CHARACTERIZATION OF SIMPLE SEQUENCE REPEATS (SSRS) MARKERS, GENETIC DIVERSITY ANALYSIS IN CULTIVATED PEANUT (*ARACHIS HYPOGAEA* L.), AND RELATED LEAF SPOT AND SPOTTED WILT RESPONSES IN THE FIELD

by

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(Under the Direction of Albert K. Culbreath)

ABSTRACT

In this research, as part of a study examining genotypic and phenotypic variation, disease reactions to Tomato spotted wilt virus (TSWV) and *Cercospora arachidicola* were evaluated in twenty-two genotypes from the U.S. and China in field trials at Tifton, GA in 2007-08. There was a continuous range of final incidence of spotted wilt from 20% to 80%; final percent defoliation by early leaf spot ranged from 10% to 97%. Disease reactions will be used in conjunction with genetic characterization of these genotypes and populations developed from crosses of selected genotypes in efforts to develop markers for resistance to Tomato spotted wilt virus and *Cercospora arachidicola*. The results of this study corroborated previous reports and confirmed that there is limited genetic diversity in the cultivated peanut. In general, the cluster in Cladogram tree followed our expectations where relationships between genotypes were known. Finally, based on genotypic and phenotypic information, two populations have been developed by crossing of Tifrunner X GT-C20 and SunOleic 97R X NC 94022.

INDEX WORDS: SSR, polymorphism, genetic diversity, leaf spot, tomato spotted wilt, TSWV, *Cercospora arachidicola*

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CHAPTER 1

Introduction – Literature Review

1. History of Peanut & Peanut Production

The cultivated peanut (*Arachis hypogaea* L.), also called a groundnut, probably originated in southern Bolivia or northern Argentina in South America. (Gregory, Krapovickas et al. 1980; Kochert, Stalker et al. 1996). Currently, peanut is an important crop in the world for human consumption and as a feed stock, and has been ranked as the third most important source of vegetable protein and fourth most important source of vegetable oil by the United Nations Food and Agricultural Organization (UN-FAO, www.fao.org).

Traditionally peanut has been grown primarily in tropical and subtropical regions throughout the world. China, India and the United States, in that order, are the largest peanut producing countries in the world. In the United States, peanuts are mainly grown in the southern regions with major peanut production areas in Georgia, Texas, Alabama, North Carolina, Florida, Virginia and Oklahoma. Among them, Georgia has the largest peanut production and accounts for approximately 44% of peanut production in the U.S.

2. Peanut Foliar Diseases

2.1 Peanut Foliar Diseases – Tomato Spotted Wilt in GA

Many factors, including diseases and insect pests, influence the peanut yield. There are several diseases which limit peanut production and profitability. Among them foliar diseases including peanut rust, tomato spotted wilt, and leaf spot diseases cause a number of yield losses in peanut. In the southeastern peanut production areas, the largest peanut production area in the U.S., the most important foliar diseases of peanut are tomato spotted wilt, caused by Tomato spotted wilt tospovirus (TSWV), and two fungal leaf spot diseases, early leaf spot caused by *Cercospora arachidicola* Deighton (teleomorph = *Mycosphaerella arachidis* Deighton) and late leaf spot caused by *Cercosporidium personatum* (Ber,. & M. A. Curtis) Deighton, (telemorph = M. berkeleyi Jenk.)(Holbrook and Anderson 1995; Culbreath 2003).

Tomato spotted wilt of peanut was first reported by Costa (Costa 1941) in Brazil. In the U.S., tomato spotted wilt of peanut was first observed in Texas in 1971 in the U.S. Since then tomato spotted wilt has become a very important disease of peanut in the southeastern peanut production area (Culbreath 1997). Tomato spotted wilt occurs every year, but severity fluctuates with year and location (Culbreath 2003). In Georgia, losses caused by tomato spotted wilt increased rapidly from the late 1980s to 1997. In 1997, losses in peanut to tomato spotted wilt were approximately $40 million for Georgia alone (Brown 2003).

Tomato spotted wilt virus is mainly transmitted by several species of thrips (Ullman, Sherwood et al. 1997). In Georgia, the two main vector species of thrips are thought to be the tobacco thrips (*Frankliniella fusca* Hinds) and the western flower thrips (*Frankliniella occidentalis* Pergande). Application of insecticides for control of thrips vectors typically has been unsuccessful for management of tomato spotted wilt in peanut (Culbreath, Todd et al. 2003). The combination of

cultivars with moderate levels of field resistance, cultural practices such as planting date, increased seeding rate, use of twin row patterns, and in-furrow applications of the insecticide phorate have been used to reduce the severity of and economic damage by tomato spotted wilt after 1997 (Brown 2003; Culbreath 2003; Cantonwine, Culbreath et al. 2006). There is no single management practice which provides adequate control for tomato spotted wilt. The integrated management system relies heavily on the use of moderately resistant cultivars, and improved resistance to TSWV appears to be the area with most potential for improving control of tomato spotted wilt.

2.2 Peanut Foliar Diseases – Leaf Spot in GA

Early and late leaf spot of peanut also occur almost every year in the state of Georgia. If not controlled on a susceptible cultivar, either of these diseases or the combination of the two can cause complete defoliation and yield losses of 50% or more. The primary cultivars planted at Georgia are susceptible to both leaf spot pathogens, and control of leaf spot diseases of peanut is heavily dependent upon the use of multiple applications of fungicides, such as chlorothalonil and tebuconazole. The fungicides are routinely used with a calendar-based schedule (Branch 1995), with standard regimes of six or seven applications per season. In addition, the intensive use of fungicides is expensive, and represents a risk of negative environmental impacts from fungicide applications. Problems with reduced sensitivity to benzimidazole (Culbreath, Stevenson et al. 2002) and ergosterol biosynthesis inhibiting fungicides (Stevenson and Culbreath 2006) in populations of both early leaf spot and late leaf spot pathogens also make leaf spot control more difficult.

Therefore, the most desirable way to improve control of leaf spot diseases and reduce the dependence on fungicides is the development of cultivars with resistance to both *C. arachidicola* and *C. personatum*. Resistance to these pathogens typically has provided moderate suppression of leaf spot epidemics rather than immunity to the diseases (Chiteka 1987; Chiyembekeza 1992; Culbreath 2003), but the rate reducing resistance can allow reduction of fungicide requirements for disease control. Several peanut breeding programs have screened many genotypes for sources of resistance, and screening for reduced levels of leaf spot in non-treated tests is used in most breeding programs in the southeastern U.S. (Holbrook and Anderson 1995; Culbreath, Todd et al. 1999; Holbrook and Stalker 2003; Holbrook, Timper et al. 2003).

Southern Runner was the first runner-type cultivar developed with resistance to *C. personatum* (Gorbet, Norden et al. 1987) and moderate resistance level in the field to TSWV (Black 1991). Georgia Green is the most popular cultivar planted in the state of Georgia with good field resistance to TSWV, but it is susceptible to *C. arachidicola* and *C. personatum* (Branch 1996). Peanut breeding programs continue to breed new cultivars with resistance to TSWV or leaf spot pathogens or both. Recently, several other cultivars or breeding lines have been observed with higher level of resistance to TSWV or leaf spot pathogens than Georgia Green, including Florida MDR-98, C-99R, Georganic, Tifguard (Cantonwine, Culbreath et al. 2006; Culbreath, Tillman et al. 2008; Holbrook, Timper et al. 2008).

3. Molecular Markers Development in Peanut

The molecular assays include RFLP, RAPD, SSR, and AFLP. These methods can be used for application depending on the principles, applications, type and amount of polymorphism

detected, as well as cost and time requirements (Karp, Isaac et al. 1998). In general, the development and identification of molecular markers has proven to be very useful in the field of breeding, genetic research and crop evolution in many species (Mohan, Nair et al. 1997). Recently, the morphological markers and PCR-based molecular markers such as isozymes, RAPD, ISSR, AFLP, and SSR have been used to estimate genetic diversities, variation identification, and to conduct phylogenetic evolutionary studies in many kinds of crops. However, almost all of those methods detected only low levels of genetic variability in peanut, especially cultivated peanut (Halward, Stalker et al. 1991; Kochert, Halward et al. 1991; Halward, Stalker et al. 1992; Stalker, Phillips et al. 1994; He and Prakash 1997; Gupta and Varshney 2000; Raina, Rani et al. 2001). SSR (simple sequence repeats) markers are co-dominant and multi-allelic in inheritance, produce higher levels of DNA polymorphism, are easy to amplify with few DNA samples, and allow better detection of diversity, even more cost efficiently than by PCR multiplex (Akkaya, Bhagwat et al. 1992; Tang, Yu et al. 2002). Even though, the development of SSR markers is a cost and labor intensive work compared with RAPDs or AFLP, but SSR markers can be very cost effective and allow high throughput in breeding activities applications after they are developed.

Meanwhile, SSR markers have been successfully applied in genetic studies in humans (Dib, Faure et al. 1996) and other mammals (Sun and Kirkpatrick 1996) as well as in plants such as soybean [Glycine max (L.)Merr.] (Rongwen, Akkaya et al. 1995) and rice (*Oryza sativa* L.) (Panaud, Chen et al. 1996). Genetic technology has been used in peanut breeding for selection of nematode resistance (Chu, Holbrook et al. 2007), and marker-assisted selection (MAS) has

become more popular within breeding programs considering more probability of selecting superior genotypes from MAS than from phenotype selection (Knapp 1998).

Although SSR markers are useful in the genetic research field, they have had limited use with the cultivated peanut because large scale DNA sequence information is needed and the number of markers should be mapped and more polymorphic markers should be developed. Hopkins et al developed and identified six sequence-tagged microsatellite (STMS) markers with high variation in cultivated peanut (Hopkins, Casa et al. 1999). Later, more then one hundred STMS markers were identified by Ferguson et al (Ferguson, Burow et al. 2004). They located and characterized the SSRs by motif and polymorphism in a diverse array of 24 cultivated peanut accessions. Moretzsohn et.al (Moretzsohn, Leoi et al. 2005) used a total of 67 new SSR markers to assess the genetic diversity of peanut. Only three of those markers were polymorphic in cultivated peanut, which corroborated previous reports that peanut presents a relatively reduced genetic variation. Later, Palmieri et al developed another 11 novel polymorphic microsatellite markers (Palmieri, Bechara et al. 2005), which detected greater gene diversity than the markers used before.

4. The Development of Genetic Diversity Analysis in Peanut

The genus Arachis contains about 70 taxa (Krapovickas A. 1994), with almost all of them being diploid, with either A or B genome. In contrast, cultivated peanut (*Arachis hypogaea* L.) is an autogamous alleotetraploid (2n = 4x =40 chromosomes) composed of A and B genomes according to cytogenetic characters. These two genome types could be found in wild-type peanut species respectively, which both are diploid genotype in genome. *Arachis hypogaea* was evolutionally obtained by hybridization between the species with two different series of

chromosomes, and then followed by spontaneous genome duplication (Halward, Stalker et al. 1991; Kochert, Stalker et al. 1996). Because of this evolutionary hypothesis of cultivated peanut origin, the genetic diversity within cultivated peanut is relatively lower compared with diploid wild-type peanut species. Even more, there are a number of rich sources of variation for agronomical traits contained in the wild diploid peanut species, which could potentially be used in peanut breeding. However, it is difficult to transfer these good agronomic traits from wild species into cultivated peanut species because of inter-specific compatibility barriers.

In the traditional breeding programs, morphological diversity was typically used for estimating variation within different species (Ayana and Bekele 1999). However, estimation of phenotypic variation has several disadvantages. Morphological characteristics may be influenced by many factors such as genotype-environment interactions, and unknown genetic control of polygenically inherited morphological and agronomic traits in addition to genetic variation (Smith 1992).

Therefore, MAS (Marker-assisted selection) has become a new gene technology which could be able to break through these traditional breeding restrictions, and increase the speed and resource efficiency of progress in peanut breeding programs. Recently, researchers have used a number of molecular assays to assess the genetic variability, which is very important for characterization of individuals, accessions, and breeding lines for the choice of parental genotypes in breeding programs (Ribaut and Hoisington 1998).

The limited genomic variability in cultivated peanut, and complicated A and B genomes have slowed the identification and characterization of molecular markers for genetic technology application (Grieshammer and Wynne 1990; Halward, Stalker et al. 1991; Kochert, Halward et al. 1991; Halward, Stalker et al. 1992; Halward, Stalker et al. 1993). The development of genomics resources in peanut also has lagged behind other economically important crops such as soybean, maize, and rice (He and Prakash 1997).

Even though the estimation of genetic similarity among genotypes is a helpful tool for crop breeding programs and genetic technology has been used in peanut breeding for selection of nematode resistance (Chu, Holbrook et al. 2007), the limited genetic diversity in the cultivated peanut limits the development and application of genetic technology in peanut breeding. The wild diploid peanut has been reported to be used in genetic mapping since the 1990s (Halward, Stalker et al. 1991; Kochert, Halward et al. 1991; Halward, Stalker et al. 1992) with different kinds of molecular markers, such as RFLP (Halward, Stalker et al. 1993; Burow, Simpson et al. 2001), and RAPD (Garcia, Stalker et al. 2005). Until now there are only 3 SSR-based genetic linkage maps reported in peanut (Moretzsohn, Leoi et al. 2005; Moretzsohn, Barbosa et al. 2009; Varshney, Bertioli et al. 2009). Among them, an AA genome linkage map was constructed using an F_2 population obtained from a cross between two diploid wild species (*A. duranensis* and *A. stenosperma*) (Moretzsohn, Leoi et al. 2005); from the same research group, another linkage map for the B-genome of Arachis was based on another F_2 population from a cross between *A.ipaensis* and *A. magna* (Moretzsohn, Barbosa et al. 2009); the other linkage map is for cultivated peanut from another research group (Varshney, Bertioli et al. 2009). It was constructed

based on a RIL mapping population comprising of 318 F_8/F_9 lines from a cross between ICGV 86031 and TAG 24.

