J	B	I	D	H	A	G	C	F
CB64B	10.78	K	E	J	B	I	D	H	A	G	C	F
CB65B	11.00		E	J	B	I	D	H	A	G	C	F
CB66A	10.78	K	E	J	B	I	D	H	A	G	C	F
CB67A	10.33	K	E	J	B	I	D	H	A	G	C	F
CB68A	11.89		E		B		D		A	G	C	F
CB69A	11.89		E		B		D		A		C	
CB70B	9.33	K	E	J		I	D	H		G		F
CB71A	9.89	K	E	J	B	I	D	H	A	G	C	F
CB72B	9.89	K	E	J	B	I	D	H	A	G	C	F
CB73A	9.00	K		J		I						
CB74A	10.22	K	E	J	B	I	D	H	A	G	C	F
CB75A	10.00	K	E	J	B	I	D	H	A	G	C	F
CB76B	8.78	K		J								
CB77A	9.56	K	E	J	B	I	D	H		G	C	F
CB78A	10.00	K	E	J	B	I	D	H	A	G	C	F
G-CER	9.83	K	E	J	B	I	D	H	A	G	C	F
G-OLD	9.11	K		J		I		H		G		
TIFGU	12.67								A			

[a] Means from untransformed data for DAI until the first three lesions appear.
[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.21 Duncan grouping for latent period (2X) in the RIL C1501 population, measured during detached leaf assay.

Genotype	Latent period (2x) [a]	Duncan grouping [b]														
CB01B	17.11				Q		S		O	P		N	R			
CB02B	19.78	K	E	J	Q	I	D	H	O	P	G	N	R	M	F	L
CB03B	22.67		E	J	B	I	D	H	A		G		C		F	
CB04A	15.33						S									
CB05A	21.33	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB06B	19.11	K		J	Q	I	S	H	O	P	G	N	R	M		L
CB07B	18.00	K			Q		S		O	P		N	R	M		L
CB08A	16.00						S						R			
CB09A	20.44	K	E	J		I	D	H	O	P	G	N	C	M	F	L
CB10B	22.00	K	E	J	B	I	D	H	A		G		C	M	F	L
CB11A	18.89	K		J	Q	I	S	H	O	P		N	R	M		L
CB12B	16.00				Q		S						R			
CB13B	22.44	K	E	J	B	I	D	H	A		G		C		F	
CB14B	16.67				Q		S		O	P			R			
CB15A	18.00	K			Q		S		O	P		N	R	M		L
CB16B	19.11	K		J	Q	I		H	O	P	G	N	R	M	F	L
CB17A	21.00	K	E	J	B	I	D	H	O	P	G	N	C	M	F	L
CB18B	18.00	K			Q		S		O	P		N	R	M		L
CB19B	22.67		E	J	B	I	D	H	A		G		C		F	
CB20A	22.22	K	E	J	B	I	D	H	A		G		C		F	L
CB21B	26.00								A							
CB22B	19.33	K		J	Q	I		H	O	P	G	N	R	M	F	L
CB23A	18.22	K		J	Q		S		O	P		N	R	M		L
CB24B	17.56				Q		S		O	P		N	R			
CB26A	19.56	K		J	Q	I		H	O	P	G	N	R	M	F	L
CB27A	22.89		E		B	I	D	H	A		G		C		F	
CB28A	19.33	K		J	Q	I		H	O	P	G	N	R	M	F	L
CB29B	20.89	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB30B	22.34	K	E	J	B	I	D	H	A		G		C		F	L
CB31B	19.55	K	E	J	Q	I		H	O	P	G	N	R	M	F	L
CB32A	24.22		E		B		D		A				C			
CB33A	22.22	K	E	J	B	I	D	H	A		G		C		F	L
CB34B	17.33				Q		S		O	P		N	R			
CB35A	21.33	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB36A	16.44				Q		S			P			R			
CB37A	24.34				B		D		A				C			
CB38B	17.33				Q		S		O	P		N	R			
CB39A	17.33				Q		S		O	P		N	R			
CB40A	16.44				Q		S			P			R			

CB41A	17.78				Q		S		O	P		N	R	M			
CB42A	16.67				Q		S		O	P			R				
CB43B	18.22	K		J	Q		S		O	P		N	R	M			L
CB44B	18.67	K		J	Q	I	S		O	P		N	R	M			L
CB45A	16.67				Q		S		O	P			R				
CB46B	19.33	K		J	Q	I		H	O	P	G	N	R	M	F	L	
CB47A	19.11	K		J	Q	I	S	H	O	P		N	R	M			L
CB48B	19.56	K	E	J	Q	I		H	O	P	G	N	R	M	F	L	
CB49A	25.50				B				A								
CB50B	20.00	K	E	J	Q	I	D	H	O	P	G	N		M	F	L	
CB51A	19.33	K		J	Q	I		H	O	P	G	N	R	M	F	L	
CB52B	24.11		E		B		D		A				C				
CB53B	20.67	K	E	J	B	I	D	H	O		G	N	C	M	F	L	
CB54B	22.89		E		B	I	D	H	A		G		C		F		
CB55A	17.33				Q		S		O	P		N	R				
CB56A	16.00				Q		S						R				
CB57B	24.11		E		B		D		A				C		F		
CB58B	23.78		E		B		D		A				C		F		
CB59B	19.56	K	E	J	Q	I		H	O	P	G	N	R	M	F	L	
CB60A	19.56	K	E	J	Q	I	D	H	O	P	G	N	R	M	F	L	
CB61B	18.67	K		J	Q	I	S		O	P		N	R	M			L
CB62B	25.17				B				A				C				
CB63A	21.11	K	E	J	B	I	D	H	A		G	N	C	M	F	L	
CB64B	19.78	K	E	J	Q	I	D	H	O	P	G	N	R	M	F	L	
CB65B	23.89		E		B		D		A		G		C		F		
CB66A	18.22	K			Q		S		O	P		N	R	M			L
CB67A	20.22	K	E	J		I	D	H	O	P	G	N		M	F	L	
CB68A	18.66	K		J	Q	I	S		O	P		N	R	M			L
CB69A	19.78	K	E	J	Q	I	D	H	O	P	G	N	R	M	F	L	
CB70B	17.33				Q		S		O	P		N	R				
CB71A	18.44	K		J	Q		S		O	P		N	R	M			L
CB72B	17.56				Q		S		O	P		N	R				
CB73A	18.00				Q		S		O	P		N	R	M			L
CB74A	17.56				Q		S		O	P		N	R				
CB75A	23.33		E		B		D	H	A		G		C		F		
CB76B	17.11				Q		S		O	P		N	R				
CB77A	18.22	K		J	Q		S		O	P		N	R	M			L
CB78A	22.00	K	E	J	B	I	D	H	A		G		C	M	F	L	
G-CER	17.56				Q		S		O	P		N	R				
G-OLD	20.44	K	E	J		I	D	H	O	P	G	N	C	M	F	L	
TIFGU	25.67								A								

[a] Means of untransformed data for DAI until sporulation is observed on the first three lesions under 2x magnification.
[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.22 Duncan grouping for latent period (16X) in the RIL C1501 population, measured during detached leaf assay.

Genotype	Latent period (16x) [a]	Duncan grouping [b]														
CB01B	15.78	K		J		I		H	O	P		N		M		L
CB02B	17.11	K	E	J		I	D	H	O	P	G	N		M	F	L
CB03B	20.89		E		B		D		A				C			
CB04A	14.22									P						
CB05A	19.33		E	J	B	I	D	H	A		G		C		F	
CB06B	16.22	K		J		I		H	O	P	G	N		M	F	L
CB07B	16.67	K	E	J		I		H	O	P	G	N		M	F	L
CB08A	14.89								O	P		N		M		L
CB09A	17.00	K	E	J		I	D	H	O	P	G	N		M	F	L
CB10B	18.00	K	E	J	B	I	D	H	O		G	N	C	M	F	L
CB11A	16.89	K	E	J		I	D	H	O	P	G	N		M	F	L
CB12B	14.78								O	P		N		M		
CB13B	18.22	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB14B	15.11								O	P		N		M		L
CB15A	16.00	K		J		I		H	O	P	G	N		M		L
CB16B	16.67	K	E	J		I		H	O	P	G	N		M	F	L
CB17A	21.44				B		D		A				C			
CB18B	16.89	K	E	J		I	D	H	O	P	G	N		M	F	L
CB19B	20.00		E		B		D		A		G		C		F	
CB20A	17.33	K	E	J		I	D	H	O	P	G	N		M	F	L
CB21B	20.45		E		B		D		A		G		C		F	
CB22B	18.00	K	E	J	B	I	D	H	O		G	N	C	M	F	L
CB23A	17.33	K	E	J		I	D	H	O	P	G	N	C	M	F	L
CB24B	14.67								O	P		N				
CB26A	16.89	K	E	J		I	D	H	O	P	G	N		M	F	L
CB27A	19.33	K	E	J	B	I	D	H	A		G		C		F	
CB28A	17.33	K	E	J		I	D	H	O	P	G	N		M	F	L
CB29B	18.67	K	E	J	B	I	D	H	A		G		C	M	F	L
CB30B	19.33	K	E	J	B	I	D	H	A		G		C		F	
CB31B	17.78	K	E	J	B	I	D	H	O	P	G	N	C	M	F	L
CB32A	19.55		E		B	I	D	H	A		G		C		F	
CB33A	19.33		E	J	B	I	D	H	A		G		C		F	
CB34B	14.67								O	P		N				
CB35A	18.89	K	E	J	B	I	D	H	A		G		C		F	L
CB36A	14.33								O	P						
CB37A	19.67		E		B		D	H	A		G		C		F	
CB38B	15.55	K		J		I			O	P		N		M		L
CB39A	15.11								O	P		N		M		L

CB40A	15.78	K		J		I		H	O	P		N		M		L
CB41A	16.22	K		J		I		H	O	P	G	N		M	F	L
CB42A	15.22	K							O	P		N		M		L
CB43B	16.22	K		J		I		H	O	P	G	N		M	F	L
CB44B	16.22	K		J		I		H	O	P	G	N		M		L
CB45A	15.33	K		J					O	P		N		M		L
CB46B	16.22	K		J		I		H	O	P	G	N		M	F	L
CB47A	16.22	K		J		I		H	O	P	G	N		M	F	L
CB48B	17.33	K	E	J		I	D	H	O	P	G	N		M	F	L
CB49A	23.00								A							
CB50B	16.67	K	E	J		I	D	H	O	P	G	N		M	F	L
CB51A	17.11	K	E	J		I	D	H	O	P	G	N		M	F	L
CB52B	22.55								A							
CB53B	17.11	K	E	J		I	D	H	O	P	G	N		M	F	L
CB54B	20.45		E		B		D		A				C		F	
CB55A	16.00	K		J		I		H	O	P	G	N		M		L
CB56A	14.89								O	P		N		M		L
CB57B	20.22		E		B		D		A		G		C		F	
CB58B	19.56		E		B	I	D	H	A		G		C		F	
CB59B	16.44	K		J		I		H	O	P	G	N		M	F	L
CB60A	16.89	K	E	J		I	D	H	O	P	G	N		M	F	L
CB61B	15.78	K		J		I		H	O	P		N		M		L
CB62B	21.78				B				A				C			
CB63A	18.00	K	E	J	B	I	D	H	O	P	G	N	C	M	F	L
CB64B	17.11	K	E	J		I	D	H	O	P	G	N		M	F	L
CB65B	17.56	K	E	J		I	D	H	O	P	G	N		M	F	L
CB66A	17.11	K	E	J		I	D	H	O	P	G	N		M	F	L
CB67A	16.89	K	E	J		I	D	H	O	P	G	N		M	F	L
CB68A	17.33	K	E	J		I	D	H	O	P	G	N		M	F	L
CB69A	17.33	K	E	J		I	D	H	O	P	G	N		M	F	L
CB70B	15.78	K		J		I		H	O	P		N		M		L
CB71A	15.33	K		J					O	P		N		M		L
CB72B	15.78	K		J		I		H	O	P		N		M		L
CB73A	14.89								O	P		N		M		L
CB74A	16.67	K	E	J		I		H	O	P	G	N		M	F	L
CB75A	22.17				B				A							
CB76B	15.11								O	P		N		M		L
CB77A	15.56	K		J		I			O	P		N		M		L
CB78A	18.00	K	E	J	B	I	D	H	O		G	N	C	M	F	L
G-CER	15.67	K		J		I		H	O	P		N		M		L
G-OLD	15.56	K		J		I			O	P		N		M		L
TIFGU	20.33		E		B		D		A		G		C		F	

[a] Means from untransformed data for DAI until sporulation is observed on the first three lesions under 16x magnification.
[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.23 Duncan grouping for DAI until lesion diameter reaches 1mm in the RIL C1501 population, during detached leaf assay.

Genotype	DAI until lesion diameter =1mm [a]	Duncan grouping [b]														
CB01B	14.89	K		J		I		H	P	O	G	N		M	F	L
CB02B	16.56	K	E	J	B	I	D	H	A		G		C	M	F	L
CB03B	19.11		E		B		D		A				C			
CB04A	12.67								P	O						
CB05A	17.33		E	J	B	I	D	H	A		G		C		F	
CB06B	15.33	K	E	J		I	D	H	P	O	G	N		M	F	L
CB07B	16.67	K	E	J	B	I	D	H	A		G		C		F	L
CB08A	13.56	K		J					P	O		N		M		L
CB09A	16.33	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB10B	17.11		E	J	B	I	D	H	A		G		C		F	
CB11A	15.33	K	E	J		I	D	H	P	O	G	N		M	F	L
CB12B	12.78								P	O		N				
CB13B	16.45	K	E	J	B	I	D	H	A		G		C	M	F	L
CB14B	14.11	K		J		I			P	O		N		M		L
CB15A	14.89	K		J		I		H	P	O	G	N		M	F	L
CB16B	14.22	K		J		I		H	P	O		N		M		L
CB17A	20.78				B				A							
CB18B	16.44	K	E	J	B	I	D	H	A		G		C	M	F	L
CB19B	18.45		E		B		D		A		G		C		F	
CB20A	15.56	K	E	J		I	D	H	P	O	G	N	C	M	F	L
CB21B	18.67		E		B		D		A				C		F	
CB22B	15.33	K	E	J		I	D	H	P	O	G	N		M	F	L
CB23A	15.11	K	E	J		I		H	P	O	G	N		M	F	L
CB24B	13.33	K							P	O		N		M		L
CB26A	15.44	K	E	J		I	D	H	P	O	G	N		M	F	L
CB27A	16.22	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB28A	16.44	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB29B	16.89	K	E	J	B	I	D	H	A		G		C		F	
CB30B	15.34	K	E	J		I	D	H	P	O	G	N		M	F	L
CB31B	18.22		E		B		D	H	A		G		C		F	
CB32A	17.11		E	J	B	I	D	H	A		G		C		F	
CB33A	16.78	K	E	J	B	I	D	H	A		G		C		F	L
CB34B	12.56								P							
CB35A	16.45	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB36A	13.33	K							P	O		N		M		L
CB37A	15.33	K	E	J		I	D	H	P	O	G	N		M	F	L
CB38B	14.33	K		J		I		H	P	O		N		M		L
CB39A	14.11	K		J		I			P	O		N		M		L
CB40A	14.67	K		J		I		H	P	O	G	N		M	F	L

CB41A	14.33	K		J		I		H	P	O		N		M		L
CB42A	13.67	K		J					P	O		N		M		L
CB43B	15.78	K	E	J	B	I	D	H	P	O	G	N	C	M	F	L
CB44B	15.56	K	E	J		I	D	H	P	O	G	N		M	F	L
CB45A	17.11	K	E	J	B	I	D	H	A		G		C		F	
CB46B	16.00	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB47A	14.44	K		J		I		H	P	O	G	N		M		L
CB48B	15.11	K	E	J		I		H	P	O	G	N		M	F	L
CB49A	20.00				B				A				C			
CB50B	16.22	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB51A	15.11	K	E	J		I		H	P	O	G	N		M	F	L
CB52B	19.33				B		D		A				C			
CB53B	18.00		E		B	I	D	H	A		G		C		F	
CB54B	17.33		E	J	B	I	D	H	A		G		C		F	
CB55A	15.33	K	E	J		I	D	H	P	O	G	N		M	F	L
CB56A	12.55								P							
CB57B	17.22		E	J	B	I	D	H	A		G		C		F	
CB58B	16.78	K	E	J	B	I	D	H	A		G		C		F	L
CB59B	16.22	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB60A	15.11	K	E	J		I		H	P	O	G	N		M	F	L
CB61B	14.56	K		J		I		H	P	O	G	N		M	F	L
CB62B	15.33	K	E	J		I	D	H	P	O	G	N		M	F	L
CB63A	17.33		E	J	B	I	D	H	A		G		C		F	
CB64B	16.44	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB65B	16.78	K	E	J	B	I	D	H	A		G		C		F	L
CB66A	16.44	K	E	J	B	I	D	H	A		G		C	M	F	L
CB67A	14.22	K		J		I		H	P	O		N		M		L
CB68A	16.78	K	E	J	B	I	D	H	A		G		C		F	L
CB69A	16.11	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB70B	15.00	K		J		I		H	P	O	G	N		M	F	L
CB71A	15.22	K	E	J		I	D	H	P	O	G	N		M	F	L
CB72B	15.33	K	E	J		I	D	H	P	O	G	N		M	F	L
CB73A	12.89								P	O		N		M		
CB74A	15.33	K	E	J		I	D	H	P	O	G	N		M	F	L
CB75A	15.56	K	E	J		I	D	H	P	O	G	N		M	F	L
CB76B	13.11								P	O		N		M		L
CB77A	14.00	K		J					P	O		N		M		L
CB78A	16.22	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
G-CER	14.84	K		J		I		H	P	O	G	N		M	F	L
G-OLD	14.22	K		J		I		H	P	O		N		M		L
TIFGU	20.22								A							

[a] Means from untransformed data for DAI until the first three lesions reach 1mm in diameter.
[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on log transformed data.

Table 3.24 Duncan grouping for sporulation index in the RIL C1501 population, during detached leaf assay.

Genotype	Sporulation index [a]	Duncan grouping [b]								
CB01B	3.45	E	B		D		A		C	
CB02B	2.67	E		I	D	H	J	G	C	F
CB03B	2.66	E		I	D	H	J	G	C	F
CB04A	3.89		B				A			
CB05A	3.00	E	B		D	H	A	G	C	F
CB06B	3.89		B				A			
CB07B	3.56		B		D		A		C	
CB08A	4.00						A			
CB09A	1.78		K	I	M		J		L	
CB10B	1.89		K	I	M	H	J		L	
CB11A	3.11	E	B		D		A	G	C	F
CB12B	3.78		B				A		C	
CB13B	2.33	E	K	I		H	J	G		F
CB14B	4.00						A			
CB15A	3.67		B		D		A		C	
CB16B	3.00	E	B		D	H	A	G	C	F
CB17A	2.22		K	I		H	J	G	L	F
CB18B	3.22	E	B		D		A		C	F
CB19B	1.78		K	I	M		J		L	
CB20A	2.67	E		I	D	H	J	G	C	F
CB21B	1.11				M				L	
CB22B	3.00	E	B		D	H	A	G	C	F
CB23A	3.22	E	B		D		A		C	F
CB24B	3.67		B		D		A		C	
CB26A	3.89		B				A			
CB27A	1.56		K		M		J		L	
CB28A	3.55		B		D		A		C	
CB29B	2.67	E		I	D	H	J	G	C	F
CB30B	1.33		K		M				L	
CB31B	3.67		B		D		A		C	
CB32A	1.78		K	I	M		J		L	
CB33A	2.56	E		I	D	H	J	G		F
CB34B	3.56		B		D		A		C	
CB35A	2.22		K	I		H	J	G	L	F
CB36A	3.89		B				A			
CB37A	0.89				M					
CB38B	3.56		B		D		A		C	
CB39A	3.33	E	B		D		A		C	F
CB40A	4.00						A			

CB41A	3.56		B		D		A		C	
CB42A	3.89		B				A			
CB43B	3.55		B		D		A		C	
CB44B	3.78		B				A		C	
CB45A	4.00						A			
CB46B	2.78	E	B	I	D	H		G	C	F
CB47A	2.89	E	B	I	D	H	A	G	C	F
CB48B	3.67		B		D		A		C	
CB49A	1.00				M					
CB50B	3.11	E	B		D		A	G	C	F
CB51A	3.34	E	B		D		A		C	F
CB52B	2.00		K	I	M	H	J	G	L	
CB53B	2.89	E	B	I	D	H	A	G	C	F
CB54B	2.33	E	K	I		H	J	G		F
CB55A	3.67		B		D		A		C	
CB56A	3.89		B				A			
CB57B	1.56		K		M		J		L	
CB58B	1.78		K	I	M		J		L	
CB59B	3.78		B				A		C	
CB60A	3.67		B		D		A		C	
CB61B	3.33	E	B		D		A		C	F
CB62B	1.22		K		M				L	
CB63A	3.44	E	B		D		A		C	
CB64B	2.56	E		I	D	H	J	G		F
CB65B	2.22		K	I		H	J	G	L	F
CB66A	3.55		B		D		A		C	
CB67A	3.33	E	B		D		A		C	F
CB68A	3.89		B				A			
CB69A	2.67	E		I	D	H	J	G	C	F
CB70B	3.67		B		D		A		C	
CB71A	3.22	E	B		D		A		C	F
CB72B	3.44	E	B		D		A		C	
CB73A	3.11	E	B		D		A	G	C	F
CB74A	4.00						A			
CB75A	0.89				M					
CB76B	3.33	E	B		D		A		C	F
CB77A	3.56		B		D		A		C	
CB78A	2.67	E		I	D	H	J	G	C	F
G-CER	3.67		B		D		A		C	
G-OLD	3.22	E	B		D		A		C	F
TIFGU	1.11				M				L	

[a] Means from untransformed data for degree of sporulation (0-4 scale) on three oldest lesions.
[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on untransformed data.

