MILLA, SUSANA RITA. Relationships and utilization of *Arachis* germplasm in peanut improvement. (Under the direction of Thomas G. Isleib and H. Thomas Stalker.)

Cultivated peanut, *Arachis hypogaea* L., is a tetraploid ($2n = 4x = 40$) species considered to be of allopolyploid origin. Its closest relatives are the diploid ($2n = 2x = 20$) annual and perennial species included with it in section *Arachis*. An understanding of taxonomic relationships among those species may allow for more efficient utilization of alien germplasm in applied peanut improvement. A total of 108 accessions, representing 26 species in section *Arachis* were genotyped with AFLP markers. Cluster and principal component analyses of the data supported previous taxonomic classifications and genome designations. Based on genetic distances and cluster analysis, "A" genome accessions 30029 (*A. helodes*), and 36009 (*A. simpsonii*), and "B" genome accession 30076 (*A. ipaensis*) were the most closely related to both *A. hypogaea* suggesting their involvement in the evolution of the tetraploid species.

Accession 10602 of *A. diogoi* possesses resistance to TSWV. Associating molecular markers with resistance would greatly aid in the transfer of resistance into high performing *A. hypogaea* backgrounds. In an attempt to find markers associated with TSWV resistance, a genetic linkage map was constructed for an F_2 population of *A. kuhlmannii* x *A. diogoi.* The map consisted of 102 AFLP markers grouped into 12 linkage groups and spanning 1068.1 cM. The map allowed the evaluation of the *Arachis* genome for associations between response to TSWV infection and the AFLP markers. Five markers, all located in the same linkage group (LG V) were closely associated ($0.0009 < P < 0.0021$) with TSWV resistance as well as several other associations believed to be

linked with minor genes conferring resistance. These markers will be studied for utilization in peanut breeding with marker-assisted selection.

Development of cultivars with moderate to high levels of field resistance to tomato spotted wilt virus (TSWV) is the most promising means of managing spotted wilt of peanuts. Breeding efficiency can be maximized by choosing parents based on their potential to produce superior progeny. Best Linear Unbiased Prediction (BLUP) is a method for estimating the breeding value of a parent based on its own performance as well as that of its relatives. The method was used in the present study to identify lines with superior ability to transmit TSWV field resistance to their progeny. BLUPs for yield, meat content, crop value, and pod brightness also were calculated. Six different weighting schemes were designed and used for index selection in order to pick lines with superior breeding values for a combination of all traits analyzed. Thirteen lines were selected with at least four of the six weighting schemes suggesting that these lines should be able to transmit to their progenies not only reduced TSWV incidence, but also increased yields and improved quality traits.

RELATIONSHIPS AND UTILIZATION OF *ARACHIS* GERMPLASM IN PEANUT IMPROVEMENT

by

SUSANA RITA MILLA

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APPROVED BY:

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BIOGRAPHY

Susana Rita Milla Comitre was born in Lima, Peru on Mother's Day in May, 1973. Being born the youngest and only girl of a conservative family of five children, her parents decided the best education she could obtain was to attend a private, Catholic, girls-only school run by German nuns. After 11 years of living in a bubble, she graduated from Santa Ursula School in December 1989. In April 1991, having passed the entrance exam for both the agricultural university (Universidad Agraria La Molina) and one of the best bio-medical schools in the country (Universidad Peruana Cayetano Heredia), the faculty at La Molina went on strike and she had no other choice than to attend Cayetano. After deciding she didn't want to wait long to start her graduate studies she obtained her bachelors degree in biological sciences a year early, in December 1994. In January 1995, she started working at the International Potato Center (CIP) in the Genetic Resources Department. Despite the lack of pay and the dreadful food at the cafeteria, she still considers her time at CIP as one of the best experiences of her life. In July 1996, she was accepted as a Master of Science student in the Crop Science Department at North Carolina State University. A month later she packed her whole life in two suitcases and moved to the U.S. She worked in tobacco genetics and breeding on the identification of RAPD markers linked to blue mold resistance in tobacco. Upon completion of her masters' degree in December 1998, she determined she wasn't ready to face the real world yet and decided to continue with her graduate studies. In January 1999, she began working towards a Ph.D. degree in the peanut breeding and genetics programs. Not having found it complicated enough to have one advisor during her

masters degree, she decided to enlist Drs. Tom Isleib and Tom Stalker as co-advisors for her Ph.D., a decision that was proven especially brilliant during the preparation of this manuscript. After 4 ½ years of work, Susana is finally ready to become “la doctora Milla.” Upon completion of her degree, she will stay at NCSU for a while longer working for Dr. Isleib in a post-doc position.

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LITERATURE REVIEW

Production and Economic Importance of Peanut

The cultivated peanut (*Arachis hypogaea* L.) is a major crop in most tropical and subtropical regions of the world (Stalker and Moss 1987). Peanut seeds are of high value because of their high contents of oil (43-54%) and protein (25-30%). Peanuts are grown in six continents; however, Asia, America and Africa are by far the biggest producers. Although India is the world leader in area under peanut cultivation, China produces more than any other country in the world. The leading countries in production are China, India, and the USA, each producing more than a million tons of peanuts annually.

Around the world, almost every part of the peanut plant is used in some way. While the seeds are used for human consumption, plant residues are valuable as fodder for cattle in many African and Asian countries. Furthermore, the roots left behind after harvest add valuable nutrition to the soil, which is especially important in less developed countries where the crop is grown under low inputs.

In the U. S., peanuts bring more than $4 billion into the country's economy each year. Unlike other countries where the bulk of the peanut production goes into manufacturing of oil and cake, in the U.S. the primary market for peanuts is edible consumption. Only 15% of U.S. production is crushed for oil. The largest single use of U.S. peanuts (40%) is manufacturing of peanut butter. About 10% of the nation's production is sold as in-shell or snack peanuts and about 25% is used by the confectionary industry for manufacturing of different types of candy. It is estimated that

Americans eat more than 600 million pounds of peanut seeds and about 700 million pounds of peanut butter each year (Peanut Council, 2003).

In North Carolina, peanuts constitute a multimillion-dollar industry. The state is the 4th largest peanut producer in the nation with above 300 million pounds produced in 2001 (FDA, 2003). Peanuts ranked 13th among all commodities and 5th among crops in total cash receipts from major farm commodities in the state (NCDA, 2003). Peanut revenues for the year 2001 totaled $88 million. Approximately 100,000 acres of the state's farmland are planted under peanuts (NCCES, 2002).

History

The South American origin of cultivated peanut was proven by the unearthing of terracotta jars containing preserved peanuts in the prehistoric cemetery of Ancón on the Peruvian coast north of Lima (Hammons, 1982). Specimens recovered from these graves date back to the period of 500 to 750 B.C. Paleobotanical artifacts discovered by Bird (1948, 1949) near the Chicama Valley have given the best dates for the occurrence of peanuts in Perú. These findings suggested the introduction of peanut associated with the first pottery dating approximately from 1200 to 1500 B.C. (Hammons, 1982). The Incas cultivated peanuts, which the Indians called *ynchic*, throughout the coastal regions of Perú (Hammons, 1994).

By the time the Spanish began their colonization of the New World, peanuts were grown as far north as Mexico. From the early stages of this colonization, exotic American plants were often collected and introduced into Europe. Although there are no records of the first introduction of peanuts, it is probable that they were carried to Europe early in

the 16th century (Hammons, 1982). During the mid 1500's, Spanish and Portuguese traders and explorers brought peanuts to Africa and Asia. In Africa peanuts were commonly grown in the western tropical region. Later on, during the slave trade, peanuts were introduced from Africa in North America and slaves planted them across the southern states of the U.S.

It was not until after the American Revolution that peanuts became a commercial product, when they were sold by small farmers for local consumption. In 1833, the first important peanut market in the U.S. opened in Wilmington, NC. Subsequently, listing of the market value of peanut in the Wilmington newspaper was initiated. Southern farmers began export trading of the crop into northern cities (Johnson, 1964). During the Civil War there was a large increase in the consumption of peanuts. Soldiers on both sides used them as food because of their rich nutritional content and their ease of storage and transport (Johnson, 1964). During the last half of the 19th century, commercial production of peanuts grew quickly (Hammons, 1994). Peanuts became a popular snack and they were sold freshly roasted by street vendors (McGill, 1973).

In the early 1900s three main events promoted the expansion of the peanut industry. First, the invention of the first mechanic picker allowed for more efficient harvesting of the crop which in turn permitted farming of larger areas. Secondly, George Washington Carver's research on peanuts at the Tuskeegee Institute lead him to the discovery of more than 300 uses for peanuts and therefore, promoted the cultivation and consumption of the crop. Finally, the destruction of the cotton crop by the boll weevil prompted farmers to look at peanut as an alternative crop. Peanut production in the U.S. grew rapidly during both World Wars due to the need for oil products (Johnson, 1964).

Later on, the development of the peanut combine allowed for further expansion of the peanut industry (Hammons, 1982).

Biosystematics and Taxonomy

Arachis hypogaea is a member of the family Leguminosae, subfamily *Fabaceae*, tribe *Aeschynomeneae*, subtribe *Stylosanthenae*. The cultivated peanut belongs to the genus *Arachis,* which comprises 69 diploid and tetraploid species native to South America (Krapovickas and Gregory, 1994). The genus has been divided into nine sections based on morphology, geographic distribution, and cross-compatibility. Sections *Caulorrhizae*, *Erectoides*, *Extranervosae*, *Heteranthae*, *Procumbentes*, *Trierectoides*, and *Triseminatae* contain only diploid species ($2n = 20$) (Stalker and Simpson, 1995). The more evolutionarily advanced tetraploids ($2n = 40$) have evolved independently only in sections *Arachis* and *Rhizomatosae* (Smartt and Stalker, 1982).

Based on differences in branching pattern and in the presence of reproductive nodes on the mainstem, *A. hypogaea* is subdivided into two subspecies: subsp. *hypogaea* and subsp. *fastigiata* Waldron (Krapovickas and Rigoni, 1960). Subspecies *hypogaea* has alternate branching pattern, no reproductive nodes on the mainstem, spreading or erect growth habit, a longer maturation period and fresh-seed dormancy. This subspecies is subdivided into botanical varieties *hypogaea* (Virginia and Runner U.S. market types) and *hirsuta* Köhler (not grown commercially in the U.S., but also known as Peruvian humpback or Chinese dragon type). Subspecies *fastigiata* has a sequential branching pattern, reproductive nodes on the mainstem, erect growth habit, earlier maturity and little or no seed dormancy. The two botanical varieties within subspecies *fastigiata* are

var. *fastigiata* (Valencia U.S. market type) and var. *vulgaris* Harz (Spanish U.S. market type). Krapovickas and Gregory (1994) later revised the classification of cultivated peanut to include the two botanical varieties *peruviana* Krapov. and W.C. Gregory (Valencia type) and *aequatoriana* Krapov. and W.C. Gregory (Zaruma type), which are classified with vars. *fastigiata* and *vulgaris* within subspecies *fastigiata*.

Botany and Reproduction

Peanut is an allotetraploid ($2n = 4x = 40$), self-pollinated, annual legume. Perhaps its most striking characteristic is geocarpy, *i.e*. its flowers develop above ground, but its fruit and seeds are produced below the soil level. The species is typically self-pollinated; however, natural hybridization may also occur at low levels (Stalker and Moss, 1987). Flowers resemble spikes and range in color from light yellow to deep orange. Inflorescences are located in the leaf axils of primary and secondary branches, but never at the same node as vegetative branches (Gregory *et al.,* 1973). The first flowers appear 4 to 6 weeks after planting with maximum flower production occurring 2 to 4 weeks thereafter. The flower contains five petals including a yellow to orange standard, two yellow to orange wings, and two petals fused to form a pale yellow keel. There are 10 anthers, two of which are not fully developed, and an elongated calyx tube containing the style attached at the base of the ovary. The stigma and anthers are enclosed in the keel, which promotes self-pollination. The stigma becomes receptive 24 hours before to 12 hours after the flower opens, while pollen becomes mature 1-8 hours before flower opening. Anthesis and pollination usually occur at about the time of sunrise.

The flower withers 5 to 7 hours after opening. Within 1 week after fertilization, a needlelike structure, called the peg, starts developing and elongates quickly. The ovary containing the fertilized ovules is located behind the tip of the peg, but distal to the peg meristem. The positively geotropic peg grows into the soil and once it reaches a depth of 2-7 cm it loses its geotropism. Subsequently, the tip orients itself horizontally and pod growth begins (Rao and Murty, 1994). Seed development occurs over three stages. The first stage is characterized by rapid growth with marked increases in both fresh and dry weight. During the second stage energy reserves are accumulated and moisture content decreases. In the third or ripening stage, moisture is lost with little or no change in dry weight (Coolbear, 1994). The mature pod contains one to five seeds. The dry pericarp of the mature pod is reticulate. The endocarp recedes as the fruit grows becoming progressively thinner as the seeds reach maturity (Coffelt, 1989). The number of days from planting to crop maturity varies depending on the cultivar and planting conditions. Runners take about 150 days, Virginias between 130 and 150 days, and Spanish types approximately 100 to 120 days to mature (Coolbear, 1994).