5. Research Objectives

From previous generalizations, it is known that there is limited genetic diversity in the genus *Arachis*, especially in cultivated peanut species *Arachis hypogaea*. Compared with diploid wild peanut species, for the resistance to major pests or pathogens, the genetic variation in cultivated peanut species is much lower (Halward, Stalker et al. 1991; Kochert, Halward et al. 1991; Halward, Stalker et al. 1992; Stalker, Phillips et al. 1994; Kochert, Stalker et al. 1996; He and Prakash 1997). Although SSR markers have been widely developed through different ways, and have been applied successfully to access discrimination and assess genetic variation, the characteristics of SSR markers have not been determined and widely applied in cultivated peanut. All of these characters involved in cultivated peanut limit the development and application of genetic technology, such as MAS (marker-assisted-selection breeding program) in the peanut. Therefore, one objective of our present project is 1) estimating SSR polymorphic markers characters through screening among 16 cultivated peanut genotypes for polymorphism, including estimating allele-length ranges, null-allele frequencies and heterozygosities; 2) detecting genetic diversity in these 16 cultivated peanut, which have a wide range of resistance to TSWV and leaf spot especially early leaf spot, should provide useful potential information for future breeding applications and resistance gene related molecular marker detection; 3) assessing the field reaction to TSWV and *C. arachidicola* of twenty-two genotypes from China and U.S.; and 4) developing a RIL population based on genotypic and phenotypic information in this study

or future cultivated peanut linkage group map construction and TSWV and/or *C. arachidicola* resistance marker detection.

The 16 genotypes used in the markers screening study also have been used in the field evaluation for resistance or susceptibility to tomato spotted wilt and leaf spot. According to a previous report, these 16 genotypes have a wide range of levels of resistance to one or more pathogens, such as TSWV, leaf spot pathogens, or both. Among them, Georgia Green (Branch 1996) is the major cultivar recently planted in the State of Georgia with good field resistance to TSWV and usually has been used as a standard resistance cultivar to TSWV; Georganic (Holbrook and Culbreath 2008) was recently released as a cultivar with high level of resistance to TSWV and moderate resistance level to early and late leaf spot pathogens; Tifguard (Holbrook, Timper et al. 2008) is another recently released cultivar with high level resistance to TSWV and root-knot nematode which is another important disease occurring in the state of Georgia and the sister line of Tifguard, C724-19-25 also has high field resistance to TSWV but lacks resistance to the nematode; Tifrunner (Holbrook Jr 2007) was released at 2007 as a new cultivar with high level field resistance to TSWV and moderate resistance to early and late leaf spot; SunOleic 97R (Gorbet and Knauft 2000) is a cultivar developed by the University of Florida with good agronomic traits, but it is considered as susceptible cultivar to TSWV and leaf spot pathogens; NC 94022 (Culbreath, Gorbet et al. 2005) is a breeding line which has been reported with high level resistance to TSWV in the field.

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Chapter 2

Characterization of Simple Sequence Repeats (SSRs) and genetic relationship analysis in cultivated peanut (*Arachis hypogaea* L.) [1]

[1] Y. Li. and A.K. Culbreath. To be submitted to *phytopathology*.

Abstract

Marker-assisted selection (MAS) has become an important part of breeding programs for many crops and has potential to break through some traditional breeding restrictions; it could efficiently increase the speed of peanut breeding programs if it can be used into peanut breeding. Even more, the marker technology has been used in peanut breeding. However, the development and identification of molecular markers and related genetic diversity analysis in peanut especially in tetraploid cultivated peanut has severely lagged behind many other economic crops because of the limitation of genetic diversity in cultivated peanut. In our research, we developed 709 SSR markers from published data and the EST database, and screened them in sixteen peanut genotypes that include commercial cultivars as well as breeding lines and germ-plasm accessions from the U.S. and China. The genotypes included have varying levels of resistance to Tomato spotted wilt virus (TSWV), *Cercospora arachidicola* (early leaf spot) and *Cercosporidium personatum* (late leaf spot). In this research study, we are going to estimate the characters of these 709 SSR markers from different research groups, and detect the genetic relationship between those 16 genotypes used in our study.

Among the screened markers, there were 556 SSR markers with PCR products. Out of 556 markers over half markers (281 markers) had excellent unambiguous amplification results. The characteristics of those markers developed from published datasets showed similar results as their previous report. Among them, 83 markers showed polymorphism within the 16 cultivated peanut genotypes with unambiguous amplification bands which indicated limited polymorphism in cultivated peanut. Whereas, 61 markers showed ambiguous amplification results can be screened more for future analysis. From PIC score and heterozygosity, we also can see limited

polymorphism in our study; the average of PIC score is 0.209; the mean of heterozygosity is 0.225; the average number of allele is 2.5 with range from 1 to 13. Those 281 markers with unambiguous amplification bands have been used to detect genetic diversity of sixteen cultivated peanut genotypes (*A. hypogaea*). These 16 genotypes are separated into different branches as expected. The sister lines-Tifguard (C724-19-15) and C724-19-25 are branched together with 0.033 genetic distance. Pedigree GP-NC WS 13 was branched together with one of parents used for the breeding. Four genotypes from China had higher similarity and grouped together, among them, two breeding lines PE1 and PE2 were grouped together with 0.070 genetic distances, whereas another two released Chinese cultivars GT-C20 and GT-C9 branched together with 0.078 genetic distances.

This research corroborated previous reports for limited genetic polymorphism in cultivated peanut. This is also the first time report of the genetic relationships among Chinese and U.S. genotype which have different levels of resistance to TSWV and *C. arachidicola*. The cladogram tree of these sixteen genotypes showed the results as expected. Even more, the U.S. genotypes used in this research also have been conducted in the filed evaluation of tomato spotted wilt and early leaf spot, and genetic relationships among these 16 genotypes vary greatly in field reactions to TSWV and *C. arachidicola*. Based on these phenotype and genotype information, two population crossed by Tifrunner X GT-C20 and SunOleic 97R X UF NC 94022 have been used to develop recombinant inbreed lines (RIL). Therefore, those polymorphic markers may be useful in the development of markers for resistance to TSWV and/or *C. arachidicola*. An AA-BB linkage group map in cultivated peanut could be highly potentially be constructed based on this research.

Keyword: SSR marker, polymorphism, genetic diversity, PIC, heterozygosity

1. Introduction

Peanut (*Arachis hypogaea* L.), also called groundnut, probably originated in southern Bolivia or northern Argentina in South America (Gregory, Krapovickas et al. 1980; Kochert, Stalker et al. 1996). Currently, it is an important crop in the world for human consumption and as a feed stock, and is the fifth most important oilseed crop in the world. It has primarily been grown in tropical and subtropical regions in the world.

Arachis contains approximately 70 species (Krapovickas and Gregory 1994), with almost all of them being diploid, with either A genome or B genomes. In contrast, cultivated peanut is tetraploid (2n = 4x = 40 chromosomes) composed of A and B genome groups according to cytogenetic characters, in which the A genome represented A chromosomes pair, and B genome represented B chromosomes pair. Evolutionarily, *A. hypogaea* was probably developed through hybridization between species with two different series of chromosomes, followed by spontaneous duplication (Halward, Stalker et al. 1991; Kochert, Stalker et al. 1996). Considering this evolution hypothesis of cultivated peanut origin, the genetic diversity within cultivated peanut is relatively low compared with diploid wild-type peanut species. However, there are a number of rich sources of variation for agronomic traits contained in the wild diploid peanut species, which could potentially been used in peanut breeding. Unfortunately, it is often difficult to transfer these good agronomic traits from wild species into cultivated peanut species because of inter-specific compatibility barriers.

In the past, morphological similarity was widely used for estimating variation within different species in breeding systems (Ayana and Bekele 1999), and the variation in morphological, physiological, and agronomic traits have also been reported in peanut. However, recently Marker-assisted selection (MAS) has become an important part of breeding programs for many crops and has potential to break through some traditional breeding restrictions; it could highly increase the speed of peanut breeding programs if there are resistance gene related markers available. In addition to improving efficiency of the selection process, it can increase the probability of selecting superior genotypes for some factors compared to phenotype selection (Knapp 1998). A number of molecular assays to assess genetic variability have been developed which can be very important for characterization of individuals and breeding lines for choosing parental genotypes in breeding programs (Ribaut and Hoisington 1998). Although the development and identification of molecular markers have proven to be very useful and helpful in the field of breeding, genetic research and crop evolution in many species (Mohan, Nair et al. 1997), the identification and characters of molecular markers for genetic technology applications in this crop have significantly lagged behind other economically important crops such as soybean, maize, and rice (Burr, Burr et al. 1988; Burr and Burr 1991; Akkaya, Bhagwat et al. 1992; Rongwen, Akkaya et al. 1995; Panaud, Chen et al. 1996; Cho, Ishii et al. 2000; Temnykh, Park et al. 2000; Gethi, Labate et al. 2002; Robertson-Hoyt, Jines et al. 2006) because of this limited genomic variability in cultivated peanut, and complicated A and B genomes. Based on studies with several kinds of molecular markers (Kochert, Stalker et al. 1996; Krishna, Zhang et al. 2004; Moretzsohn, Leoi et al. 2005; da Cunha, Nobile et al. 2008), the genetic variation in peanut especially in tetreploid cultivated peanut (AABB) generally has also been reported to be

very low (Grieshammer and Wynne 1990; Halward, Stalker et al. 1991; Kochert, Halward et al. 1991; Halward, Stalker et al. 1992; Halward, Stalker et al. 1993; Mohan, Nair et al. 1997; Krishna, Zhang et al. 2004).

Recently, the morphological markers and PCR-based molecular markers such as RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), and SSR (simple sequence repeat) have been used to estimate genetic diversities, variation identification, and phylogenetic evolutionary studies in many kinds of crops (Burr, Burr et al. 1988; Burr and Burr 1991; Akkaya, Bhagwat et al. 1992; Rongwen, Akkaya et al. 1995; Panaud, Chen et al. 1996; Cho, Ishii et al. 2000; Temnykh, Park et al. 2000; Gethi, Labate et al. 2002; Robertson-Hoyt, Jines et al. 2006). However, almost all methods have indicated a low level of genetic variability in the cultivated peanut (Halward, Stalker et al. 1991; Kochert, Halward et al. 1991; Halward, Stalker et al. 1992; Stalker, Phillips et al. 1994{Raina, 2001 #36; He and Prakash 1997; Gupta and Varshney 2000; Raina, Rani et al. 2001). Compared with other kinds of markers such as AFLPs and RAPDs, SSR (simple sequence repeats) markers are co-dominant and multi-allelic in inheritance, produce higher level of DNA polymorphism, are easy to amplify with few DNA samples, and allow better detection of diversity, even more cost efficiently by multiplex PCR (Akkaya, Bhagwat et al. 1992; Tang, Kishore et al. 2003). Even more, several research group results suggested that DNA markers produce a higher level of DNA polymorphism than other DNA markers (AFLPs and RAPDs) in cultivated peanut (Hopkins, Casa et al. 1999; He, Meng et al. 2003). Although SSR markers are useful in the genetic research (Rongwen, Akkaya et al. 1995; Dib, Faure et al. 1996; Panaud, Chen et al. 1996; Sun and Kirkpatrick 1996), they have had limited use with the cultivated peanut (Kochert, Halward et al. 1991; Hopkins, Casa et al.

1999; Palmieri, Hoshino et al. 2002; Moretzsohn, Hopkins et al. 2004; He, Meng et al. 2005; Moretzsohn, Leoi et al. 2005; Palmieri, Bechara et al. 2005). This is due to limited large scale DNA sequence information, and number of markers that have been screened and mapped. Therefore, more polymorphic markers are needed and a dense linkage group map also will play an import role to increase the development of marker technology in cultivated peanut.

Several research groups have developed hundreds of different SSR markers and screened them within different species. Six sequence-tagged microsatellite (STMS) markers with high variation in cultivated peanut were developed and identified (Hopkins, Casa et al. 1999). Ferguson et al (Ferguson, Burow et al. 2004) located and characterized hundreds of SSRs by motif and estimated polymorphism in a diverse array of 24 cultivated peanut accessions. Later 11 novel polymorphic microsatellite markers were developed by Palmieri et al (Palmieri, Bechara et al. 2005), where they detected greater genetic diversity than the markers used before. Meanwhile, Guohao He tested the microsatellite markers performance in the cultivated peanut (He, Meng et al. 2003). From his research, 19 microsatellite markers out of 56 different microsatellite markers showed polymorphism among several different resource botanical peanut variety (He, Meng et al. 2003). Moretzsohn et al. (Moretzsohn, Hopkins et al. 2004; Moretzsohn, Leoi et al. 2005) developed a total of 338 microsatellite markers from SSR-enriched genomic libraries, EST (expressed sequence tags), and by "data-mining" sequences available in Genebank. They all showed good percentage polymorphism in A-genome wild type peanut, and those polymorphic markers also have been used in the linkage group mapping construction of AA genome. Due to the lack of abundant polymorphic molecular markers and morphological variation, there are only 3 genetic mapping results reported in peanut, two were done with inter-specific diploid hybrids

and another was done in an inter-specific tetraploid hybrid breeding system (Halward, Stalker et al. 1993; Burow, Simpson et al. 2001; Moretzsohn, Leoi et al. 2005). Meanwhile, the marker technology also got developed to some extent in peanut breeding, it has been used in peanut breeding for selection of nematode resistance (Chu, Holbrook et al. 2007), and RAPD markers have been developed for resistance to *Puccinia arachidicola*, (peanut rust) in peanut (Mondal, Badigannavar et al. 2008).

In all, SSR markers have been widely developed through different ways, and have been applied successfully to assess genetic variation to some extent. In this research, majority polymorphic markers detected by different research group discussed above have been selected and screened with sixteen cultivated peanut genotypes with a range of reactions to Tomato spotted wilt virus (tomato spotted wilt), *Cercospora arachidicola* (early leaf spot) *and Cercosporidium personatum* (late leaf spot). The objective of this project was 1) to estimate the marker performance from allele-length ranges, average PIC score and heterozygosity among 16 genotypes of cultivated peanut; 2) to detect the genetic diversity relationship of these 16 genotypes of cultivated peanut which have showed a wide range of field reactions to TSWV and the early leaf spot pathogen; 3) to explore potential genotypes which could be used as hybridization for recombinant inbred line (RIL) population development so that a dense cultivated peanut genetic linkage map could be constructed based on our study in the future.