Table 3.25 Duncan grouping for sporulation percentage in the RIL C1501 population, during detached leaf assay.

Genotype	Sporulation (%) [a]	Duncan grouping [b]																								
CB01B	98.15				E				B				D				A				C		F			
CB02B	79.59	W	K	V	E	U	J	T	Z	S	I	R		Q	H	P	Y	O	G	N	A	M	F	L	X	
CB03B	68.08	W	K	V		U		T	Z	S		R	B	Q		P	Y	O		N	A	M		L	X	
CB04A	97.22				E		J		B		I		D		H		A		G		C		F			
CB05A	93.18		K		E		J		B		I		D		H		A		G		C		F	L		
CB06B	94.76		K		E		J		B		I		D		H		A		G		C	M	F	L		
CB07B	88.99		K		E		J		B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L		
CB08A	99.78								B								A									
CB09A	76.28	W	K	V		U	J	T	Z	S	I	R	B	Q	H	P	Y	O		N	A	M		L	X	
CB10B	67.71	W		V		U		T	Z	S		R	B	Q		P	Y	O		N	A	M			X	
CB11A	87.48	W	K	V	E	U	J	T	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L		
CB12B	98.20				E				B				D				A		G		C		F			
CB13B	68.36	W		V		U		T	Z	S		R	B	Q		P	Y	O		N	A	M		L	X	
CB14B	96.48				E				B		I		D		H		A		G		C		F			
CB15A	90.99		K		E		J		B		I		D		H	P	A	O	G	N	C	M	F	L		
CB16B	72.01	W	K	V		U	J	T	Z	S	I	R	B	Q		P	Y	O		N	A	M		L	X	
CB17A	70.82	W	K	V	E	U	J	T	Z	S	I	R	D	Q	H	P	Y	O	G	N		M	F	L	X	
CB18B	90.74		K		E		J		B		I		D		H		A		G		C	M	F	L		
CB19B	51.87	W		V					Z				B				Y				A				X	
CB20A	66.90	W		V		U		T	Z	S		R	B	Q		P	Y	O		N	A				X	
CB21B	26.75												B								A					
CB22B	85.40	W	K	V	E	U	J	T	B	S	I	R	D	Q	H	P	Y	O	G	N	C	M	F	L	X	
CB23A	94.13		K		E		J		B		I		D		H		A		G	N	C	M	F	L		
CB24B	83.30		K		E		J		B		I		D		H	P	A	O	G	N	C	M	F	L		
CB26A	95.79		K		E		J		B		I		D		H		A		G		C		F			
CB27A	56.87	W		V		U		T	Z	S			B				Y				A				X	
CB28A	89.55		K		E		J		B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L		

CB29B	56.35	W		V		U		T	Z				B				Y				A				X
CB30B	44.90								Z				B				Y				A				X
CB31B	85.14	W	K	V	E	U	J	T	B	S	I	R	D	Q	H	P	Y	O	G	N	C	M	F	L	X
CB32A	77.33	W	K	V		U	J	T	Z	S	I	R		Q	H	P	Y	O	G	N	A	M		L	X
CB33A	64.11	W		V		U		T	Z	S		R	B	Q			Y				A				X
CB34B	88.57	W	K	V	E	U	J	T	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	X
CB35A	70.12	W		V		U		T	Z	S		R	B	Q		P	Y	O		N	A	M		L	X
CB36A	98.40				E				B				D				A				C				
CB37A	35.54								Z				B								A				
CB38B	83.79	W	K	V	E	U	J	T	Z	S	I	R	D	Q	H	P	Y	O	G	N	C	M	F	L	X
CB39A	88.81	W	K	V	E	U	J	T	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	X
CB40A	100.00																A								
CB41A	86.08		K	V	E	U	J	T	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	
CB42A	97.17				E				B				D				A		G		C		F		
CB43B	97.95				E				B				D		H		A		G		C		F		
CB44B	96.39				E				B				D				A		G		C		F		
CB45A	96.75				E		J		B		I		D		H		A		G		C		F		
CB46B	86.64		K		E		J	T	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	
CB47A	86.90	W	K	V	E	U	J	T	B	S	I	R	D	Q	H	P	Y	O	G	N	C	M	F	L	X
CB48B	84.57		K	V	E	U	J	T	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	
CB49A	19.91												B												
CB50B	63.25	W		V		U		T	Z	S		R	B	Q		P	Y				A				X
CB51A	83.58		K	V	E	U	J	T	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	
CB52B	66.06	W		V		U		T	Z	S		R	B	Q		P	Y	O			A				X
CB53B	96.83				E				B				D				A		G		C		F		
CB54B	73.55	W		V		U		T	Z				B				Y				A				X
CB55A	94.21		K		E		J		B		I		D		H		A		G		C	M	F	L	
CB56A	95.06		K		E		J		B		I		D	Q	H	P	A	O	G	N	C	M	F	L	
CB57B	52.43	W		V		U			Z				B				Y				A				X
CB58B	52.40	W		V					Z				B				Y				A				X
CB59B	97.63				E				B		I		D		H		A		G		C		F		

CB60A	80.73	W	K	V		U	J	T	Z	S	I	R		Q	H	P	Y	O	G	N	A	M	F	L	X
CB61B	97.06				E		J		B		I		D		H		A		G		C		F		
CB62B	50.18	W		V		U		T	Z				B				Y				A				X
CB63A	70.17	W	K	V		U	J	T	Z	S		R	B	Q		P	Y	O		N	A	M		L	X
CB64B	73.91	W	K	V		U	J	T	Z	S	I	R	B	Q		P	Y	O		N	A	M		L	X
CB65B	63.29	W		V		U		T	Z	S		R	B				Y				A				X
CB66A	98.32				E				B				D				A				C				
CB67A	91.92		K		E		J		B		I	R	D	Q	H	P	A	O	G	N	C	M	F	L	
CB68A	90.28		K		E		J		B		I		D		H		A		G		C		F		
CB69A	76.45	W	K	V	E	U	J	T	Z	S	I	R		Q	H	P	Y	O	G	N	A	M	F	L	X
CB70B	97.61				E				B				D				A		G		C		F		
CB71A	81.30	W	K	V	E	U	J	T	Z	S	I	R		Q	H	P	Y	O	G	N	A	M	F	L	X
CB72B	91.22		K		E		J	T	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	
CB73A	91.55		K		E		J	T	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	
CB74A	99.13								B								A				C				
CB75A	49.09	W							Z				B				Y				A				X
CB76B	89.12		K		E		J		B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	
CB77A	86.86		K		E	U	J	T	B	S	I	R	D	Q	H	P	A	O	G	N	C	M	F	L	
CB78A	62.31								Z				B				Y				A				
G-CER	98.41								B				D				A				C				
G-OLD	91.38		K		E		J		B		I		D		H		A	O	G	N	C	M	F	L	
TIFGU	42.90								Z				B				Y				A				

[a]Means from untransformed data for percentage of sporulating lesions on four leaflets, 29 DAI.

[b]Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on $(\sin^{-1}\sqrt{x})^2$ transformed data.

Table 3.26 Duncan grouping for sporulation percentage in the RIL C1501 population, during detached leaf assay.

Genotype	Diseased leaf area (%) [a]	Duncan grouping [b]														
CB01B	11.39		E		B		D		A				C			
CB02B	4.54	K	E	J		I	D	H		O	G	N	C	M	F	L
CB03B	3.23	K		J		I				O		N		M		L
CB04A	10.20		E		B	I	D	H	A		G		C		F	
CB05A	5.42	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB06B	5.37	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB07B	10.28		E		B	I	D	H	A		G		C		F	
CB08A	11.04		E		B		D		A		G		C		F	
CB09A	3.56	K		J		I		H		O	G	N		M		L
CB10B	3.93	K		J		I		H		O	G	N		M	F	L
CB11A	4.81	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB12B	10.34		E	J	B	I	D	H	A		G		C		F	
CB13B	7.82	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB14B	10.41		E	J	B	I	D	H	A		G		C		F	
CB15A	7.07	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB16B	3.79	K	E	J		I		H		O	G	N		M	F	L
CB17A	7.19	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB18B	10.96		E		B		D		A		G		C		F	
CB19B	3.77	K	E	J		I		H		O	G	N		M	F	L
CB20A	5.88	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB21B	2.10									O		N				
CB22B	6.98	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB23A	7.48	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB24B	12.90				B				A							
CB26A	8.05	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB27A	2.85	K		J						O		N		M		L
CB28A	3.50	K		J		I		H		O		N		M		L
CB29B	5.49	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB30B	5.10	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB31B	3.15	K		J		I		H		O		N		M		L
CB32A	3.03	K		J		I				O		N		M		L
CB33A	4.25	K	E	J	B	I	D	H		O	G	N	C	M	F	L
CB34B	7.58	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB35A	5.78	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB36A	11.32		E		B		D		A				C		F	
CB37A	3.74	K	E	J		I		H		O	G	N		M	F	L
CB38B	4.08	K	E	J		I	D	H		O	G	N		M	F	L

CB39A	8.63	K	E	J	B	I	D	H	A		G		C	M	F	L
CB40A	12.34				B				A				C			
CB41A	11.02		E	J	B	I	D	H	A		G		C		F	
CB42A	10.55		E		B	I	D	H	A		G		C		F	
CB43B	8.58	K	E	J	B	I	D	H	A		G		C		F	L
CB44B	8.69	K	E	J	B	I	D	H	A		G		C		F	L
CB45A	6.37	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB46B	6.82	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB47A	6.29	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB48B	12.85								A							
CB49A	2.48									O		N		M		
CB50B	5.13	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB51A	6.38	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB52B	2.12									O						
CB53B	4.67	K	E	J	B	I	D	H		O	G	N	C	M	F	L
CB54B	4.62	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB55A	7.36	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB56A	12.34				B		D		A				C			
CB57B	2.99	K								O		N		M		L
CB58B	3.11	K		J		I		H		O		N		M		L
CB59B	8.29	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB60A	4.64	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB61B	9.17	K	E	J	B	I	D	H	A		G		C		F	L
CB62B	7.22	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB63A	5.79	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB64B	5.20	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB65B	4.85	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB66A	8.75	K	E	J	B	I	D	H	A		G		C		F	L
CB67A	10.42		E		B		D	H	A		G		C		F	
CB68A	9.78	K	E	J	B	I	D	H	A		G		C		F	L
CB69A	6.93	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB70B	10.66		E		B		D	H	A		G		C		F	
CB71A	6.98	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB72B	10.53		E		B	I	D	H	A		G		C		F	
CB73A	10.98		E		B		D		A				C		F	
CB74A	10.78	K	E	J	B	I	D	H	A		G		C		F	
CB75A	7.86	K	E	J	B	I	D	H	A		G	N	C	M	F	L
CB76B	11.59		E		B		D		A				C			
CB77A	6.56	K	E	J	B	I	D	H	A	O	G	N	C	M	F	L
CB78A	3.85	K		J		I		H		O	G	N		M	F	L
G-CER	9.43		E	J	B	I	D	H	A		G		C		F	
G-OLD	10.17		E		B	I	D	H	A		G		C		F	
TIFGU	2.74									O		N		M		L

[a]Means from untransformed data for percent leaf area affected by late leaf spot lesions, 29 days after inoculation (DAI).
[b]Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on square root transformed data.

Table 3.27 Duncan grouping for lesion diameter of 5 largest late leaf spot lesions in the RIL C1501 population, during detached leaf assay.

Genotype	Lesion diameter [a]	Duncan grouping [b]															
CB01B	0.238	K	E	J		I	D	H	P	Q	O	G	N	C	M	F	L
CB02B	0.223	K		J		I		H	P	Q	O	G	N		M	F	L
CB03B	0.202				R				P	Q	O		N		M		
CB04A	0.295				B					A							
CB05A	0.212	K		J					P	Q	O		N		M		L
CB06B	0.228	K		J		I		H	P	Q	O	G	N		M	F	L
CB07B	0.270		E		B	I	D	H		A		G		C		F	
CB08A	0.263	K	E	J	B	I	D	H		A		G		C		F	L
CB09A	0.220	K		J		I		H	P	Q	O		N		M		L
CB10B	0.240	K	E	J	B	I	D	H	P	Q	O	G	N	C	M	F	L
CB11A	0.233	K	E	J		I		H	P	Q	O	G	N		M	F	L
CB12B	0.289		E		B		D			A				C			
CB13B	0.222	K		J		I		H	P	Q	O	G	N		M	F	L
CB14B	0.292				B		D			A				C			
CB15A	0.256	K	E	J	B	I	D	H		A		G		C	M	F	L
CB16B	0.228	K		J		I		H	P	Q	O	G	N		M	F	L
CB17A	0.157				R												
CB18B	0.274		E		B		D	H		A		G		C		F	
CB19B	0.226	K		J		I		H	P	Q	O	G	N		M	F	L
CB20A	0.216	K		J		I			P	Q	O		N		M		L
CB21B	0.221	K		J		I		H	P	Q	O	G	N		M		L
CB22B	0.244	K	E	J	B	I	D	H	P	A	O	G	N	C	M	F	L
CB23A	0.251	K	E	J	B	I	D	H	P	A	O	G	N	C	M	F	L
CB24B	0.252	K	E	J	B	I	D	H		A	O	G	N	C	M	F	L
CB26A	0.229	K		J		I		H	P	Q	O	G	N		M	F	L
CB27A	0.206				R				P	Q	O		N		M		L
CB28A	0.237	K	E	J		I	D	H	P	Q	O	G	N		M	F	L
CB29B	0.252	K	E	J	B	I	D	H		A	O	G	N	C	M	F	L
CB30B	0.221	K		J		I		H	P	Q	O	G	N		M		L
CB31B	0.222	K		J		I		H	P	Q	O	G	N		M	F	L
CB32A	0.203				R				P	Q	O		N		M		
CB33A	0.256	K	E	J	B	I	D	H		A		G		C	M	F	L
CB34B	0.264	K	E	J	B	I	D	H		A		G		C		F	
CB35A	0.238	K	E	J		I	D	H	P	Q	O	G	N	C	M	F	L
CB36A	0.297									A							
CB37A	0.204				R				P	Q	O		N		M		
CB38B	0.250	K	E	J	B	I	D	H	P	A	O	G	N	C	M	F	L
CB39A	0.268		E	J	B	I	D	H		A		G		C		F	

CB40A	0.261	K	E	J	B	I	D	H		A		G		C		F	L
CB41A	0.252	K	E	J	B	I	D	H		A	O	G	N	C	M	F	L
CB42A	0.294				B					A				C			
CB43B	0.251	K	E	J	B	I	D	H	P	A	O	G	N	C	M	F	L
CB44B	0.240	K	E	J	B	I	D	H	P	Q	O	G	N	C	M	F	L
CB45A	0.258	K	E	J	B	I	D	H		A		G		C	M	F	L
CB46B	0.244	K	E	J	B	I	D	H	P	A	O	G	N	C	M	F	L
CB47A	0.246	K	E	J	B	I	D	H	P	A	O	G	N	C	M	F	L
CB48B	0.234	K	E	J		I		H	P	Q	O	G	N		M	F	L
CB49A	0.194				R				P	Q							
CB50B	0.227	K		J		I		H	P	Q	O	G	N		M	F	L
CB51A	0.239	K	E	J	B	I	D	H	P	Q	O	G	N	C	M	F	L
CB52B	0.184				R					Q							
CB53B	0.211	K		J					P	Q	O		N		M		L
CB54B	0.223	K		J		I		H	P	Q	O	G	N		M	F	L
CB55A	0.274		E		B		D	H		A		G		C		F	
CB56A	0.295				B					A							
CB57B	0.196				R				P	Q	O						
CB58B	0.223	K		J		I		H	P	Q	O	G	N		M	F	L
CB59B	0.245	K	E	J	B	I	D	H	P	A	O	G	N	C	M	F	L
CB60A	0.221	K		J		I		H	P	Q	O	G	N		M		L
CB61B	0.257	K	E	J	B	I	D	H		A		G		C	M	F	L
CB62B	0.207	K			R				P	Q	O		N		M		L
CB63A	0.218	K		J		I		H	P	Q	O		N		M		L
CB64B	0.242	K	E	J	B	I	D	H	P	A	O	G	N	C	M	F	L
CB65B	0.210	K			R				P	Q	O		N		M		L
CB66A	0.239	K	E	J	B	I	D	H	P	Q	O	G	N	C	M	F	L
CB67A	0.246	K	E	J	B	I	D	H	P	A	O	G	N	C	M	F	L
CB68A	0.259	K	E	J	B	I	D	H		A		G		C	M	F	L
CB69A	0.244	K	E	J	B	I	D	H	P	A	O	G	N	C	M	F	L
CB70B	0.268		E	J	B	I	D	H		A		G		C		F	
CB71A	0.263	K	E	J	B	I	D	H		A		G		C		F	L
CB72B	0.295				B					A							
CB73A	0.254	K	E	J	B	I	D	H		A		G	N	C	M	F	L
CB74A	0.278		E		B		D			A		G		C		F	
CB75A	0.225	K		J		I		H	P	Q	O	G	N		M	F	L
CB76B	0.278		E		B		D			A				C		F	
CB77A	0.256	K	E	J	B	I	D	H		A		G		C	M	F	L
CB78A	0.219	K		J		I		H	P	Q	O		N		M		L
G-CER	0.272		E		B	I	D	H		A		G		C		F	
G-OLD	0.244	K	E	J	B	I	D	H	P	A	O	G	N	C	M	F	L
TIFGU	0.198				R				P	Q	O		N				

[a] Means from untransformed data for mean diameter of five largest lesions.
[b] Means within a column with the same letter do not differ at P=0.05 as determined by analyses of variance and Duncan's multiple range test procedures on untransformed data.

Table 3.28 Correlation coefficients between the four Florida (1-10) scale ratings (FSRI-IV) for disease severity and area under disease progress (AUDPC) due to late leaf spot in the RIL C1501 population in 2011 and 2012.

Year		2012 [b]				
	Source	FSRI	FSRII	FSRIII	FSRIV	AUDPC
2011 [a]	FSRI	-0.279***	-0.260***	-0.211***	-0.263***	-0.268***
	FSRII	0.242***	0.269***	0.319***	0.230***	0.299***
	FSRIII	0.096	0.201***	0.247***	0.187*	0.218***
	FSRIV	0.239***	0.350***	0.388***	0.326***	0.374***
	AUDPC	0.169**	0.244***	0.300***	0.214***	0.269***

[a] *r* – values for Florida scale ratings taken at DAP (FSRI), DAP (FSRII), DAP (FSRIII), 141 DAP (FSRIV), area under disease progress values (AUDPC) in 2011.

[b] *r* – values for Florida scale ratings taken at DAP (FSRI), DAP (FSRII), DAP (FSRIII), 141 DAP (FSRIV), area under disease progress values (AUDPC) in 2012

*, **, *** = Significant at 0.05, 0.01 and 0.001 probability levels, respectively.

Table 3.29 Correlation coefficients between percent diseased leaf area, infection frequency measured during two lateral stem assays and corresponding disease severity ratings ,taken on Florida (1-10)scale, and disease progress values (AUDPC) due to late leaf spot in field trial 2011.

	2011				2011		
LSAI	FSRII [g]	FSRIV [i]	AUDPC [j]	LSAII	FSRIII [h]	FSRIV	AUDPC
Diseased leaf area (%) [a]	0.681***	0.604***	0.684***	Diseased leaf area (%) [d]	0.678***	0.750***	0.748***
Infection frequency [b]	0.389***	0.417***	0.408***	Infection frequency [e]	0.661***	0.720***	0.724***
Defoliation (%) [c]	0.505***	0.431***	0.539***	Defoliation (%) [f]	0.674***	0.691***	0.747***

[a, b, c] *r* – values for percent leaf area affected by late leaf spot (LLS), number of lesions per unit leaf area (cm^2), and percent defoliation, respectively, in first lateral stem assay (LSAI), 118-120 days after planting (DAP).

[d, e, f] *r* – values for percent leaf area affected by LLS, number of lesions per unit leaf area (cm^2), and percent defoliation, respectively, in second lateral stem assay (LSAII), 132-134 DAP.