Centers of Origin and Diversity

The center of origin for the genus *Arachis* is the Matto Grosso region of Brazil (Gregory *et al*., 1980). Wild species are found in South America, in a large region bound by the Amazon River to the north, the Río de la Plata to the south, the Andes mountains to the west, and the Atlantic Ocean to the east (Valls *et al*., 1985). Because considerable overlaps in distribution occur between species in several sections of the genus, species most likely diverged early in the evolutionary history of the genus (Valls *et al*., 1985).

The center of origin of the cultivated peanut is believed to be on the eastern slopes of the Andes of southern Bolivia and northern Argentina because its putative progenitor species have been found only in this region (Krapovickas, 1968). Seven primary centers of diversity have been described for cultivated peanut: (1) Guaraní region (Paraguay-Paraná river basins and southwestern Brazil) for var. *fastigiata* and var. *vulgaris*; (2) Goiás and Minas Gerais region of Brazil (Jocantis-São Francisco river basin) also for var. *fastigiata* and var. *vulgaris*; (3) Rondonia and northwestern Matto Grosso region of Brazil (headwaters of the Amazon River) for var. *hypogaea*; (4) Bolivian region (eastern slopes of the Andes) for var. *hypogaea*; (5) Peruvian region (upper Amazon and west coast) for vars. *hirsuta*, *fastigiata* and *peruviana;* (6) northeastern Brazil for var. *fastigiata*; and (7) Ecuadorian region for var. *aequatoriana* (Gregory and Gregory, 1976; Stalker and Simpson, 1995).

Africa has been described as a secondary center of diversity for cultivated peanut by Gibbons *et al*. (1972). Natural hybridization among types introduced to Africa from Brazil followed by selection is thought to be responsible for the variation in the African collection (Gibbons *et al*., 1972).

Evolution and Genome Donors

Little is known about the origin and subsequent domestication of peanut (Kochert *et al*., 1996). The identity of the progenitor species of the cultivated peanut has been one of the questions that captured the attention of peanut scientists during most of the last century. In 1936, Husted observed the presence of two pairs of chromosomes that could be easily differentiated from the others: one pair (A) that was conspicuously small and

another pair (B) that had an unusually long secondary constriction. It was also observed that chromosome association at meiosis was usually of 20 bivalents with an occasional multivalent. Based on these findings, Husted proposed that *A. hypogaea* was an allopolyploid which had arisen through the natural hybridization of two distinct but related species. However, the possibility that *A. hypogaea* could be an autotetraploid formed by chromosome doubling followed by the fragmentation or loss of some chromosomes was not discarded (Husted, 1936).

Breeding experiments established that *A. hypogaea* and *A. monticola* Krapov. and Rigoni belonged essentially to the same biological species, the former being domesticated and the latter its wild counterpart (Hammons, 1970). The close biological relationship between the two species suggests that they descended from the same progenitor species. Gregory and Gregory (1976) hypothesized that an interspecific hybrid between a perennial (such as *A. cardenasii* Krapov. and W.C. Gregory) and an annual (such as *A. duranensis* Krapov. and W.C. Gregory) species of section *Arachis* would have a karyotype similar to that of *A. hypogaea-monticola*. They postulated that *A. hypogaea* originated through the chromosome doubling of such a hybrid.

Smartt *et al*. (1978) studied mitotic chromosome preparations of several *Arachis* wild species. Their results indicated that there were several potential donor species of the A genome, while only *A. batizocoi* Krapov. and W.C. Gregory could be the donor of the B genome because it was the only species within section *Arachis* that lacked the small A chromosome pair. Based on morphological and phytogeographical considerations, *A. cardenasii* was proposed as the most likely contributor of the A genome. In cross-compatibility studies of crosses between *A. batizocoi* and several other diploid species of

section *Arachis,* a low frequency of bivalents and a high frequency of univalents were found (Singh and Moss, 1984). These supported the hypothesis that among diploid species of the section *Arachis*, *A. batizocoi* was the only species with a unique genome (B).

Subsequent research on hybrids between *A. hypogaea* and inter- (AB) and intracluster (AA) amphidiploids showed that mean bivalent associations were significantly higher in *A. hypogaea* x AABB amphidiploids than those from *A. hypogaea* x AAAA amphidiploids (Singh, 1988). These results supported the hypothesis of an amphidiploid origin of *A. hypogaea* involving two diploid species, one with A and the other with B genomes.

Arachis correntina Krapov. and W.C. Gregory was also used to develop hybrids with *A. hypogaea* (Murty and Jahnavi, 1986). Pachytene chromosome morphology of *A. correntina* corresponded well with the A genome of *A. hypogaea*. Therefore, they proposed *A. correntina* as a possible donor of the A genome. Krishna and Mitra (1988) analyzed total seed protein profiles and based on arachin patterns, concluded that *A. hypogaea and A. monticola* were closely related to *A. cardenasii, A. duranensis*, and *A. batizocoi*. Moreover, *A. duranensis* showed the highest number of similar bands to *A. monticola* than any other A genome species.

Stalker *et al*. (1990) were the first to contradict the hypothesis that *A. batizocoi* was the donor of the B genome. Their analysis of isozymes led to the conclusion that this species was too distantly removed from all other species in section *Arachis,* and therefore it was not likely to have been involved in the evolution of *A. hypogaea*. Seed storage

protein (Bianchi-Hall *et al*., 1993) and additional isozyme (Lu and Pickersgill, 1993; Stalker *et al*., 1994) studies confirmed these results.

With the development of RFLPs, RAPDs and other high throughput molecular marker systems, large amounts of molecular data have been generated which has allowed researchers to make more precise comparisons among diploid and tetraploid genomes. Kochert *et al*. (1991) analyzed RFLP patterns of diploid and tetraploid *Arachis* species and found that *A. ipaensis* Krapov. and W.C. Gregory*, A. duranensis*, and *A. spegazzinii* (name changed to *A. duranensis* by Krapovickas and Gregory, 1994) were the species which more consistently shared bands with *A. monticola*. Furthermore, when they attempted to reconstruct the tetraploid RFLP banding pattern based on combinations of diploid patterns, the combinations *A. ipaensis* x *A. duranensis* and *A. ipaensis* x *A. spegazzinii* were the ones that most often reconstituted the tetraploid genome. Similar results were obtained by Hilu and Stalker (1995), who used RAPD markers to study inter- and intraspecific variation among nine species of section *Arachis* and reported close relationship between *A. duranensis* and *A. hypogaea-A. monticola.* Fernandez and Krapovickas (1994) also conducted extensive cytological analysis of several wild peanut species. According to their results based on karyotype, morphology, and geographical distribution, *A. duranensis* and *A. ipaensis* were the most probable ancestors of *A. hypogaea*.

Kochert *et al*. (1996) were the first to combine cytological and molecular marker data to study evolution in the genus *Arachis*. The two genomes (A and B) in *A. hypogaea* were found to be different enough to be distinguished by RFLP patterns specific for each genome. When these patterns were compared with those of the diploid species, *A.*

ipaensis emerged as the species most closely related to the B genome of the cultivated peanut. Next, RFLP patterns from other accessions were examined in order to find the ones that, when combined with those of *A. ipaensis,* would reconstitute patterns characteristic of *A. hypogaea*. *Arachis duranensis* was found to be the most likely donor of the A genome. To support this evidence, a hybrid between *A. duranensis* and *A. ipaensis* was produced. RFLP patterns from this hybrid were indistinguishable from those of peanut. The chloroplast restriction fragment pattern of *A. duranensis* was found to be identical to that of *A. hypogaea*, while that of *A. ipaensis* showed three differences, which indicated that *A. duranensis* was the female parent. The karyotype of *A. ipaensis* was found to possess those characteristics that had previously supported *A. batizocoi* as the donor of the B genome. Based on molecular marker and cytological data, *A. duranensis* and *A. ipaensis* were proposed as the present-day species most closely related to the progenitors of domesticated peanut (Kochert *et al*., 1996).

Utilization of Wild Species for Peanut Improvement

The section *Arachis* includes cross-compatible diploid annual and perennial species and only two tetraploid species: the cultivated peanut (*A. hypogaea*) and its putative wild progenitor (*A. monticola*) (Gregory and Gregory, 1976). Wild species of *Arachis* are important sources of genes of interest for cultivar improvement. Several *Arachis* species have significantly higher levels of resistance to disease and insect pests than those found in the cultivated species (Stalker and Moss, 1987). High levels of resistance or immunity to early leafspot (*Cercospora arachidicola* Hori), late leafspot (*Cercosporidium personatum* Deighton), Cylindrocladium black rot (*Cylindrocladium*

parasiticum Crous, M.J. Wingfield, & Alfenas), rust (*Puccinia arachidis* Speg.), corn earworm (*Helicoverpa zea* Bodie), fall armyworms (*Spodoptera frugiperda* J. E. Smith), lesser cornstalk borer (*Elasmopalpus lignosellus* Zeller), potato leafhoppers (*Empoasca fabae* Harris), nematodes (*Meloidogyne* spp.), mites (*Tetranychus* spp.), tobacco thrips (*Frankliniella fusca* Hinds), groundnut rosette virus, tomato spotted wilt virus, peanut mottle virus, and peanut stunt virus have been reported in *Arachis* species (see Stalker and Moss, 1987; Lynch and Mack, 1995; Stalker and Simpson, 1995). In addition to insect and disease resistances, other traits for which *Arachis* wild species would be valuable sources of novel alleles include protein and oil composition and drought resistance (Stalker and Moss, 1987). However, cross-incompatibilities that restrict gene transfer have precluded the widespread use of *Arachis* wild species in cultivar development. There are several strategies that can be used for introgression of genes from wild relatives into *A. hypogaea*. Direct hybridization is possible between *A. hypogaea* and diploid species in the secondary gene pool. The resulting triploid hybrids ($2n = 3x = 30$) are highly sterile, but fertility can be restored by doubling the number of chromosomes ($2n = 6x = 60$) with colchicine. The hexaploid hybrids can then be selfed for several generations until tetraploidy is eventually restored by spontaneous elimination of chromosomes (Stalker, 1992). An alternate strategy for gene introgression is to bring wild diploid species to the same ploidy level as cultivated peanut prior to hybridization by either doubling their chromosome number to produce autotetraploids or by doubling the chromosomes of AA x BB interspecific hybrids, backcrossing allotetraploids to the cultigen (Simpson, 1991).

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I. TAXONOMIC RELATIONSHIPS AMONG *ARACHIS* SPECIES AS REVEALED BY AFLP MARKERS

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ABSTRACT

Cultivated peanut, *Arachis hypogaea* L., is a tetraploid ($2n = 4x = 40$) species considered to be of allopolyploid origin. Its closest relatives are the diploid ($2n = 2x = 20$) annual and perennial species included with it in section *Arachis*. Species in section *Arachis* represent an important source of novel alleles for the improvement of cultivated peanut. The AFLP technique was used to determine intra- and inter-specific relationships among and within 108 accessions of 26 species of this section. A total of 1328 fragments were generated with 8 primer combinations. From those, 239 unambiguous bands ranging in size from 65 to 760 bp were scored as binary data. Genetic distances among accessions ranged from 0 to 0.50. Average distances among diploid species (0.30) were much higher than that detected between tetraploid species (0.05). Cluster analysis using different methods and principal component analysis were performed. The resulting grouping of accessions and species supports previous taxonomic classifications and genome designations. Based on genetic distances and cluster analysis, "A" genome accessions KG 30029 (*A. helodes*), and KSSc 36009 (*A. simpsonii*), and "B" genome accession KGBSPSc 30076 (*A. ipaensis*) were the most closely related to both *A. hypogaea* and *A. monticola* suggesting their involvement in the evolution of the tetraploid species.

INTRODUCTION

Genus *Arachis* L. is comprised of 69 diploid and tetraploid species native to South America, including the cultivated peanut, *Arachis hypogaea* L. (Krapovickas and Gregory, 1994). The genus has been divided into nine sections based on morphology, geographic distribution, and cross-compatibility. Section *Arachis* is of special interest because it includes *A. hypogaea* and its putative wild progenitor, *A. monticola* Krapov. and Rigoni (Gregory and Gregory, 1976). Cultivated peanuts can be crossed with other species in section *Arachis* but not with species in other sections (Gregory and Gregory, 1979). Therefore, biosystematic information for species in section *Arachis* would be useful for germplasm utilization (Stalker *et al.*, 1991).

Section *Arachis* is distinguished from other sections of the genus by having taproots, no rhizomes, vertical pegs, and flowers with no red veins in the back of the standard (Gregory *et al.*, 1973). The section includes 25 cross-compatible diploid annual and perennial species and the two tetraploid species, *A. hypogaea* and *A. monticola.* Wild species in this section are widely distributed in central and southern Brazil, Argentina, Bolivia, Paraguay and Uruguay (Valls *et al.*, 1985). On the basis of cytological evidence, three different genomes have been designated in section *Arachis*. The "A" genome is characterized by the presence of a distinctly small chromosome pair (Husted, 1936) and is represented by most of the diploid species (Smartt *et al.*, 1978). The "B" genome lacks the small A chromosome pair, possesses a chromosome pair with a secondary constriction (Husted, 1936), and is represented by only a few species. The "D" genome presents a unique karyotype and is known only in *A. glandulifera* (Stalker, 1991).