2. Materials and methods

2.1 Plant materials

Plant materials were provided from USDA-ARS and University of Georgia peanut programs at the University of Georgia Coastal Plain Experiment Station in Tifton, GA in 2006. A diverse array of 16 genotypes tested in this study is listed in Table 2.5. Genotypes examined included commercial cultivars as well as breeding lines and germ-plasm accessions from the U.S. and China. These genotypes included some lines that have been previously evaluated for resistance to TSWV and leaf spot pathogens, and had varying levels of resistance to TSWV, *C. arachidicola* and *C. personatum*. According to the previous reports, Georgia Green (Branch 1996) is the predominant cultivar planted in the state of Georgia with a moderate level of field resistance to TSWV and usually has been used as standard moderate resistance cultivars to TSWV (Culbreath, Todd et al. 1999; Culbreath, Todd et al. 1999; Culbreath, Tillman et al. 2008), Georganic (Holbrook and Culbreath 2008), and Georgia-01R (Branch 2002), are cultivars with resistance to TSWV and moderate levels of resistance to the early and late leaf spot pathogens (Holbrook, Timper et al. 2008; Holbrook, Timper et al. 2008); Tifguard and a near-isogenic sister line – C724-19-25 also have field resistance to TSWV but different susceptibility to nematode (Holbrook, Timper et al. 2008), Tifrunner (Holbrook and Culbreath 2007) was released in 2007 as a new cultivar with field resistance to TSWV and moderate resistance to early and late leaf spot; SunOleic 97R (Gorbet and Knauft 2000) is a cultivar developed by the University of Florida with good agronomic traits including high (>80%) oleic acid oil composition, but is susceptible to TSWV (Culbreath, Gorbet et al. 2005) and leaf spot pathogens. The breeding line NC 94022 has been reported to have a high level of field resistance to TSWV(Culbreath, Gorbet et al. 2005).

2.2 DNA extraction

Leaves of 16 genotypes for total DNA extraction were collected from greenhouse grown plants at Tifton. The fresh leaf tissues were frozen at -80℃ as soon as possible, and then ground in liquid nitrogen with a mortar and pestle. The total genomic DNA was extracted according to modified CTAB methods (Tang, Yu et al. 2002) from those frozen leaves. DNA concentration and quality was determined by use of a spectrophotometer (Spectronic Instruments) at 260nm wavelength and a ratio of 260nm/280nm determined. Afterwards, DNA was diluted in sterile water to 10ng/ul for PCR reaction.

2.3 SSR fluorescent markers

A total of 709 pairs of SSR markers were used for screening for polymorphisms in this study. Among them, 97 pairs of primers were newly developed from ESTs by Dr. S. Knapp's lab, University of Georgia, Athens. An additional 612 pairs of primers were chosen from 6 different research groups' published data according to their screening performance. These markers are from different resources shown in Table 2.6. For these 709 markers, the forward primer of all these primers were added different fluorescent phosphoramidite to the 5' end of the oligo-nucleotide to be used for multiplex mixture analysis in the marker screening process. Three kinds of fluorescently labeled markers were used in this study. Among them 237 primers were labeled with 6-FAM (6-carboxyfluorescein), 236 primers were labeled as HEX (hexachloro-carboxyfluorescein), and the remaining 236 primers were labeled as TAMRA (tetramethyl rhodamine) (Hopkins, Casa et al. 1999; Tang, Yu et al. 2002).

Initially, SSR markers were screened on 1.5% agarose gels for utility, functionality, and length estimation using the bulk DNA of 4 randomly chosen cultivated peanut and 4 randomly chosen

wild type peanut samples. The pre-screening results are presented in Table 2.2. After screening for functional SSR markers, 153 SSR markers were deleted because of no amplification, leaving 556 (78.4%) SSR markers to be screened for polymorphism in the 16 genotypes.

2.4 PCR amplification

An 11.5µl volume reaction system in 384-wells plate was used for 'touchdown' PCR reaction, containing 1 µl of forward and reverse primer; 2µl of sample to be prepared as template; 1.15µl of 10 X PCR buffer; 0.25µl of dNTP; 0.1µl of Taq enzyme. The 'touchdown' thermal cycle of the PCR reaction was decided by the melting temperature (Tm) for different kinds of primers separately to be applied with either 52℃ or 56℃ 'touchdown' cycle reaction. The 'touchdown' amplification program is as follows: 94 ℃ for 1 min to allow samples to denature, followed by 6 cycles of 94℃ for 30s, 62℃ for 30s and 72℃ for 30s, the annealing temperatures were decreased 1℃ per cycle in subsequent cycles till the temperature reached 52 ℃ or 56℃ for the different kinds of 'touch down' program. Products were subsequently amplified for 36 cycles at 94℃ for 20s, 56℃ for 20s, and 72℃ for 30s.

2.5 Electrophoresis and detection of fluorescent products

After PCR reaction, the products were first checked with 1.5% agarose gel to ensure successful amplifications. According to the record of those markers fluorescent labels and expected amplification length check in the pre-screening, those amplicons with different labels and amplification length were diluted 60- to 100- fold. Then those six different diluted amplicons with different color and amplification length were mixed into one well by 1 uL with 9 ul of formamide with a GeneScan 500 internal lane standard labeled with ROX. GeneScan Filter Set

D and the ROX 500 internal-lane were used for analyses of amplicons labeled with FAM, HEX, and TAMARA. The functional SSR markers (556 SSR markers) screening results were analyzed by Gene Mapper 4.0 based on the multiplexes amplicon mixtures with different fluorescent label and amplification length. In the end, the results were entered into an Excel 2007 spreadsheet for future analysis.

2.6 Statistical analysis method

The screening results for the 556 markers were recorded as 1 for presence of the amplification band, 0 for absence of the amplification band for all markers. According to the amplification quality and reliability within the 16 genotypes, the markers were divided into 1-5 different rating groups, where 1= Excellent, amplification results indicated perfect amplification with unambiguous product within all 16 genotypes; 2 = Good, amplification results indicated clear peak with magnification within GeneMapper 4.0; 3 = Fair, indicated clear peak with high magnification and 1-2 genotypes with null amplification results; 4 = Poor, indicated bad amplification results and 2-4 genotypes with null amplification results; 5 = No good, indicated that more than half genotypes had null amplification results. Examples of excellent and poor amplification results observed in GeneMapper 4.0 are shown in Figure 2.1.

PIC value is defined by Botstein (Botstein, White et al. 1980) as a closely related diversity measure, is a measure of the polymorphism of a marker (SSR marker) for linkage. The formula for this estimation of PIC score is:

$$\hat{PIC}_l = 1 - \sum_{u=1}^{k} \tilde{p}_{lu}{}^2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\tilde{p}_{lu}{}^2 \tilde{p}_{lv}{}^2$$

Where l=index for marker 'l'; $\tilde{p}_{lu}$ = proportion of marker 'l' alleles which are of allele type 'u'; $\tilde{p}_{lv}$ = proportion of marker 'l' alleles which are of allele type 'v'; k=number of alleles types present for marker 'l'， (Shete, Tiwari et al. 2000).

Another estimator, heterozygosity (H) was estimated according to the formula:

$$H = 1 - \sum_{i=1}^{n} p_i^2$$

Where P_i is the frequency of i^{th} allele in the genotypes population (Shete, Tiwari et al. 2000). The estimation of PIC, amplified allele number, allelic frequency and observed heterozygosity were obtained by software GeneMarker. (Liu and Muse 2005)

2.7 Genetic relationship analysis

Among these 556 screened markers, 281 markers with excellent amplification results and unambiguous product within all 16 genotypes were used to analyze the diversity relationship in this study.

Estimators of genetic diversity within these 16 genotypes were based on the shared alleles distance (Dps) in pairwise comparisons. The estimates of genetic distance were obtained by the parameter: [1- Dps], the MicroSat software was used to perform the calculation of genetic distance estimate within the 16 genotypes. The cladogram trees were obtained by PHLIP program with neighbor-joining method based on the genetic distance matrix obtained as described above. Then trees were drawn using Tree View.

3. Results

3.1 Screening of markers in 16 cultivated peanut genotypes

After bulk DNA pre-screening with 4 bulked cultivated peanut and wild type peanut genotypes, there were 153 pairs of primers found which did not work in our screening, and 556 pairs of primers left for polymorphism screening in 16 cultivated peanut genotypes. Among 556 markers with utility, 80 pairs of primers should be used with 52℃ 'touchdown' and others can be used with 56℃ 'touchdown' PCR reaction. An overview of marker amplification quality and polymorphism screening results are shown in Table 2.1 and Table 2.2. In our study, not all markers clearly showed unambiguous amplification bands, whereas some markers showed no amplification band within several genotypes. Out of 556 functional markers in screening, only 281 markers, accounting for approximately 50.5% of those tested, gave reliable unambiguous results, which are used in the analysis of genetic diversity within these 16 genotypes in case other markers could cause estimate errors in genetic distance estimation.

After polymorphism screening within 16 genotypes using 556 functional SSR markers, a total of 235 (42.27%) markers showed polymorphism within the 16 genotypes. The polymorphism showed in this screening is relatively high compared with other DNA markers (RFLPs and RAPDs) in the cultivated peanut (He, Meng et al. 2005). However, only 83 markers out of 281 excellent markers (29.5%) showed polymorphism within 16 genotypes, whereas there are 235 markers (42.3%) out of 556 markers in the screening showed polymorphism within 16 genotypes. The polymorphism screening result based only on excellent quality marker is severely lower than the estimate from the overall screening results, because ambiguous bands and no amplification within several genotypes caused higher estimate of polymorphism. Therefore, in our study,

except those markers with ambiguous bands and no amplification within some genotypes, there are totally 461 markers with reliable amplification bands observed in GeneMapper (exclude poor and no-good quality markers in Table 2.1. Of these 461 markers, 174 (37.74%) had polymorphism within these 16 genotypes. Therefore, considering the estimate error from polymorphism screening itself, we can conclude that the polymorphism within these 16 genotypes in our study is between 29.54% and 37.74%.

3.2 SSR marker characters

Average heterozygosity estimated from 556 pairs of SSR markers was 0.225 among the 16 cultivated peanut genotypes. Heterozygosity for individual SSR markers ranged from 0.000 to 0.922. Characters indicators including Average No. of alleles, PIC score, and heterozygoity for different research groups are shown in Table 2.3.

As with most previous reports, all those average values including allele number, PIC, and heterozygosity are very low, except with markers from Dr. Krishna research group. In our study only 2 markers from their research study were used to screen polymorphism in our research, and one marker showed polymorphism results without clear unambiguous bands. The information of Allele No. as 4, PIC as 0.664 and heterozygosity as 0.703 does not reflect overall marker performance. Whereas the average No. of alleles overall is 2.509 with a range from 1 to 13 among 16 cultivated peanut genotypes (Table 2.3). 57.81% of the markers have only 1 allele in our screening. Overall screening results in our study keep consistent as the markers polymorphism screening results from different research groups. Overall results either from

marker polymorphism performance, PIC, or heterozygosity indicated that there is very limited genetic diversity within these 16 genotypes in our study.

Among those 125 excellent and good quality polymorphism markers, the most frequent repeat family identified was binucleotide GA with accounting for 26.4% similar to that reported by Ferguson et al (Ferguson, Burow et al. 2004), followed by binucleotide CT(10.4%), and trinucleotide TAA (9.6%) show in Table 2.4. Our research validated that the binucleotide and trinucleotide repeat motifs were the most abundant type of SSRs, and binucleotide GA repeat motif showed higher polymorphism compared with other trinucleotide and polynucleotide repeat motifs (Moretzsohn, Leoi et al. 2005).

3.3 Genetic relationship analysis of 16 Arachis hypogaea cultivated peanut

The genetic distance matrix was estimated by shared allele distance in pairwise comparisons of 16 *Arachis hypogaea* cultivate peanut genotypes using only those 281 excellent quality markers with unambiguous band (Figure 2.1). The genetic distance within those 16 genotypes can be observed from 0.002 to 0.169. Among those 16 genotypes, the smallest genetic distances were between Tifton8 and Tifrunner with genetic distance of 0.002 and the largest genetic distance is 0.169 between Georganic and GTC9. Tifguard and C 724-19-25 had genetic distance of 0.033.

A cladogram of the 16 *Arachis hypogaea* genotypes was constructed based on the genetic distance matrix (Figure 2.2) with neighbor-joining methods shown in Figure 2.3. There is no significant cluster between considering all of them belong to *A. hypogaea.* But several small clusters (cluster A, B, C, D, E in Figure 2.3) were branched together in the cladogram tree under

our expectations. Sister lines Tifguard and C 724-19-25 also as expected were grouped together in cluster A. Two Virginia type peanuts (GPNCWS13 and NC6) were grouped together. Georgia Green (GaGreen) were grouped together with released cultivar SunOleic in cluster C. Cluster D consists of two Chinese breeding lines (PE1 and PE2) and another U.S. released cultivar (Tennessee Red-TNRed); cluster E includes two Chinese released cultivars (GTC20 and GTC9) and U.S. released cultivar (Spancross). The smallest genetic distance between Tifton8 and Tifrunner also had been branched together from the cladogram tree; whereas the largest genetic distance between Georganic and GT-C9 belonged to the different branches from the cladogram tree.

4. Discussion

4.1 SSR markers screening in 16 cultivated peanut genotypes

Considering these markers are from different research groups, the polymorphism of markers could also be different from their research, because of the different genotypes used for polymorphism screening. In much of the related research, the cultivated peanuts used for polymorphism screening were mainly from South America (He, Meng et al. 2003; Ferguson, Burow et al. 2004; Moretzsohn, Hopkins et al. 2004; Moretzsohn, Leoi et al. 2005). In our study, 12 genotypes are from U.S. and another 4 genotypes are from China. In our study, the polymorphism of the markers developed by Ferguson et al. (Ferguson, Burow et al. 2004) did not show the same high polymorphism results (110 polymorphism markers out of 192 amplified well markers) reported. Only 71 markers (19.27%) our of 192 designed markers showed polymorphism within 16 cultivated genotypes in our study. Among those 71 markers, only half of them (37 markers) amplified unambiguous bands within all 16 genotypes. The lower

polymorphism in our study could be caused by the cultivated peanut genotypes used in screening, because in their research, 24 genotypes used in screening are from different sources and different market types, so they may have been more diverse compared with genotypes in our study.