[g, h] *r* – values for corresponding disease severity ratings, FSRII (110 DAP) and FSRIII (130 DAP), closest in time to LSAI and LSAII respectively in 2011.

[I, j] *r* – values for fourth disease severity rating (FSRIV) and disease progress values (AUDPC) in 2011.

*, **, *** = Significant at 0.05, 0.01 and 0.001 probability levels, respectively.

Table 3.30 Correlation coefficients between percent diseased area, infection frequency, and percent defoliation due to late leaf spot measured, in the RIL C1501 population, during two lateral stem assays in field season 2011.

		LSAII		
		Diseased leaf area (%) [d]	Infection frequency [e]	Defoliation (%) [f]
LSAI	Diseased leaf area (%) [a]	0.576***	0.557***	0.538***
	Infection frequency [b]	0.336***	0.322***	0.348***
	Defoliation (%) [c]	0.400***	0.349***	0.451***

[a, b, c] *r* – values for percent leaf area affected by late leaf spot (LLS), number of lesions per unit leaf area (cm^2), and percent defoliation, respectively, in first lateral stem assay (LSAI), 118-120 days after planting (DAP).

[d, e, f] *r* – values for percent leaf area affected by LLS, number of lesions per unit leaf area (cm^2), and percent defoliation, respectively, in second lateral stem assay (LSAII), 132-134 DAP.

*, **, *** = Significant at 0.05, 0.01 and 0.001 probability levels, respectively.

Table 3.31 Correlation coefficients among the components of resistance to LLS measured during two lateral stem assays.

LSAI	Diseased leaf area (%) [a]	Infection frequency [b]	Defoliation (%) [c]	LSAII	Diseased leaf area (%) [d]	Infection frequency [e]	Defoliation (%) [f]
Diseased leaf area (%) [a]	-	0.399***	0.448***	Diseased leaf area (%) [d]	-	0.951***	0.710***
Infection frequency [b]	0.399***	-	0.297***	Infection frequency [e]	0.951***	-	0.694***
Defoliation (%) [c]	0.448***	0.297***	-	Defoliation (%) [f]	0.710***	0.694***	-

[a, b, c] *r* – values for percent leaf area affected by late leaf spot (LLS), number of lesions per unit leaf area (cm^2), and percent defoliation, respectively, in first lateral stem assay (LSAI), 118-120 days after planting (DAP).

[d, e, f] *r* – values for percent leaf area affected by LLS, number of lesions per unit leaf area (cm^2), and percent defoliation, respectively, in second lateral stem assay (LSAII), 132-134 DAP.

*, **, *** = Significant at 0.05, 0.01 and 0.001 probability levels, respectively.

Table 3.32 Correlation coefficients between components of resistance to late leaf spot (LLS) measured in growth chamber and fourth disease severity ratings (Florida 1-10 scale rating, FSRIV) and disease progress values (AUDPC) measured in field trials across 2 growing seasons (2011-2012).

		2011		2012	
		FSRIV [a]	AUDPC	FSRIV [b]	AUDPC
Detached leaf assay	Diseased leaf area (%) [c]	0.010	-0.109	0.240***	0.263***
	Infection frequency [d]	-0.097	-0.184**	0.088	0.120
	Incubation period [e]	-0.079	-0.057	-0.158*	-0.183**
	Latent period (16x) [f]	-0.226***	-0.086	-0.373***	-0.357***
	Latent period (2x) [g]	-0.274***	-0.129*	-0.385***	-0.406***
	Sporulation index [h]	0.340***	0.246***	0.494***	0.504***
	Sporulation (%) [i]	0.350***	0.237***	0.486***	0.469***
	Lesion diameter (mm) [j]	0.100	0.003	0.302***	0.326***
	DAI until lesion diameter=1mm [k]	-0.143*	-0.055	-0.221***	-0.229***

[a ,b] *r*- values for fourth Florida 1-10 scale ratings (FSRIV), 141 days after planting in 2011 and 2012.
[c] *r*– values for percent leaf area affected by LLS, 29 days after inoculation (DAI).
[d] *r* – values for number of lesions per unit leaf area (cm^2), 29 DAI.
[e] *r* – values for mean DAI until the first three lesions appear.
[f, g] *r* – values for mean DAI until sporulation is observed on the first three lesions, under 2x and 16x magnifications respectively
[h] *r* – values for mean degree of sporulation (0-4 scale) on three oldest lesions.
[i] *r* – values for mean percentage of sporulating lesions on four leaflets, 29 DAI.
[i] *r* – values for mean diameter of five largest lesions, 29 DAI.
[j] *r* – values for mean DAI until the first three lesions reach 1mm in diameter.
*, **, *** = Significant at 0.05, 0.01 and 0.001 probability levels, respectively.

Table 3.33 Correlation coefficients among the components of resistance to LLS measured by detached leaf assay.

Detached leaf assay	Diseased leaf area (%)	Infection frequency	Incubation period	Latent period (16x)	Latent period (2x)	Sporulation index	Sporulation (%)	Lesion diameter (mm)
Diseased leaf area (%) [a]	-	-	-	-	-	-	-	-
Infection frequency [b]	0.818***	-	-	-	-	-	-	-
Incubation period [c]	-0.493***	-0.399***	-	-	-	-	-	-
Latent period (16X) [d]	-0.573***	-0.420***	0.653***	-	-	-	-	-
Latent period (2X) [e]	-0.617***	-0.414***	0.535***	0.834***	-	-	-	-
Sporulation index [f]	0.489***	0.292***	-0.358***	-0.628***	-0.752***	-	-	-
Sporulation (%) [g]	0.512***	0.314***	-0.347***	-0.629***	-0.695***	0.799***	-	-
Lesion diameter (mm) [h]	0.624***	0.295***	-0.532***	-0.701***	-0.698***	0.546***	0.500***	-
DAI until lesion diameter=1mm [i]	-0.556***	-0.405***	0.742***	0.748***	0.635***	-0.431***	-0.427***	-0.681***

[a] *r* – values for percent leaf area affected by late leaf spot (LLS), 29 days after inoculation (DAI).
[b] *r* – values for number of lesions per unit leaf area (cm^2), 29 DAI.
[c] *r* – values for mean DAI until the first three lesions appear.
[d, e] *r* – values for mean DAI until sporulation is observed on the first three lesions, under 16x and 2x magnifications respectively
[f] *r* – values for mean degree of sporulation (0-4 scale) on three oldest lesions.
[g] *r* – values for mean percentage of sporulating lesions on four leaflets, 29 DAI.
[h] *r* – values for mean diameter of five largest lesions, 29 DAI.
[i] *r* – values for mean DAI until the first three lesions reach 1mm in diameter.

*, **, *** = Significant at 0.05, 0.01 and 0.001 probability levels, respectively.

Table 3.34 Correlation coefficients between the components of resistance to late leaf spot measured by lateral stem and detached leaf assays.

		LSAI			LSAII		
		Diseased leaf area (%) [a]	Infection frequency [c]	Defoliation (%) [e]	Diseased leaf area (%) [b]	Infection frequency [d]	Defoliation (%) [f]
Detached leaf assay	Diseased leaf area (%) [g]	-0.086	-0.074	-0.214***	0.014	0.008	-0.068
	Infection frequency [h]	-0.128*	-0.072	-0.217***	-0.066	-0.084	-0.130**
	Incubation period [i]	-0.079	-0.022	0.024	-0.057	-0.055	-0.028
	Latent period (16x) [j]	-0.078	0.017	0.086	-0.176**	-0.168**	-0.073
	Latent period (2x) [k]	-0.123	-0.049	0.068	-0.239***	-0.233***	-0.119
	Sporulation index [l]	0.207***	0.106	-0.044	0.320***	0.299***	0.201*
	Sporulation (%) [m]	0.190*	0.017	-0.032	0.273***	0.255***	0.197*
	Lesion diameter (mm) [n]	0.001	0.026	-0.170*	0.104	0.098	-0.001
	DAI until lesion diameter=1mm [o]	-0.037	0.027	0.078	-0.119	-0.109	0.008

[a, b] *r* – values for percent leaf area affected by late leaf spot (LLS), 118-120 days after planting (DAP) in first lateral stem assay (LSAI), and second lateral stem assay (LSAII), respectively.

[c, d] *r* – values for number of lesions per unit leaf area (cm^2), 118-120 DAP in LSAI and 132-134 DAP in LSAII, respectively.

[e, f] *r* – values for percent defoliation, calculated by counting the nodes (n), potential number of leaflets (n*4), and leaflets actually present(p), as defoliation percentage =((n*4) – p)* 100/n*4, 118-120 DAP in LSAI and 132-134 DAP in LSAII, respectively.

[g] *r* – values for percent leaf area affected by LLS, 29 days after inoculation (DAI).

[h] *r* – values for number of lesions per unit leaf area (cm^2), 29 DAI.

[i] *r* – values for mean DAI until the first three lesions appear.

[j, k] *r* – values for mean DAI until sporulation is observed on the first three lesions, under 16x and 2x magnifications respectively

[l] *r* – values for mean degree of sporulation (0-4 scale) on three oldest lesions.

[m] *r* – values for mean percentage of sporulating lesions on four leaflets, 29 DAI.

[n] *r* – values for mean diameter of five largest lesions, 29 DAI.
[o] *r* – values for mean DAI until the first three lesions reach 1mm in diameter.
*, **, *** = Significant at 0.05, 0.01 and 0.001 probability levels, respectively.

CHAPTER 4

CONSTRUCTION OF A SSR- AND SNP-BASED GENETIC MAP AND QUANTITATIVE TRAIT LOCUS (QTL) ANALYSIS FOR LATE LEAF SPOT RESISTANCE IN CULTIVATED PEANUT (*Arachis hypogaea* L.)[2]

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Abstract

Late leaf spot (LLS), caused by *Cercosporidium personatum*, is one of the most destructive, economically important, and widespread foliar diseases of peanut. Yield losses due leaf spot diseases may range from 10% to 80%, varying geographically, between seasons, and due to availability of chemical control. A recombinant inbred line (RIL) population, derived from a cross between Gregory and Tifguard, consisting of 78 lines segregating for LLS resistance, was used for quantitative trait locus (QTL) analysis. Phenotyping data for the population was collected over two years, under natural epidemic in field trials and artificial disease epiphytotics in the growth chamber. A set of 447 simple sequence repeat (SSR) and 25 single nucleotide polymorphism (SNP) markers was used for parental screening, out of which 108 SSRs were polymorphic (24.16%). Segregation data from these markers were used to generate a linkage map with 94 loci (76 SSR and 18 SNP) distributed into 19 LGs. QTL analysis, using composite interval mapping (CIM), was performed on the genotyping and phenotyping data to identify 7 QTL (explaining 10.90-38.4% variation (PVE)) for field and growth chamber datasets. Although all the significant QTL appeared to have major effects on LLS resistance (PVE >10%), the magnitude of the effects of QTL may have been overestimated due to the small size of the population and low marker density on the linkage map.

Introduction

Cultivated peanut (*Arachis hypogaea* L.) is a major legume crop and its kernels are primarily crushed for oil or used for direct consumption in several different parts of the world (Tillman and Stalker, 2010). Peanut kernels are a rich source of oil, protein, and various minerals and vitamins (Savage and Keenan, 1994). After oil extraction,

peanut cake can be used as an animal feed (Savage and Keenan, 1994) and the haulms make a relatively palatable and protein-rich forage for cattle (Cook and Crosthwaite, 1994). Peanut grows mainly in tropical and sub-tropical regions of the world. World annual production of peanut is about 38.6 mt, with Asia, Africa and the Americas accounting for 68%, 24%, and 8%, respectively. China is the world's largest producer of peanut and contributes 42% of the world production, followed by India at 18%. The USA contributes only about 4% to the total world production. However, the trends for yield are different. Average yield for peanut in the U.S. is 3.7 t/ ha, more than twice the average yields in India and the world at 1.7 t/ ha and 1.8 t/ ha, respectively. On the other hand, average peanut yields in China are much higher than the world average and slightly lower than the U.S., at 3.4 t / ha (FAOSTAT, 2011). These variations in yields across countries may be attributed to the level of farm mechanization, management of biotic and abiotic stresses, and investment in crop improvement and research efforts (Dwivedi *et al.*, 2007).

Among biotic stresses, early leaf spot (ELS), caused by *Cercospora arachidicola* S. Hori, and late leaf spot (LLS), caused by *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton, are the most destructive and economically important foliar diseases affecting peanut (*Arachis hypogaea* L.) throughout the world (Shokes and Culbreath, 1997). Yield losses due to these diseases may range from 10% to 80%, varying geographically, among seasons, and with availability of chemical control (McDonald *et al.*, 1985; Miller *et al.*, 1990; Shokes and Culbreath, 1997). Fungicide applications can reduce or limit yield losses due to these diseases, but they represent a significant cost for producers, and may be cost-prohibitive for small-scale farmers in developing countries.

The two leaf spots occur wherever peanut is grown (Jackson and Bell, 1969; Porter *et al.,* 1984) and are often found together. Sometimes, one pathogen may be more predominant than the other, in a certain location or year, or a time of the year. This variation in occurrence of leaf spots may be influenced by changing weather patterns, cultivar selection and other management inputs (Nutter *et al.*, 1995).

Several authors (Jackson and Bell, 1969; Jenkins, 1938; McDonald *et al.*, 1985; Woodroof, 1933) have described symptoms of early and late leaf spots. Host genotype and weather conditions can influence the disease symptoms of leaf spots. In general the symptoms of early and late leaf spots start as small necrotic flecks that later develop into light brown to black sub-circular lesions on the leaflets. The symptoms extend beyond the leaflets, and disease lesions may be observed on petioles, stems, stipules, and pegs. Lesions of early leaf spot are usually surrounded by a pale yellow halo. However, this is not considered a reliable diagnostic character because the halo may be influenced by the host genetics, nutritional status of host, or weather conditions, and late leaf spot lesions may have a halo as well. In general, the lesions produced by *C. arachidicola* (CA) are circular to irregular in shape, brown in color and slightly raised on the adaxial leaflet surface, where most of sporulation occurs. They are light brown and usually smooth on the abaxial leaflet surface with fruiting structures randomly distributed on the adaxial surface. The lesions produced by *C. personatum* (CP) are circular in shape, dark brown to black and smooth on the adaxial leaflet surface, black and rough or granular on the abaxial leaflet surface, where most of the sporulation occurs. LLS lesions occur as circular rings of fruiting structures on the abaxial surface of leaflets. In both the leaf spots, as the severity of disease increases, the symptoms develop from chlorosis, through

necrosis, coalesced lesions to defoliation of the plant. Severe leaf spot attack may lead to total defoliation of the plant. Numerous pods may be lost from such defoliated plants while digging and inverting the plant. The amount of yield loss depends on how early the defoliation starts. Digging these defoliated plants before their expected maturity is usually recommended to avoid huge yield losses. However, digging early may result in poorer quality kernels.

Chemical control is historically the most prevalent method of peanut leaf spot management in the southeastern US and represents about 20% of the total variable production costs (Tillman and Stalker, 2010). As mentioned in several previous studies, it is not a sustainable disease management strategy in many developing countries (Gibbons, 1980; Subrahmanyam *et al.*, 1982).

Host resistance offers great scope in this scenario and breeding for resistance to early and late leaf spots has been a major objective in peanut breeding programs since the early 1980s (Chiteka *et al.*, 1988; Fehr, 1987; Norden *et al.*, 1982). However, incorporation of LLS disease resistance in suitable cultivars developed using conventional breeding methods has been challenging due to the polygenic and complex inheritance of the trait (Dwivedi *et al.*, 2002; Nevill, 1982; Sharief *et al.*, 1978). Although high levels of disease resistance have been identified in wild *Arachis* spp. (Abdou *et al.*, 1974; Dwivedi *et al.*, 2002; Hassan and Beute, 1977; Jackson and Bell, 1969; Pande and Rao, 2001; Sharief *et al.*, 1978), harnessing these resistance sources for widespread cultivar development is cumbersome. Owing to the additional challenges, such as linkage drag, associated with using unadapted germplasm, there has been limited

success in utilizing these resistance sources directly for peanut cultivar development (Gowda *et al.*, 2002).

In contrast to the substantial diversity that exists for various morphological, physiological, and agronomic traits in *Arachis hypogea*, relatively low genetic diversity has been observed in this species. This low-level genetic variation in cultivated peanut is mainly attributed to its evolution through a recent single polyploidization event (Kochert *et al.*, 1996) Another reason is the restricted gene flow between diploid relatives and tetraploid cultivated peanut. Moreover, years of conventional breeding and selection have led to a narrowed genetic base. Several researchers (Cuc *et al.*, 2008; Koppolu *et al.*, 2010; Singh *et al.*, 1998; Young *et al.*, 1996) have also discussed the lack of a suitable molecular marker system in peanut.

Various marker systems including, Restriction Fragment Length Polymorphisms (RFLPs) (Halward *et al.*, 1991; Herselman *et al.*, 2004; Kochert *et al.*, 1991), Random Amplification of Polymorphic (RAPDs) (Garcia *et al.*, 1995; Halward *et al.*, 1992; Raina *et al.*, 2001), Amplified Fragment Length Polymorphisms (AFLPs) (He and Prakash, 2001; Herselman *et al.*, 2004) and Simple Sequence Repeats (SSRs) (He *et al.*, 2003; Pandey *et al.*, 2012a) have been used in the past for detecting polymorphisms in cultivated peanut. Microsatellites or SSR markers are often preferred over other classes of molecular markers because they are multi-allelic, co-dominant, relatively abundant and provide extensive genome coverage (Gupta and Varshney, 2000). In peanut, SSRs are also the most informative and useful marker system available for genetic applications (Gupta and Varshney, 2000; Pandey *et al.*, 2012a). More than 4000 SSR markers are now available in peanut (Gautami *et al.*, 2012a; Pandey *et al.*, 2012a) owing to the

extensive efforts of various research groups across the globe (Pandey *et al.*, 2012a). However, less than 38% of these have been found to be polymorphic among *A. hypogaea* lines (Pandey *et al.*, 2012a). These SSR markers are a useful tool to study the genetic variability in peanut germplasm of both cultivated and wild species. Therefore, SSRs were the markers of choice for this QTL mapping study.

Single nucleotide polymorphisms (SNPs) are another marker system with potential for higher polymorphism detection and high-throughput applications in cultivated peanut (Guo *et al.*, 2011; Pandey *et al.*, 2012b; Paterson *et al.*, 2004). Therefore, a set of 25 SNP markers, including 15 SNPs from an Illumina GoldenGate SNP array developed at the University of Georgia (Peggy Ozias-Akins, pers. comm.) for tetraploid peanut, and 10 SNPs from an Illumina GoldenGate SNP array developed at the University of California-Davis (Nagy *et al.*, 2012; Pandey *et al.*, 2012b) based on tentative orthologous genes (TOGs) between diploid genotypes, was also used for mapping. Since such a small set of SNPs was used for genotyping the segregating RILs, high-throughput assay platforms such as BeadXpress and GoldenGate assays from Illumina Inc. (San Diego, CA) would not be economical. Therefore, a more flexible and cost-effective option (Hiremath *et al.*, 2012), Competitive Allele Specific PCR (KASPar) assay from KBiosciences (Hertfordshire, UK) (www.kbioscience.co.uk) was used.

Materials and Methods

Plant material

The C1501 population consisted of 78 RILs derived from the cross Gregory x Tifguard. Gregory is a high yielding virginia-type peanut cultivar but is susceptible to early leaf spot (Isleib *et al.*, 1999), whereas Tifguard is a result of the efforts to combine

resistance to root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood race 1) and tomato spotted wilt tospovirus (TSWV), the causal agent of tomato spotted wilt, in a single peanut cultivar (Holbrook *et al.,* 2008) and is moderately resistant to the early leaf spot (Li *et al*., 2012 The single seed descent (SSD) method was used to advance the population from F_2 to $F_{4,}$ followed by advancing the material from $F_{4:5}$ to $F_{7:8}$ by harvesting individual plants and increasing seed in winter nurseries over the next two years. The plant material was advanced from $F_{7:8}$ to $F_{7:10}$ by bulk harvesting plots from 2010-2012. Field phenotyping was done on $F_{7:9}$ and $F_{7:10}$ generations in 2011 and 2012 respectively, whereas the growth chamber study was conducted on $F_{7:9}$ generation.

The C1501 RIL population was evaluated for late leaf spot resistance at the University of Georgia Coastal Plain Experiment Station, Gibbs Farm in Tifton for two consecutive years. Planting dates were May 25, 2011 and May 22, 2012. Experimental design for the field trials in both years was a randomized block design with three replications. Planting was done in 2.79 m^2 rectangular plots that consisted of two rows (1.52 m each). Forty seeds were planted in each of these plots, such that there were 20 seeds at uniform distance per row. Plots were irrigated and sprayed to control insects throughout the season as and when required. Low intensity spotted wilt (caused by TSWV) was observed in some plots, but white mold (caused by *Sclerotium rolfsii* Sacc.) was controlled by a single spray of Folicur, a.i. tebuconazole (Folicur 3.6F, Bayer Crop Protection, Research Triangle Park, NC) and prothioconazole (Proline, Bayer Crop Protection), in June 2011 and June 2012, respectively. The leaf spot epidemics occurred naturally in both years. Although ELS and irregular leaf spot (Cantonwine *et al.*, 2010)

were observed early in the season, the LLS epidemic soon took over and *C. personatum* was the predominant pathogen at the time of harvest during both years.