Little is known about the origin of domesticated peanut. Domesticated peanut is an allotetraploid (AABB) considered to have originated from a single hybridization event between two diploids (Husted, 1936). Based on morphological, cytological, cross-compatibility and molecular marker evidence, *A. cardenasii* Krapov. and W.C. Gregory (Smartt *et al.,* 1978; Singh and Moss 1982)*, A. villosa* Benth. (Kirti *et al.* 1983; Raina and Mukai 1999), *A. correntina* Krapov. and W.C. Gregory (Murty and Jahnavi 1986) and A. *duranensis* Krapov. and W.C. Gregory (Seetharam *et al.* 1973; Gregory and Gregory 1976; Singh 1988; Kochert *et al.* 1991; Singh *et al.* 1996; Kochert *et al.* 1996) have been proposed as the donor of the A genome. *Arachis batizocoi* Krapov. and W.C. Gregory (Smartt *et al.* 1978; Singh and Moss 1984; Singh 1988; Klosova *et al.* 1983) and *A. ipaensis* Krapov. and W.C. Gregory (Kochert *et al.* 1991, 1996; Fernandez and Krapovickas 1994) have been proposed as possible B genome donors. The single hybridization event that gave rise to *A. hypogaea* isolated it reproductively from their genome donors and other species in the section. As a consequence of this reproductive isolation, cultivated peanut possesses a narrow genetic base. Low levels of genetic polymorphism for biochemical and molecular markers have been found within the species in a series of studies (Halward *et al.* 1991, 1992; Kochert *et al.* 1991; Paik-Ro *et al.* 1992; Bianchi-Hall 1993; Lu and Pickersgill 1993; Singh *et al.* 1994; Stalker *et al.* 1994; Hilu and Stalker 1995; He and Prakash 1997). On the other hand, extensive polymorphism occurs in the closely related *Arachis* species. Several agronomically useful characters including resistance and tolerance to pests and diseases, have been found in species of section *Arachis* (Stalker and Simpson 1995). Successful gene introgression from wild to cultivated crop species relies on the development of hybrids with genomic

configurations that promote exchange of genetic material through pairing of homoeologous chromosomes (Nagpal *et al.* 1996). The level of speciation and phylogenetic relationships among diploid species of section *Arachis* remains unclear (Raina *et al.* 2001). A better understanding of these relationships is crucial for the use of desirable traits from the wild species in peanut improvement programs.

A variety of molecular marker techniques have been used to determine taxonomic relationships and genetic variation of crop species and their wild relatives. Among these methods, the amplified fragment length polymorphism (AFLP) method (Zabeau and Vos 1993; Vos *et al.* 1995) has been successfully used to analyze inter- and intra-specific genetic diversity in a wide range of crop species (Hill *et al.* 1995; Powell *et al.* 1996). The major advantage of the AFLP technique over other marker technologies is that it enables simultaneous analysis of a large number of marker loci throughout the genome (Powell *et al.* 1996). Other benefits of the technique include high reproducibility, high levels of polymorphism detection, and no prior knowledge of the genome being studied is required (Prabhu and Gresshoff 1994; Lu *et al.* 1996). Consequently, AFLPs are ideally suited for the study of genetic diversity within gene pools of *Arachis* species for which little information currently exists (Tomkins *et al.* 2001).

In the present study we have examined accessions from almost all species in section *Arachis* in order to (1) determine the extent of inter- and intra-specific variation and infer phylogenetic relationships, (2) gain information on the genomic origin of cultivated peanut by evaluating genetic distances, and (3) determine the potential of AFLP markers to discriminate between species and accessions.

MATERIALS AND METHODS

Plant Material and DNA Extraction

A total of 108 genotypes representing 26 species of section *Arachis* were evaluated in this study (Table 1). Seeds of peanut cultivars and wild species were obtained from the *Arachis* germplasm collection maintained at North Carolina State University. Entries representing botanical varieties were obtained from the USDA/ARS Plant Genetic Resources Conservation Unit (Griffin, GA).

Two to three young unopened leaves were collected from two plants of each accession, bulked, and used for DNA extraction using the CTAB method of Afanador *et al.* (1993) with the modification that a Fast Prep FP120 (Thermo Savant, Holbrook, NY) machine was used to grind the tissue. DNA was quantified using a Hoefer fluorometer (Hoefer Scientific Instruments, San Francisco, CA).

AFLP Analysis

AFLP fingerprinting was performed as described by Myburg and Remington (2000). All primers and adaptors were obtained from Sigma Genosys (The Woodlands, TX) with the exception of the labeled primers. Labeled primers were obtained from LI-COR Inc. (Lincoln, NE).

Digestion of DNA. Approximately 500 ng of DNA was simultaneously digested with *Eco*RI and *Mse*I at 37°C for 1.5 hr using 12U *Eco*RI, 8U *Mse*I, and 6 µl of 5X restriction-ligation buffer (50 mM Tris-acetate, 50 mM magnesium acetate, 250 mM

potassium acetate, 25 mM DTT, and 250 ng/µl BSA) in a final volume of 30 µl. DNA was run on a 0.8% agarose gel to check for complete digestion.

Adaptor Ligation. Adaptor ligation was performed by adding 5 pmol *Eco*RI adaptor, 50 pmol *Mse*I adaptor, 10 mM ATP, 0.5U of T4 DNA ligase, and 1 µl 5X restriction-ligation buffer to 20 µl of each double-digested DNA sample (25 µl final volume) and incubating overnight at 37°C.

Pre-amplification. A pre-amplification step was performed with primers complementary to the adaptor sequences carrying an additional selective nucleotide. A 1:10 dilution of the digested and adapter-ligated DNA was used as a template for this step. PCR reactions were carried out in a total volume of 20 µl containing 2 µl of 10X PCR buffer (100 mM Tris-HCl pH 8.3, 15 mM $MgCl_2$, 500 mM KCl), 2.5 mM of each dNTP, 30 ng primer E01-A, 30 ng primer M02-C, 1.2 U *Taq* DNA polymerase (Qiagen, Valencia, CA), and 5 µl of DNA. PCR amplifications were carried out in a PTC-100 programmable thermal controller (MJ Research Inc., Reno, NV) using the following temperature profile: 28 cycles of 15 s at 94°C, 30 s at 60°C, and 60 s + 1 s/cycle of extension at 72°C; followed by one cycle of 2 min at 72°C. Upon completion of amplification, 15 µl of each sample were diluted 1:20 with low TE (10 mM Tris-HCl pH 8.0 and 0.1 mM EDTA). The remaining 5 µl of each sample were checked on a 0.8% agarose gel where a smear was visible.

Selective Amplification and Polyacrylamide Gel Electrophoresis (PAGE). For the selective amplification, primers with three selective nucleotides were used (Table 2). EcoRI primers were labeled with a fluorescent near-infrared group (IRD-700 or IRD-

800). The PCR amplification mixture (20 µl final volume) was comprised of 2 µl of 10X PCR buffer, 2.5 mM of each dNTP, 30 ng unlabeled *Mse*I+3 primer, 5 ng labeled EcoRI+3 primer, 1.2 U *Taq* DNA polymerase, and 5 µl of diluted pre-amplification product. Selective amplification was carried out in a PTC-100 programmable thermal controller using the following temperature profile: 13 cycles of 10 s at 94°C, 30 s at 65°C – 0.7°C/cycle after the first cycle, and 60 s at 72°C; followed by 25 cycles of 10 s at 94°C, 30 s at 56°C, and 60 s + 1 s/cycle of extension at 72°C; followed by one cycle of 2 min at 72°C. After amplification, samples were denatured by adding 10 µl of loading dye (95% deionized formamide, 20 mM EDTA, and 0.8 mg/ml bromophenol blue), heating at 94°C for 3 min, and chilling on ice. AFLP fragments were separated by PAGE on a LI-COR 4200 DNA Analyzer Sequencer on 25 cm gels using 8% denaturing polyacrylamide gels (7 M ultra pure Urea, 0.8X TBE, and 8% Long Ranger acrylamide (BioWhittaker Molecular Applications, Rockland, ME)). Near-infrared labeled size standards (LI-COR Inc.) were loaded on each gel for sizing of the AFLP fragments.

Data Analysis

The AFLP-Quantar 1.0 (Keygene Products B.V., Wageningen, The Netherlands) software package was used to score only distinct, major, reproducible bands. Presence or absence of each AFLP fragment was scored as a binary unit character (present = 1, absent = 0). A genetic distance matrix was obtained using the computer program PAUP* 4.0b2a (Swofford 1998) and subsequently used to construct dendrograms using both the unweighted pair group method average (UPGMA; Sokal and Michener 1958) and the

neighbor-joining (NJ; Saitou and Nei 1987) clustering procedure. In addition, genetic distances were estimated based on the log of the shared allele coefficient (Jin and Chakraborty 1993). The generated matrix was then analyzed using the Fitch clustering procedure of PHYLIP 1.0 package (Felsenstein 1993). The robustness of the phylogenetic trees was evaluated by comparing dendrograms obtained from different methods and by bootstrap analysis (Felsenstein 1985) with 1000 replicates using PAUP* 4.0b2a. Principal component analysis was performed based on the variance-covariance matrix of the data using the PRINCOMP procedure of SAS 8.2.

RESULTS

AFLP analysis

A total of 1328 AFLP bands were generated using eight primer combinations. From these fragments, 239 unambiguous bands ranging in size from 65 to 760 bp were scored (Fig. 1). The number of scored loci amplified by each primer varied from 14 to 47 with an average of 30 per reaction.

To evaluate the reproducibility of the banding patterns, two completely independent AFLP fingerprints were generated for 10 samples. Mean reproducibility values (calculated as the percentage of bands that were identical in the two repeats) were very high and ranged from 96% to 100% for the eight primer combinations used (Table 2).

Genetic Distance Matrix and Cluster Analysis

Estimates of genetic distance among the 108 accessions ranged from 0 to 0.50. Average genetic distances among diploid species (0.30) were much higher than that detected between tetraploid species (0.05) (Table 3). Differences between diploid species were substantial. Among them, *A. benensis* and *A. helodes* shared the greatest distance (with an average of 0.42), and *A. palustris* and *A. praecox* shared the shortest distance (with an average of 0.09). The mean genetic distance among accessions within each species was 0.16 for A genome species, 0.13 for B genome species and 0.07 for the only D genome species. The tetraploid species showed not only the shortest distance between species (0.05), but also the shortest distances within species with 0.01 for *A. hypogaea* and 0.02 for *A. monticola*.

Dendrograms were produced with different methods and each had the same eleven main clusters. The species relationships obtained from the NJ method (Fig. 2) were similar to those obtained using the UPGMA and the Fitch methods except for minor differences in branch lengths and a few inconsequential topological rearrangements. The taxa fell into eleven groups that, for the most part, are consistent with previously defined cytogenetic genome groups and inter-species relationships.

Cluster I contains accessions of nine of the A genome species and is divided into six sub-clusters. Sub-cluster 1 contains accessions of *A. kuhlmannii* and *A. stenosperma.* Sub-cluster 2 includes accessions of *A. kuhlmannii* and *A. helodes.* Sub-cluster 3 contains accessions of *A. kempff-mercadoi*, *A. kuhlmannii*, and *A. herzogii,* but also accessions of *A. diogoi* and *A. helodes.* Sub-cluster 4 consists of *A. villosa*. Sub-cluster 6 contains

accessions of *A. cardenasii*, *A. correntina* and *A. diogoi*. Sub-cluster 5 includes two accessions of *A. cardenasii* and two accessions of *A. correntina*.

Cluster II is composed of all accessions of A genome species *A. duranensis*, one of *A. kuhlmannii* and one of *A. magna.* Cluster III contains four sub-clusters where sub-cluster 1 contains tetraploid (AABB) species *A. monticola* and *A. hypogaea*, and one accession of A genome species *A. helodes.* Sub-cluster 2 consists only of one accession of B genome species *A. ipaensis*. Sub-cluster 3 is comprised of B genome species *A. williamsii, A. magna* and *A. valida*. Sub-cluster 4 consists of one accession of A genome species *A. simpsonii.* Cluster IV includes only accessions of B genome species *A. hoehnei* (bootstrap value of 100%). In cluster V are all accessions of the 18 chromosome A genome species *A. decora, A. praecox* and *A. palustris* (bootstrap value of 100%), with the exception of accession 9953 of *A. decora* which is included by itself in cluster VI. Cluster VII is made up of A genome species *A. trinitensis* and *A. benensis* and one accession of *A. diogoi* (bootstrap value of 100%). Cluster VIII contains B genome species *A. cruziana* and *A. batizocoi* (bootstrap value of 100%). Cluster IX consists of three accessions of D genome species *A. glandulifera.* The remaining accession of *A. glandulifera* is by itself in cluster X. One accession of *A. batizocoi* comprises cluster XI. The *A. helodes*, *A. magna, A. kuhlmannii* and *A. cardenasii* accessions were highly variable, showing large amounts of intraspecific variation.

Principal Component Analysis

The 239 band scores were subjected to principal component analysis to visualize genetic relationships among the species studied (Fig. 3). The first three PCs accounted for

42% of the total variation observed and separated the different genomic groups. The first PC clearly discriminated diploid from tetraploid species. The second PC separated, for the most part, A from B genome species. A plot generated with the first two PCs showed that most of the accessions clustered into four well-defined groups: a tight cluster of tetraploid species, a cluster of B and D genome species, a cluster of A genome species, and a cluster of accessions of *A. hoehnei* and of 18-chromosome A genome species. The tight grouping of the tetraploid accessions reflects the lack of variation present among these accessions, which was also revealed with the cluster analysis. Plotting of the third PC did not add clarification in the separation of the data (data not shown).