Out of 56 designed markers developed from microsatellite enriched library by He et al., 19 markers (34%) were reported as polymorphism markers in their research (He, Meng et al. 2003). A similar trend was observed showed in our study, where 22 markers showed polymorphism, and 13 markers had an unambiguous reliable amplification band within all 16 genotypes in our study. Although, those 24 genotypes reported by He et al. consisted of different botanical species including *hypogaea, hirsute, fastigiata, peruviana, aequatoriana, vulgais*, they are all from South America. The country origin could restrict the markers polymorphism performance. Therefore, the markers polymorphism performance are very similar with our research study using 16 genotype mainly from U.S. Similar polymorphism results also observed for the markers developed by Moretzsohn et al. ((Moretzsohn, Hopkins et al. 2004)). In their study, they reported that 3 markers out of 56 designed markers had polymorphism within 5 cultivated peanut lines (Moretzsohn, Hopkins et al. 2004). Sixty-six of 271 designed markers had polymorphism within six *A. hypogaea* accessions mainly from Brazil.

Among 6 polymorphism markers obtained by Hopkins et al. with the library screening method, not all had polymorphism in our screening. It could be possible that the country of origin of the genotypes used in screening could be responsible for 2 markers which did not show polymorphism in our study, but showed polymorphism in their study. The similar result for Dr. Krishina research group, 48 cultivated Valencia peanut genotypes could show polymorphism in

their research, but we only used two markers out of 18 SSR markers in their study. In our study, one marker did not show application result, so only one marker was left for 16 cultivated peanut genotypes screening, which showed polymorphism within 16 genotypes in our study. The markers developed by Dr. Palmieri research group are from *Arachis pintoi* ((Palmieri, Hoshino et al. 2002; Palmieri, Bechara et al. 2005), which may be responsible for polymorphic markers in their study not showing polymorphism in our research among 16 *Arachis hypogaea* accessions.

The 97 newly developed markers were developed from EST (expressed sequence tags) library, 40 markers showed polymorphism, but only 16 markers had unambiguous amplification bands within all 16 genotypes; therefore, it was hard to evaluate markers polymorphism performance here considering some apparent polymorphisms could be pseudopolymorphism caused by null amplification results with some genotypes.

4.2 SSR marker characters

Our research corroborated that the binucleotide and trinucleotide repeat motif were the most abundant type of SSRs, and binucleotide GA repeat motif showed higher polymorphism compared with other trinucleotide and polynucleotide repeat motif(Moretzsohn, Leoi et al. 2005). The most frequent repeat family identified was binucleotide GA which also reported by Ferguson et al (Ferguson, Burow et al. 2004), followed by binucleotide CT(10.4%), and trinucleotide TAA (9.6%) . All of the information from different research groups keeps consistent as the marker polymorphism results. All of these results either from marker polymorphism performance, PIC, or heterozygosity indicated that there is very limited genetic diversity within these 16 genotypes in our study.

4.3 Genetic diversity analysis with 16 Arachis hypogaea cultivated peanut

The genetic distance matrix were estimated by shared allele distance in pairwise comparisons of 16 *Arachis hypogaea* cultivated peanut genotypes using only those 281 excellent quality markers with unambiguous band Figure 2.1. A cladogram of the 16 *Arachis hypogaea* genotypes was constructed based on the genetic distance matrix (Figure 2.2) with neighbor-joining methods shown in Figure 2.3. One of close genetic relationship is from Tifguard and C 724-19-25 as expected in cluster A, since those are sister lines and have been reported as near-isogenic (Holbrook, Timper et al. 2008). Even though they both were developed by crossing 'C-99R' with 'COAN', they contain some extent agronomic trait difference in the resistance reaction of TSWV and peanut root-knot nematode, Tifguard is a released cultivar with a high level of resistance to the peanut root-knot nematode, whereas, C7241925 is susceptible to the nematode. The largest genetic distance is from Georganic and GTC9 considering these genotypes are from different countries and from breeding programs with different objectives. In cluster D GTC20 and GTC9 branched together with U.S. released cultivar Spancross, they all belong to the Spanish type (Hammons 1970) (B.Z. Guo, personal communication) and there is no significant difference of field reaction to TSWV and C. *arachidicola* (Li Y. unpublished data). Another two Chinese breeding lines PE1 and PE2 were grouped together in cluster E with U.S. release cultivar TNRed which is out of expectation to some extent. However it could be presumed that TNRed has similar morphology trait with those two Chinese breeding lines, but two Chinese breeding lines belong to Viginia type, whereas TNRed is a released Valensia peanut. NC 6 and GPNPWIS-13 in cluster B is also not surprising since NC 6 was a parent used in development of GP-NC WS 13 (Stalker, Beute et al. 2002). However, there is no apparent close relation between Tifton8 and

Tifrunner, which had the smallest genetic distance with each other. Tifunner was developed from a cross of a component line of the cultivar Florunner and PI 203396 (Holbrook, Timper et al. 2008), whereas Tifton-8 was developed from a virginia-type plant in the spanish-type genotype, PI 261976 (Coffelt, Hammons et al. 1985).

5. Conclusion

This research project provided a reliable polymorphism marker screening results within 16 *Arachis hypogaea* genotypes which widely used in the southeast peanut production region, and gave a confirmation of genetic relationship within those sixteen genotypes. The polymorphism marker screening results corroborated previous reports of limited genetic diversity in cultivated peanut *Arachis hypogaea*. Although many SSR markers used in our study have been developed and screened in other *Arachis* genotypes, polymorphisms are not consistent and did not show the same polymorphism within different genotypes.

This is also the first time report of the genetic relationships among Chinese and U.S. genotype which have different levels of resistance to TSWV and C. *arachidicola*, even more, the U.S. genotypes used in this research also have been conducted in the filed evaluation of tomato spotted wilt and early leaf spot, and genetic relationships among these 16 genotypes vary greatly in field reactions to TSWV and C. *Arachidicola* (Li Y. unpublished data) so that those polymorphic markers may be useful in the development of markers for resistance to TSWV and/or C. *Arachidicola*. In addition, all of these sixteen genotypes also have been used in the field evaluation to detect the reaction to TSWV and C. *arachidicola* in the two years filed evaluation (Li Y., unpublished data). Based on the field evaluation and genetic relationship

results using simple sequence repeat (SSR) markers in this study, the four genotypes were used to develop two recombinant inbred line (RIL) populations. Finally, the genetic relationship analysis already gave reliable information for RIL populations development together with field evaluation results (Li Y. unpublished data); this research provided reliable polymorphism SSR markers for cultivated peanut linkage group mapping construction and markers potentially related to the resistance gene of TSWV and/or C. *Arachidicola.*

6. References

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Figure 2.1 - Amplified fragment patterns of two different markers within Tifrunner (above) and GTC20 (below), indicating different quality rating results for 2 markers with 1 (above) and 3 (below)

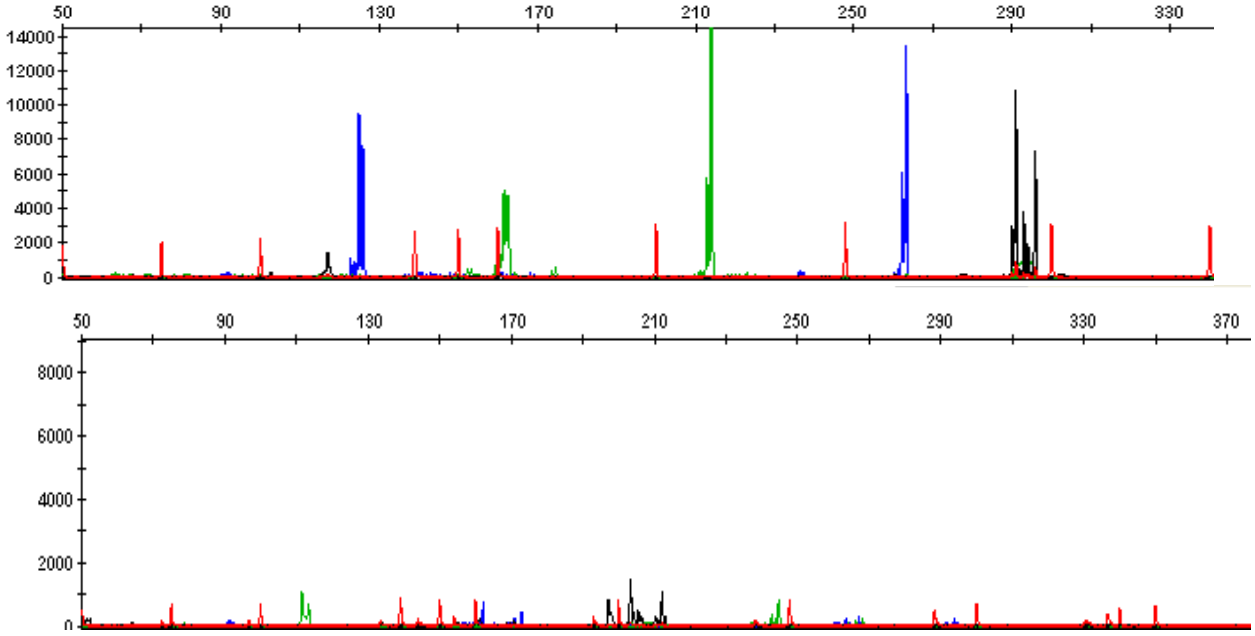

Figure 2.2 - Genetic relationship of 16 cultivated peanut genotypes estimating from 281 excellent SSR markers

	Tifrunner	GTC20	GTC9	Georganic	Tifguard	C7241925	GaGreen	GPNCWS13	NC6	UFNC94022	Spancross	TNRed	Tifton8	PE1	PE2	SunOleic
Tifrunner	0.000	0.150	0.168	0.053	0.086	0.059	0.095	0.094	0.089	0.128	0.131	0.153	0.002	0.122	0.137	0.089
GTC20	0.150	0.000	0.078	0.151	0.140	0.142	0.116	0.157	0.149	0.141	0.106	0.141	0.150	0.130	0.111	0.106
GTC9	0.168	0.078	0.000	0.169	0.160	0.167	0.130	0.160	0.157	0.140	0.132	0.153	0.168	0.122	0.113	0.122
Georganic	0.053	0.151	0.169	0.000	0.110	0.087	0.097	0.119	0.099	0.131	0.132	0.149	0.054	0.127	0.128	0.101
Tifguard	0.086	0.140	0.160	0.110	0.000	0.033	0.071	0.088	0.103	0.128	0.127	0.129	0.085	0.106	0.128	0.078
C7241925	0.059	0.142	0.167	0.087	0.033	0.000	0.069	0.082	0.090	0.125	0.118	0.126	0.058	0.101	0.128	0.077
GaGreen	0.095	0.116	0.130	0.097	0.071	0.069	0.000	0.115	0.105	0.095	0.114	0.119	0.094	0.088	0.112	0.055
GPNCWS13	0.094	0.157	0.160	0.119	0.088	0.082	0.115	0.000	0.063	0.110	0.114	0.149	0.093	0.087	0.123	0.113
NC6	0.089	0.149	0.157	0.099	0.103	0.090	0.105	0.063	0.000	0.092	0.101	0.136	0.088	0.094	0.118	0.102
UFNC94022	0.128	0.141	0.140	0.131	0.128	0.125	0.095	0.110	0.092	0.000	0.125	0.134	0.129	0.120	0.126	0.101
Spancross	0.131	0.106	0.132	0.132	0.127	0.118	0.114	0.114	0.101	0.125	0.000	0.117	0.130	0.105	0.108	0.106
TNRed	0.153	0.141	0.153	0.149	0.129	0.126	0.119	0.149	0.136	0.134	0.117	0.000	0.152	0.120	0.108	0.114
Tifton8	0.002	0.150	0.168	0.054	0.085	0.058	0.094	0.093	0.088	0.129	0.130	0.152	0.000	0.121	0.136	0.089
PE1	0.122	0.130	0.122	0.127	0.106	0.101	0.088	0.087	0.094	0.120	0.105	0.120	0.121	0.000	0.070	0.094
PE2	0.137	0.111	0.113	0.128	0.128	0.128	0.112	0.123	0.118	0.126	0.108	0.108	0.136	0.070	0.000	0.103
SunOleic	0.089	0.106	0.122	0.101	0.078	0.077	0.055	0.113	0.102	0.101	0.106	0.114	0.089	0.094	0.103	0.000

Figure 2.3 - The cladogram tree of 16 cultivated peanut genotypes based on the estimation of genetic relationship

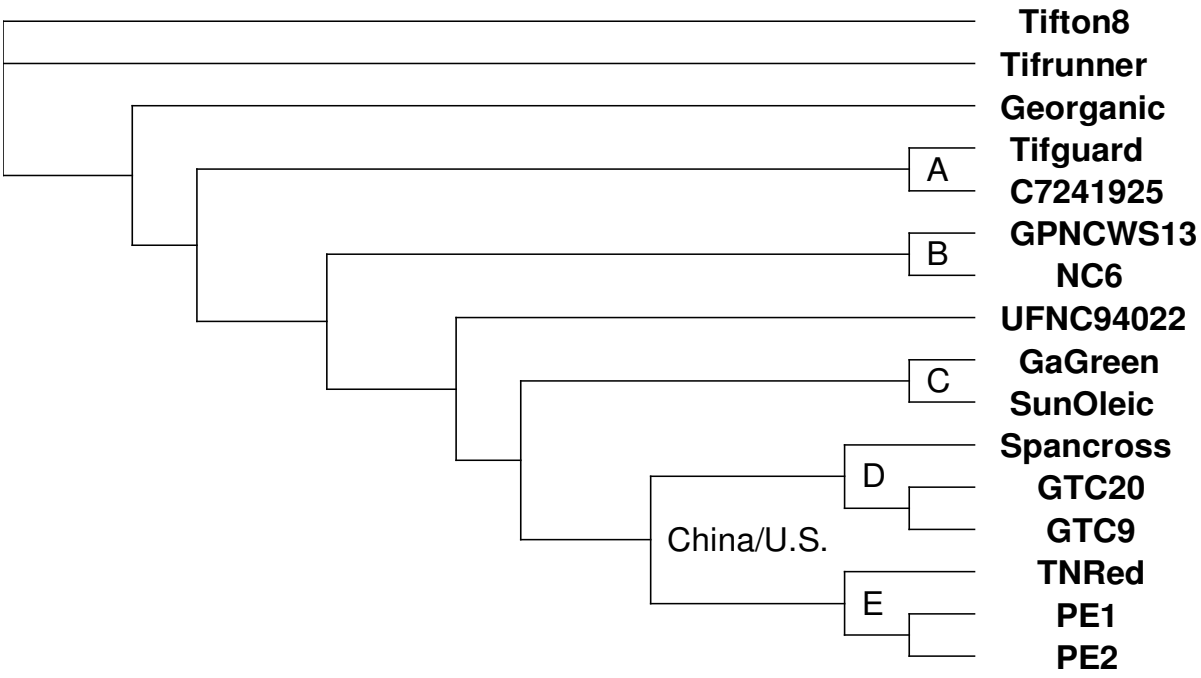

Table 2.1 - Overview of markers quality information

Quality index	No. of Markers in Screening	Percentage of Quality Markers in Screening	No. of Markers showing polymorphism in Screening
Excellent	281	50.53 %	83
Good	95	17.09 %	42
Fair	85	15.29 %	49
Poor	28	5.04 %	16
No-good	67	12.05 %	45
Total	556	100%	235