Phenotyping

Florida 1-10 scale rating

Late leaf spot severity per plot was evaluated four times in each year, using the Florida 1-10 scale rating system, where 1 = no leaf spot; 2 = very few lesions on the leaves and none on the upper canopy; 3 = few lesions on the leaves and very few on the upper canopy; 4 = some lesions with more on the upper canopy and 5% defoliation; 5 = lesions noticeable even on upper canopy and 20% defoliation; 6 = lesions numerous and very evident on upper canopy and 50% defoliation; 7 = lesions numerous on upper canopy and 75% defoliation; 8 = upper canopy covered with lesions and 90% defoliation; 9 = very few leaves remaining and those covered with lesions and 98% defoliation; and 10 = plants completely defoliated and dead (Chiteka *et al.*, 1988). Ratings were taken at 82, 110, 130 and 141 days after planting (DAP) in 2011 and 111, 121, 132, and 141 DAP in 2012. To compare disease progress, area under the disease progress curve (AUDPC) values were calculated for each plot from the disease ratings each year (Shaner and Finney, 1977). The disease progress values were standardized (stAUDPC) by dividing AUDPC values by the number of days between the first and the last rating of each season, to account for differences in the duration of LLS epidemics (Singh *et al.*, 2011; Woodward *et al.*, 2008; Woodward *et al.*, 2010).

Lateral stem assay

Lateral stem assay (LSA) was used to assess the late leaf spot epidemic more objectively by separately measuring the attributes, defoliation and disease severity, that

constitute the Florida 1-10 scale rating. Three lateral branches were randomly collected from each plot, twice during the 2011 season, first at 118-120 DAP and then at 132-134 DAP. The sample collection from three replications was staggered over three days such that the samples from the first, second and third replications were collected on the first, second, and third day, respectively. The lateral stems were brought to the laboratory where the number of nodes (n) and total number of leaflets present were counted (p). The potential number of leaflets was calculated by n*4, and number of missing leaflets by subtracting the actual number of leaflets present (p) from the potential number of leaflets (n*4). Percent defoliation was calculated from these data (Aquino *et al.*, 1995). All the leaflets were manually removed from the rachis and covered with a clear plastic sheet with sporulation side (abaxial side) up next to a blue background. The leaflets were scanned at 300 dpi using a flatbed scanner (Canon CanoScan LiDE 210) and stored as .tiff or .png files. Leaflet images were processed using ASSESS 2.0 image analysis software (American Phytopathological Society, St. Paul, MN) to give the percent lesion area (LAP) (Erickson *et al.*, 2003) and infection frequency (i.e. number of lesions per cm^2) (Cantonwine *et al.*, 2008).

Detached leaf assay

The C1501 RIL population was evaluated for the components of resistance to LLS in a growth chamber study. The $F_{7:9}$ generation plant material was raised in the greenhouse. Four to five seeds for each line were sown in 15-cm pots filled with potting mix and sterilized field soil in a 1:1 ratio. When the plants were 9 weeks old, a DLA was set up in the growth chamber by carefully excising the first or second fully expanded leaf at the base of the petiole from a randomly selected plant in each pot. The experimental

design was a randomized complete block with three replications and a control. The control consisted of uninoculated detached leaf samples of the whole population. The study was carried out in March-April 2012 over a period of 30 days. All plants of one line, CB25A died in the greenhouse, so it was not included in the study. In addition, an alternate source of seed for Gregory (certified seed from 2007, designated "Gregory-certified"), the susceptible parent, was evaluated in this study. Leaf sample collection for the first, second and third replications were scheduled on three consecutive days, respectively. Therefore, each of the three replications had individual initiation, inoculation and harvest dates during the experiment. After being detached from the plant, the cut ends of leaves were dipped in a gel-based formulation of indole butyric acid (IBA) (CLONEX Rooting Gel), and placed individually in 16 ml test tubes containing autoclaved water. The tubes with detached leaves were arranged randomly in test tube racks, placed in a transparent plastic tub with clear plexiglass on top, and put in a growth chamber. The conditions in the growth chamber were set at 22-24°C, 95% -100% RH, and a 10/14 h light/dark photoperiod to provide an optimal environment for CP germination and infection (Alderman and Nutter Jr, 1994; Butler *et al.*, 1994; Shew *et al.*, 1988; Sommartya and Beute, 1986; Wadia and Butler, 2007). Two inches of standing water was maintained in the tubs to achieve very high humidity. Water was added to test tubes and tubs as needed (Cantonwine *et al.*, 2008). Detached leaves were misted with autoclaved water three times in the first 24 hours to help them recover from excision shock and water stress. After this period, leaf samples were observed for any signs of wilting. None of the samples had to be removed because of wilting, and inoculations were administered following this step.

Peanut leaves with sporulating LLS lesions from natural infections were collected from the University of Georgia Coastal Plain Experiment Station Rigdon Farm, Tifton, GA, in 2011. Sporulating lesions were cut out, and stored in test tubes at 4°C for 5 to 8 months. These served as the inoculum source for the detached leaf experiment. For inoculation, conidial suspensions were prepared by stirring these leaf discs, bearing CP conidia, in 0.005% Tween 20 to suspend the conidia, and standardized to a concentration of 1.0×10^4 conidia ml^{-1} using a hemacytometer as described by Cantonwine *et al.* (2008).

Since the inoculations of three replications were performed over three separate days, fresh conidial suspensions were prepared each day. Inoculation was achieved by individually spraying each leaf sample for 1 second using an atomizing sprayer held at 30 cm from the leaf. A sterile solution of 0.005% Tween 20 was used on the control samples (Cantonwine *et al.*, 2008).

The leaf samples were observed daily for the development of initial late leaf spot lesions for the first 11 days after inoculation (DAI) in each replication and then on every second day until harvest (29 DAI). During this period, the DAI for the appearance of symptoms and sporulation were recorded for the first three lesions on each leaf sample for estimating incubation period (IP) and latent period (LP), respectively. Days after inoculation until the first three lesions reached 1mm in diameter were recorded as another component that represented an interaction of incubation period with lesion diameter (designated as "IP (1mm)") (E.G. Cantonwine, personal communication, 2012). The final values of IP, LP and IP (1mm) for each sample were recorded as mean values for the three lesions. LP estimations were made at two different magnifications (2X and

16X) and recorded as LP (2X) and LP (16X), respectively. The leaves were removed from test tubes at 29 DAI and percent sporulation (SPp) and sporulation index (SI) were measured. Numbers of sporulating and non-sporulating lesions were counted to calculate percent lesions with sporulation on each leaflet and the mean of four leaflets was recorded as the SPP for that sample. A 0-4 scale, where 0= few or no stromata; 1= few stromata with little sporulation; 2= stromata over more than half of the lesion, with moderate sporulation; 3= stromata over most of the lesion, moderate to high sporulation; 4= stromata over entire lesion with heavy sporulation (Chiteka *et al.*, 1988; Subrahmanyam *et al.*, 1982; Subrahmanyam *et al.*, 1985), was used to score sporulation on three oldest lesions in each leaf sample and the mean was recorded as SI for that leaf sample. Leaflets were removed from the rachis and prepared for imaging as described under the LSA method described above. Leaflet images were processed using ASSESS 2.0 image analysis software to give the LAp (Erickson *et al.*, 2003), infection frequency (i.e. number of lesions per cm^2) (Cantonwine *et al.*, 2008), and mean lesion diameter of the five largest lesions for each leaf sample. IF was calculated by measuring the number of lesions and total leaf area (cm^2) separately by using 'count' and 'leaf area' options, respectively in ASSESS 2.0. To calculate mean lesion diameter (LD), the five largest lesions were delineated using 'freehand' option and 'length' and 'width at centroid' were checked under 'feature extraction options'. Since most lesions were not perfectly round, the software generated a dataset with different 'long axis' and 'short axis' values for each lesion. Diameter of each lesion was recorded as the mean of 'long axis' and 'short axis' and the mean of five lesions was recorded as mean lesion diameter for that leaf sample. Alternatively, area of each lesion could be measured using the software and diameter

calculated from the formula area= $\pi d^2/4$. Equivalent diameter values were obtained using either method. However, the former was used in this study.

Day 29 was chosen as the cutoff date for the experiment so that defoliation of the leaf samples would not occur, and biased evaluation of percent sporulation, number of lesions etc. could be prevented since when a lesion sporulates, it continues to sporulate until the leaf abscises. In that case, it would be difficult to assess if the lesion sporulation increased, decreased or stopped entirely after defoliation (Ricker *et al.*, 1985).

Statistical analyses

Analysis of variance (ANOVA) was performed on field disease severity ratings and components of resistance to late leaf spot measured during lateral stem and detached leaf assays, to test the significance of differences between genotypes. Data were appropriately transformed, as needed, to meet the assumptions of ANOVA. Frequency distribution histograms were plotted to graphically represent the variation for traits (Figure 4.1)

DNA extraction

$F_{7:9}$ generation plants of the C1501 RIL population were raised in the greenhouse at Tifton, GA. Total genomic DNA was isolated from fresh unfurled leaves from the two parents and 78 lines using a modified cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980). Fluorometric quantitation of DNA was done using Picogreen dye (Invitrogen, Eugene, Oregon, USA) and fluorescence was read with a FluoroCount (Packard/Perkin-Elmer) microplate reader. For a few lines, DNA was extracted using DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions and quantified using Nanodrop 1000 (Thermo Scientific,

Wilmington, DE, USA). The DNA quality for all samples was checked on 1% agarose gels. For genotyping, DNA samples were diluted with sterile water and standardized to a concentration of ~ 5 ng/ul.

Marker nomenclature and genotyping

Simple Sequence Repeats (SSRs)

Depending on the in-house availability, SSR markers were selected from amongst several publications (Gautami *et al.*, 2012b; Guo *et al.*, 2012; Khedikar *et al.*, 2010; Qin *et al.*, 2012b; Ravi *et al.*, 2011; Sujay *et al.*, 2012) and used for screening parents of the C1501 population. A set of 447 SSR primer pairs, including expressed sequence tags (EST)-SSR (Guo *et al.*, 2012) and genomic SSRs (Gautami *et al.*, 2012b; Pandey *et al.*, 2012a; Qin *et al.*, 2012b; Ravi *et al.*, 2011; Sujay *et al.*, 2012), was screened on Gregory and Tifguard. In this study, updated 'universal' nomenclature (Guo *et al.*, 2012) was used for all SSRs (Table 4.1). PCR reactions were performed in 20 µl volumes, containing 2 µl of forward and reverse primers, 2 µl if 10X PCR buffer, 2 µl of 10% PVP, 0.04 µl of each dNTP (100mM), 1.2 µl of $MgCl_2$, 0.2 µl of BSA (100mM) , 0.2 µl of Taq polymerase, 2 µl of genomic DNA (5ng/ µl) and 10.8 µl of water.

PCR amplifications for SSR markers were carried out in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA) using a touchdown program, starting with 95°C for 5 min, followed by 6 cycles of 95°C for 30s, 64°C (dropping 1°C /cycle) for 30s and 72°C for 30s, followed by 30 cycles of 95°C for 30s, 58°C for 30s and 72°C for 30s, final extension was performed at 72°C for 7 min. PCR products were run on 1.5% agarose gels to check for amplification and contamination. These PCR products were then diluted using sterile water. The final mixture for fragment analysis in each

well of a 96-well semi-skirted plate was constituted by 1 µl of the diluted PCR product, 9 µl of Hi-Di formamide (Applied Biosystems, Foster City, CA) and ~ 0.20 µl of the ROX™ dye-labeled size standard (GGF500R). SSR markers were genotyped on an ABI3730XL Capillary DNA Sequencer (Applied Biosystems, Foster City, CA) using forward primers labeled with FAM, HEX, or TAMRA fluorophores (Table 4.1).

SSRs that were polymorphic between parents were used to genotype the 78 RILs of the C1501 population. For population genotyping, PCR reaction volumes were reduced to 10 µl, halving the amount of each reagent. Marker multiplexing was performed based on differences in fluorescent labels and allele size ranges. Occasionally, markers with the same label were multiplexed together provided that more than 50 bp separated their allele ranges. Screening for amplification and length polymorphisms was done using Gene Mapper 4.0 (Applied Biosystems, Foster City, CA).

Single Nucleotide Polymorphisms (SNPs)

A KASPar assay (KBioscience Ltd., Hoddesdon, UK), KASP by design, was developed for a set of 25 SNP markers that were polymorphic between parents. This set included 15 SNPs from Illumina GoldenGate SNP array developed at the University of Georgia (Peggy Ozias-Akins, pers. communication, 2012) for tetraploid peanut, and 10 SNPs from Illumina GoldenGate SNP array developed at the University of California-Davis (Nagy *et al.*, 2012; Pandey *et al.*, 2012b) based on tentative orthologous genes (TOGs) between diploid genotypes. In this study, updated 'GKAM' (Groundnut KASPar Assay Markers) nomenclature (Khera *et al.*, 2013) was used for the SNPs from the tetraploid array and 'AdSNP' for the SNPs from the diploid array (Table 4.2).

Thermocycling and endpoint genotyping for the KASPar assay were performed on a Roche LC-480 (Roche Applied Science Indianapolis, IN) using two 5' oligos labeled with FAM and HEX fluorophores. Each 5 µl volume reaction contained 2.5 µl of KASP Genotyping Mix, 0.07 µl of primer assay mix, 1.63 µl of water and 0.8 µl of genomic DNA template (5 ng/µl). The following thermal cycling program was used: hot start or activation at 95°C for 15 min, followed by 9 cycles of 94°C for 20s and 61°C for 60s, the annealing temperature dropped at the rate of 0.6°C/cycle, followed by 27 cycles at 94°C for 10s and 55°C for 60s and 2 cycles of 94°C for 20 s and 57°C for 60s. Pre- or post-melt cycles were at 30°C for 1s and cooling to 25°C during plate reading. If the signals did not separate correctly, three additional cycles of 94°C for 20 s and 57°C for 60s were performed. Roche LC-480 allowed for endpoint genotyping based on dual color hydrolysis probes (FAM and HEX) and automated scatterplot analysis. To ensure accuracy, all the automated genotype calls were manually checked and ambiguous data points with inadequate clustering were scored as missing data.

Map construction

Marker genotyping data, from 117 SSRs and 24 SNPs, obtained on the RIL C1501mapping population were used for the linkage analysis using JoinMap 3.0 (Ooijen and Voorrips, 2002). Genotyping data from 141 loci was input as a *.mcd* file in JoinMap3.0. Under 'locus genotype frequency' tab, loci were sorted in descending order for their missing data. Sixteen loci that had greater than 33% of their genotypic data missing were excluded at this step. A chi-squared (χ^2) test was performed to examine segregation distortion in marker data. Seven loci that showed significant distortion at $P<0.0001$ were excluded. Out of the remaining 118 markers, 55 (46.6%) showed

significant (P<0.05) segregation distortion. However, considering the limited number of polymorphic markers, the distorted markers were also used for grouping. Markers were placed into linkage groups with the ‘‘LOD Groupings’’ and ‘‘Create Groups for Mapping’’ command using the Kosambi mapping function (Kosambi, 1943). Linkage groups (LGs) were established using variable LOD ranging from 4.0-10.0 and a maximum recombination fraction (θ) of 0.40. Marker order in groups was established using the ‘‘Calculate Map’’ command. Linkage maps were drawn using MapChart for Windows, version 2.1 (Voorrips, 2002) (Figure 4.2).

LGs in the C1501 map were assigned to chromosomes (A-subgenome or B-subgenome) based on common markers between this map and a recent consensus map available for peanut (Gautami *et al.*, 2012a). The groups were assigned to a chromosome only if they had two or more markers in common. If more than one LG in the C1501 map was found similar to a single chromosome on the consensus map, they were named as subparts of the same chromosome (e.g. a06-i, a06-ii). The LGs that had markers in common with more than one chromosome (usually two) of the consensus map were designated to both chromosomes. Such LGs were given a hyphenated name with both the constituent chromosomes (e.g. a09-b04) and the chromosome order in the name was based on the number of common markers, the one with the higher number of common markers occurring first. For example, LG ‘a09-b04’ had 4 and 2 markers in common with chromosomes a09 and b04, respectively. One LG, named as ‘a07-b07**’, had the same markers in common with two different groups in the consensus map. When either no subgenome could be assigned to a LG, due to lack of sufficient common markers or

limited information for some loci, the LG was designated as LGXX, where XX refers to a serial number.

QTL analysis

Genotyping and phenotyping data obtained on the C1501 RIL population were subjected to QTL analyses, using the composite interval mapping (CIM) method (Zeng, 1993; Zeng, 1994), in WinQTLCart version 2.5 mapping software (Wang *et al.*, 2010). Forward and backward regression was used for mapping. Significance thresholds were determined by permutations (Churchill and Doerge, 1994; Doerge and Churchill, 1996), at 1000 times with a significance level of $P = 0.05$. QTL analyses were performed separately on the field and growth chamber datasets. Maximum likelihood graphs were plotted for significant QTL (Figure 4.3). Each field scoring and disease resistance component was mapped as an individual trait. The field data was also mapped separately for both years. Graphic representations of the LGs with marked QTL positions were furnished using MapChart for Windows, version 2.1 (Voorrips, 2002) (Figure 4.2).

Results

Phenotyping

The C1501 population was extensively phenotyped for LLS resistance, in field and growth chamber, over a period of 2 years. For field phenotyping, four disease severity ratings (Florida 1-10 scale) were taken each year during 2011 and 2012. Results from ANOVA revealed that genotypic differences were significant (P=0.05) for each of the four Florida scale disease severity ratings, and standardized disease progress values stAUDPC in 2011 and 2012, except for the third Florida scale rating in 2011 (FSRIII) (Table 4.3). Among the components of resistance to LLS measured during detached leaf

assay, viz. incubation period (IP), latent period (LP), lesion diameter (LD), DAI until LD=1mm, sporulation (%), sporulation index (SI), diseased leaf area (%), and infection frequency (IF), all but IF showed significant differences between RILs (Table 4.4).

Gregory and Tifguard showed contrasting phenotypes for LLS resistance and the RILs exhibited segregation for most of the resistance traits (Table 4.5)

Marker polymorphism

A total of 447 SSR markers were screened on Gregory and Tifguard and 108 (24.16%) were polymorphic. In addition to these 108 polymorphic SSRs, a set of 24 SNP markers, known to be polymorphic for the parents were used for genotyping the RIL C1501population. While genotyping the mapping population, segregation data was scored at two loci each for 9 SSRs (GM0030, GM0066, GM0068, GM0377, GM0429, GM 1076, GM 1577, GM 2007, and GM0415). Therefore, segregation data for 141 loci (117 SSR loci and 24 SNP loci) was available for genetic mapping.

Genetic map construction and comparison to consensus map

At the beginning, marker segregation data for 141 loci (SSRs and SNPs) was available. Sixteen loci that had greater than 33% of their genotypic data missing were excluded at this step. Genotyping data from the remaining 125 loci were evaluated for distorted segregation with the chi- squared test. Seven loci that were significantly distorted for segregation ratio at $P< 0.0001$ were excluded; however, 55 (46.6%) loci that showed significant distortion at $P<0.05$ were retained for mapping due to the scarcity of polymorphic markers. A total of 118 loci were subjected to grouping using a minimum LOD score of 4.0 and recombination frequency of 0.4. While 24 loci remained unlinked, a linkage map with 94 (76 SSR and 18 SNP) loci distributed into 19 LGs, covering

206.06 cM of total map distance, was generated (Table 4.6). The map consisted of 63 codominant and 31 dominant loci.

The genetic map was compared with a recent consensus map available for tetraploid peanut (Gautami *et al.*, 2012a). LGs in the C1501 map were named based on the common markers with the consensus map. Two of the 19 LGs could not be designated to any specific chromosome or LG on the consensus map and were therefore named as LG18 and LG19. While LG19 did not have any common markers with the consensus map, LG18 had two markers, each corresponding to a different chromosome (b02 and b08). So, these LGs were not assigned to any specific chromosomes due to insufficient information. However, LG19 had 3 markers in common with LGJ21 of the integrated linkage map generated by Qin, *et al.* (2012). The markers followed the same map order in both groups and LGJ21 could not be assigned a subgenome in that study either (Qin, *et al.*, 2012). In general, the C1501 map and the consensus map were congruent for grouping and map order, with few exceptions. In the C1501 map, the number of markers per LG ranged from 2 (a04, a06-i, a06-ii, b02, b07-ii, a08-b07**, LG18) to 22 (a09-b04) and the length of LGs ranged from 1.4 cM (a06-ii) to 26.4 cM (b03-i) with an average map distance of 10.8 cM. The average map density ranged from 0.65 cM (a05) to 6.60 cM (b03-i) (Table 4.7).