Genome Donors

As an approach to elucidate the most probable genome donors to the tetraploid species, the average distance between each diploid accession and all tetraploid accessions as a group was calculated (Table 4). Average distances were then compared in order to find the accessions most closely related to the group of tetraploid accessions. Accessions 30029 (*A. helodes*, distance = 0.09), 36009 (*A. simpsonii*, distance = 0.24), and 30067 (A. duranensis, distance = 0.27) ranked first, second and third, respectively, among all A genome accessions. Accession 30076 (*A. ipaensis*, distance = 0.21) ranked first among all B genome accessions. Based on the cluster analysis, accessions 30029 and 30076 grouped tightly with the tetraploid accessions. Moreover, clustering of 30029 with the tetraploid accessions was strongly supported by bootstrap analysis (100%).

DISCUSSION

Despite the lack of polymorphism observed in the tetraploid species, the AFLP technique was useful in assessing genetic diversity and relationships among accessions in section *Arachis*. The presence of unique AFLP markers among various *Arachis* species indicated the usefulness of the approach for fingerprinting purposes. Moreover, the repeatability of the technique was very high, ranging from 96 to 100%. AFLPs have been recognized as an efficient marker system that is as reliable as RFLP and SSR at a lower cost, and more reliable than RAPD markers (Powell *et al.* 1996).

Both cluster and principal component analyses showed well-defined groupings of the species, and provided additional support for previous groupings based on taxonomic classification and genome designations. Results from this experiment indicate that the AFLP technique provides increased resolution over the approach of Stalker (1990), which was based on morphological data. Discrepancies in clustering can be explained by differences resulting from the observation of morphological characters vs. genetic characters. Changes in the DNA sequence and changes in morphological characters do not exhibit a one-to-one correlation (Futuyma 1986). Furthermore, our results indicate that the AFLP technique is more robust than the RAPD technique used by Halward *et al*. (1992) and Raina *et al*. (2001). Some of the discrepancies in clustering between RAPD and AFLP analyses may have resulted from the difference in number of accessions included in each of these studies. The larger number of accessions analyzed in our experiments allows for the closing up of "gaps" in the branching pattern, which results in

minor modifications in the clusters generated and slight differences in the estimates of relatedness among species.

Significant amounts of variation were observed both between and within species. Inter-specific variation was higher than intra-specific variation for most species. B genome species showed less interspecific variation than A genome species. Interestingly, 18-chromosome A genome species showed less inter- and intra-specific variation than their 20-chromosome counterparts. Moreover, these species seem to be as closely related to B and D genome species (average distance 0.29 and 0.28, respectively) as to the 20-chromosome A genome species (average distance 0.30). These results pose a question about their true genomic identity.

For species *A. helodes, A. diogoi,* and *A. magna,* intraspecific variation was high and as a result, not all accessions of these species clustered together. This result is not surprising for *A. helodes* given the fact that accessions exhibit a high degree of morphological variability (Stalker, 1990). *Arachis magna* accessions 30092 and 30093 were described as a separate species based on morphological traits (Krapovickas and Gregory, 1994). However, our results found these accessions were not only very different but accession 30092 clustered tightly with *A. duranensis* accessions (clustering supported by a bootstrap value of 80%). This discrepancy was previously observed by Kochert *et al.* (1996). Possibilities such as introgression from other species caused by cross-pollination or adaptation to a new environment as a result of selection pressure may account for these results. A similar pattern was observed for *A. batizocoi* accession 9484, which was removed from other accessions of this species. Because this accession was collected in the 1950's and maintained in nurseries for the past 45 years, it seems likely that selection

has occurred for adaptation to its new environment, which resulted in detectable genetic change. This issue raises the question of how much and what kind of selection pressure is being exerted on wild species accessions maintained through decades in U.S. collections.

In general, the AFLP data grouped accessions into similar species groups or species-species associations. For example, *A. kuhlmanni* clustered with *A. kempff-mercadoi*, *A. cardenasii* with *A. correntina*, and *A. benensis* with *A. trinitensis*. Crosses between these pairs of species show high levels of chromosome pairing (), implying that they are closely related. Additional data on cross-compatibility, geographical distribution, and climatic adaptation may provide added insight into these relationships.

Because A. *glandulifera* has a D genome, it was expected to form a discrete genetic cluster apart from both A and B genome species. Although the first two principal components did not provide separation between the B and D genomes in the PC analysis, cluster analysis showed D accessions clearly removed from other accession in the section. D genome accessions were supported as the outgroup by a bootstrap value of 100%

The AFLP profiles for accessions of *A. monticola* were almost identical to those for accessions of *A. hypogaea* (correlation 88%, genetic distance 0.05). These findings support the hypothesis that both tetraploid species originated by hybridization of the same two progenitor species and that *A. monticola* is either the direct ancestor of cultivated peanut or a weedy escape from cultivation. The average distance for A genome species to *A. hypogaea* was 0.39, for B species this distance was 0.37, and for D genome species 0.42.

The A genome and B genome species seemed to be almost equally related to the cultivated peanut. This result is expected because *A. hypogaea* is believed to be an amphidiploid produced by the hybridization of an A genome species and a B genome species, and *A. glandulifera* is not believed to have been involved in its evolution (Stalker, 1991). B genome accessions 30076 (*A. ipaensis*) and 1118 (*A. williamsii*), and A genome accessions 30029 (*A. helodes*), 30067 (*A. duranensis*) and 36009 (*A. simpsonii*) were most closely associated with *A. hypogaea*. *Arachis ipaensis* has been previously proposed as the B genome donor (Fernandez and Krapovickas 1994; Kochert *et al.* 1996) and *A. duranensis* as the A genome donor to the tetraploid *Arachis* species. Although accessions of *A. williamsii*, *A. helodes* and *A. simpsonii* may be closely associated with *A. hypogaea*, geographic distribution of these three species (Fig. 4) does not support their involvement in the evolution of cultivated peanut. *Arachis helodes* and *A. simpsonii* were collected from Mato Grosso, Brazil, and *A. williamsii* from Beni, Bolivia, locations removed from the hypothesized center of origin of *A. hypogaea* in southern Bolivia (Krapovickas, 1968).

Information on genetic relatedness would be extremely useful in terms of which species to use for peanut improvement. For example, *A. helodes* accession 30029 appears to be closely related to *A. hypogaea*. Transfer of specific genes from this accession into cultivated peanut should be more easily achieved than that from other more distantly related species. On the other hand, the primers used in this study revealed unique banding patterns for most species, indicating the wide genetic base of the *Arachis* species. Accessions with the most distinct DNA profiles are likely to contain the greatest number of novel alleles. Estimates of genetic relatedness can be useful for management of

germplasm for conservation of genetic resources, selection of parents for hybridization, and reducing the number of accessions needed to ensure sampling a broad range of genetic variability in breeding programs.

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Figure 1: AFLP fingerprint generated from genomic DNA isolated from 108 genotypes of section *Arachis* using primer combination E-ACT/M-CAC. Arrows indicate fragments scored. Standard band sizes appear on the left.

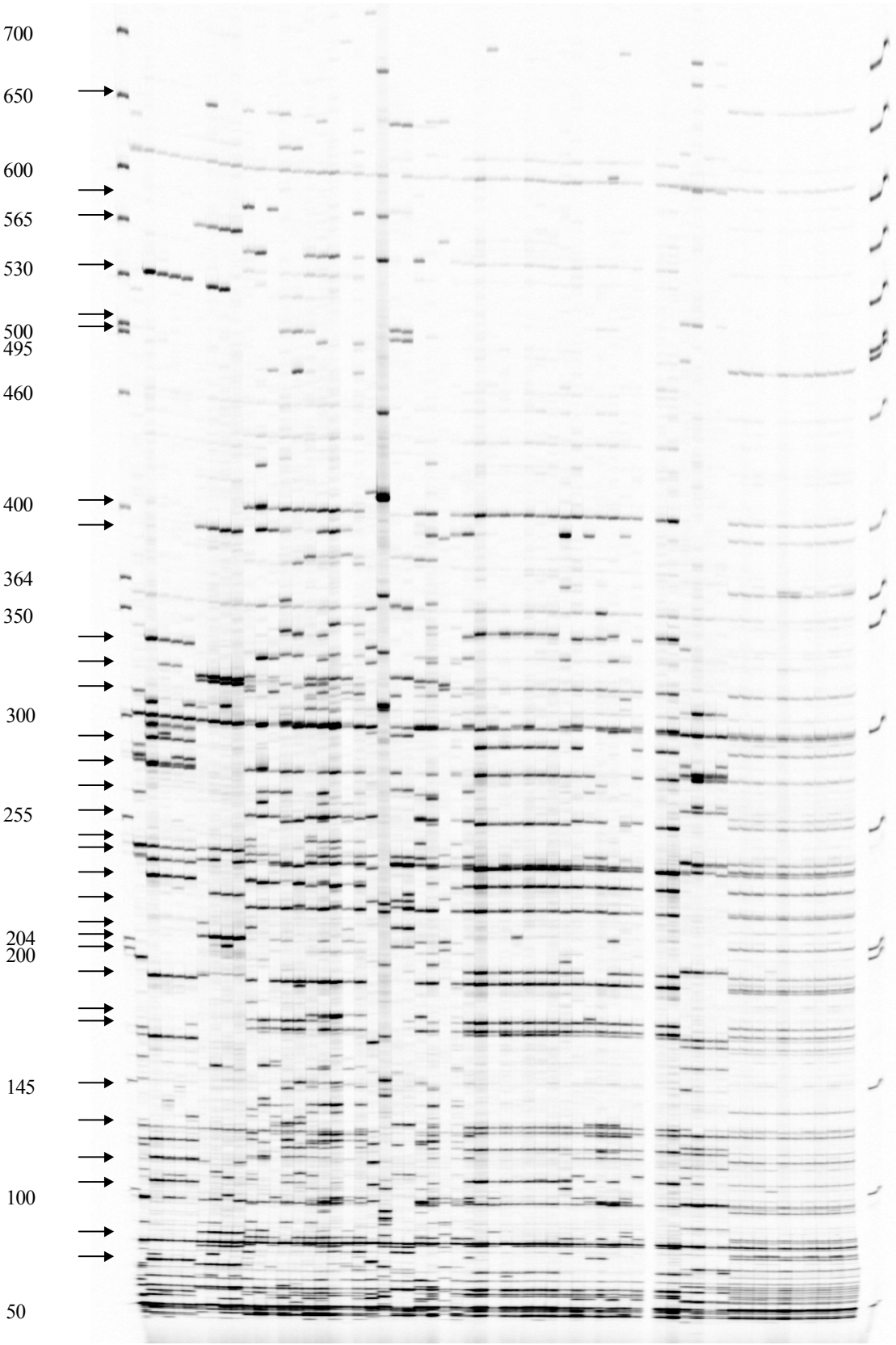
700
650
600
565
530
500
495
460
400
364
350
300
255
204
200
145
100
50

Figure 2: Dendrogram of *Arachis* genotypes based on AFLP polymorphisms. Phenetic relationships were derived from pairwise genetic distance estimates between 108 genotypes. Cluster analysis was performed using the neighbor-joining method. The names of the genotypes are given next to their branches starting with the accession number followed by an abbreviation for the species name. Numbers in parenthesis indicate bootstrap values (%) obtained from 1000 replicate analyses.