Table 2.2 - Overview of SSR marker characters

SSR ID	No. of Marker Designed	No. (Per.)of Polymorphism Markers out of Designed Markers	No. (Per.) of Excellent and Good Quality Polymorphism Marker out of Designed Markers	Resources
GM421-GM612	192	71 (36.98%)	37 (19.27%)	Ferguson et al. 2004
GM365-GM420	56	22 (39.29%)	13 (23.21%)	He et al. 2003
GM339-GM344	6	4 (66.67%)	3 (50%)	Hopkins et al. 1999
GM345,GM346	2	1 (50%)	0 (0%)	Krishna et al. 2004
GM271-GM338	67	8 (11.94%)	6 (8.96%)	Moretzsohn et al. 2004
GM1-GM271	271	85 (31.37%)	49 (18.08%)	Moretzsohn et al. 2005
GM347-	7	2 (28.57%)	1(14.29%)	Palmieri et al. 2002
GM364	11	2 (18.18%)	0 (0%)	Palmieri et al. 2005
GM613-GM709	97	40 (41.24%)	16 (16.49%)	Steve J. Knapp et al. 2006
Total	709	235 (33.16%)	125 (17.6%)	

Table 2.3 - Average No. of alleles, PIC score, and heterozygosity of 235 polymorphism markers within 16 genotypes

SSR ID in our screening	Citation	Average of Allele No.	Range of Allele No.	Average of PIC	Average of heterozygosity
GM421-GM612	Ferguson et al. 2004	2.52	1-12	0.215	0.235
GM365-GM420	He et al. 2003	2.38	1-9	0.223	0.246
GM339-GM344	Hopkins et al. 1999	3.83	1-8	0.374	0.394
GM345,GM346	Krishna et al.2005	4	4	0.644	0.703
GM1-GM271	Moretzsohn et al. 2005	2.96	1-13	0.253	0.269
GM271-GM338	Moretzsohn et al. 2004	1.3	1-5	0.047	0.051
GM347-	Palmieri et al. 2002	2	1-4	0.148	0.164
GM364	Palmieri et al. 2005	2.5	1-6	0.224	0.238
GM613-GM709	Steve J. Knapp et al. 2006	2.26	1-11	0.186	0.202
Average		2.5	1-13	0.209	0.225

Table 2.4 - Characters of 125 excellent and good polymorphism markers by repeat family

Repeat Family	No. of polymorphic marker with excellent and good quality	Percentage of Repeat Motif
aac	2	1.60
aac/ga	1	0.80
aat	1	0.80
ag	10	8.00
agc/aac	1	0.80
ata	3	2.40
att	1	0.80
c	1	0.80
ca/aaag	1	0.80
cca/acc	1	0.80
cga/ag/ga	1	0.80
cgg	1	0.80
ct	13	10.40
ct/ca	3	2.40
ct/ga	1	0.80
ct/ta	1	0.80
ctc/aac	1	0.80
ctcact	1	0.80
ctt	4	3.20
ctt/ctg	1	0.80
cttt	1	0.80
ga	33	26.40
ga/aa	1	0.80
ga/gaa	1	0.80
ga/gg	1	0.80
ga/gt	2	1.60
ga/ttc	1	0.80
gag/ctt/tc	1	0.80
gat	1	0.80
gcc	1	0.80
gt/tatt	1	0.80
ta	1	0.80
ta/ga	1	0.80
taa	12	9.60
tac/ga	1	0.80
tat	1	0.80
tatc	1	0.80
tc	9	7.20
tca	1	0.80
tg	2	1.60
tgg	1	0.80
ttg	1	0.80
ttg/aga	1	0.80

Table 2.5 - The list of 16 genotypes used in the SSR marker screening

S. No.	Genotype	Country Origin	Market Type
1	Tifton 8	U.S. germplasm	Virginia
2	C724-19-25	U.S. breeding line	Runner type
3	Georgia Green	U.S. cultivar	Runner type
4	Georganic (C-11-2-39)	U.S. cultivar	Runner type
5	Spancross	U.S. cultivar	Spanish
6	Tifguard (C724-19-15)	U.S. cultivar	Runner type
7	NC-6	U.S. cultivar	Virginia
8	SunOleic 97R	U.S. cultivar	Runner type
9	Tifrunner	U.S. cultivar	Runner type
10	UF NC 94022	U.S. breeding line	Runner type
11	PE-2	Chinese breeding line	Virginia
12	PE-1	Chinese breeding line	Virginia
13	GTC-20	Chinese cultivar	Spanish
14	GTC-9	Chinese cultivar	Spanish
15	Tennessee Red	U.S. cultivar	Valencia
16	GPNCWS13	U.S. cultivar	Virginia

Table 2.6 - 709 Marker information after bulk DNA functional screening

Markers	Number of Markers Designed	Marker yielding amplification[1]	Resources
GM421-GM612	192	158 (82.3%)	Ferguson et al. 2004
GM365-GM420	56	47 (83.9%)	He et al. 2003
GM339-GM344	6	6 (100%)	Hopkins et al. 1999
GM345,GM346	2	1 (50%)	Krishna et al.2005
GM1-GM271	271	186 (68.6%)	Moretzsohn et al. 2005
GM271-GM338	67	56 (83.6%)	Moretzsohn et al. 2004
GM347-	7	4 (57.1%)	Palmieri et al. 2002
GM364	11	4 (36.4%)	Palmieri et al. 2005
GM613-GM709	97	94 (96.9%)	Steve J. Knapp et al. 2006
Total	709	556 (78.4%)	

[1]Indicate what "marker yielding amplification means ie. Number of functional markers used in screening. Numbers in parenthesis are percentage of functional markers.

Chapter 3

Variability in Field Response of Peanut Genotypes from the U.S. and China to Tomato Spotted Wilt Virus and *Cercospora arachidicola* [2]

[2] Y. Li. and A.K. Culbreath. To be submitted to *plant disease*.

Abstract

Tomato spotted wilt, caused by Tomato spotted wilt virus (TSWV), transmitted by several kinds of thrips, and early leaf spot caused by *Cercospora arachidicola*, are among the most important diseases of peanut (*Arachis hypogaea*) in the southeastern United States. In field trials in 2007 and 2008, 22 genotypes, planted in the field at Tifton were evaluated for tomato spotted wilt and early leaf spot reactions. There was a near-continuous range of final incidence of spotted wilt from 18.22% to 78.68%. Among genotypes, UF NC 94022, Georganic, C689-6-2, Georgia-01R, C724-19-25, C209-6-13, C11-154-61, C 12-3-114-58, and Tifguard were among the most resistant genotypes for spotted wilt, whereas GTC20, GTC9 and PE2 were the most susceptible. Final percent defoliation by early leaf spot ranged from 10% to 97% for both years. In average, genotypes C689-2, Georgia-01R, C12-3-114-58, C11-154-61, Tifguard and Georganic showed resistance to early leaf spot pathogen, whereas NC-6, Spancross, GT-C9, GT-C20 and PE-2 are susceptible to early leaf spot pathogen. There were three cultivars and three breeding lines which were classified as resistant to both TSWV and *C. arachidicola*; whereas there are 3 genotypes from China susceptible to both TSWV and *C. arachidicola*. This could be caused by different breeding objective. Disease reactions will be used in conjunction with genetic characterization of these genotypes and recombinant inbred line populations developed form crosses of selected genotypes in efforts to develop markers for resistance to TSWV and *C. arachidicola*.

Keywords: Tomato spotted wilt, TSWV, early leaf spot, *Cercospora arachidicola*, multiple pathogen resistance, susceptible

1. Introduction

Peanut (*Arachis hypogaea* L.), also known as groundnut, is an important crop in the world for human consumption, livestock feed, and oil. It is mainly grown in tropical and subtropical regions of the world with China, India and the U.S. being the top three peanut producing countries. Diseases are major yield limiting factors in all areas where peanuts are grown.

Two of the most prevalent and severe diseases of peanut worldwide are early leaf spot caused by *Cercospora arachidicola* S. Hori, (teleomorph = Mycosphaerella arachidis Deighton) and late leaf spot caused by *Cercosporidium personatum* (Ber. & M. A. Curtis) Deighton, (telemorph = M. berkeleyi Jenk.) (Culbreath, Todd et al. 2003; Smith and Littrell 1980). In the U. S., most of the peanuts are grown in the southern states where environmental conditions are often favorable for leaf spot epidemic development. One or both of the leaf spot diseases occurs in all peanut producing states, and multiple applications of fungicides are necessary for control on the susceptible cultivars currently used.

Tomato spotted wilt, caused by Tomato spotted wilt virus (TSWV), is another serious problem in peanut in the southeastern U.S. Spotted wilt of peanut was first reported by Costa (Costa 1941) in Brazil, and was observed in the U.S. in Texas in 1971 (Halliwell and Philley 1974). It has since become one of the most important diseases in peanut production areas in the southeastern U.S. (Culbreath 1997). Tomato spotted wilt occurs every year with fluctuations in severity within years and locations (Culbreath, Todd et al. 2003; Smith and Littrell 1980). Losses to tomato spotted wilt in peanut increased steadily from the late 1980s to 1997, with annual loss caused by spotted wilt as high as $40 million in Georgia alone (Williams-Woodward 2001; Brown 2003).

TSWV is transmitted by several species of thrips (Ullman, Sherwood et al. 1997), but control of thrips has not shown much promise for management of spotted wilt in peanut. Currently, management of spotted wilt is dependent upon the use of cultivars such as Georgia Green with a moderate level of field resistance (Culbreath, Todd et al. 1996), and cultural practices which reduce the incidence and severity of spotted wilt (Brown, Todd et al. 1996; Culbreath, Todd et al. 1999; Brown, Todd et al. 2000; Brown 2003). New cultivars such as AP-3 (Gorbet 2007), Georgia-02C (Branch 2003) , and Tifguard (Holbrook, Timper et al. 2008) have greater levels of field resistance than Georgia Green, but still can be severely affected. Higher levels of field resistance have been reported in the cultivar Georganic (Culbreath, Todd et al. 1997; Culbreath, Todd et al. 1999) and breeding line UF NC 94022 (Culbreath, Gorbet et al. 2005), but these lines are not acceptable for standard commercial production.

The development and use of resistant cultivars is one of the most desirable ways to manage both leaf spot diseases and tomato spotted wilt, even if resistance in not complete (Chiteka 1987; Chiyembekeza 1992; Culbreath, Todd et al. 2003). Currently, peanut breeding programs focus on extensively screened lots of germ-plasm to look for sources of resistance and to make selections of resistant lines once crosses have been made and breeding lines are available. Field selection for resistance to leaf spot pathogens and TSWV requires considerable space, and relatively large numbers of plants. Identification of resistant lines is dependent upon environmental conditions and natural fluctuations in disease severity that may not be controllable.

Even though, mechanical transmission of TSWV to peanut is possible, but tomato spotted wilt occurrence in the field under the natural inoculations can be different than with the mechanical

transmission of TSWV. Previous reports have indicated that there is not significant difference in the susceptibility to the virus in peanut based on mechanical transmission (Pereira 1993; Hoffmann 1998). However, large differences in tomato spotted wilt epidemics in the field are reported among cultivars and breeding lines (Culbreath, Gorbet et al. 2005). Therefore, the primary breeding selection methods for resistance to TSWV is still conducted in the field, and is dependant upon natural transmission.

Development of molecular markers for assisting in selection for resistance to TSWV and the leaf spot pathogens could increase the efficiency of breeding programs. Marker assisted selection has been used in development of nematode resistant peanut cultivars 'COAN' (Simpson and Starr 2001) and Tifguard (Holbrook, Timper et al. 2008), but markers are not currently available for resistance to either of the leaf spot pathogens or TSWV in peanut. The objective of this study was to compare field susceptibility of several diverse peanut genotypes, including cultivars, germ-plasm lines and advanced breeding to TSWV and leaf spot pathogens to relate to genotypic characterization of many of these lines in an effort to develop markers for resistance to TSWV and leaf spot pathogens. Although field resistance to TSWV, and/or leaf spot pathogens, has been reported for several of these lines, responses have not been evaluated for others. In addition it is desirable to have direct comparison of genotypes used in the genotypic character work in field studies in which they were exposed to the same conditions and same pathogen populations. Of particular interest in the field characterizations was the relative performance of each of these lines compared to Georgia Green, the predominant runner-type cultivar grown in the southeastern U.S.

2. Materials and methods

2.1 Plant materials

Twenty-two genotypes were evaluated in this study. Sources and general information about the lines are provided in Table 3.1. The lines include commercial cultivars as well as breeding lines and germ-plasm accessions. Several genotypes were reported previously to have varying levels of resistance to one or more of TSWV, *Cercospora arachidicola* and *Cercosporidium personatum* either in the field under natural inoculation conditions or with mechanical inoculation.