QTL analysis

LLS traits measured in the field showed continuous variation across two years (Figure 4.1) indicating that these traits are controlled by more than one gene. The traits, FSRIV and stAUDPC (2011), also exhibited transgressive segregation with phenotypic values exceeding those of the susceptible parent, Gregory. However, a closer

examination of the pods from these Gregory plots revealed that homogeneity of those plots had been compromised by some possible seed contamination event during advancement of lines. Therefore, an alternate source of Gregory, called "Gregory-new" in this study, was planted as a reference in the field trial alongside the RIL 1501 population in 2012. From the phenotyping results, it was evident that transgressive segregation observed during the 2011 (Figure 4.1) was an anomaly that arose due to off-type plants in Gregory plots. QTL analysis using the CIM method was performed on 18 traits, 7 from the two-year Florida (1-10) scale data (FSRII, FSRIV, stAUDPC (2011), and FSRII-IV, stAUDPC (2012)), 2 from LSA data (LSAII_LAp, and LSAII_IF), and 9 from the growth chamber data (IP, IP (1mm), LP (2X), LP (16X), LP(2X)-IP, SPp, SI, LD, and LAp). Only those traits that showed significant differences between genotypes were included. Also, the first Florida scale ratings taken during both years were not included because of the insufficient segregation for disease resistance at that time. A total of 7 QTL were found for the 18 traits analyzed from field and growth chamber phenotyping datasets (Figure 4.2). Distributions of QTL across linkage groups are shown in Figure 4.2. Since each of the Florida 1-10 disease scale ratings were mapped as different traits, the identified QTL were related to disease severity in general. Standardized AUDPC values, that represented the total disease damage per plot, were also mapped for both years (2011 and 2012). Five of the identified QTL were found for the two-year FSR data, and one each for LSA and DLA data (Table 4.8). According to the analysis, all 7 QTL were major effect QTL (Collard *et al.*, 2005) with their estimated phenotypic variance explained ranging from 10.90 to 38.40% (Table 4.8). Positive

additive effects were observed for all traits with significant QTL, indicating that the resistant parent Tifguard contributed the resistant alleles.

Discussion

A low-density map of cultivated peanut was constructed by mapping 94 loci (76 SSRs and 18 SNPs) onto 19 LGs and covering 206.06 cM of map distance. Forty-seven of the mapped loci showed segregation distortion (SD). The loci showing SD were not evenly distributed among LGs, with a09-b04, a05, a03, and LG19 harboring 21, 7, 5, and 3 distorted loci, respectively. The remaining LGs had two or fewer distorted markers each. This tendency of distorted loci to cluster together in certain regions of the genome has been previously reported in several crop studies (Liu *et al.*, 2010). Possible reasons for such a high number of distorted loci in this study may include, population type (Wang *et al.*, 2003), size and residual heterozygosity of lines, relatively higher number of dominant loci (~33%) (Xian-Liang *et al.*, 2006), and errors in genotyping (Sibov *et al.*, 2003).

The high amount of SD observed in LGa09-b04 may be attributed to the presence of an alien introgression in this region. Tifguard has been reported to carry an alien introgression region, spanning one-third to one-half of chromosome 9A (LGa09-b04 in the C1501 map), derived from *A. cardenasii* (Burow *et al.*, 1996) that harbors a dominant root-knot nematode resistance gene (*Rma*) (Nagy *et al.*, 2010). The F_4 generation of the C1501 population was genotyped and phenotyped for nematode resistance in order to genetically map the alien introgression described above. However, the C1501 population exhibited complete suppression of recombination and fine mapping of the *Rma* region could not be accomplished (Nagy *et al.*, 2010). Several researchers have observed that

such populations that segregate for alien introgressions are often affected by suppressed recombination and segregation distortion (Nagy *et al.*, 2010).

Segregation distortion is believed to produce false linkages between markers, bias the estimation of position and effect of QTL while reducing the statistical power of QTL mapping. However, contrary to this belief, several researchers have reported that inclusion of distorted markers is a preferable strategy to avoid reducing marker density and missing important QTL information (Liu *et al.*, 2010). In general, it is a trade-off between precision and the number of QTL that may be mapped, depending on whether the distorted loci are included or excluded from mapping. In the present study, considering the paucity of polymorphic markers, only very highly distorted ($P< 0.0001$) markers were excluded from mapping. Due to similar reasons, distorted markers have been incorporated in several mapping studies in peanut (Gautami *et al.*, 2012a; Khedikar *et al.*, 2010; Sujay *et al.*, 2012; Varshney *et al.*, 2009).

The C1501 map had 59 markers in common with the referenced consensus map. However, 17 of these loci (GM0005, GM0007, GM0030, GM0068, GM0089, GM0098, GM0126, GM 367, GM0377, GM0408, GM0429, GM0544, GM1076, GM1899, GM1986, GM2233, and GM 2531) were assigned to more than one LG in the consensus map (Gautami *et al.*, 2012a) because they were located on different LGs among different component maps. Three of these markers, GM0126, GM0367, and GM0377 have been previously reported to amplify more than one locus in the same genome (Foncéka *et al.*, 2009).

LG assignment of these 17 markers was based on the LG designation of their neighboring markers in the C1501 map. For example, GM0367 is designated to both a03

and b03 LGs in the consensus map. However, in the C1501 map, GM0367 was assigned to a03 LG because it had 4 of its neighboring markers in common with LGa03 of the consensus map and no markers in common with LGs b03-i or b03-ii. Following this procedure, all of these markers were assigned to one or more (hyphenated names) LGs and they were in agreement with the consensus map. In fact, all 59 common markers, except one (GM1609), were in congruence with the consensus map for LG designation. However, some ambiguities were seen in the order of a few markers. The lack of congruence in map order for some loci can be attributed to the fact that the map order in the consensus map was generated by integrating data from several different populations, varying for the number of lines and progeny type (Gautami *et al.*, 2012a). The accuracy of estimating genetic distances and marker order increases with the number of individuals in a mapping population (Collard *et al.*, 2005). Therefore, population size is a critical factor in obtaining reliable linkage maps. Other possible reasons for these map order conflicts may include, the large number of distorted markers, and errors in genotyping (Feltus *et al.*, 2006; Gustafson *et al.*, 2009; Varshney *et al.*, 2007).

QTL mapping has now become routine in many species as a method to achieve higher genetic gains for quantitative traits in fewer generations than conventional breeding. It helps to identify marker-trait associations, that can be used in genotypic selection, and demarcate the genomic regions responsible for conferring favorable or unfavorable alleles for quantitative trait phenotypes. Owing to the low-level genetic variation and scarcity of polymorphic markers, QTL mapping for complex traits in cultivated peanut is still in its infancy. Various traits that have been mapped in peanut using QTL analysis include, abiotic stress (drought tolerance) (Gautami *et al.*, 2012b;

Ravi *et al.*, 2011), disease resistance (LLS, rust, and TSWV) (Khedikar *et al.*, 2010; Qin *et al.*, 2012; Sujay *et al.*, 2012), and nutritional quality (oleic and linoleic acid content) (Sarvamangala *et al.*, 2011). In the case of LLS resistance, two studies in cultivated peanut are available (Khedikar *et al.*, 2010; Sujay *et al.*, 2012). A total of 28 QTL for LLS, with PVE ranging from 10.1 to 67.8%, have been reported in these studies. While no major QTL for LLS was found by Khedikar *et al.* (2010), a significant QTL that explains 62.34% of the phenotypic variation has been reported in the study by (Sujay *et al.*, 2012). However, all these mapping studies have genetic maps based on SSRs alone. Due to their amenability to high- throughput genotyping and cost-effective platforms, SNPs have the potential to replace SSRs as markers of choice for peanut genotyping in the near future (Guo *et al.*, 2011; Pandey *et al.*, 2012b; Paterson *et al.*, 2004). Therefore, an attempt was made in this study to incorporate a small number of SNPs along with SSRs to construct a genetic map for QTL mapping.

Among 18 traits that were subjected to QTL analysis, only 7 QTL for 6 traits (Table 4.8) were detected. Statistical power of QTL detection is greatly reduced by small population size and low marker density (Collard *et al.*, 2005). Although all the significant QTL appeared to have major effect on LLS resistance (PVE >10%), their effects may have been overestimated due to the small size of the population and low marker density. Since the different Florida scale ratings were mapped as separate traits, 5 out of 7 identified QTL essentially represented the phenotypic variation based on FSR alone. From 2011 field scoring data, a single QTL, named 11_FSRIV*(* denotes that log transformed data were used), was identified for the final field rating (*ln*(FSRIV)). This QTL is located on LGa03, explains 12.85% of the variation of this trait, and has a

maximum logarithm of odds (LOD) of 3.10. Based on common markers, LGa03 is parallel to LG AhIII* of the map developed by Sujay *et al.,* (2012) and a single QTL ($QTL_{R5\text{-}LLS}15$) on this LG, with a PVE of 4.28%, in close vicinity to the QTL (11_FSRIV*) was reported. There were not enough common markers to determine if these two QTL were same or different. Another QTL, named 11_stAUDPC* was detected for the standardized disease progress values (*ln*(stAUDPC)) from 2011 field scoring data. This QTL is located on LGa09-b04, explains 12.49% of the variation of this trait, and has a maximum LOD of 2.73. Another QTL for LSAII_IF** (square root transformed data were used for analysis) measured during LSAII, with a PVE of 38.4% was detected on LG a09-b04. LGa09-b04 is comparable to LG AhXIII* of (Sujay *et al.*, 2012), which harbors 3 QTL for LLS ($QTL_{R4\text{-}LLS}06$, $QTL_{R5\text{-}LLS}10$, $QTL_{R5\text{-}LLS}11$). $QTL_{R4\text{-}LLS}06$ and $QTL_{R5\text{-}LLS}10$ represent the same locus mapped in two different populations and 11_stAUDPC* appears to have mapped in close proximity to this region. Again, it is difficult to ascertain if these are the same QTL, owing to lack of sufficient common markers between the two LGs. While 2 QTL (12_FSRIVa and 12_FSRIVb) were identified for the final Florida scale rating of the season (FSRIV), a single QTL (12_stAUDPC) was detected for standardized disease progress values (stAUDPC) among the traits measured during the second year field trial (2012). Both 12_FSRIVa and 12_stAUDPC mapped on the same LG, a06-i, having maximum LOD scores of 2.59 and 2.69, and explaining 11.01 and 12.25 % variation in the traits, respectively. Since LGa06-i has only two markers, both the QTL are positioned at one end. Precise location of these QTL cannot be determined unless additional markers are added on this LG. Therefore, it cannot be said if both these QTL are the same. Another QTL, 12_FSRIVb,

is located on LGb05, with a PVE of 10.9% and maximum LOD of 2.7. Again, b05 has only three markers, the QTL appears at one end of the LG, and additional markers will be needed to locate the accurate position of this QTL. Since two significant QTL (12_stAUDPC and 12_FSRIVa) were detected in the same region, LGa06-i (flanked by GM1916 and GM0408 markers); it is likely that this region is important for LLS resistance. On the other hand, FSRIV and stAUDPC measurements are not mutually exclusive and the two QTL falling in the same region could be a result of redundant phenotypic information. Another QTL for the DLA component LP (2X)-IP, that represents delay in sporulation, with a PVE of 13.11%, was detected on LG b03-i. Due to lack of common markers, no comparisons could be made with the map by Sujay *et al.* (2012). Genotype x environement interaction can influence the phenotype in quantitative traits. This may be the reason behind the corresponding QTL for field scores from two years mapping at different locations in the genome.

In general, Gregory had higher disease susceptibility scores than Tifguard for all traits. Positive additive effects were observed for all the traits with significant QTL, indicating that the alleles that confer the disease resistance were most likely derived from the resistant parent, Tifguard. A similar trend was reported in the most recent mapping study for LLS resistance (Sujay *et al.*, 2012), where 8 of the 13 QTL identified were derived from the resistant parent.

In a small population such as the RIL C1501, statistical power to detect QTL is reduced and tendency to overestimate the effects of significant QTL is increased. Therefore, the QTL identified in this study will need to be validated in larger populations, before assessing their potential applications in peanut disease resistance breeding. With

the enhanced statistical power in a larger population, QTL for the different components of resistance may be identified. The linkage map will need to be enriched with more SSR and SNP markers to improve the precision of QTL mapping and identification of useful marker-trait associations.

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Table 4.1 Sources and nomenclature for SSRs mapped on the C1501 linkage map. (Adapted from Guo *et al.*, 2011).

Universal Name‡	Alternate name†	Primer Label	Reference
GM0001	Ah1TC0A01	HEX	Moretzsohn *et al.*, 2005
GM0005	Ah1TC1B02	TAMRA	Moretzsohn *et al.*, 2005
GM0007	Ah1TC1D02	FAM	Moretzsohn *et al.*, 2005
GM0012	Ah1TC1G04	FAM	Moretzsohn *et al.*, 2005
GM0014	Ah1TC2A02	TAMRA	Moretzsohn *et al.*, 2005
GM0022	Ah1TC2D06	FAM	Moretzsohn *et al.*, 2005
GM0030	Ah1TC3B05	FAM	Moretzsohn *et al.*, 2005
GM0066	Ah1TC5D06	TAMRA	Moretzsohn *et al.*, 2005
GM0068	Ah1TC6E01	FAM	Moretzsohn *et al.*, 2005
GM0089	Ah2TC9H08	HEX	Moretzsohn *et al.*, 2005
GM0098	Ah2TC11F12	TAMRA	Moretzsohn *et al.*, 2005
GM0126	AS1RI1F06	TAMRA	Moretzsohn *et al.*, 2005
GM0232	gi-29824925	TAMRA	Moretzsohn *et al.*, 2005
GM0237	pPGPSeq2F10	HEX	Moretzsohn *et al.*, 2005
GM0248	pPGPSeq4B11	FAM	Moretzsohn *et al.*, 2005
GM0339	Ah4-04	FAM	Hopkins *et al.*, 1999
GM0367	PM3	HEX	He *et al.*, 2003
GM0371	PM15	TAMRA	He *et al.*, 2003
GM0377	PM35	FAM	He *et al.*, 2003
GM0386	PM65	HEX	He *et al.*, 2003
GM0389	PM119	TAMRA	He *et al.*, 2003
GM0405	PM204	HEX	He *et al.*, 2003
GM0408	PM210	FAM	He *et al.*, 2003
GM0415	PM238	HEX	He *et al.*, 2003
GM0424	pPGPseq2B10	HEX	Ferguson *et al.*, 2004
GM0429	pPGPseq2E6	HEX	Ferguson *et al.*, 2004
GM0434	pPGPseq2G4	HEX	Ferguson *et al.*, 2004
GM0500	pPGSseq10D4	HEX	Ferguson *et al.*, 2004
GM0503	pPGSseq11D4	FAM	Ferguson *et al.*, 2004
GM0507	pPGSseq11G3	HEX	Ferguson *et al.*, 2004
GM0508	pPGSseq11G7	TAMRA	Ferguson *et al.*, 2004
GM0544	pPGSseq15D3	HEX	Ferguson *et al.*, 2004
GM0565	pPGSseq17E3	HEX	Ferguson *et al.*, 2004
GM0568	pPGSseq17G6	HEX	Ferguson *et al.*, 2004
GM0591	pPGSseq19D6	HEX	Ferguson *et al.*, 2004
GM0625	GA26	TAMRA	Ma *et al.*, 2006
GM0630	GA32	FAM	Ma *et al.*, 2006

GM0650	GA60	HEX	Ma *et al.*, 2006
GM0665	GA88	TAMRA	Ma *et al.*, 2006
GM0679	GA133	TAMRA	Ma *et al.*, 2006
GM0695	GA166	TAMRA	Ma *et al.*, 2006

‡ SSR nomenclature used in the present study.

†Alternate SSR nomenclature used in literature.

Table 4.2 Sources and nomenclature for SNPs used in KASPar assay developed for the C1501 population.

SNP Source Assay	MarkerID	SequenceID	Gregory	Tifguard
SNPs from *duranensis* (2x array)	AdSNP92	Ah2n_TOG898887	G	A
	AdSNP739	DurSNP_c4523-75	H	G
	AdSNP672	DurSNP_c3667-602	H	C
	AdSNP38	Ah2n_TOG896007	H	G
	AdSNP901	DurSNP_c6790-512	H	T
	AdSNP391	DurSNP_c1441-514	G	H
	AdSNP972	DurSNP_c8038-140	H	C
	AdSNP584	DurSNP_c27472-132	A	G
	AdSNP124	Ah2n_TOG900848	H	A
	AdSNP344	DurSNP_c13004-120	H	A
SNPs from 4x array	GKAM0005	alltetpeanut3_c11006_489	AG	AA
	GKAM0014	alltetpeanut3_c15479_204	AG	AA
	GKAM0028	alltetpeanut3_c21978_528	AC	AA
	GKAM0030	alltetpeanut3_c2270_1149	CC	AC
	GKAM0035	alltetpeanut3_c25917_539	AG	GG
	GKAM0037	alltetpeanut3_c26954_935	AG	GG
	GKAM0044	alltetpeanut3_c29348_426	GG	AG
	GKAM0047	alltetpeanut3_c30765_1315	GG	CC
	GKAM0073	alltetpeanut3_c83979_675	AC	CC
	GKAM0077	alltetpeanut3_c86075_741	CG	GG
	GKAM0078	alltetpeanut3_c8914_506	CC	AC
	GKAM0079	alltetpeanut3_c9035_362	AG	GG
	GKAM0089	alltetpeanut3_c995_385	AC	CC
	GKAM0092	alltetpeanut3_rep_c83385_529	GG	AG
	GKAM0094	alltetpeanut3_rep_c83454_1132	AC	CC

Table 4.3 Analysis of variance for field disease severity readings on the Florida 1-10 scale, taken on the C1501 RIL population during 2011 and 2012 growing seasons.

	2011[a]	2012[b]
FSRI	<.0001 [d]	<.0001 [c]
FSRII	0.0079 [c]	<.0001 [c]
FSRIII	0.1311 [e]	<.0001 [c]
FSRIV	<.0001 [c]	<.0001 [d]
AUDPC	0.0204 [c]	<.0001 [c]
stAUDPC	0.0206 [c]	<.0001 [c]

[a] *p* – values for Florida scale ratings taken at 82 DAP (FSRI), 110 DAP (FSRII), 130 DAP (FSRIII), 141 DAP (FSRIV), area under disease progress values (AUDPC) and disease progress values standardized over duration of epidemic (stAUDPC) in 2011.
[b] *p* – values for Florida scale ratings taken at 111 DAP (FSRI), 121 DAP (FSRII), 132 DAP (FSRIII), 141 DAP (FSRIV), area under disease progress values (AUDPC) and disease progress values standardized over duration of epidemic (stAUDPC) in 2012.
[c] From analysis of log transformed data.
[d] From analysis of untransformed data, ANOVA conditions could not be satisfied.
[e] From analysis of untransformed data, ANOVA conditions satisfied.

Table 4.4 Effect of peanut genotype on components of resistance measured during detached leaf assay in the RIL C1501 population.

Source	DLA [a]
Diseased leaf area (%) [b]	<.0001 [k]
Infection frequency [c]	0.0578 [k]
Incubation period [d]	0.0118 [k]
Sporulation index [e]	<.0001 [l]
Sporulation (%) [f]	<.0001 [k]
DAI until lesion diameter=1mm [g]	<.0001 [k]
Latent period (16x) [h]	<.0001 [k]
Latent period (2x) [i]	<.0001 [k]
Lesion diameter [j]	<.0001 [m]

[a] *p* – values for components of resistance to late leaf spot (LLS) measured during detached leaf assay (March-April 2012) in growth chamber

[b] *p* – values for percent leaf area affected by LLS, 29 days after inoculation (DAI) in DLA

[c] *p* – values for number of lesions per unit leaf area (cm^2), 29 DAI in DLA

[d] *p* – values for mean DAI until the first three lesions appear.

[e] *p* – values for mean degree of sporulation (0-4 scale) on three oldest lesions.

[f] *p* – values for mean percentage of sporulating lesions on four leaflets, 29 DAI.

[g] *p* – values for mean DAI until the first three lesions reach 1mm in diameter.

[h, i] *p* – values for mean DAI until sporulation is observed on the first three lesions, under 2x and 16x magnifica0tions respectively.

[j] *p* – values for mean diameter of five largest lesions, 29 DAI.

[k] From analysis of log transformed data.

[l] From analysis of untransformed data, ANOVA conditions could not be satisfied.

[m] From analysis of untransformed data, ANOVA conditions satisfied.