9484bati
30091glan
30099glan
30098glan
30100glan
(100)
36024cruz
30079bati
(100)
30083bati
30081bati
30082bati
(100)
1117trin
30005diog
860bene
(88)
35007bene
35005bene
35006bene
(100)
9953deco
9955deco
12900deco
(100)
6416prae
6536palu
13023palu
30006hoeh
(98)
9094hoeh
9140hoeh
9146hoeh
36009simp
30093magn
1118will
(100)
9153vali
9157vali
30011vali
30076ipae
30029helo
(100)
7264mont
30062mont
21768mont
(86)
21769mont
339960hypo
(95)
NC4hypo
12518hypo
261924hypo
339954hypo
497615hypo
590455hypo
NMVaICchypo
501296hypo
A1hypo
30077dura
(88)
30070dura
7988dura
30072dura
30074dura
30067dura
30092magn
30069dura
36002dura
30034kuhl
15101dura
30060dura
10038dura
36006dura
36036dura
21766dura
21763dura
21767dura
30064dura
36032card
36035card
7830corr
9530corr
10017card
36000corr
19616corr
30106diog
30001diog
7897corr
36015card
36019card
862vill
22585vill
30084kemp
7639kuhl
30031helo
10602diog
8888kuhl
30085kemp
35001kemp
30089kemp
36029herz
30088kemp
6331helo
9470kuhl
8916kuhl
9017sten
6404kuhl
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13672sten
7762sten
13256sten
7377sten
12575sten
13796sten

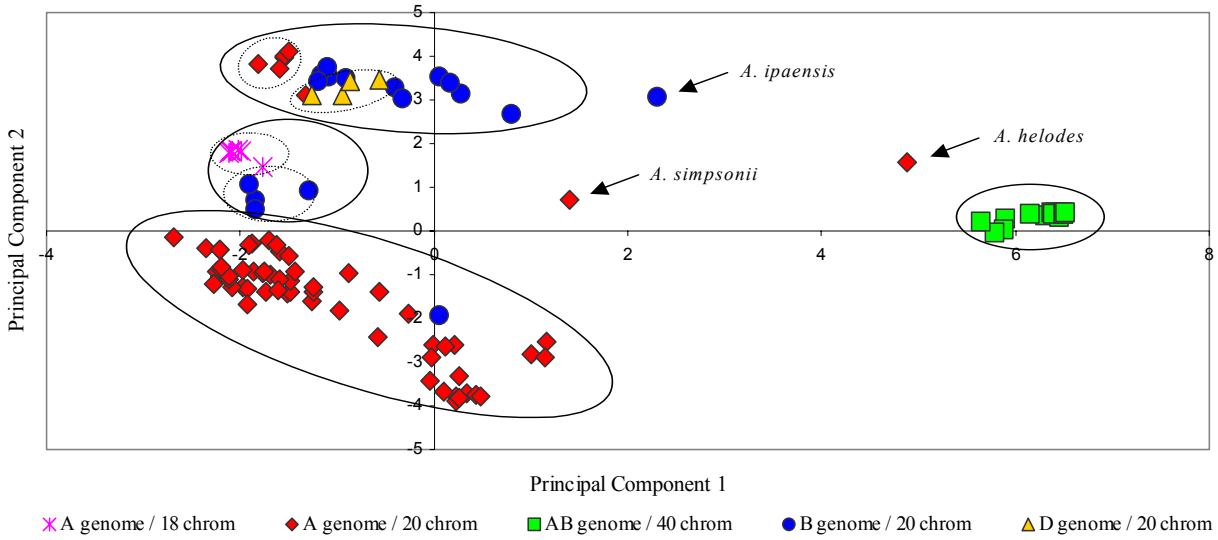

Figure 3: Principal Component plot of 108 *Arachis* genotypes for two principal components estimated with 239 AFLP markers using the variance-covariance matrix of the data. Genome designations and chromosome numbers are indicated.

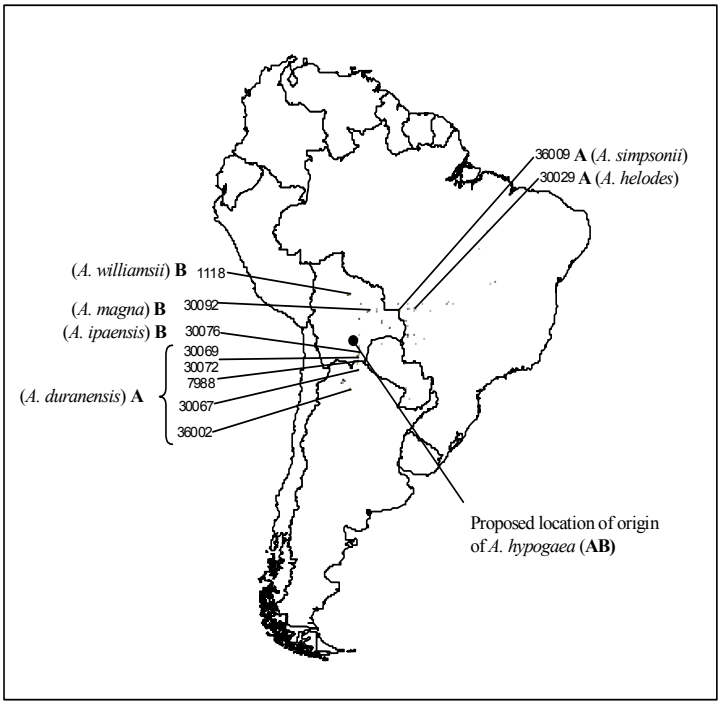

Figure 4: Approximate geographical locations of original collection sites for several accessions of wild *Arachis* species.

Table 1. Number of chromosomes and genome designation of 108 accessions representing 26 species of section *Arachis* analyzed for AFLP variation.

Species	Chrom #	Genome	Accessions
A. batizocoi	20	B	9484, 30079, 30081, 30082, 30083
A. benensis	20	A	860, 35005, 35006, 35007
A. cardenasii	20	A	10017, 36015, 36019, 36032, 36035
A. correntina	20	A	7830, 7897, 9530, 19616, 36000
A. cruziana	20	B	36024
A. decora	18	A	9953, 9955, 12900
A. diogoi	20	A	10602, 30001, 30005, 30106
A. duranensis	20	A	7988, 10038, 15101, 21763, 21766 21767, 30060, 30064, 30067, 30069, 30070, 30072, 30074, 30077, 36002, 36005, 36006, 36036
A. glandulifera	20	D	30091, 30098, 30099, 30100
A. helodes	20	A	6331, 30029, 30031
A. herzogii	20	A	36029
A. hoehnei	20	B	9094, 9140, 9146, 30006
A. ipaensis	20	B	30076
A. kempff-mercadoi	20	A	30084, 30085, 30088, 30089, 35001
A. kuhlmanni	20	A	6404, 7639, 8888, 8916, 9214, 9470, 30008, 30034
A. magna	20	B	30092, 30093
A. monticola	40	AB	7264, 21768, 21769, 30062, 30063
A. palustris	18	A	6536, 13023
A. praecox	18	A	6416
A. simpsonii	20	A	36009
A. stenosperma	20	A	408, 7377, 7762, 9017, 10309, 12575, 13256, 13672, 13796
A. trinitensis	20	A	1117
A. valida	20	B	9153, 9157, 30011
A. villosa	20	A	862, 22585
A. williamsii	20	B	1118
A. hypogaea var. *aequatoriana*	40	AB	Grif 12518, PI 497615
var. *fastigiata*			PI 339960, NM Valencia C
var. *hirsuta*			PI 501296
var. *hypogaea*			PI 339954, NC 4
var. *peruviana*			PI 590455, A1
var. *vulgaris*			PI 261924

Table 2. Combinations of primers used for AFLP analysis of 108 *Arachis* accessions.

Primer Combination	TB[a]	PB[b]	PPB[c]	SB[d]	SRSB[e]	R[f]
E-AAC + M-CAT	197	180	0.91	14	375-625	0.98
E-ACG + M-CAA	168	156	0.93	29	160-635	0.98
E-ACT + M-CAA	183	172	0.94	39	180-760	0.97
E-ACT + M-CAC	160	152	0.95	47	065-655	0.96
E-ACT + M-CAG	156	149	0.96	17	085-680	1.00
E-ACT + M-CAT	175	161	0.92	32	145-625	0.97
E-AGT + M-CTA	162	150	0.93	29	170-615	0.99
E-ATC + M-CGC	127	117	0.92	32	125-695	0.99

[a] TB: total number of bands

[b] PB: number of polymorphic bands

[c] PPB: percentage of polymorphic bands

[d] SB: number of scored bands

[e] SRSB: size range of scored bands

[f] R: repeatability

Table 3: Estimated genetic distance values between (upper triangle) and within (diagonal) the different species of section *Arachis* based on mean character differences between individuals.

Genome	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	D	AB	AB	
Chromosome #	20	20	20	18	20	20	20	20	20	20	18	18	20	20	20	20	20	20	20	20	20	20	20	20	40	40	
Species	*benensis*	*cardenasii*	*correntina*	*decora*	*diogoi*	*duranensis*	*helodes*	*herzogii*	*kempff-merc.*	*kuhlmannii*	*palustris*	*praecox*	*simpsonii*	*stenosperma*	*trinitensis*	*villosa*	*batizocoi*	*cruziana*	*hoehnei*	*ipaensis*	*magna*	*valida*	*williamsii*	*glandulifera*	*hypogaea*	*monticola*	Average distance
benensis	**0.06**	0.39	0.39	0.30	0.31	0.42	0.42	0.40	0.38	0.37	0.33	0.34	0.38	0.37	0.11	0.39	0.39	0.35	0.34	0.35	0.34	0.32	0.33	0.34	0.48	0.47	0.36
cardenasii		**0.19**	0.19	0.28	0.24	0.24	0.28	0.25	0.24	0.23	0.29	0.29	0.29	0.23	0.38	0.21	0.39	0.38	0.30	0.33	0.28	0.35	0.30	0.35	0.38	0.36	0.30
correntina			**0.16**	0.29	0.22	0.26	0.27	0.21	0.21	0.21	0.30	0.30	0.33	0.21	0.38	0.17	0.39	0.37	0.28	0.33	0.29	0.37	0.33	0.34	0.40	0.38	0.30
decora				**0.18**	0.28	0.31	0.35	0.29	0.27	0.27	0.15	0.17	0.31	0.29	0.29	0.27	0.33	0.33	0.26	0.30	0.27	0.30	0.26	0.28	0.44	0.42	0.29
diogoi					**0.26**	0.30	0.31	0.24	0.23	0.24	0.30	0.30	0.33	0.25	0.30	0.24	0.40	0.37	0.29	0.33	0.29	0.34	0.32	0.34	0.41	0.39	0.30
duranensis						**0.11**	0.32	0.33	0.28	0.26	0.32	0.33	0.28	0.28	0.37	0.25	0.42	0.39	0.30	0.38	0.26	0.37	0.33	0.37	0.33	0.31	0.32
helodes							**0.34**	0.26	0.27	0.27	0.36	0.36	0.32	0.28	0.40	0.25	0.40	0.38	0.36	0.31	0.32	0.37	0.32	0.37	0.30	0.30	0.33
herzogii								.[c]	0.18	0.21	0.30	0.30	0.36	0.25	0.39	0.21	0.39	0.41	0.32	0.36	0.34	0.36	0.36	0.38	0.40	0.39	0.32
kempff-merc.									**0.17**	0.20	0.28	0.28	0.29	0.22	0.35	0.20	0.37	0.36	0.28	0.32	0.29	0.34	0.32	0.35	0.39	0.37	0.29
kuhlmannii										**0.20**	0.28	0.28	0.29	0.21	0.35	0.19	0.38	0.36	0.26	0.34	0.28	0.35	0.31	0.34	0.39	0.37	0.29
palustris											**0.05**	0.09	0.31	0.30	0.32	0.27	0.32	0.30	0.27	0.31	0.26	0.30	0.26	0.28	0.45	0.43	0.30
praecox												.[c]	0.31	0.30	0.32	0.27	0.32	0.28	0.26	0.33	0.27	0.30	0.28	0.29	0.45	0.44	0.30
simpsonii													.[c]	0.31	0.36	0.29	0.35	0.33	0.30	0.23	0.25	0.30	0.24	0.33	0.26	0.22	0.30
stenosperma														**0.09**	0.33	0.20	0.37	0.36	0.28	0.34	0.28	0.37	0.33	0.33	0.41	0.39	0.30
trinitensis															.[c]	0.36	0.41	0.35	0.31	0.33	0.31	0.31	0.31	0.31	0.45	0.43	0.34
villosa																**0.10**	0.36	0.35	0.26	0.33	0.27	0.35	0.30	0.31	0.38	0.36	0.28
batizocoi																	**0.22**	0.21	0.35	0.34	0.35	0.33	0.30	0.28	0.46	0.45	0.36
cruziana																		.[c]	0.32	0.31	0.32	0.30	0.27	0.30	0.44	0.43	0.34
hoehnei																			**0.11**	0.34	0.29	0.34	0.29	0.32	0.43	0.42	0.31
ipaensis																				.[c]	0.27	0.24	0.16	0.29	0.22	0.21	0.30
magna																					**0.31**	0.28	0.23	0.31	0.33	0.31	0.29
valida																						**0.08**	0.22	0.28	0.39	0.38	0.33
williamsii																							.[c]	0.26	0.30	0.28	0.29
glandulifera																								**0.07**	0.42	0.41	0.33
hypogaea																									**0.01**	0.05	0.38
monticola																										**0.02**	0.36

[c] = Species for which only one accession was analized

Table 4. Genetic distances between the tetraploid *Arachis* sect. *Arachis* species and their most closely related accessions.

			to monticola [a]		***to hypogaea*** [b]		to both species[c]	
accession	species	genome	distance[d]	rank[e]	distance[d]	rank[e]	distance[d]	rank[e]
30067	*duranensis*	A	0.2659	4	0.2761	4	0.2710	4
30069	*duranensis*	A	0.2669	5	0.2891	5	0.2780	5
30072	*duranensis*	A	0.2964	8	0.3205	9	0.3085	8
30074	*duranensis*	A	0.2976	9	0.3303	11	0.3139	11
36002	*duranensis*	A	0.2701	6	0.2916	6	0.2809	6
30029	*helodes*	A	0.0899	1	0.0908	1	0.0903	1
30092	*magna*	A	0.3030	11	0.3193	8	0.3111	9
36009	*simpsonii*	A	0.2237	3	0.2609	3	0.2423	3
30076	*ipaensis*	B	0.2074	2	0.2228	2	0.2151	2
1118	*williamsii*	B	0.2850	7	0.2975	7	0.2912	7

[a] includes accessions 7264, 21768, 21769, 30062, 30063

[b] includes accessions PI339954, NC4, PI501296, PI339960, NMValC, PI261924, Grif12518, PI497615, PI590455, A1

[c] includes all accesions in [a] and [b]

[d] calculated as the average distance between each diploid accession and the tetraploid accessions as a group

[e] rank over all 108 accessions analyzed

II. IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH TOMATO SPOTTED WILT VIRUS (TSWV) RESISTANCE IN A GENETIC LINKAGE MAP OF *ARACHIS KUHLMANNII* x *ARACHIS DIOGOI*

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ABSTRACT

Arachis diogoi Hoehne is a wild relative of peanut (*A. hypogaea* L.) of special interest to breeders because accession GKP 10602 of this species possesses resistance to several diseases of peanut, including tomato spotted wilt virus (TSWV). In an attempt to associate markers with TSWV resistance, a genetic linkage map was constructed using an F_2 population of *A. kuhlmannii* Krapov. and W. C. Gregory x *A. diogoi.* A total of 13 *Eco*RI/*Mse*I primer combinations were used to screen 179 individuals. The map consisted of 102 AFLP markers grouped into 12 linkage groups and spanning 1068.1 cM. Markers were randomly distributed throughout the genome with an average distance between adjacent markers of 13.7 cM. The map allowed us to scan the *Arachis* genome for associations between response to TSWV infection and the AFLP markers. Five markers, all located in the same linkage group (LG V) were closely associated ($0.0009 < P < 0.0021$) with TSWV resistance. Another 10 markers were also associated with resistance although at a lower significance level ($P \leq 0.05$). All these markers will be studied for utilization in peanut breeding with marker-assisted selection.