2.2 Experimental design

Two field experiments were conducted in adjacent areas of the same field at the University of Georgia, Coastal Plain Experiment Station, Belflower Farm, Tifton, GA in 2007 and 2008. Soil type was Tifton loamy sand. The field used in each year had been planted to corn (*Zea mays* L.) the previous year. In each year, one experiment was planted in April to maximize potential for development of tomato spotted wilt epidemics (Culbreath, Todd et al. 2003), and one was planted in May to reduce potential for spotted wilt (Culbreath, Todd et al. 2003) and increase the likelihood of evaluation of leaf spot epidemics.

The field trials were conducted using randomized complete block designs with 4 replications in the experiments planted in the middle of April and 3 replications for the experiments planted in the middle of May. Planting dates were 23 April and 23 May in 2007, and 18 April and 20 May in 2008. Seeding rate was 3.3 seed/m of row. Sparse seeding rate was used to maximize pressure of tomato spotted wilt (Culbreath, Todd et al. 2003) and to allow tomato spotted wilt severity

evaluations of individual plants as described by Baldessari (Baldessari 2008). Only April planted trials were used for the spotted wilt evaluations, but both early planting in April and late planting trials in May were used for leaf spot evaluations.

2.3 Disease assessment

Tomato spotted wilt severity was evaluated for each plant in the April-planted experiments on July 25, 2007 and July 21, 2008. Severity of tomato spotted wilt was assessed using a 0 to 5 severity scale adapted from that used by Baldessari (Baldessari 2008) based on visual determination of presence symptoms and estimation of the degree of stunting (reduction in plant height, width, or both) for symptomatic plants (Table 3.2). Genotype comparisons were made for total incidence of spotted wilt (severity ratings of 1 or greater), as well as incidence of plants with severity ratings in classifications of 2 or greater, 3 or greater, 4 or greater and 5.

Leaf spot severity was evaluated on 25 September 2007, and 26 September 2008. Leaf spot severity was evaluated for each plot using the Florida 1-to-10 scale where 1 = no leaf spot; 2=very few lesions on the leaves and none on upper canopy; 3 = very few lesions on upper canopy; 4 = some lesions with more on upper canopy, and 5% defoliation; 5 = noticeable lesions on upper canopy with 20% defoliation; 6 = numerous lesions on upper canopy with 50% significant defoliation; 7 = numerous lesions on upper canopy with 75% defoliation; 8 = Upper canopy covered with lesions with 90% defoliation; 9 = very few leaves covered with lesions remain and some plants completely defoliated; 10 = plants dead. Final leaf spot ratings were converted to estimates of percent defoliation according to the function:

Percent defoliation = 99.7714 / (1+exp (-(Florida scale leaf spot rating - 6.0672) / 0.7975))

2.4 Statistical analysis

Data were analyzed using Proc Mixed with ddfm = satterth option on the model statement (SAS v.8.3, SAS Institute, Cary, NC), unless otherwise stated. Incidence of tomato spotted wilt and final defoliation by leaf spot estimated from Florida scale ratings were used as response variables. Genotype was considered a fixed variable, and replication and year were treated as random effects. Main effects and interactions as well as specific treatment effects were considered significant if $P \leq 0.05$. Fisher's least significant difference (LSD) was computed using standard error and t values of adjusted degrees of freedom got from ddfm = satterth option. If the interaction effect between year and genotype was not significantly different ($P > 0.05$), the data were pooled and presented averaged across years; if the interaction was significant ($P \leq 0.05$), the data were analyzed separately and are presented by year.

2.5 Resistance classification

Genotypes were classified as susceptible, moderately susceptible, moderately resistant, and resistant, based on tomato spotted wilt incidence and leaf spot intensity compared to that in Georgia Green, the standard runner-type cultivar in the southeastern U.S.

3. Results

3.1 Tomato spotted wilt assessment.

There was no significant interaction between year and genotype for the total incidence of tomato spotted wilt (severity of 1 or greater), but there were significant interactions for the incidence of severity categories, ≥ 2, ≥ 3, ≥ 4 and 5 (results not shown). Therefore, data were pooled across

years for the analysis of incidence of severity ≥1, but incidence of plants in all other severity ratings categories are presented for each year at 2007 and 2008. For the incidence of severity ≥1, UF NC 94022 had the lowest numerical ranking for total incidence of tomato spotted wilt, but did not differ from that of thirteen genotypes. Total incidence ranked highest in GTC-20, one of released cultivar from China. There were no significant differences among GTC-20, GTC-9 and PE-2 (Table 3.3). Across both years, only UF NC 94022 had total incidence of tomato spotted wilt lower than that of Georgia Green.

For incidence of tomato spotted wilt in all other severity classes, ≥2, ≥3, ≥4 and 5, there was a significant interaction between year and genotype. Therefore data are presented for each year at 2007 and 2008 separately. UF NC 94022 still had lower incidence of tomato spotted wilt than Georgia Green in each of the spotted wilt severity classes in both years. Several additional genotypes had incidence of tomato spotted wilt that ranked lower than Georgia Green in all severity classes, and were significantly lower in some of the severity classes in both years (Table 3.3).

Incidence of tomato spotted wilt in genotypes, GTC-9, GTC-20, and PE-2 was greater than that of Georgia Green in all severity classes in both years. Incidence of tomato spotted wilt in SunOleic 97R, Tifton 8, and PE-2 typically ranked higher than that of Georgia Green and was significantly higher in one or more of those genotypes than that of Georgia in one or more severity classes in each year (Table 3.3).

3.2 Leaf spot assessment.

Leaf spot epidemics were severe in both trials in both years. Early leaf spot developed and caused noticeable defoliation in all genotypes evaluated (Table 3.5). In 2008, leaf spot evaluation was not made in GTC-20 in the early planted trial because of severe stunting and mortality from tomato spotted wilt by the time of leaf spot evaluation. There was a significant year X genotype interaction for percent defoliation for both early and late planted trials (Table 3.4). Therefore, means of percent defoliation are presented by trial and year.

Percent defoliation in Georganic, Georgia-01R, Tifguard, C 12-3-114-58, C 11-154-61 and C 689-6-2 was lower than in Georgia Green in all four trials (Table 3.5), and was lower in Tifton 8, C 724-19-25, Tifrunner, C 209-6-13 and Georgia-02C in trail B for both years and trial A in only 2008 (Table 3.5). Defoliation in GTC-20 and SunOleic 97R was greater than in Georgia Green for both trials in 2007, and defoliation in Spancross and GTC-9 was greater than in Georgia Green for both trials in 2007 and only trial A in 2008. There is no genotype which showed higher percent defoliation than Georgia Green for trial B in 2008.

For the late planted peanuts, most genotypes had similar relative results in the early planted trials. In general, the late planted peanuts had lower levels of defoliation by leaf spot than early planted peanuts. However, it should be noted that leaf spot evaluations for both tests were made the same day. Therefore younger plants at time of evaluation may have been partial explanation for lower levels of defoliation in some genotypes in the later planted trials.

A summary of the disease responses of the genotypes compared to previous reports of resistance to either pathogen is listed in Table 3.6. There were three cultivars and three breeding lines

which were classified as resistant to both TSWV and *C. arachidicola*, whereas two Chinese release cultivars (GT-C20 and GT-C9) and one Chinese breeding line PE-2 were susceptible to both TSWV and *C. arachidicola*, the other Chinese breeding line PE-1 was moderate susceptible to both TSWV and *C. arachidicola* (Table 3.6).

4. Discussion

These results corroborated previous reports of better field resistance to TSWV in UF NC 94022 (Culbreath, Gorbet et al. 2005), Georganic (Culbreath, Todd et al. 1999; Culbreath, Gorbet et al. 2005), Tifguard (Holbrook, Timper et al. 2008; Holbrook, Timper et al. 2008), C724-19-25 (Holbrook, Timper et al. 2008; Holbrook, Timper et al. 2008), Tifrunner (Holbrook and Culbreath 2007) and Georgia-01R (Culbreath, Tillman et al. 2008) than in Georgia Green. Previous reports on SunOleic 97R had higher incidence to tomato spotted wilt in Georgia Green in most cases (Culbreath, Todd et al. 1999; Culbreath, Gorbet et al. 2005), but there were other genotypes from China that showed more severe tomato spotted wilt epidemic, therefore SunOleic 97R was reported as moderately susceptible to TSWV in this study, and the other three U.S. cultivars (NC-6, Spancross, and Tifton8) and one Chinese breeding line PE-1 were similar in incidence in fields with moderate epidemics. Field reaction to tomato spotted wilt had not been reported previously for most of the breeding lines. Breeding lines C 689-6-2, C 209-6-13, C 12-3-114-58, and C 11-154-61 have promising levels of field resistance to TSWV, but genotypes from China PE-2, GTC-9, and GTC-20 are very susceptible based on these results.

For those genotypes from China, there is no report of spotted wilt in peanut in China; therefore, resistance to TSWV would not have been selected for by Chinese breeders. In this experiment,

the leaf spot evaluations were hindered by high incidence and severe symptoms of spotted wilt. However, susceptibility to C. *arachidicola* was obvious even when only the results of the later planted trials are considered. In addition, the resistance of leaf spot was also not a primary objective with these varieties. These genotypes were released in China with high resistance to *Aspergillus flavus* (B.Z. Guo, personal communication) and high oil concentration (B.Z. Guo, personal communication).

The similar results for the reports of early leaf spot, the results in this study also corroborated previous reports of better field resistance to early leaf spot pathogen *C. arachidicola* in Georgia-01R (Culbreath, Tillman et al. 2008), Georganic (Culbreath, Todd et al. 1999; Culbreath, Gorbet et al. 2005) and Tifrunner (Holbrook and Culbreath 2007). This is the first report of resistance to *C. arachidicola* in Tifguard and its sister line C-725-19-25. A preliminary report from another study indicates Tifguard also has a moderate level of resistance to *C. personatum* (Culbreath et al, 2009). This is especially noteworthy in that both of these genotypes have maturity similar to that of Georgia Green, and shorter than most cultivars with appreciable resistance to either *C. arachidicola* or *C. personatum*. Although field resistance to TSWV in UF NC 94022 has been reported (Culbreath, Gorbet et al. 2005), this is the first report of reaction to either of the leaf spot pathogens for this genotype. This is also the first report of field response to early leaf spot pathogen *C. arachidicola* for most of the breeding line including C 11-154-61, C12-3-114-58, C689-6-2, C724-19-25, C209-6-13gen04-14; among them C11-154-61, C12-3-114-58, C689-6-2 all showed low percent defoliation and are reported as resistant genotypes, whereas C724-19-25 and C209-6-13 were reported as moderate resistant and moderate susceptible genotype to *C. arachidicola* respectively. However, the resistance classification of tomato spotted wilt and early

leaf spot both were severe because those genotypes from China were more susceptible to TSWV and *C. arachidicola* in our study. Considering different breeding objective between China and the U.S., therefore, we could consider hybridization between these genotypes with the genotype breed here with high resistance to spot wilt and leaf spot so that we could get varieties with better resistance to TSWV and *C. arachidicola* in lines that have the resistance to *A. flavus* and higher oil concentration.

5. Conclusion

The results of this study combined with previous results with some of these genotypes indicate that the genotypes included in this study represent a wide range of field reactions to the two pathogens. This information should be useful for formulating disease management strategies for those genotypes that either have been released or have potential for release as commercial cultivars. In addition, these trials included fourteen of a panel of sixteen genotypes for which genetic diversity was characterized using simple sequence repeat (SSR) markers (Yan Li thesis unpublished) and the four parents used to develop two recombinant inbred line (RIL) populations. Based on results of these field trials, the parents, UF NC 94022 and SunOleic 97R used to develop one population differ markedly in their field resistance to TSWV. The parents Tifrunner and GTC-20 used to develop a second population differ greatly in field resistance to both TSWV and *C. arachidicola*. Information from the combination of the field and genetic characterization of these genotypes and populations developed from crosses from selected lines should be useful in mapping resistance genes and in developing genetic markers for identification of resistance to one or both of these pathogens.

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Table 3.1 - The list of 22 genotypes used for the field evaluation for reaction to tomato spotted wilt and early leaf spot

Entry	Genotype	Source	Market type
1	Tifton 8	U.S. germplasm	Virginia
2	C724-19-25	U.S. breeding line	Runner type
3	Georgia Green	U.S. cultivar	Runner type
4	Georganic (C-11-2-39)	U.S. cultivar	Runner type
5	Spancross	U.S. cultivar	Spanish
6	Tifguard (C724-19-15)	U.S. cultivar	Runner type
7	NC-6	U.S. cultivar	Virginia
8	SunOleic 97R	U.S. cultivar	Runner type
9	Tifrunner	U.S. cultivar	Runner type
10	UF NC 94022	U.S. breeding line	Runner type
11	PE-2	Chinese breeding line	Virginia
12	PE-1	Chinese breeding line	Virginia
13	GTC-20	Chinese cultivar	Spanish
14	GTC-9	Chinese cultivar	Spanish
15	gen 04-14	U.S. breeding line	Runner type
16	C 689-6-2	U.S. breeding line	Runner type
17	C 209-6-13	U.S. breeding line	Runner type
18	Georgia-01R	U.S. cultivar	Runner type
19	C 11-154-61	U.S. breeding line	Runner type
20	C 12-3-114-58	U.S. breeding line	Runner type
21	Georgia-02C	U.S. cultivar	Runner type
22	AP-3	U.S. cultivar	Runner type

Table 3.2 - The rating scale of severity of tomato spotted wilt caused by TSWV (Adapted from Baldessari, 2008 (Baldessari 2008))

Severity rating	Disease severity	Plant size relative to typical healthy plants
0	No symptoms	
1	Plants with foliar symptoms, with no stunting or only slight stunting	80-100%
2	Noticeable stunting	60-79%
3	Marked stunting	40-59%
4	Very marked stunting	30-40%
5	Severe stunting	0-20%

Table 3.3 - Effect of peanut genotype on incidence of tomato spotted wilt in five severity classes, Tifton, Georgia, 2007-2008