Table 4.5 Late leaf spot phenotyping data on Gregory, Tifguard, and the C1501 RIL population.FSR (Florida 1-10 scale rating)

Phenotyping Method	Trait†	Year	Mean				Variation in RILs	σ^2
			Gregory (old)	Gregory*	Tifguard	RILs		
Florida (1-10) scale	FSRI (82 DAP)	2011	1.08	.	1.25	1.29	1.00-3.17	0.10
	FSRII (110 DAP)	2011	1.67	.	1.33	2.31	1.33-3.83	2.31
	FSRIII (130 DAP)	2011	4.58	.	2.67	4.78	3.50-6.83	0.43
	FSRIV (141 DAP)	2011	5.67	.	4.25	6.17	4.75-8.33	0.91
	stAUDPC	2011	2.61	.	1.89	3.01	2.24-4.17	0.21
	FSRI (111 DAP)	2012	2.67	3.58	1.58	2.66	1.67-4.50	0.28
	FSRII (121 DAP)	2012	4.33	5.75	3.50	4.42	3.00-6.17	0.44
	FSRIII (132 DAP)	2012	5.58	7.50	4.17	5.56	4.00-8.83	0.59
	FSRIV (141 DAP)	2012	8.58	9.67	6.33	8.17	5.50-9.92	1.00
	stAUDPC	2012	5.11	6.56	3.83	5.07	3.51-6.94	0.47
Lateral Stem Assay I	Lesion area (%)(LAp)	2011	0.40	.	0.26	1.17	0.23-5.07	0.77
	Infection frequency (IF)(number of lesions/cm2)	2011	0.18	.	0.16	0.58	0.12-6.22	0.55
	Defoliation (%) (DEF)	2011	24.07	.	17.68	18.47	5.01-38.29	32.00
Lateral Stem Assay II	LAp	2011	2.10	.	0.76	3.03	0.72-9.48	3.27
	IF	2011	0.92	.	0.33	1.36	0.32-4.26	0.66
	DEF	2011	34.05	.	31.66	41.00	22.38-71.34	144.58
Detached Leaf	Sporulation index (SI)	2012	3.22	3.67	1.11	2.98	0.89-4.00	0.76

Assay								
	IF	2012	3.66	2.62	1.65	2.49	0.91-4.34	0.52
	Incubation period (IP)(days)	2012	9.11	10.67	12.67	10.25	8.44-12.33	0.85
	Latent period (LP2X)(days)	2012	20.44	17.56	25.67	19.47	14.00-25.50	6.40
	Latent period (LP16X)(days)	2012	15.56	15.33	20.33	17.33	14.22-23.00	4.25
	LP (2X)-IP	2012	11.33	7.72	15.22	9.21	1.89-14.89	4.87
	IP (1mm)	2012	14.22	14.11	20.22	15.74	12.55-20.77	2.93
	Sporulation (%)	2012	91.38	97.31	42.90	79.87	19.91-100	335.13
	Lesion diameter (LD) (mm)	2012	0.24	0.27	0.20	0.24	0.10-0.29	0.00
	LAp	2012	10.17	9.65	2.74	7.05	2.10-12.90	9.13

†First Florida scale rating (FSRI) was not included for both years (2011 and 2012) because disease scoring early in the season tends to be confounded by presence of irregular leaf spot (ILS)
*alternate seed source for Gregory ("Gregory-new" for field phenotyping data and "Gregory-certified" for growth chamber phenotyping data).

Table 4.6 Details of linkage map with 94 loci based on the C1501 population.

Locus	LGs	Position (cM)
GM0126	a02	0.00
GM0507	a02	4.599
GM0695	a02	5.037
GM0508	a02	8.747
GKAM0077	a02	10.706
GM0232	a03	0.00
GM0415a	a03	2.673
GM0001	a03	10.773
GM0371	a03	11.807
GM0339	a03	14.914
GM2215	a03	15.305
GKAM0044	a03	16.865
GM0367	a03	18.018
GM0568	a04	0.00
GM2246	a04	3.034
GM0630	a05	0.00
GM0386	a05	0.445
GM0237	a05	0.612
GKAM0078	a05	1.085
GM0500	a05	1.682
GM0068b	a05	3.124
GM1577b	a05	3.894
GKAM0028	a05	5.196
GM1916	a06-i	0.00
GM0408	a06-i	12.791
GM0544	a06-ii	0.00
GM0377b	a06-ii	1.395
GM1937	a07	0.00
GM1986	a07	0.771
GM0405	a07	6.386
GM1990	a07	7.756
GM1922	a07	7.759
GM0089	a07	9.426
GM0030a	a08-b07**	0.00
GM0098	a08-b07**	10.301
GKAM0005	a09-b04	0.00
AdSNP584	a09-b04	0.295
GM1076a	a09-b04	1.903
AdSNP38	a09-b04	2.505
GKAM0079	a09-b04	2.53
GM0650	a09-b04	2.667
AdSNP672	a09-b04	2.687
GM1416	a09-b04	2.691
GM1834	a09-b04	2.691

GM2839	a09-b04	2.691
AdSNP972	a09-b04	2.691
GM0665	a09-b04	2.692
GM0377a	a09-b04	3.141
GKAM0037	a09-b04	3.145
AdSNP124	a09-b04	3.151
GM0389	a09-b04	3.491
AdSNP92	a09-b04	3.497
GM1291	a09-b04	3.538
GM0565	a09-b04	3.541
GM0007	a09-b04	4.053
GM0066b	a09-b04	4.294
GM1047	a09-b04	5.994
GM0503	a10	0.00
GKAM0092	a10	4.045
GM2531	a10	12.5
GM0012	a10	15.684
GM1501	b01-a01	0.00
GM1864	b01-a01	6.724
GM1992	b01-a01	12.24
GM2233	b01-a01	12.814
AdSNP901	b01-a01	13.192
GM0022	b01-a01	13.725
GM2606	b01-a01	14.917
GM0005	b02	0.00
GM0434	b02	6.592
GM1609	b03-i	0.00
GM0014	b03-i	6.954
GM0424	b03-i	18.392
GM1733	b03-i	26.396
GM1996	b03-ii	0.00
GM0415b	b03-ii	7.238
GM2388	b03-ii	11.047
GM0591	b05	0.00
GM1577a	b05	4.886
GM0068a	b05	5.038
GM1953	b07-i	0.00
GM0429a	b07-i	1.821
GM2067	b07-i	16.42
GM1899	b07-i	20.035
GM1076b	b07-ii	0.00
GM0030b	b07-ii	11.677
GM0625	LG18	0.00
GM0679	LG18	9.45
GM1521	LG19	0.00
GM0248	LG19	3.433

GM1495	LG19	4.869
GKAM0035	LG19	6.124
AdSNP344	LG19	6.163
GKAM0094	LG19	8.366

** Common markers were found in two different groups in the consensus map.

Table 4.7 Salient features of linkage groups of the C1501 linkage map

Linkage group	No. of mapped loci	Map length (cM)	Map density (cM)
a02	5	10.71	2.14
a03	8	18.18	2.27
a04	2	3.03	1.52
a05	8	5.20	0.65
a06-i	2	12.79	6.40
a06-ii	2	1.40	0.70
a07	6	9.43	1.57
a08-b07	2	10.31	5.16
a09-b04	22	5.99	3.00
a10	4	15.68	3.92
b01-a01	7	14.92	2.13
b02	2	6.59	3.30
b03-i	4	26.39	6.60
b03-ii	3	11.05	3.68
b05	3	5.04	1.68
b07-i	4	20.04	5.01
b07-ii	2	11.68	5.84
LG18	2	9.45	4.73
LG19	6	8.37	1.39

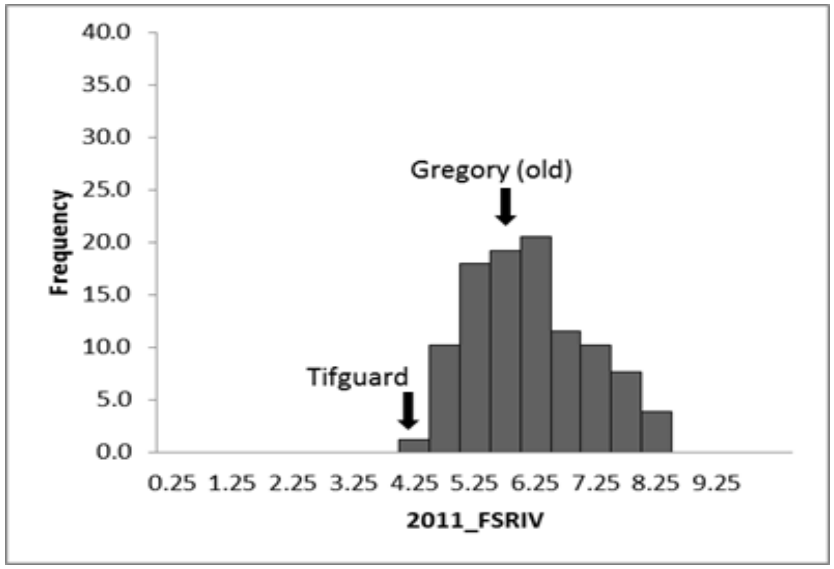
Table 4.8 Quantitative trait loci for late leaf spot resistance identified by composite interval mapping (CIM) method

Trait	Year	QTL name	Linkage group	Marker interval	Position (cM)	Highest LOD score (threshold)	Phenotypic variation (r^2 %)	Additive effect
FSRIV*	2011	11_FSRIV*	a03	GM0415a/GM0001	10.68	3.10(2.5)	12.85	0.0520
stAUDPC*	2011	11_stAUDPC*	a09-b04	AdSNP38/GM0650	2.68	2.78(2.4)	12.49	0.0525
FSRIV	2012	12_FSRIVa	a06-i	GM1916/GM0408	0.01	2.59(2.5)	11.01	0.4086
		12_FSRIVb	b05	GM0591/GM1577a	0.01	2.70(2.5)	11.90	0.3485
stAUDPC	2012	12_stAUDPC	a06-i	GM1916/GM0408	0.01	2.69(2.5)	12.25	0.2636
LSAII_IF**	2011	L2_IF**	a09-b04	AdSNP584/GM1076a	1.31	3.25(2.4)	38.40	0.2287
DLA_LP-IP	2012	DL_LP-IP	b03-i	GM1609/GM0014	3.01	2.61(2.4)	13.11	0.9787

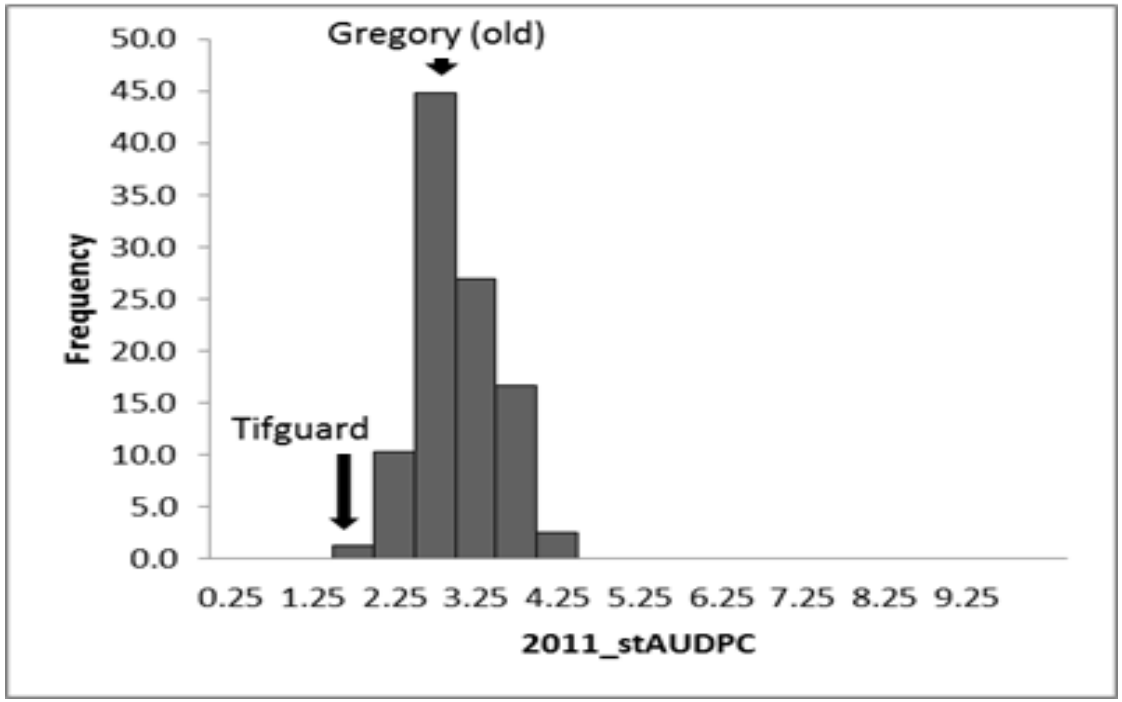
*log transformed data was used for analysis

** square root transformed data was used for analysis

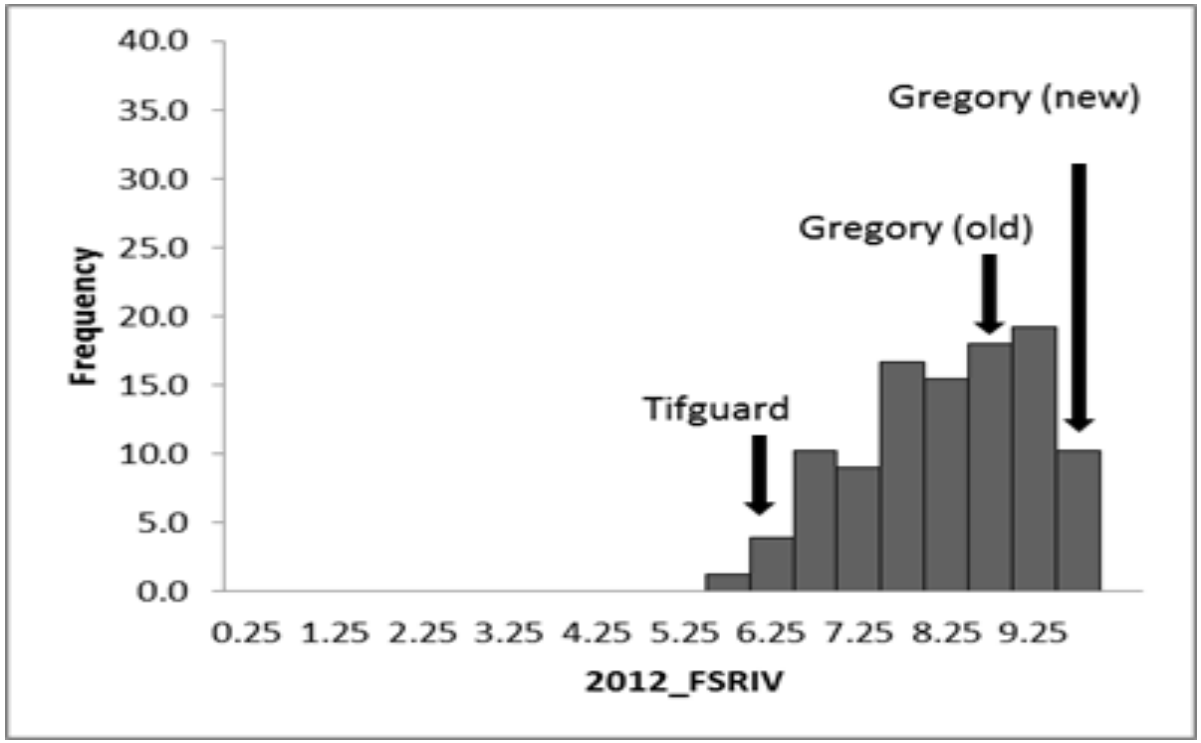
+ve additive effect means that resistance alleles were derived from the resistant parent Tifguard.


40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Frequency
Gregory (old)
Tifguard
0.25 1.25 2.25 3.25 4.25 5.25 6.25 7.25 8.25 9.25
2011_FSRIV

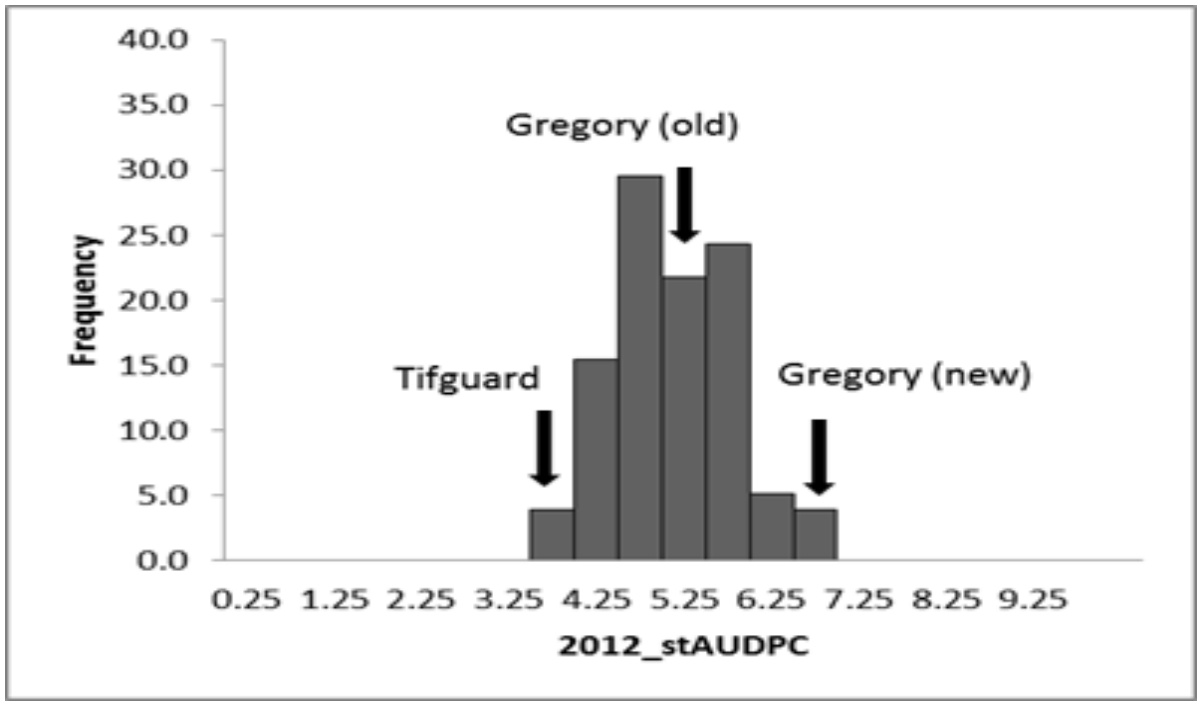
(A)


Gregory (old)
Tifguard
Frequency
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0.25 1.25 2.25 3.25 4.25 5.25 6.25 7.25 8.25 9.25
2011_stAUDPC

(B)

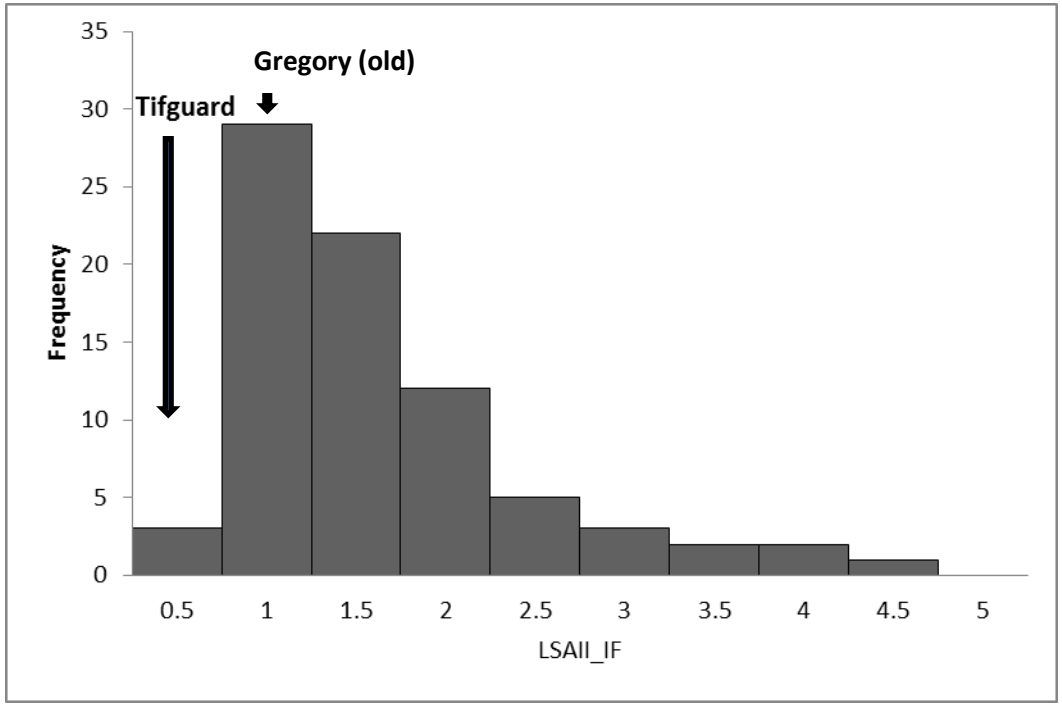

40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Frequency
Gregory (new)
Gregory (old)
Tifguard
0.25 1.25 2.25 3.25 4.25 5.25 6.25 7.25 8.25 9.25
2012_FSRIV

(C)

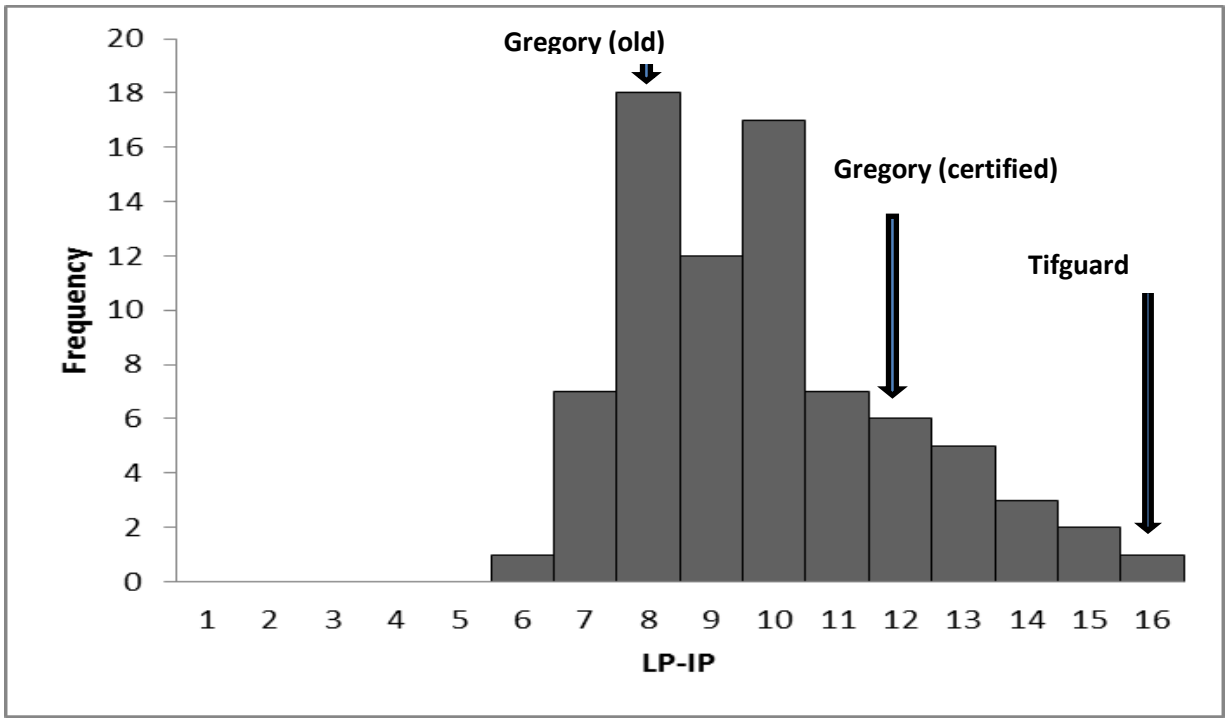

40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Frequency
Gregory (old)
Tifguard
Gregory (new)
0.25 1.25 2.25 3.25 4.25 5.25 6.25 7.25 8.25 9.25
2012_stAUDPC

(D)

.