Keywords: AFLP, mapping, TSWV resistance, *Arachis diogoi*, peanut.

INTRODUCTION

Tomato spotted wilt virus (TSWV) is the causal agent of spotted wilt of peanuts (*Arachis hypogaea* L.). The disease has been progressively increasing in severity since the mid 1980s and is currently a major limiting factor to peanut production in the U.S. TSWV infection has been shown to dramatically reduce seed weight and yield (Culbreath *et al*. 1992). In cases of severe epidemics, yield reductions of as high as 95% have been observed (Black 1987, Black *et al*. 1986). Pod and seed production are affected by the growth stage in which the plant becomes systemically infected. Infection early during the growing season often results in severe stunting, wilting and seedling death (Culbreath *et al*. 1992). On the other hand, infection later in the season usually results in poor quality seeds that must be crushed for oil because they are not suitable for the edible market.

TSWV is vectored in nature only by thrips, of which tobacco thrips (*Frankliniella fusca* Hinds) and western flower thrips (*F. occidentalis* Pergande) are the primary vectors in peanuts in the U.S. Tobacco thrips are mostly responsible for transmission early in the season, while western flower thrips are the primary vector late in the growing season (Reed and Sukamto 1995). TSWV has the ability to replicate within the vector, allowing it to be transmitted for long periods of time (Ullman *et al*. 1993). Therefore, adult viruliferous thrips can infect large numbers of plants during their lifespan.

Although TSWV is vectored only by thrips, chemical control of thrips usually has not resulted in a reduction of spotted wilt incidence (Todd *et al*. 1994). There are few effective cultural and chemical practices for management of the disease (Culbreath *et al.* 1994). Although several factors have been shown to provide some suppression, no single

measure by itself has been effective under heavy disease pressure. Cultivar selection is the most important component for reducing the risk of spotted wilt (Hurt *et al.* 2003). Therefore, breeding for resistant cultivars appears to have the most potential for minimizing the risk of losses to spotted wilt (Culbreath *et al.* 1999, 2000). Several cultivars with field resistance to TSWV have been released; however, none possess true resistance to the virus *per se* (Hoffman *et al*. 1998). As opposed to resistance in *A. hypogaea*, high levels of resistance to TSWV have been identified in several of the wild diploid *Arachis* relatives of peanut in artificial inoculations (Lyerly *et al*. 2002). These recently identified sources of resistance can ultimately be used for the incorporation of resistance genes into improved peanut cultivars. Accession GKP 10602 of *A. diogoi* Hoehne has high levels of resistance to different isolates of TSWV and represents a possible donor of TSWV resistance for cultivar imrpovement (Lyerly *et al.* 2002). The utilization of the genetic variation present in *A. diogoi* for applied peanut breeding is feasible due to its cross compatibility with peanut (Smartt and Gregory 1967).

Genetic linkage maps may be useful tools for localizing genes controlling both simple and complex traits. When the map position of a gene is known, the presence of that gene can be determined by nearby molecular markers rather than waiting for gene expression (Paterson *et al.* 1991a). Marker-assisted selection (MAS), is especially effective where markers co-segregate with the trait(s) of interest and can be used to introgress a specific gene(s) into a desired genetic background. Moreover, markers can increase the probability of obtaining a suitable recovery of the recurrent parent genome and decrease the time required to achieve that recovery (Openshaw *et al*. 1994). A persistent problem in plant breeding is the linkage of desirable traits to undesirable genes.

Backcrossing results in the transfer of not only the gene(s) of interest, but also additional linked genes (a phenomenon known as linkage drag) (Tanksley *et al*. 1989). Furthermore, information on linkage maps allows the identification of recurrent parent and donor parent markers. Selection for the former and against the latter allows for a more efficient recovery of the portion of the recurrent parent genome that is not linked to the gene(s) of interest.

Biotechnological developments have expanded the range of plant DNA polymorphism assays for linkage mapping, gene targeting, and assisted breeding (see Powell *et al*. 1996). These techniques include randomly amplified polymorphic DNA (RAPD) (Williams *et al*. 1990), simple sequence repeats (SSR or microsatellites) (Tautz 1989), and amplified fragment length polymorphisms (AFLP) (Vos *et al*. 1995). The last is especially advantageous because it has the capacity to inspect a greater number of loci than other PCR-based techniques. Moreover, the AFLP technique does not require prior knowledge of sequence information, which makes its application relatively easy. Genetic linkage maps based on AFLP markers have been constructed for several species, for example *Allium* (van Heusden *et al*. 2000), peach (Lu *et al*. 1998), lettuce (Jeuken *et al*. 2001), and alfalfa (Barcaccia *et al*. 1999). The objectives of the present study were to (1) construct a linkage map based on AFLP markers in an F_2 population of *A. kuhlmannii* x *A. diogoi,* and (2) identify markers associated with resistance to TSWV.

MATERIALS AND METHODS

Plant Material and DNA Extraction

Arachis diogoi accession GKP 10602 (PI 276235) was used as the pollen parent in crosses with *A. kuhlmannii* accession VRGeSv 7639 (Grif 7571) (Lyerly, 2000). Accession 7639 was chosen as the maternal parent due to its high susceptibility to the TSWV virus. Hybrids were made in a greenhouse during 1999 and an F_1 plant (coded 1C) from this cross was propagated vegetatively in order to obtain a sufficient number of F_2 seed to analyze in this study. For this purpose, twenty cuttings were taken from the plant, dipped in rooting hormone (Rootone, Dragon Corp., Roanoke, VA), inserted into flats filled with sand, and placed under a mist system for 4 to 6 weeks to develop roots. The cuttings were then transplanted into 10 plant space-isolated plots at the Sandhills Research Station at Jackson Springs, NC in the summer of 2002 and seeds harvested by hand-sifting soil the following fall.

A total of 200 seeds of the *A. kuhlmannii* x *A. diogoi* F_2 population were planted during the winter of 2002-03, of which 179 germinated. Plants were grown in the greenhouse in plastic pots containing potting mix Metro Mix (Scuffs-Sierra Horticultural Co., Marysville, OH). Approximately four weeks after planting, two or three young unopened leaves were collected from each plant and used for DNA extraction using the CTAB method of Afanador *et al.* (1993) with the modification that a Fast Prep FP120 (Thermo Savant, Holbrook, NY) machine was used to grind the tissue. DNA was quantified by fluorometry using a Hoefer fluorometer (Hoefer Scientific Instruments, San Francisco, CA).

TSWV Inoculations

The virus isolate (TSWV 10) used in this study was obtained from J. Moyer, Dept. of Plant Pathology, North Carolina State University. The isolate was originally collected from naturally infected peanut plants during 1991 by staff in the Plant Pathology Department. *Emilia sonchifolia* L. was used for maintenance of the isolate and *Nicotiana benthamiana* Domin. Was used to increase the virus load prior to plant inoculations. All inoculations were carried out in ice-cold Tris buffer (0.01M Tris with 0.01M Na_2SO_3 and 0.01% cysteine HCl added immediately before use). Grinding materials and buffer were kept on ice before and during inoculation. Approximately 3-5 g of symptomatic tissue was collected from infected plants (*E. sonchifolia* plants for *N. benthamiana* inoculation, and *N. benthamiana* plants for peanut inoculation) and ground in inoculation buffer with a mortar and pestle. After grinding the tissue, silicon carbide (carborundrum, 600-800 mesh) was added to the suspension to facilitate wounding in order to provide an entryway for the virus into the plant tissue. The inoculum was then rubbed on two leaves of each plant with a cotton swab. Plants were subsequently rinsed with water. Six plants of *A. diogoi* and *A. kuhlmannii* were included in each set of inoculations as resistant and susceptible checks, respectively. Inoculated plants were monitored for 4-6 wk for symptom development. Plants were classified as having systemic infection (1) or no systemic infection (0).

AFLP Analysis

AFLP fingerprinting was performed as described by Myburg and Remington (2000). All primers and adaptors were obtained from Sigma Genosys (The Woodlands,

TX) with the exception of the labeled primers, which were obtained from LI-COR Inc. (Lincoln, NE).

Digestion of DNA. Approximately 500 ng of DNA was simultaneously digested with *Eco*RI and *Mse*I at 37°C for 1.5 hours using 12U *Eco*RI, 8U *Mse*I, and 6 µl of 5X restriction-ligation buffer (50 mM Tris-acetate, 50 mM magnesium acetate, 250 mM potassium acetate, 25 mM DTT, and 250 ng/µl BSA) in a final volume of 30 µl. DNA was run on a 0.8% agarose gel to check for complete digestion.

Adaptor Ligation. Adaptor ligation was performed by adding 5 pmol *Eco*RI adaptor, 50 pmol *Mse*I adaptor, 10 mM ATP, 0.5U of T4 DNA ligase, and 1 µl 5X restriction-ligation buffer to 20 µl of each double-digested DNA sample (25 µl final volume) and incubating overnight at 37°C.

Pre-amplification. A pre-amplification step was performed with primers complementary to the adaptor sequences carrying an additional selective nucleotide. A 1:10 dilution of the digested and adapter-ligated DNA was used as a template for this step. PCR reactions were carried out in a total volume of 20 µl containing: 2 µl of 10X PCR buffer (100 mM Tris-HCl pH 8.3, 15 mM $MgCl_2$, 500 mM KCl), 2.5 mM of each dNTP, 30 ng primer E01-A, 30 ng primer M02-C, 1.2 U *Taq* DNA polymerase (Qiagen, Valencia, CA), and 5 µl of DNA. PCR amplifications were carried out in a PTC-100 programmable thermal controller (MJ Research Inc., Reno, NV) using the following temperature profile: 28 cycles of 15 s at 94°C, 30 s at 60°C, and 60 s + 1 s/cycle of extension at 72°C; followed by one cycle of 2 m at 72°C. Upon completion of amplification, 15 µl of each sample were diluted 1:20 with low TE (10 mM Tris-HCl pH

8.0 and 0.1 mM EDTA). The remaining 5 µl of each sample were checked on a 0.8% agarose gel where a smear was visible.

Selective Amplification and PAGE. For the selective amplification primers with three selective nucleotides were used (Table 1). EcoRI primers were labeled with a fluorescent near-infrared group (IRD-700 or IRD-800). The PCR amplification mixture (20 µl final volume) was comprised of: 2 µl of 10X PCR buffer, 2.5 mM of each dNTP, 30 ng unlabeled *Mse*I+3 primer, 5 ng labeled EcoRI+3 primer, 1.2 U *Taq* DNA polymerase, and 5 µl of diluted pre-amplification product. Selective amplification was carried out in a PTC-100 programmable thermal controller using the following temperature profile: 13 cycles of 10 s at 94°C, 30 s at 65°C – 0.7°C/cycle after the first cycle, and 60 s at 72°C; followed by 25 cycles of 10 s at 94°C, 30 s at 56°C, and 60 s + 1 s/cycle of extension at 72°C; followed by one cycle of 2 m at 72°C. After amplification, samples were denatured by adding 10 µl of loading dye (95% deionized formamide, 20 mM EDTA, and 0.8 mg/ml bromophenol blue), heating at 94°C for 3 m, and chilling on ice. AFLP fragments were separated by PAGE on a LI-COR 4200 DNA Analyzer Sequencer on 25 cm 8% denaturing polyacrylamide gels [7 M ultra pure Urea, 0.8X TBE, and 8% Long Ranger acrylamide (BioWhittaker Molecular Applications, Rockland, ME)]. Near-infrared labeled size standards (LI-COR Inc., Lincoln, NE) were loaded on each gel for sizing of the AFLP fragments.

Scoring of Data and Marker Nomenclature. Primer combinations were selected on the basis of the level of polymorphism and the quality of amplification when analyzing the two parents and a set of 30 F_2 individuals from the population developed by

Lyerly (2000) (data not shown). All the AFLP markers were scored as either presence (1) or absence (0) of clear and unambiguous polymorphic bands, using the AFLP-Quantar 1.0 (Keygene Products B.V., Wageningen, The Netherlands) software package. The AFLP marker name was designated according to the primers used to amplify the DNA: E followed by two numbers refers to the *Eco*RI primer and M followed by two numbers refers to the *Mse*I primer, e.g., E38M59. Scored markers were numbered in descending order of molecular weight; therefore, the last one or two numbers of the marker code refer to the fragment position in the gel.