	≥1	≥2		≥3		≥4		5	
Genotype	Pool	2007	2008	2007	2008	2007	2008	2007	2008
UF NC 94022	18.22*	11.55*	3.46*	6.91*	2.33*	4.05*	0.00*	3.05*	0.00*
Georganic	18.61	15.36*	8.71*	11.89*	3.49*	9.61	1.56*	4.33	1.56*
C 689-6-2	20.29	15.12*	13.72*	11.61*	9.18	5.97*	3.04*	4.40	0.86*
Georgia-01R	21.59	18.60	10.56*	13.99	5.23*	7.50	1.09*	3.75	1.09*
C724-19-25	21.64	21.30	4.39*	18.73	3.60*	17.11	1.78*	9.30	1.00*
C 209-6-13	23.08	15.30*	10.55*	13.05	5.43*	10.80	2.63*	6.94	1.85*
Tifguard	23.09	16.76*	10.06*	13.69	8.44	9.32	2.38*	7.54	1.62*
C 12-3-114-58	25.86	16.79*	16.40*	13.54	9.59	7.83	6.53	3.92	3.75
C 11-154-61	27.16	23.36	17.25*	16.10	9.77	7.24	4.22*	3.62	2.05*
Tifrunner	31.52	27.92	6.13*	15.09	2.56*	10.77	1.78*	4.42	0.00*
Georgia-02C	34.30	18.79	24.82	13.92	16.37	11.84	9.17	9.84	5.24
gen 04-14	34.68	19.18	20.32	13.54	14.06	8.60	10.64	2.74*	6.22
AP-3	34.82	33.67	13.90*	27.09	10.15	15.60	7.57	9.77	3.44
Georgia Green[§]	37.53	32.50	27.88	26.73	15.47	16.97	12.84	12.06	8.88
NC-6	46.22	42.71	24.50	35.42	16.43	19.79	12.08	12.50	8.51
PE-1	50.49	45.49	31.74	37.65	25.45*	32.15*	18.68	25.80*	16.85*
Tifton 8	54.07	33.35	34.35	26.63	25.78*	16.63	15.77	8.72	9.09
Spancross	54.18	43.76	32.83	30.55	20.73	19.30	14.44	14.38	10.14
SunOleic 97R	55.00	42.65	40.49*	34.29	25.77*	25.36	15.73	12.68	5.13
GTC-9	75.82*	79.71*	45.68*	67.73*	34.32*	58.16*	27.87*	37.75*	19.95*
PE-2	78.52*	73.31*	62.55*	66.52*	57.78*	51.33*	44.55*	37.12*	33.56*
GTC-20	78.68*	66.61*	53.38*	54.10*	40.32*	42.67*	28.34*	37.70*	16.07*
LSD	19.235	15.177	9.630	14.021	8.560	10.773	7.773	8.742	6.014

[1]Symbol § indicates the genotype used as a standard for comparisons.

[2]Severity classes >1, >2, >3, >4, and 5 represent the incidence (percentage of the total population) with: 1) no to light stunting; 2) noticeable stunting; 3) marked stunting; 4) very marked stunting and severe stunting (Baldessari 2008).

3An asterisk indicates there is significant difference of the marked genotypes compared with standard comparison genotype - Georgia Green.

Table 3.4 - Interaction between year and genotypes for early leaf spot

	Covariance	Ratio	Estimate	Standard Error	Z Value	Pr Z
Defoliation in early planting	year*gen	0.9257	175.89	76.2230	2.31	0.0105
Defoliation in late planting	year*gen	0.4134	91.7065	50.3995	1.82	0.0344

Table 3.5 - Effect of peanut genotype on percent defoliation caused by early leaf spot (*Cercospora arachidicola*), Tifton, GA, 2007-2008

		Trial A[1]		Trial B[2]	
		Defoliation		Defoliation	
		2007	2008	2007	2008
4	Georganic (C-11-2-39)	40.42[a]	19.04[a]	28.97[a]	9.61[a]
10	Tifrunner	78.17	33.56[a]	28.16[a]	37.29[a]
20	Georgia-01R	46.13[a]	10.65[a]	11.54[a]	18.2[a]
21	C 11-154-61	32.47[a]	13.98[a]	13.32[a]	18.2[a]
22	C 12-3-114-58	32.14[a]	13.43[a]	11.69[a]	18.2[a]
18	C 689-6-2	53.70[a]	2.28[a]	5.48[a]	19.82[a]
11	UF NC 94022	67.01	41.12	66.68	79.33
1	Tifton 8	71.41	17.29[a]	15.1[a]	39.38[a]
6	Tifguard (C724-19-15)	22.59[a]	16.25[a]	19.14[a]	34.36[a]
12	PE-2	79.64	94.94[b]	74.94	65.79[a]
2	C724-19-25	71.41	17.16[a]	19.14[a]	51.14[a]
17	gen 04-14	88.51	13.05[a]	66.88	55.44[a]
9	SunOleic 97R	94.76[b]	19.49[a]	84.45[b]	80.86
19	C 209-6-13	81.69	39[a]	24.77[a]	61.39[a]
24	AP-3	82.99	41.12	62.34	67.68[a]
13	PE-1	82.89	60.2	49.06	73.5[a]
7	NC-6	85.31	61.96	75	86.25
23	Georgia-02C	80.53	38.69[a]	15.59[a]	71.98[a]
5	Spancross	94.48[b]	87.59[b]	91.51[b]	80.63
3	Georgia Green § 4	76.7	53.83	59.45	87.1
15	GTC-9	95.38[b]	91.7[b]	90.25[b]	93.97
14	GTC-20	92.45[b]	*[3]	97.69[b]	97.08
	LSD	15.38	14.26	21.05	12.87

[1]Trial A was planted in April in both years.

[2]Trial B was planted in May in both years.

[3]Asterisks indicate no rating is reported because of the influence of spotted wilt.

[4]Symbol § indicates the genotype used as a standard for comparisons.

[5]Uppercase 'a' indicates the percent defoliation is significant lower than standard comparison genotype - Georgia Green.

[6]Uppercase 'b' indicates the percent defoliation is significant higher than standard comparison genotype - Georgia Green.

Table 3.6 - The summary of field response of twenty-two peanut genotypes to tomato spotted wilt, caused by Tomato Spotted Wilt Virus, and early leaf spot caused by *Cercospora arachidicola*, Tifton, GA 2007-2008

Genotype	Field response to tomato spotted wilt	Previous reports of response to tomato spotted wilt	Previous report citation	Field response to early leaf spot	Previous reports of response to early leaf spot	Previous report citation
Georgia-01R	R	MR/R	Branch Crop Sci, 2002 Cantonwine et al 2006	R	R	Branch Crop Sci. 2002 Cantonwine et al, 2006
Tifguard (C724-19-15)	R	R	Holbrook et al., 2008	R	*	
C 11-154-61	R	*		R	*	
C 12-3-114-58	R	*		R	*	
C 689-6-2	R	*		R	*	
Georganic (C-11-2-39)	R	R	Culbreath et al, 1999, 2005	R	R	Cantonwine 2006 and 2008
C724-19-25	R	R	Holbrook et al, 2008	MR	*	
Tifrunner	MR	R	Cantonwine et al, 2006	MR	MR	Cantonwine et al 2006, Branch 2008
UF NC 94022	R	R	Culbreath et al.	MS	*	

C 209-6-13	R	*		MS	*	
Georgia Green	MR	MR	Culbreath et al, 1995, 1999	MS	S	Monfort et al 2004
Georgia-02C	MR	R	Branch Peanut Sci 2008	MS	MR	Branch Peanut Sci, 2008
AP-3	MR	R		MS	S	Culbreath et al 1999, 2008
gen 04-14	MR	*		MS	*	
PE-1	MS	*		MS	*	
SunOleic 97R	MS	S	Culbreath et al, 1999	MS	S	
NC-6	MS	*		S	MR/MS	Green & Wynne, 1987
Spancross	MS	*		S	*	
Tifton 8	MS[1]	*[2]	*	MR	MR	Coffelt et. al, 1985
GT-C20	S	*		S	*	
GT-C9	S	*		S	*	
PE-2	S	*		S	*	

[1] R = resistant, MR= moderately resistant, MS = Moderately susceptible, S = Susceptible.

[2]Asterisks indicate no previous report for that genotype.

Chapter 4

Characterization of Simple Sequence Repeats (SSRs) in the parents of 2 RIL populations under development in *Arachis hypogaea* L. [3]

[3] Y. Li. and A.K. Culbreath. To be submitted to *phytopathology*.

Abstract

In our research, 4 genotypes were chosen as 2 population groups according to previous report of different response to TSWV and leaf spot or both, they are Tifrunner, GTC20, NC 94022, SunOleic 97R; among them one population is crossed between Tifrunner and GTC20, another one is obtained by hybridization between SunOleic 97R and NC 94022.

Totally 709 SSR markers were used to screen the genetic variety between the parents of this two population which are under RIL development. According to our research, the genetic distances between these two populations are 0.15 and 0.101 respectively. Totally, there are 162 (29.14%) polymorphic markers between Tifrunner and GTC20, and 124 (22.30%) polymorphic markers between SunOleic 97R and NC 94022.

In the 2 years field evaluation program, GTC 20 shown highly susceptibility to TSWV and (early) leaf spot pathogen, whereas, Tifrunner was reported as moderate resistance to TSWV and (early) leaf spot pathogen. In another population, SunOleic is the most popular cultivars released by the University of Florida and reported as susceptible to TSWV by other researchers, but it was reported as moderate susceptible to TSWV, considering several other genotypes from China shown stronger occurrence of tomato spotted wilt in our field evaluation research.

Recently, these two populations have been developed into F4/5 generation, the F2 hybrid seeds already have been check for the hybridization in the process of RIL population development. We are going to use these polymorphic markers to screen the RIL population as a future goal of this project to try to found some markers which could be related to resistance gene of TSWV and leaf

spot pathogen or both, and also hope one dense linkage group in the cultivated peanut could be constructed with these polymorphic markers and some other markers which have been screened in Dr. Knapp's research lab (unpublished data).

Key words: *Arachis hypogaea* L., SSR, polymorphism, RIL, hybridization

1. Introduction

Peanut (*Arachis hypogaea* L.), also called groundnut, probably originated in southern Bolivia or northern Argentina in South America (Gregory, Krapovickas et al. 1980; Kochert, Stalker et al. 1996). It has primarily been grown in tropical and subtropical regions in the world, and is an important crop in the world for human consumption and feed stock, and is the fifth most important oilseed crop in the world; even more the peanut has become into more and more important economic crops in the world.

The genus *Arachis* contains about 70 species (Krapovickas A. 1994), with almost all of them being diploid, with either A genome or B genomes. In contrast, cultivated peanut is an allotetraploid (2n = 4x = 40 chromosomes) composed of A and B genome groups according to cytogenetic characters in which the A genome represents A chromosome pairs, and the B genome represents B chromosome pairs. *A. hypogaea* probably developed by hybridization between the species with two different series of chromosomes, and then followed by spontaneous duplication (Halward, Stalker et al. 1991; Kochert, Stalker et al. 1996). Considering the evolution hypothesis of cultivated peanut origin, the genetic diversity within cultivated peanut is relatively lower compared with diploid wild-type peanut species. However, there are a number of

rich sources of variation for agronomical traits contained in the wild diploid peanut species, which could potentially been used into peanut breeding. However, it is hard to transfer these good agronomical traits from wild species into cultivated peanut species because of inter-specific compatibility barriers.

Marker-assisted selection (MAS) has become into a new gene technology which could be able to break through these traditional breeding restriction, and increase the speed of peanut breeding program. And recently, researchers have used a number of molecular assays to assess the genetic variability, which is very important for characterization of individuals, accessions, and breeding lines for the choice of parental genotypes in breeding programs (Ribaut and Hoisington 1998). However, the limited genomic variability in cultivated peanut, and complicated A and B genomes have slowed the identification and characterization of molecular markers for genetic technology application (Grieshammer and Wynne 1990; Halward, Stalker et al. 1991; Kochert, Halward et al. 1991; Halward, Stalker et al. 1992; Halward, Stalker et al. 1993). Although genetic technology has been used recently in peanut breeding for selection of nematode resistance (Chu, Holbrook et al. 2007), the limited genetic diversity in the cultivated peanut hinders the development and application of genetic technology in peanut breeding.

The wild diploid species of peanut have been reported to be used in genetic mapping since 1990s (Halward, Stalker et al. 1991; Kochert, Halward et al. 1991; Halward, Stalker et al. 1992) with different kinds of molecular markers, such as RFLP (Halward, Stalker et al. 1993; Burow, Simpson et al. 2001), and RAPD(Garcia, Stalker et al. 2005). There are only 3 SSR-based genetic linkage maps reported in peanut (Moretzsohn, Leoi et al. 2005; Moretzsohn, Barbosa et

al. 2009; Varshney, Bertioli et al. 2009). Among them, an AA genome linkage map was constructed using an F_2 population obtained from a cross between two diploid wild species (*A. duranensis* and *A. stenosperma)* (Moretzsohn, Leoi et al. 2005). From the same research group, another linkage map for B-genome of *Arachis* was based on another F_2 population from a cross between *A. ipaensis and A. magna*.

In general, the development and identification of molecular markers have proven to be very useful in the fields of breeding, genetic research and crop improvement in many species (Mohan, Nair et al. 1997). In spite of limited genotypic variability in peanut compared to other crops, there is a wide range of field resistance to important pathogens such as TSWV, *C. arachidicola*, and *C. personatum*. Development of molecular markers for assisting in selection for resistance to TSWV and the leaf spot pathogens of peanut could increase the efficiency of breeding programs. Marker assisted selection has been used in development of nematode resistant peanut cultivars 'COAN', and Tifguard, but markers are not currently available for resistance to either of the leaf spot pathogens or TSWV in peanut. Therefore, in this research we developed 2 recombinant inbred line (RIL) populations. From genotype standpoint, objectives were to find polymorphic markers between the parents of these two populations so that the markers could be used in mapping cultivated peanut linkage groups. Meanwhile, in another portion of this study, the phenotype characteristics field response to tomato spotted wilt caused by TSWV and leaf spot caused by C. *arachidicola* have been recorded for these genotypes. Therefore, results from these two populations could also can be applied into QTL detection analysis of TSWV and spotted wilt pathogen resistance.