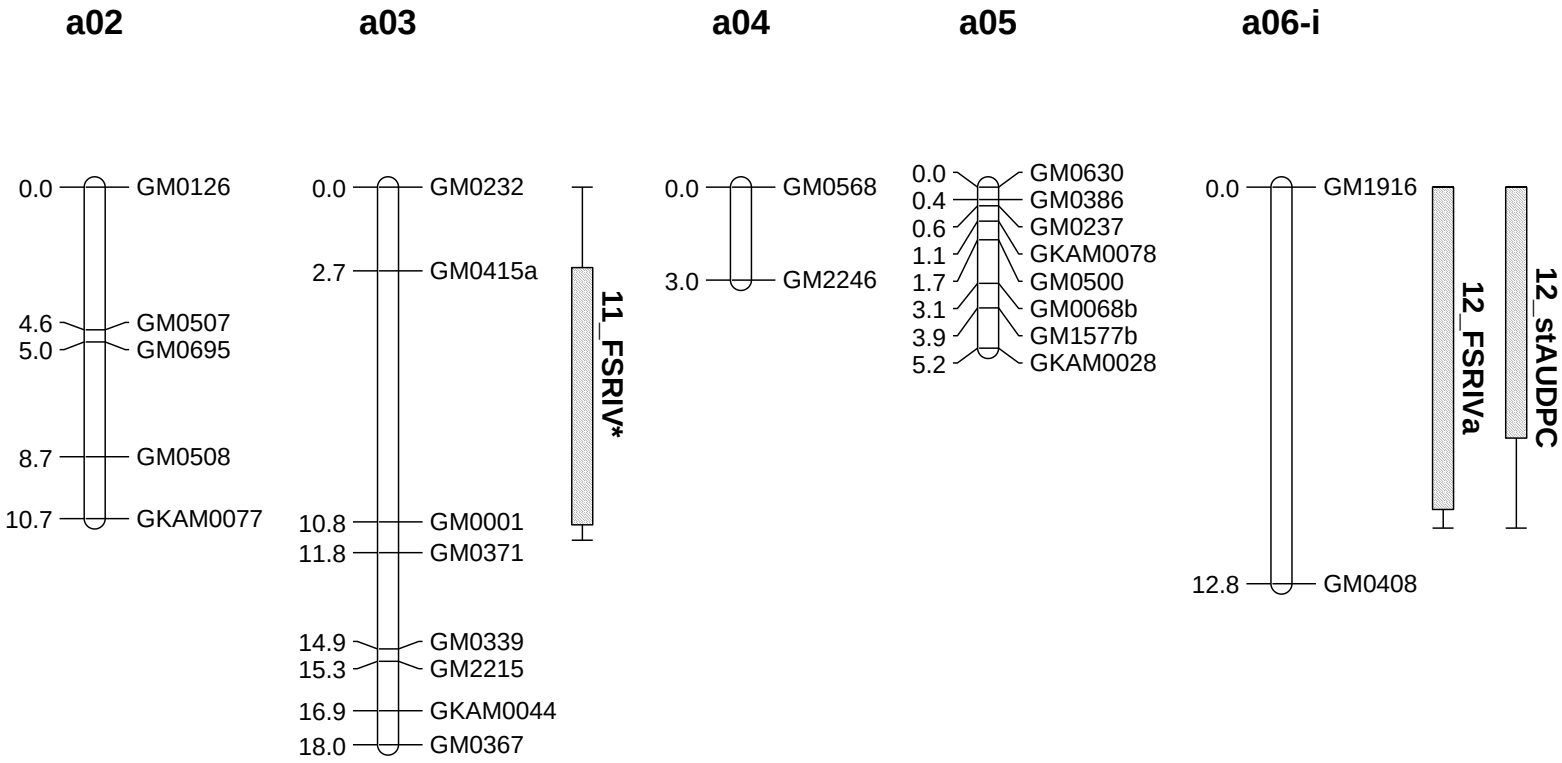

Gregory (old)
Tifguard
Frequency
35
30
25
20
15
10
5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
LSAII_IF

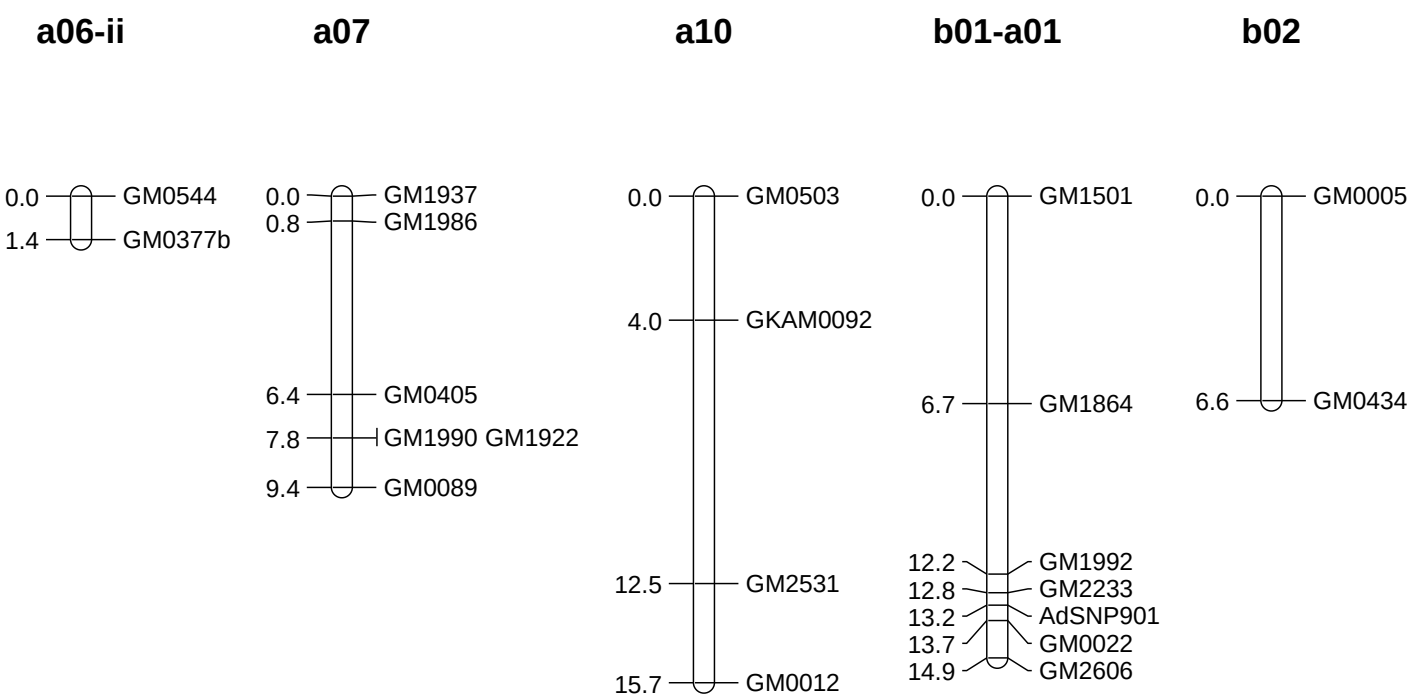
(E)

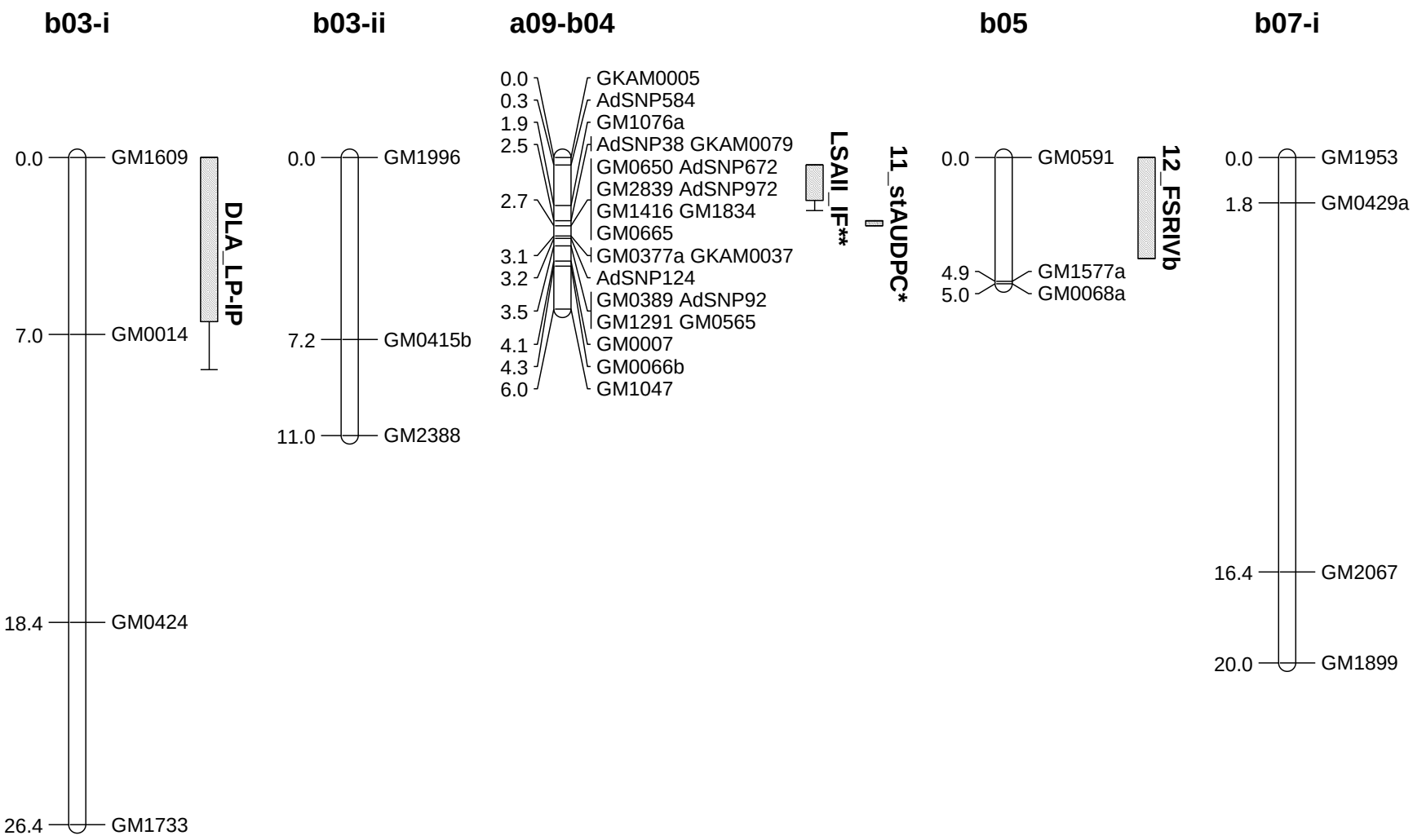


(F)

Figure 4.1 Frequency distribution for late leaf spot (LLS) resistance in the RIL C1501 population (Gregory x Tifguard). Arrows represent phenotypic values of the parents. (A) 2011_FSRIV (141 DAP), (B) 2011_stAUDPC, (C) 2012_FSRIV (141 DAP), (D) 2012_stAUDPC, (E) LSAII_IF, and (F) DLA_LP-IP


a02
a03
a04
a05
a06-i
0.0 GM0126
4.6 GM0507
5.0 GM0695
8.7 GM0508
10.7 GKAM0077
0.0 GM0232
2.7 GM0415a
10.8 GM0001
11.8 GM0371
14.9 GM0339
15.3 GM2215
16.9 GKAM0044
18.0 GM0367
11_FSRIV*
0.0 GM0568
3.0 GM2246
0.0 GM0630
0.4 GM0386
0.6 GM0237
1.1 GKAM0078
1.7 GM0500
3.1 GM0068b
3.9 GM1577b
5.2 GKAM0028
0.0 GM1916
12.8 GM0408
12_FSRIVa
12_stAUDPC


a06-ii
a07
a10
b01-a01
b02
0.0
GM0544
1.4
GM0377b
0.0
GM1937
0.8
GM1986
6.4
GM0405
7.8
GM1990 GM1922
9.4
GM0089
0.0
GM0503
4.0
GKAM0092
12.5
GM2531
15.7
GM0012
0.0
GM1501
6.7
GM1864
12.2
GM1992
12.8
GM2233
13.2
AdSNP901
13.7
GM0022
14.9
GM2606
0.0
GM0005
6.6
GM0434


b03-i
0.0
GM1609
7.0
GM0014
18.4
GM0424
26.4
GM1733
DLA_LP-IP
b03-ii
0.0
GM1996
7.2
GM0415b
11.0
GM2388
a09-b04
0.0
0.3
1.9
2.5
2.7
3.1
3.2
3.5
4.1
4.3
6.0
GKAM0005
AdSNP584
GM1076a
AdSNP38 GKAM0079
GM0650 AdSNP672
GM2839 AdSNP972
GM1416 GM1834
GM0665
GM0377a GKAM0037
AdSNP124
GM0389 AdSNP92
GM1291 GM0565
GM0007
GM0066b
GM1047
LSAII_IF**
11_stAUDPC*
b05
0.0
GM0591
4.9
5.0
GM1577a
GM0068a
12_FSRIVb
b07-i
0.0
GM1953
1.8
GM0429a
16.4
GM2067
20.0
GM1899

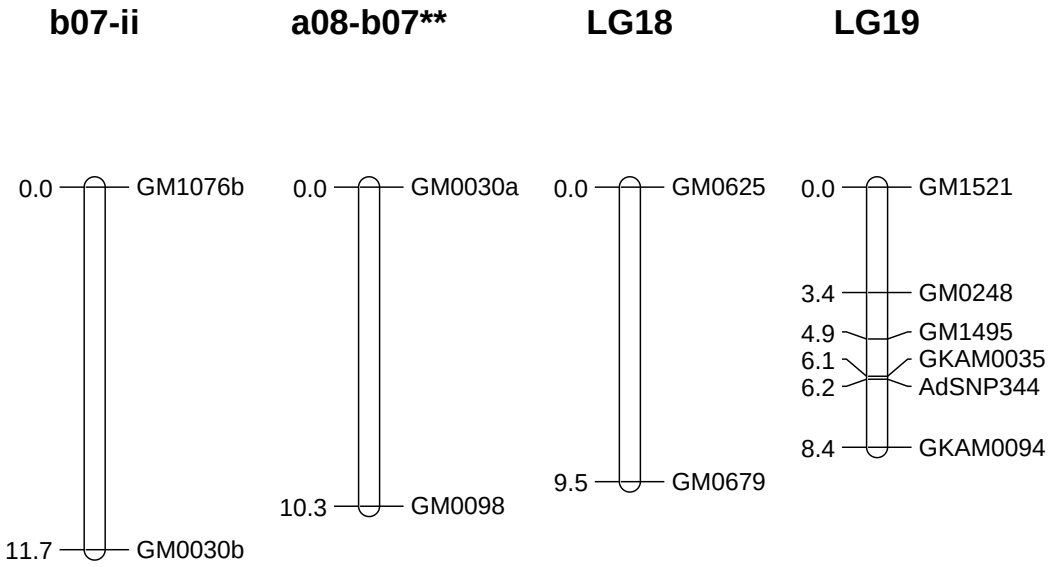
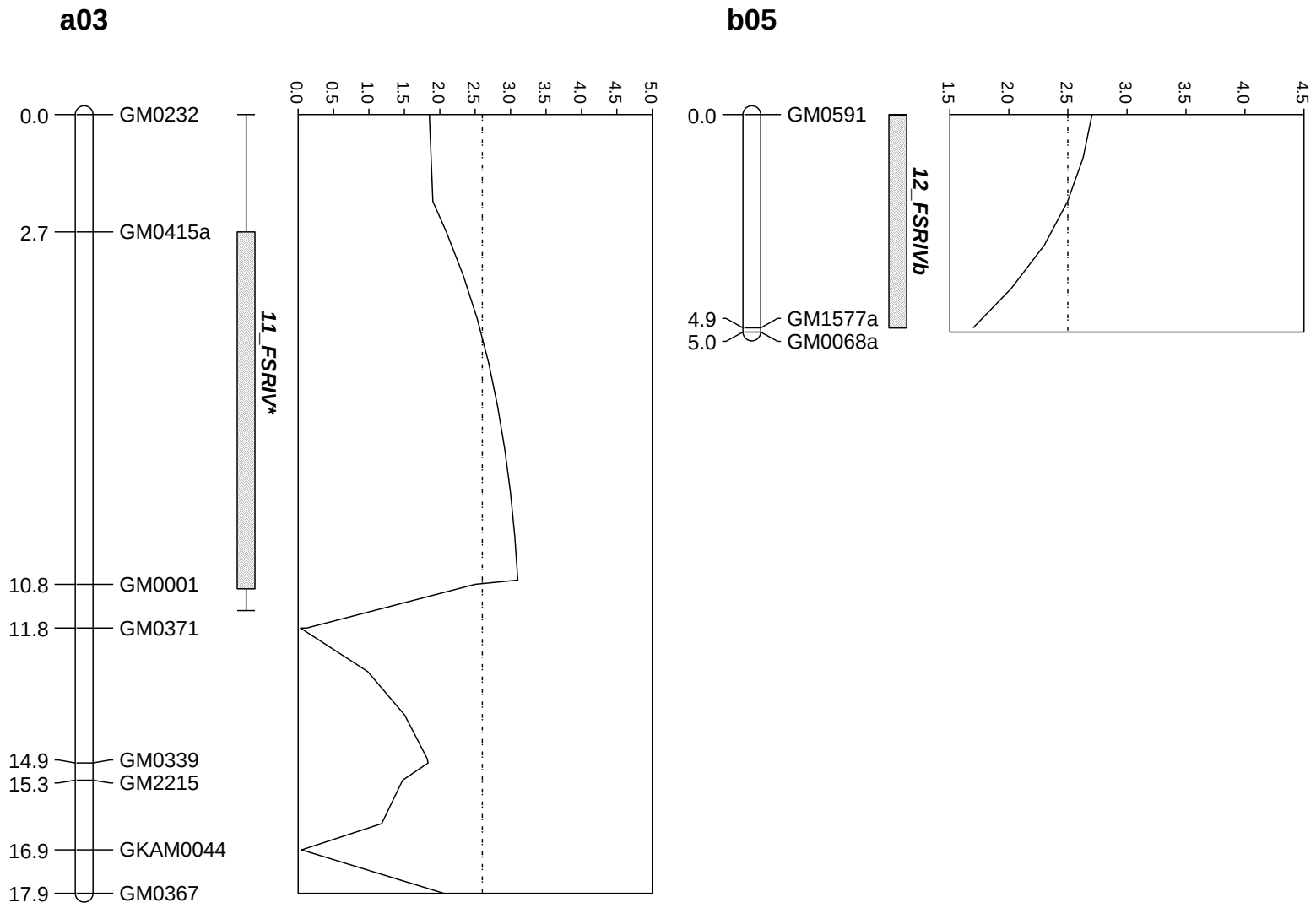

b07-ii
a08-b07**
LG18
LG19
0.0
GM1076b
11.7
GM0030b
0.0
GM0030a
10.3
GM0098
0.0
GM0625
9.5
GM0679
0.0
GM1521
3.4
GM0248
4.9
GM1495
6.1
GKAM0035
6.2
AdSNP344
8.4
GKAM0094