Data Analysis

Linkage analysis and ordering of the AFLP loci were done using MAPMAKER 3.0/EPX (Lander *et al.* 1987). Initially, a minimum LOD score of 3.0 and a maximum recombination frequency of 0.25 were established as thresholds for grouping markers. Subsequently, marker loci within each linkage group were ordered using the 'Compare' and 'Try' functions. The 'Ripple' function was then used to confirm the final order of the marker loci. Recombination fractions were converted to map distances in cM using the Kosambi mapping function (Kosambi 1944). Chi square goodness of fit tests were used to compare single locus segregation against the expected 3:1 ratio. Additionally, tests of independence were performed in order to evaluate the null hypothesis that there were no significant differences in TSWV infection (S, R) between the two genotypes (0, 1) at each marker locus.

RESULTS

Analysis of AFLP Markers

A total of 13 primer combinations were used to analyze 179 F_2 individuals of the *A. kuhlmannii* x *A. diogoi* hybrid. The number of bands generated per primer combination ranged from 34 to 122 with an average of 79 (Table 1). The number of polymorphic bands ranged from 26 to 96, which resulted in an average degree of polymorphism of 74%. Although many detectable polymorphic products were observed, many of these showed overlapping banding patterns and were difficult to score. Therefore, only 179 of the fragments were scored in the progeny and included in the analyses.

Segregation Distortion

Chi-square tests (d.f. = 1) were performed to test the null hypothesis of a 3:1 segregation of the markers. At the 1% significance level, 84 (47%) AFLP markers had aberrant segregation ratios. These markers were excluded from linkage analysis with the exception of seven markers, which were significantly associated with TSWV resistance. When the significance level was lowered to 5%, 16 additional markers showed segregation distortion. These markers were retained for linkage analysis. Most of the AFLP markers showing significant deviations ($0.01 < P < 0.05$) were skewed towards the *A. kuhlmannii* parent. Loci with distorted segregation patterns were generally scattered among linkage groups. However, 25% of markers with significant segregation distortion remained unassigned to any linkage group.

Linkage Analysis and Map Coverage

A total of 102 AFLP markers were used to construct a genetic linkage map using MAPMAKER (V3.0) software of which 80 were placed in 12 linkage groups using a LOD score of 3.0 and a maximum recombination value of 0.25 (Fig. 1). These markers covered 1068.1 cM of the *Arachis* genome, with an average interval of 13.7 cM between adjacent markers (Table 2). Two of the linkage groups were classified as 'minor' since they contained only two markers each. The other linkage groups contained from 4 to 15 markers and ranged in distance from 49.9 to 292.7 cM. The number of markers within each linkage group should be directly correlated with the length of the group if the markers are randomly distributed throughout the genome (Foisset *et al.* 1996). The correlation between the size of the linkage groups and their number of loci for our data was extremely high (r = 0.94), which is in agreement with the previous statement. Twenty-two markers remained unassigned to any linkage group. Unlinked markers are either artifacts segregating in Mendelian ratios by chance, or they represent regions with very few markers (Cervera *et al.* 2001).

The structure of the linkage groups was confirmed by running MAPMAKER only with markers showing Mendelian segregation. The assignment of the Mendelian markers was identical to that obtained when loci with biased segregation were included in the analysis. The order of the Mendelian markers remained relatively unchanged.

TSWV Evaluations

Based on our observations, levels of resistance varied across plants of *A. diogoi* accession 10602. Symptoms in this accession included slightly wrinkled or misshapen

leaves and the appearance of tiny brown spots. However, compared with cultivated varieties of peanut, *A. diogoi* exhibited greatly reduced symptoms. Out of the 179 progeny inoculated with the virus, 68 did not develop any symptoms. On the other hand, 108 F_2 plants showed symptoms ranging from the characteristic chlorotic spots or ringspots to stunting of the emerging leaves, wrinkled leaves, and defoliation.

Genomic segments exhibiting a significant difference ($P \leq 0.05$) in TSWV inoculation response between alternate marker classes were identified in five different linkage groups (14 total) (Fig. 1). The most significant effect was on LG V. Interestingly, all five markers showing the strongest association with TSWV resistance ($P = 0.01$) fell into this group within a distance of 62.7 cM. All of these markers, with the exception of E32M61-5, originated from *A. diogoi*.

DISCUSSION

Using only 13 primer combinations, we obtained 1023 selectively amplified fragments. Of those, 61-86% were polymorphic between the two parents. One of the most important advantages of the AFLP technique is the high number of loci that can be screened per experiment. Many more polymorphic DNA markers can be found with this technique than with any of the other PCR-based marker systems (Vos *et al.* 1995; Zabeau and Vos 1993). AFLPs have a clear advantage over RAPDs and microsatellite markers in terms of the number of sequences amplified per reaction and their reproducibility.

The genetic linkage map of *A. kuhlmannii* x *A. diogoi* comprises 102 markers. There are six large linkage groups of at least 60 cM, six smaller linkage groups ranging from 18.6 to 56.5 cM, and 22 unlinked markers. Two of the linkage groups (LG VIII and LG XI) are quite small, containing only two markers each. These groups may eventually come together with one of the larger linkage groups as additional markers are added to the map. Once the map is completed, it should consist of 10 linkage groups corresponding to the haploid chromosome number of diploid *Arachis* species.

A high rate of segregation distortion, affecting 48% of the loci, was found among the AFLP markers in this study. The frequency of distorted markers is much larger than that observed in other interspecific F_2 populations in *Arachis* (Halward *et al.* 1993). Hybrid disgenesis and segregation distortion have been observed in mapping experiments involving interspecific hybrids in other genera (Korol *et al.* 1994; Riesberg and Linder 1999). For instance, skewed segregation has been reported in many interspecific crosses of tomato, with the degree of skewness being greater in wider crosses than in those between closely related species, and also greater in F_2 than in backcross populations (Chen and Foolad 1999; Patterson *et al.* 1991b). There may be several biological explanations for segregation distortion; however, chromosomal non-homologies are the most likely explanation in our case. The relatively low levels of fertility in the hybrids (Lyerly 2000) and the occurrence of plants with aberrant morphology, such as severe stunting and deformed leaves, provide some evidence to support this hypothesis. The problem of including markers with segregation distortion is that they increase the chance of Type I errors of false linkage. Moreover, estimates of map distance for distorted markers may be inaccurate (Cloutier *et al.* 1997). Although we included only markers

that deviated at the 5% and not at the 1% level for linkage analysis, it is important to note that distances displayed in the map may not be completely accurate.

The observed segregation ratio of resistant to susceptible plants for our F_2 population was skewed toward the *A. kuhlmannii* parent. Moreover, it did not fit the expected ratio for the two major resistance genes conditioning resistance as proposed by Lyerly (2000). These observations suggest the involvement of minor genes in the control of TSWV resistance in *A. diogoi* accession 10602. Significant associations between genetic markers and TSWV resistance were detected on five of the twelve linkage groups (LG V, LG VII, LG VIII, LG IX, and LG X). Given the threshold significance of some of the associations, it is likely that a few of these could have occurred simply by chance. However, it is worth noting that LG V not only has more than half of the markers associated with TSWV resistance, but also includes all five markers exhibiting the strongest association ($0.0009 < P < 0.0021$). This linkage group has the most significant effect on TSWV resistance, and is therefore, a likely hot spot for the detection of genes conditioning TSWV resistance. These markers will aid in transferring the TSWV resistance present in *A. diogoi* accession 10602 into cultivated peanut.

The genetic linkage map produced in this study constitutes a basic framework for adding other markers and for tagging major genes and QTLs controlling traits of interest in *Arachis*. Although the map was constructed using a population derived from the cross between two wild *Arachis* species, it should be also a useful tool for applied peanut breeding programs. For genera in which a species was domesticated, the largest amount of genetic variation exists not between cultivated types but among their wild counterparts. However, breeders have been somewhat hesitant to use wild species

because in the transfer of traits from wild species into cultivars desirable genes are often linked to undesirable, deleterious ones, a phenomenon referred to as linkage drag (Tanksley *et al.* 1989). Even after several generations of backcrossing followed by selection, the genes of interest can remain linked to DNA segments large enough to carry hundreds of undesirable genes (Young and Tanksley 1989). Molecular linkage maps have provided a method to monitor and facilitate interspecific gene transfer while reducing linkage drag (Tanksley *et al.* 1989; de Vicente and Tanksley 1993). In the case of peanut where diploid x tetraploid hybrids are sterile and ploidy levels need to be manipulated to restore fertility and recover tetraploid progenies, markers will be highly useful to insure that target genes are incorporated into the *A. hypogaea* genome. The map produced in this study will serve as the basis for future work to develop a saturated linkage map of peanut. The development of a complete linkage map in *Arachis* would greatly assist breeders to tag and follow the introgression of specific chromosome segments carrying desirable genes from wild species into breeding lines of peanut.

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Figure 1. Genetic linkage map based on 179 individuals from an F_2 population of the interspecific cross *Arachis kuhlmannii* x *Arachis diogoi*. Linkage groups were produced by MAPMAKER (V3.0) software with a minimum LOD score of 3.0 and a maximum recombination frequency of 0.25. AFLP markers are indicated on the right and genetic distance estimates in centimorgans are indicated on the left. The extensions * and ** represent markers associated with TSWV resistance at P = 0.05 and P = 0.01, respectively.

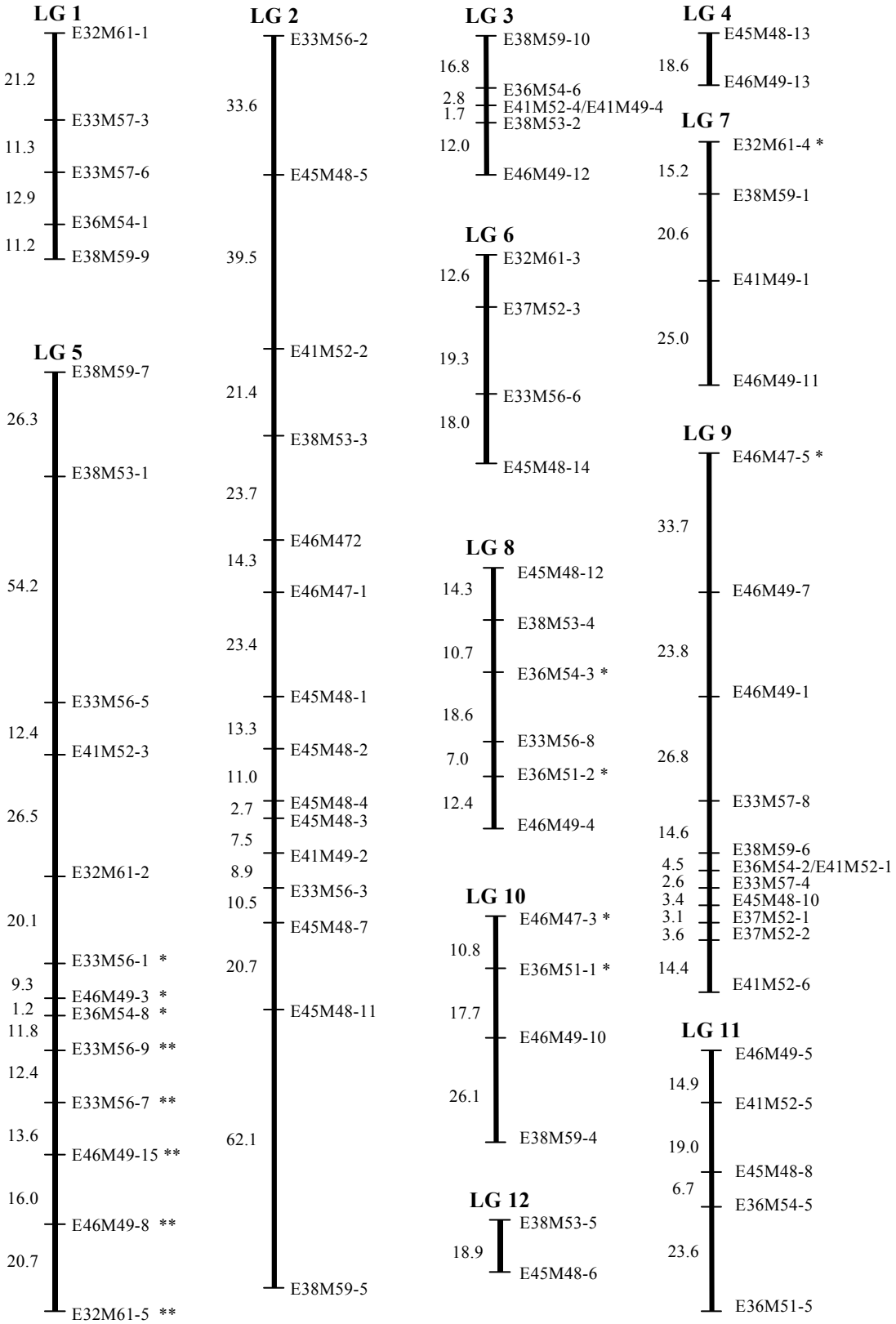
LG 1
E32M61-1
21.2
E33M57-3
11.3
E33M57-6
12.9
E36M54-1
11.2
E38M59-9
LG 2
E33M56-2
33.6
E45M48-5
39.5
E41M52-2
21.4
E38M53-3
23.7
E46M472
14.3
E46M47-1
23.4
E45M48-1
13.3
E45M48-2
11.0
E45M48-4
2.7
E45M48-3
7.5
E41M49-2
8.9
E33M56-3
10.5
E45M48-7
20.7
E45M48-11
62.1
E38M59-5
LG 3
E38M59-10
16.8
E36M54-6
2.8
E41M52-4/E41M49-4
1.7
E38M53-2
12.0
E46M49-12
LG 4
E45M48-13
18.6
E46M49-13
LG 5
E38M59-7
26.3
E38M53-1
54.2
E33M56-5
12.4
E41M52-3
26.5
E32M61-2
20.1
E33M56-1 *
9.3
E46M49-3 *
1.2
E36M54-8 *
11.8
E33M56-9 **
12.4
E33M56-7 **
13.6
E46M49-15 **
16.0
E46M49-8 **
20.7
E32M61-5 **
LG 6
E32M61-3
12.6
E37M52-3
19.3
E33M56-6
18.0
E45M48-14
LG 7
E32M61-4 *
15.2
E38M59-1
20.6
E41M49-1
25.0
E46M49-11
LG 8
E45M48-12
14.3
E38M53-4
10.7
E36M54-3 *
18.6
E33M56-8
7.0
E36M51-2 *
12.4
E46M49-4
LG 9
E46M47-5 *
33.7
E46M49-7
23.8
E46M49-1
26.8
E33M57-8
14.6
E38M59-6
4.5
E36M54-2/E41M52-1
2.6
E33M57-4
3.4
E45M48-10
3.1
E37M52-1
3.6
E37M52-2
14.4
E41M52-6
LG 10
E46M47-3 *
10.8
E36M51-1 *
17.7
E46M49-10
26.1
E38M59-4
LG 11
E46M49-5
14.9
E41M52-5
19.0
E45M48-8
6.7
E36M54-5
23.6
E36M51-5
LG 12
E38M53-5
18.9
E45M48-6