2. Materials and methods

2.1 Plant materials

A total of 16 cultivated peanut genotypes have been involved into the polymorphism screening as in Table 4.1, all plant materials were provided from USDA-ARS and University of Georgia peanut programs at the University of Georgia Coastal Plain Experiment Station in Tifton, GA in 2006. Among these 16 genotypes, 4 genotypes were chosen as the parents of 2 RIL populations under development recently according to the country origin and the resistance level to TSWV or leaf spot pathogen. The hybrid of F2 seed have been checked for the hybridization with several polymorphic markers shown in this research. Even more, around 20 markers also have been used to check for the segregation in the F3 generation of both populations. Among these 4 parental genotypes, Tifrunner (Holbrook Jr 2007) was released 2007 as a new cultivar with a high level field of resistance to TSWV and moderate resistance to early and late leaf spot; SunOleic 97R (Gorbet and Knauft 2000) is a cultivar developed by the University of Florida with good agronomic traits, but it is considered as susceptible cultivar to TSWV and leaf spot pathogen. NC 94022 (Culbreath, Gorbet et al. 2005) is a breeding line which has been reported to have a high level of resistance to TSWV; GTC20 is a released variety from China with high resistance to *A. flavus* invasion (B.Z. Guo, personal communication).

2.2 DNA extraction

Leaves of genotypes for total DNA extraction were collected from greenhouse grown samples. The fresh leaf tissues collected from greenhouse were frozen at -80℃ as soon as possible to keep fresh, then crushed in liquid nitrogen with a mortar and pestle in the lab. The total genomic DNA was extracted according to modified CTAB methods (Tang, Yu et al. 2002) from those fresh

leaves. DNA concentration and quality was determined by Spectrophotometer (Spectronic Instruments) at 260nm wavelength and ratio with 260nm/280nm. In the end, DNA was diluted to 10ng/ul with sterile water for PCR reaction.

2.3 SSR fluorescent markers

There were 709 pairs of SSR markers totally for polymorphisms screening all together, the forward primers of their markers were labeled by adding flurophores (6FAM, HEX, TAM) to the 5' end to facilitate multiplexing at next step (Tang, Kishore et al. 2003). These markers are from different resources shown in Table 4.2. At first, SSR markers were screened on 1.5% agarose gels for utility, functionality, and length estimation using the bulk DNA of 4 randomly chosen cultivated peanut and 4 randomly chosen wild type samples. After screening for functional SSR markers, 153 SSR markers were deleted because of no amplification, leaving 556 (78.4%) SSR markers to be screened for polymorphism within 16 genotypes with different level resistance to TSWV or leaf spot pathogen or both.

2.4 PCR amplification

An 11.5ul volume reaction system in 384-wells plate was used for 'touchdown' PCR reaction, containing 1 ul of forward and reverse primer; 2 ul of sample to be prepared as template; 1.15 ul of 10 X PCR buffer; 0.25 ul of dNTP; 0.1 ul of Taq enzyme. The 'touchdown' thermal cycle of PCR reaction was decided by the thermal Temperature (Tm) for different kinds of primers separately to be applied with either 52 or 56 'touchdown' cycle reaction. The 'touchdown' amplification program is as follows: 94° for 1 min to allow samples to denature, followed by 6 cycles of 94° for 30s, 62° for 30s and 72° for 30s, the annealing temperatures were decreased 1°

per cycle in subsequent cycles till the temperature reached 52° or 56° for the different kinds of 'touch down' program. Products were subsequently amplified for 36 cycles at 94° for 20s, 56° for 20s, and 72° for 30s.

2.5 Electrophoresis and detection of fluorescent products

After amplification, products were first checked with 1.5% agarose gel to ensure that the amplifications were successful and complete and that the good amplification products had been recorded. Then, the amplicons with different labels were diluted 60- to 100- fold according to the concentration and color of different amplification products. The six different diluted amplicons with different color and amplification length were mixed into one well by 1 uL with 9 ul of formamide with GeneScan 500 internal lane standard labeled with ROX. GeneScan Filter Set D and the ROX 500 internal-lane were used for analyses of amplicons labeled with 6FAM, HEX, and TAM. The functional SSR markers (556 markers) screening results were analyzed by Gene Mapper 4.0 into multiplexes of 6 primers for polymorphism analysis based on color multiplexes and amplification length. Results were entered into an Excel 2007 spreadsheet.

2.6 Statistical analysis method

PIC value, defined by Botstein D (Botstein, White et al. 1980) as a closely related diversity measure, is a measure of the polymorphism of a marker (SSR marker) for linkage. The formula for this estimation of PIC score is

$$\hat{PIC}_l = 1 - \sum_{u=1}^{k} \tilde{p}_{lu}{}^2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\tilde{p}_{lu}{}^2 \tilde{p}_{lv}{}^2$$

Where l=index for marker 'l'; $\tilde{p}_{lu}$ = proportion of marker 'l' alleles which are of allele type 'u'; $\tilde{p}_{lv}$ = proportion of marker 'l' alleles which are of allele type 'v'; k=number of alleles types present for marker 'l'， (Shete, Tiwari et al. 2000). Another estimator heterozygositys (H) was estimated according to the formula:

$$H = 1 - \sum_{i=1}^{n} p_i^2$$

Where P*i* is the frequency of *i*th allele in the genotypes population (Shete, Tiwari et al. 2000). The estimation of PIC, amplified allele number, allelic frequency and observed heterzygosity were obtained by software GeneMarker (Liu and Muse 2005).

3. Results and discussion

For the population developed from the cross of Tifrunner and GTC-20, 162 markers have polymorphisms, whereas only 124 markers showed polymorphisms between Sun Oleic 97 R and UF NC 94022 (Table 4.3). Among those polymorphic markers between Tifrunner and GTC-20, 68 markers are labeled as FAM, 48 markers were labeled as HEX, and 46 markers were labeled as TAMRA. For those polymorphic markers between population of Sun Oleic 97 R crossed by UF NC 94022, 49 are labeled as FAM, 45 as HEX, and 30 as TAMRA (Table 4.4). Among those polymorphism markers within two populations, it showed that the distributions of markers label were not even in both populations. Usually the markers labeled with FAM and HEX always shown better polymorphism detection results compared with TAMRA labeled markers, which caused a hypothesis deduction that whether TAMRA label could probably influence the PCR reaction to some extent.

Among those polymorphic markers, as in Table 4.5, 10 markers for the population of Tifrunner and GTC-20 had bad quality, which indicated either one or both have no amplification results, and 31 markers showed unclear amplification results either from one or both. For the population from SunOleic 97 R crossed by UF NC 94022, 3 makers are marked as "no good" for quality, and 25 markers showed unclear amplification results. Therefore, the markers classified as no good or poor could either be re-screened or deleted for the later resistance markers selection within those two populations.

Meanwhile, the phenotype characteristic of these 4 genotypes as parents of 2 under developed RIL population also have been observed in a 2-years long field evaluation result (Chapter 3). The field evaluations of these four genotypes were summarized as in Table 4.6. The field evaluation results are mostly consistent with previous reports except SunOleic, this could be caused by different levels comparing. In our field evaluation, the 4 genotypes from China (Chapter 2) were susceptible to TSWV and C. arachidicola. Tomato spotted wilt is not a problem in peanut in China, so there would have been no selection for such kind of resistance requirement in China. Even though leaf spot also occurs in China, but these four genotypes were not selected for leaf spot resistance.

4. Conclusion

Two RIL populations are under development at Tifton, and are now in the F4/5 generation. The RIL population may be used for linkage group mapping and QTL analysis of TSWV and leaf spot pathogen resistance. In this research, approximately one hundred markers have been tested

for polymorphism within two populations separately, and approximately two-thousand also have been screened for polymorphism within the parents of these two populations. Although the percentage of the markers showing polymorphism within two populations both is not high, there will be some other polymorphic markers which could be applied with cultivated peanut linkage group mapping construction. Meanwhile additional markers will be tested for polymorphism between the parents of these two populations. The phenotype characteristics also have been recorded for the F3 generation of these two RIL populations, so QTL detection of resistance genes related to TSWV and/or *C. arachidicola* could be possible within these two populations.

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Figure 4.1 - Amplified fragment patterns of two different markers within Tifrunner (above) and GTC20 (below), indicating different quality rating results for these 2 makers with 1 (above) and 3 (below)

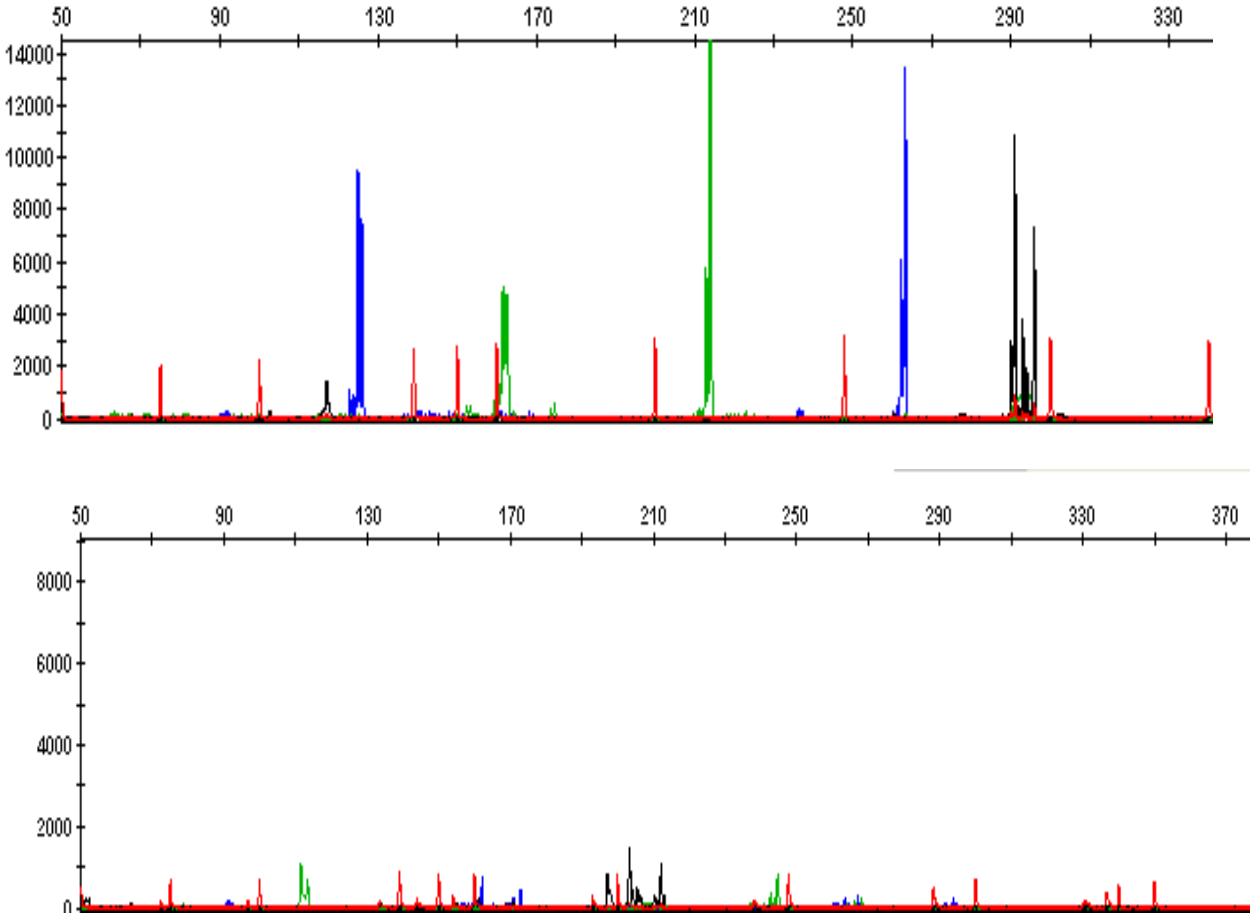

Table 4.1 - The list of 16 genotypes used in the SSR marker screening

S. No.	Genotype	Country of origin	Market Type
1	Tifton 8	U.S.	Runner
2	C724-19-25	U.S.	Runner
3	Georgia Green	U.S.	Runner
4	Georganic (C-11-2-39)	U.S.	Runner
5	Spancross	U.S.	Spanish
6	Tifguard (C724-19-15)	U.S.	Runner
7	NC-6	U.S.	Viginia
8	SunOleic 97R *	U.S.	Runner
9	Tifrunner *	U.S.	Runner
10	UF NC 94022 *	U.S.	Runner
11	PE-2	China	Bunch?
12	PE-1	China	Bunch?
13	GTC-20 *	China	Bunch?
14	GTC-9	China	Bunch?
15	Tennessee Red	U.S.	??
16	GPNCWS13	U.S.	??

* indicated that they are the parents of two populations under RIL development

Table 4.2 - Marker information after bulk DNA functional screening

Markers	Markers Designed	Markers yielding amplification	Resources
GM421-GM612	192	158 (82.3%)	Ferguson et al. 2004
GM365-GM420	56	47 (83.9%)	He et al. 2003
GM339-GM344	6	6 (100%)	Hopkins et al. 1999
GM345,GM346	2	1 (50%)	Krishna et al.2005
GM1-GM271	271	186 (68.6%)	Moretzsohn et al. 2005
GM271-GM338	67	56 (83.6%)	Moretzsohn et al. 2004
GM347-GM364	7	4 (57.1%)	Palmieri et al. 2002
	11	4 (36.4%)	Palmieri et al. 2005
GM613-GM709	97	94 (96.9%)	Steve J. Knapp et al. 2006
Total	709	556 (78.4%)	

Table 4.3 - Markers performance within two populations

	Tifrunner X GTC-20	SunOleic 97 R X UF NC 94022
Polymorphism	162	124
Monomorphism	394	432
percentage of polymorphism	29.14%	22.30%

Table 4.4 - The distribution of flurophore label of polymorphic makers within two populations

	Tifruner X GTC-20	SunOleic 97 R X UF NC 94022
FAM Labeled Marker	68	49
HEX Labeled Marker	48	45
TAMRA Labeled Marker	46	30

Table 4.5 - The distribution of quality of polymorphic markers within two populations

	Excellent	Good	Fair	Poor	No good
Tifrunner X GTC20	55	30	35	30	12
SunOleic 97R X NC 94022	36	32	26	23	7

Table 4.6 - The field evaluation results summary for reaction to TSWV and *Cercospora arachidicola* of parental lines used to develop two recombinant inbred line populations.

	Tifrunner	GTC 20	SunOleic 97R	NC 94022
Spotted Wilt	MR	S	MS	R
(Early) Leaf Spot	MR	S	S	MS