Figure 4.2 Map positions of each significant quantitative trait locus (QTL) on each linkage group (LG), associated with late leaf spot (LLS) resistance in the RIL C1501 population (Gregory x Tifguard). 11_FSRIV* (141 DAP), 11_stAUDPC*, 12_FSRIVa (141 DAP), 12_FSRIVb (141DAP), 12_stAUDPC, LSAII_IF**, DLA _LP-IP. '*', '**' denote that log and square root transformed values were used for analyses, respectively.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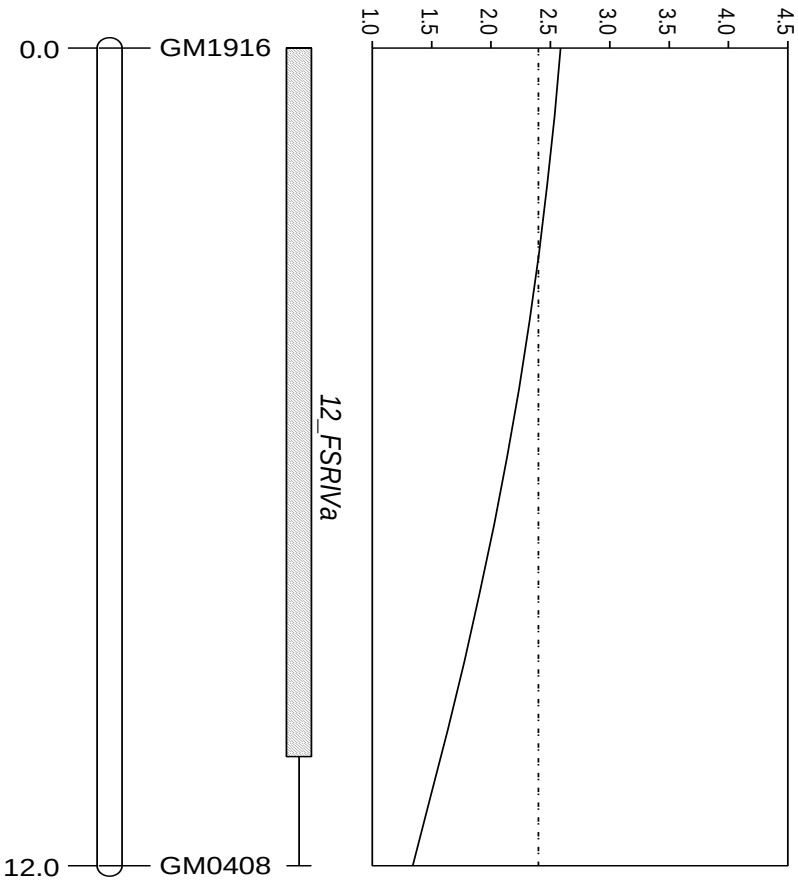
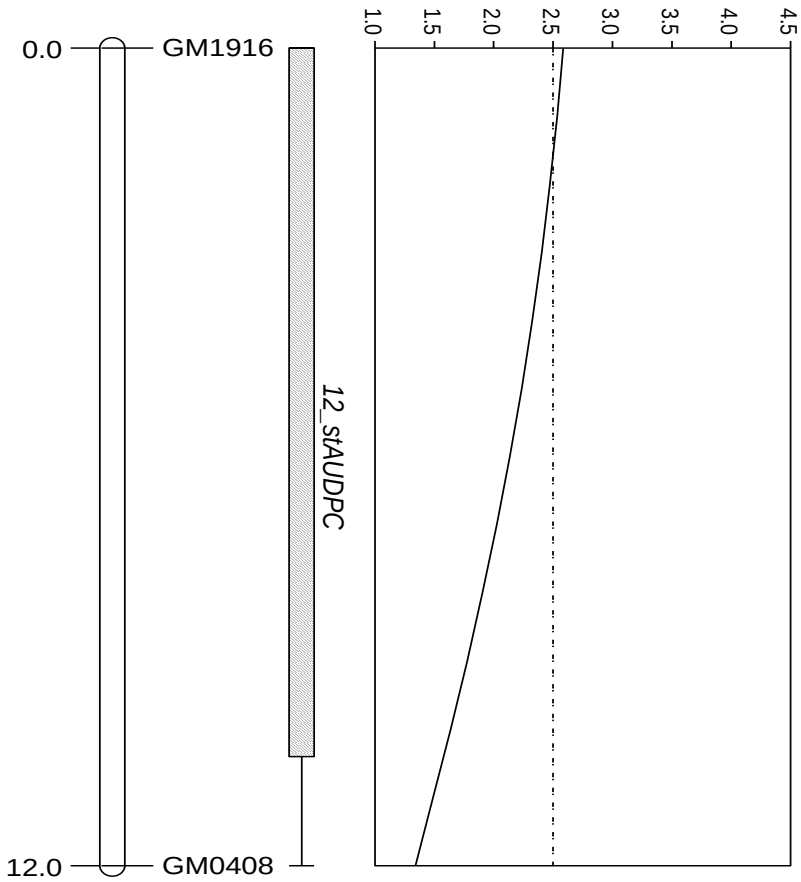
0.0
GM0232
2.7
GM0415a
10.8
GM0001
11.8
GM0371
14.9
GM0339
15.3
GM2215
16.9
GKAM0044
17.9
GM0367
11_FSRIV*
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
b05
0.0
GM0591
4.9
GM1577a
5.0
GM0068a
12_FSRIVb
1.5
2.0
2.5
3.0
3.5
4.0
4.5

a06-i


0.0
GM1916
12_FSRIVa
12.0
GM0408
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5

a06-i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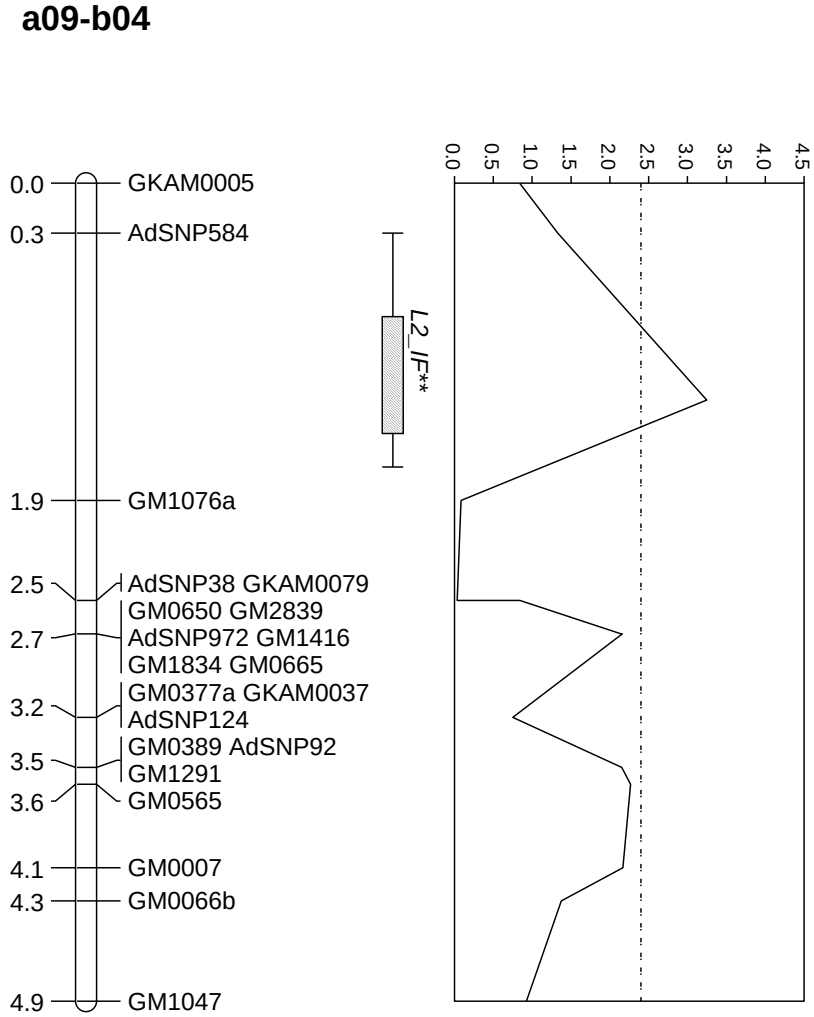
GM1916
12_stAUDPC
12.0
GM0408
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5


a09-b04
0.0
0.3
1.9
2.5
2.7
3.2
3.5
3.6
4.1
4.3
4.9
GKAM0005
AdSNP584
GM1076a
AdSNP38 GKAM0079
GM0650 GM2839
AdSNP972 GM1416
GM1834 GM0665
GM0377a GKAM0037
AdSNP124
GM0389 AdSNP92
GM1291
GM0565
GM0007
GM0066b
GM1047
L2_IF**
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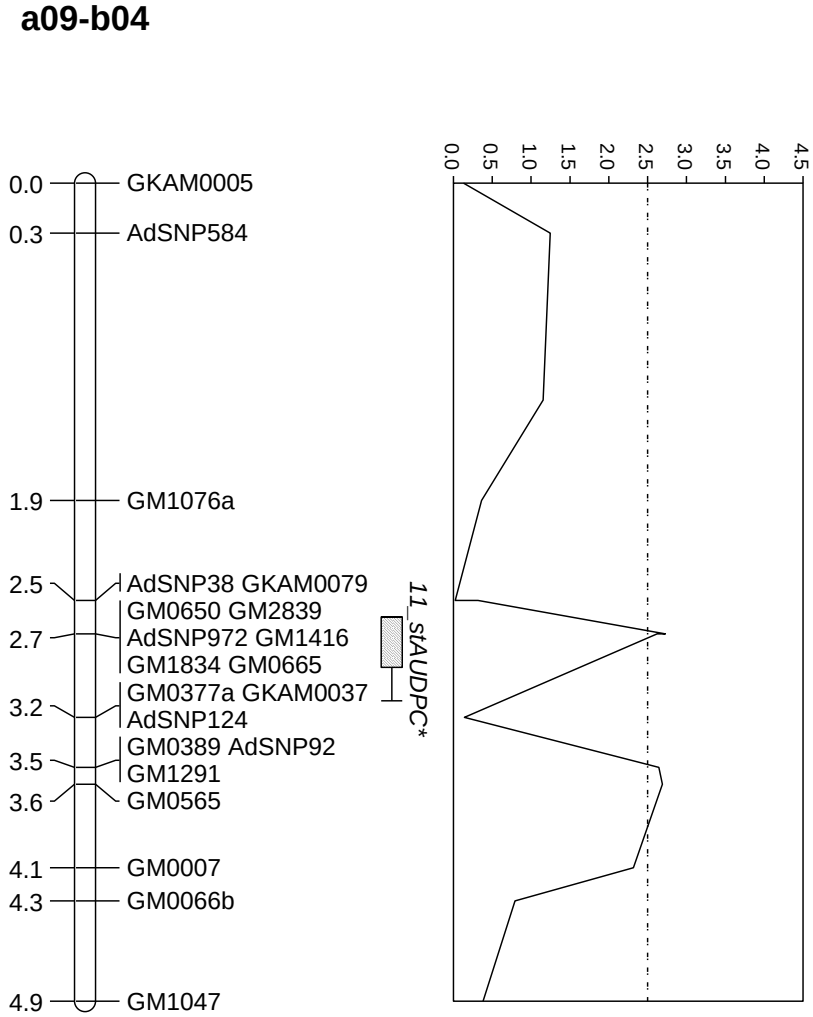
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5


a09-b04
0.0
0.3
1.9
2.5
2.7
3.2
3.5
3.6
4.1
4.3
4.9
GKAM0005
AdSNP584
GM1076a
AdSNP38 GKAM0079
GM0650 GM2839
AdSNP972 GM1416
GM1834 GM0665
GM0377a GKAM0037
AdSNP124
GM0389 AdSNP92
GM1291
GM0565
GM0007
GM0066b
GM1047
11_stAUDPC*
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5

b03-i

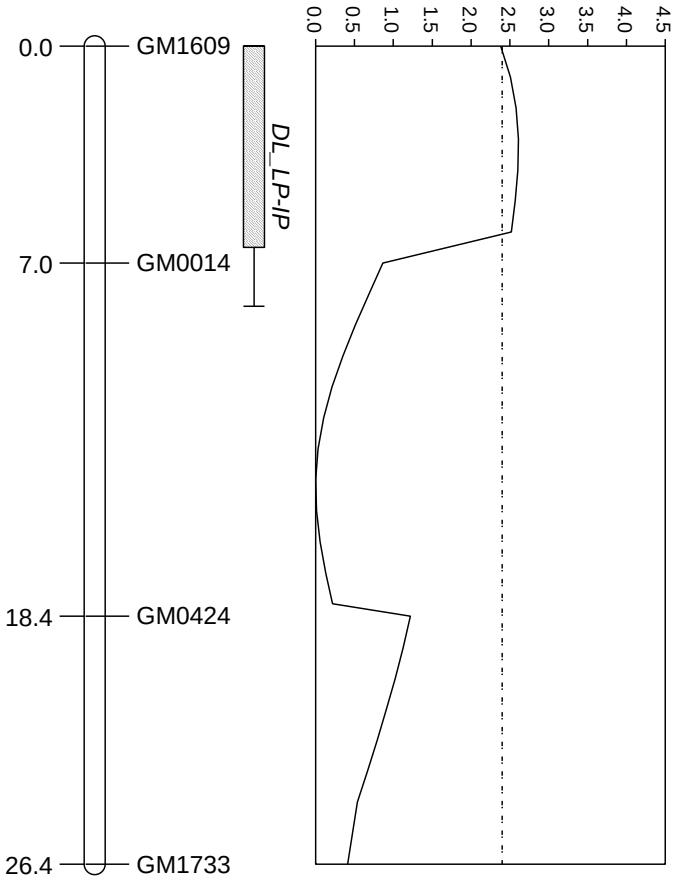

0.0
GM1609
7.0
GM0014
18.4
GM0424
26.4
GM1733
DL_LP-IP
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5

Figure 4.3 Maximum likelihood plots along linkage groups, identifying genomic regions of quantitative trait loci associated with late leaf spot (LLS) resistance in the RIL C1501 population (Gregory x Tifguard). '*' and '**' denote that log and square root transformed values were used for analyses, respectively. Positions of loci are given in centimorgan.

CHAPTER 5

SUMMARY

Three different phenotyping methods including, Florida (1-10) scale, lateral stem assay, and detached leaf assay were tested to address the concerns of reliable and detailed phenotyping of LLS in a mapping population. Data obtained from these methods were statistically analyzed and comparisons were made. Although each of the components recorded with different phenotyping methods conveys relevant information about the disease, it is not possible to measure all components in large populations. According to results from various statistical analyses, ease and efficiency of measurement, feasibility and objectivity, the following order of priority was proposed: percent sporulation (SPp) or sporulation index (SI)> latent period (LP)> days after inoculation until lesion diameter=1mm (IP (1mm))> percent lesion area (LAp)> average lesion diameter (LD)> incubation period (IP)> infection frequency (IF) for detached leaf assay; LAp >IF> percent defoliation (DEF) for lateral stem assay.

Among the three methods of phenotyping, both the Florida 1-10 scale and detached leaf assay were mostly able to differentiate among RILs segregating for LLS resistance. Lateral stem assay needs to be further refined to be useful in population phenotyping. While the Florida (1-10) scale is easier to use in large populations and provides general information on disease severity and defoliation, detached leaf assay, though tedious to carry out, provides comprehensive information on components of disease resistance and helps to dissect the basis of resistance for each segregating line in

the population. For example, it was observed that in few lines (CB75A, CB62B, CB52B, CB49A), LLS lesions appeared quite early in the experiment (low IP), but these lesions exhibited delayed sporulation (high LP). Therefore, the difference between LP and IP for these lines is high. A possible hypothesis for such observations could be that resistance to LLS in these lines is achieved *via* arrested sporulation, such that the lines were susceptible to CP infection but have an underlying mechanism to prevent secondary inoculum production. It is difficult to detect such subtleties in field scoring methods as they are based on the overall disease response of a genotype and do not provide any information on how different components contribute to produce that response.

The present study provides detailed comparisons of available leaf spot phenotyping methods in peanut. To the best of our knowledge, this is the first report of integrating electronic imaging and disease analysis in a peanut mapping population. In addition, two new components of disease resistance including, IP (1mm) and LP-IP are being proposed. The proposed priority order for components of resistance may serve as a general guideline for future research involving LLS phenotyping. However, the choice of a phenotyping method and components would vary depending upon objectives of the study, number of genotypes to be evaluated, and availability of resources (skill, labor, and time).

A low-density map of cultivated peanut was constructed by mapping 94 loci (76 SSRs and 18 SNPs) into 19 LGs and covering 206.06 cM of map distance. Forty-seven of the mapped loci showed segregation (SD) distortion. The loci showing SD were not evenly distributed among LGs, with a09-b04, a05, a03, and LG19 harboring 21, 7, 5, and 3 distorted loci, respectively. The high amount of SD observed in LGa09-b04 may be

attributed to the presence of an alien introgression, in Tifguard, in this region, historically derived from *A. cardenasii* (Nagy *et al.*, 2010). Several researchers have observed that such populations that segregate for alien introgressions are often affected by suppressed recombination and segregation distortion (Nagy *et al.*, 2010).

The C1501 map had 59 markers in common with the referenced consensus map. LGs in the C1501 map were named based on the common markers with the consensus map. Two of the 19 LGs could not be designated to any specific chromosome or LG on the consensus map and were therefore named as LG18 and LG19. Following this procedure, all of these markers were assigned to one or more (hyphenated names) LGs and they were in agreement with the consensus map. In fact, all 59 common markers, except one (GM1609), were in congruence with the consensus map for LG designation. However, some ambiguities were seen in the order of few markers.

Among 18 traits that were subjected to QTL analysis, only 7 QTL ((explaining 10.90-38.4% variation (PVE)) for 6 traits were detected. Statistical power of QTL detection is greatly reduced by small population size and low marker density (Collard *et al.*, 2005). Although, all the significant QTL appeared to have major effect on LLS resistance (PVE >10%), their effects may have been overestimated due to the small size of the population and low marker density. Since the different Florida scale ratings were mapped as separate traits, 5 out of 7 identified QTL essentially represented the phenotypic variation based on FSR alone. One QTL each for the lateral stem and detached leaf assays was identified.

In a small population such as the RIL C1501, statistical power to detect QTL is reduced and tendency to overestimate the effects of significant QTL is increased.

Therefore, the QTL identified in this study will need to be validated in larger populations, before assessing their potential applications in peanut disease resistance breeding. With the enhanced statistical power in a larger population, QTL for the different components of resistance may be potentially identified. The linkage map will need to be enriched with more SSR and SNP markers to improve the precision of QTL mapping and identification of useful marker-trait associations.

References

Collard, B., M. Jahufer, J. Brouwer and E. Pang. 2005. An Introduction to Markers, Quantitative Trait Loci (QTL) Mapping and Marker-Assisted Selection for Crop Improvement: The Basic Concepts. Euphytica 142: 169-196.

Nagy, E.D., Y. Chu, Y. Guo, S. Khanal, S. Tang, Y. Li, W.B. Dong, P. Timper, C. Taylor and P. Ozias-Akins. 2010. Recombination Is Suppressed in an Alien Introgression in Peanut Harboring *Rma,* a Dominant Root-Knot Nematode Resistance Gene. Molecular Breeding 26: 357-370.

Sujay, V., M. Gowda, M. Pandey, R. Bhat, Y. Khedikar, H. Nadaf, B. Gautami, C. Sarvamangala, S. Lingaraju and T. Radhakrishan. 2012. Quantitative Trait Locus Analysis and Construction of Consensus Genetic Map for Foliar Disease Resistance Based on Two Recombinant Inbred Line Populations in Cultivated Groundnut (*Arachis hypogaea* L.). Molecular Breeding 30: 773-788.