Table 1. Primer combinations used and polymorphism rates for AFLP analysis of 179 F_2 individuals from the cross *Arachis kuhlmannii* x *A. diogoi.*

Primer combination	TB[a]	PB[b]	PPB[c]	SB[d]	SRSB[e]
E-AAC/M-CTG	98	60	61	19	92-521
E-AAG/M-CGC	78	60	77	34	63-539
E-AAG/M-CGG	53	44	83	29	72-512
E-ACC/M-CCA	83	57	69	44	58-476
E-ACC/M-CCT	74	55	74	25	69-532
E-ACG/M-CCC	34	26	76	20	60-500
E-ACT/M-CCG	41	34	83	11	96-379
E-ACT/M-CTA	88	58	66	28	106-480
E-AGG/M-CAG	64	45	70	18	44-502
E-AGG/M-CCC	64	55	86	16	67-322
E-ATG/M-CAC	117	86	74	28	60-517
E-ATT/M-CAA	122	96	79	20	218-597
E-ATT/M-CAG	107	76	71	30	109-421
TOTAL	1023	752	74	322	44-597

[a] TB: total number of bands

[b] PB: number of polymorphic bands

[c] PPB: percentage of polymorphic bands

[d] SB: number of scored bands

[e] SRSB: size range of scored bands

Table 2. Distribution of genetic markers along the twelve linkage groups of the *Arachis kuhlmannii* x *A. diogoi* map.

Linkage group	NL[a]	Size[b]	AI[c]	correl[d]
I	5	56.5	11.3	
II	15	292.7	19.5	
III	6	33.4	5.6	
IV	2	18.6	-	
V	13	224.5	17.3	
VI	4	49.9	12.5	
VII	4	60.8	15.2	
VIII	6	62.9	10.5	
IX	12	131.2	10.9	
X	4	54.6	13.7	
XI	5	64.1	12.8	
XII	2	18.9	-	
TOTAL	78	1068.1	13.7	0.94

[a] NL = number of loci

[b] Size = length in cM

[c] AI = average interval between adjacent markers

[d] Correlation between number of loci in the group and length of the group

III. BEST LINEAR UNBIASED PREDICTION OF BREEDING VALUE FOR TOMATO SPOTTED WILT VIRUS (TSWV) INCIDENCE IN VIRGINIA-TYPE PEANUTS

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ABSTRACT

Spotted wilt, caused by the tomato spotted wilt virus (TSWV) has progressively become more prevalent in the Virginia-Carolina peanut (*Arachis hypogaea* L.) production area. Management tactics for control of spotted wilt are limited. Development of cultivars with moderate to high levels of field resistance to TSWV is the most promising means of managing the disease. Breeding efficiency can be maximized by choosing parents based on their potential to produce superior progeny. Best linear unbiased prediction (BLUP) is a method for estimating the breeding value of a parent based on its own performance as well as that of its relatives. BLUP was used to identify lines with superior ability to transmit TSWV field resistance to their progeny. The data set used included 118 breeding lines, 12 cultivars and one *hirsuta*-type (*A. hypogaea* subsp. *hypogaea* var. *hirsuta* Köhler) accession. Data on TSWV incidence were obtained from trials representing three locations and six years and on agronomic traits from trials at three locations and 13 years. Because only estimates of broad-sense heritability (H) were available, BLUPs were computed using a range of estimates for narrow sense heritability (h^2). BLUPs obtained with different estimates of h^2 were highly correlated ($r > 0.85$), indicating that BLUPs are not critically affected by inaccurate estimates of h^2. Breeding values predicted by BLUP were moderately correlated ($0.54 < r < 0.83$) with line means estimated from a fixed-effect model. Specific lines with high breeding values for TSWV resistance included a set of lines resistant to early leafspot (*Cercospora arachidicola* Hori) and the *hirsuta* accession, PI 576636. BLUPs for yield, meat content, crop value, and pod brightness were also calculated. Six different weighting schemes

used for index selection in order to pick lines with superior breeding values for a combination of all traits analyzed. Thirteen lines were selected with at least four of the six weighting schemes, suggesting that these lines should be able to transmit to their progenies not only reduced TSWV incidence, but also increased yields and improved quality traits.

Keywords: *Arachis hypogaea* L., BLUP, breeding value, TSWV incidence.

INTRODUCTION

Spotted wilt of peanut, caused by the tomato spotted wilt virus (TSWV), is currently one of the major limiting factors in peanut production in the U.S. In the Virginia-Carolina growing region, spotted wilt has been gradually increasing in severity since the mid 1990s. Incidence and damage in peanuts was the highest in both states during 2002 (Hurt *et al.*, 2003).

Symptoms of spotted wilt in peanut vary from severe stunting to elaborate concentric ring spots on individual leaflets, and even plant death. The first symptoms of the virus usually appear a few weeks after planting, and newly symptomatic plants emerge thereafter for the remainder of the growing season. The growth stage at which the plant is infected determines the degree of yield reduction (Culbreath *et al.*, 1992). Plants that are infected early in the season are the most affected, showing severe stunting and producing very few or no seed. However, reductions in both quantity and quality of pods and seed are also observed in plants infected at later growth stages (Culbreath *et al.*, 1992).

TSWV is vectored in nature by several species of thrips (Thysanoptera). The virus is acquired by immature thrips feeding on infected host plants and then transmission occurs primarily through feeding activities of adults. TSWV has the ability to replicate within the vector, allowing it to transmit the virus for long periods of time. Therefore, viruliferous adult thrips are capable of infecting many plants (Ullman *et al.*, 1993). Even though TSWV is vectored only by thrips, control of thrips with insecticide applications has proved ineffective in reducing the incidence of spotted wilt (Todd *et al.*, 1994). There

are few effective cultural and chemical practices for management of the disease (Culbreath *et al.*, 1994). Although several factors have been shown to provide some suppression, no single measure by itself has been effective under heavy disease pressure. From all known factors that can be manipulated to reduce the risk of spotted wilt – including peanut cultivar, planting date, plant population, in-furrow insecticide application, and tillage practices, cultivar selection appears to have the most potential for minimizing the risk of losses to spotted wilt (Culbreath *et al.*, 1999, 2000; Hurt *et al.*, 2003). None of the virginia cultivars released to date have a high level of field resistance and they may suffer significant damage under extremely intense epidemics. Cultivars with higher levels of resistance would be of great benefit across the Virginia-Carolina growing region. Moreover, cultivars are needed that combine TSWV resistance with good yield and quality.

Parent selection for the development of populations with high expected mean performance and which have genetic variation for desirable traits has been a problem historically faced by plant breeders. Identification of parental combinations that meet those two criteria increases the probability of recovering superior genotypes for cultivar development. The conventional method of selecting parents is based on their own performance. Observed performances are then used to calculate midparent values (MPV), or the mean of the parental means, to predict cross combination means. This method of parental selection poses some obvious disadvantages such as performance estimate biases when not all genotypes are evaluated or when data is missing in some environments (Panter and Allen, 1995b). Moreover, the efficiency of phenotypic selection in discriminating among superior individuals is reduced as the heritability decreases, and

becomes very inefficient for traits with low heritability values (Falconer, 1989). Furthermore, performance testing of new genetic material is one of the most important and also most expensive aspects of plant breeding programs. Selecting superior lines is usually accomplished by testing a large group of lines across several locations and years. Statistical methods that maximize the accuracy of the estimate of performance of a line from fewer environments would be extremely useful for plant breeders (Panter and Allen, 1995b).

Henderson (1975) described the use of a mixed model to calculate the best linear unbiased predictions (BLUP) of breeding values of potential parents based on observed data and the known variance-covariance structure among fixed and random effects. Genetic effects are considered to be random in the model while environmental effects are considered to be fixed. Henderson's method uses the genetic relationships among individuals as the variance-covariance among genetic effects, and assumes that correlation between data on different individuals is caused only by additive genetic variance (Henderson, 1975). By using genetic relationships among individuals, related individuals contribute to the predicted values for one another. Information from relatives can contribute to the predicted breeding value for an individual for which there is little or no data. Moreover, the magnitude of that contribution is dependent on the extent of the relationship between the two individuals (Panter and Allen, 1995a).

The BLUP procedure could be widely applicable in crop breeding programs because no additional experiments are required for obtaining the predictions. Instead, they are made from data that is routinely generated in a breeder's testing program, including performance data and estimates of genetic relationship among lines (Bernardo,

1996b). Best linear unbiased prediction has been widely used in livestock breeding (Henderson, 1975) and to a lesser degree, in forest tree breeding (White and Hodge, 1988). Among crop species, BLUPs have been useful to estimate breeding values to identify superior cross combinations in maize (Bernardo, 1994, 1995, 1996a, b, c), soybean (Panter and Allen, 1995a, b), peach (de Souza *et al.*, 1998a, b, 2000), sugarcane (Chang and Milligan, 1992), peanut (Pattee *et al*., 2001), and oil palm (Purba *et al.*, 2001). The objective of this study is to investigate the use of BLUP for selection of lines with superior ability to transfer decreased TSWV incidence in combination with five other important agronomic and quality traits in peanut.

MATERIALS AND METHODS

Experimental Materials

The material analyzed included 118 breeding lines from the North Carolina State University peanut breeding program, 12 virginia type cultivars and one var. *hirsuta* (*A. hypogaea* subsp. *hypogaea* var. *hirsute* Köhler) accession (Table 1). Plants were grown and harvested under standard recommended procedures for peanut production in North Carolina. TSWV trials were conducted using wide plant spacing (25-51 cm between seeds) and no insecticide.

Evaluations

Spotted wilt was evaluated using a disease incidence rating where the number of severely stunted, chlorotic, wilted or dead plants was counted for each plot two times during the growing season. That number was then converted to a percentage of the total number of plants per plot. For TSWV incidence, genotypes were evaluated over 18 tests in 7 year-by-location combinations. Not all genotypes were included in all tests, so replication ranged from 1 to 15 tests with a mean of 3.

Data on yield (lbA^{-1}), meat content (% of kernels from 500g of clean unshelled pods), extra large kernels (% of extra large kernels, *i.e*. seeds that ride a 8.4 x 19.0 mm slotted screen, based on the 2002 federal grade sheet for virginia-type peanuts, from 500g of clean unshelled pods), pod brightness (Hunter L scale), and crop value ($\$A^{-1}$) were compiled. These data consisted of the lines' means from each test in which the line occurred. Because some lines had been tested for yield and quality more extensively than others, there was a wide range in the number of records for each line (Table 1). In total, genotypes were evaluated for yield and quality over 84 tests in 30 year-by-location combinations.

Statistical Analysis

The mixed model procedure (PROC MIXED) in SAS (SAS Institute, 2001b) was used for the analysis of the unbalanced data set to calculate means for genotypes adjusted to a common environmental effect. The following additive genetic mixed model was used to predict the additive genetic effect for each individual:

$$Y = \mu + X\beta + Z\alpha + \varepsilon \qquad \text{[Eq. 1]}$$

Where,

Y is a vector of observations,

β is a vector of fixed effects,

α is a vector of additive genetic effects,

ε is a vector of error terms, and

X and *Z* are incidence matrices that associate specific effects with individual observations.

The variance-covariance matrix for the random effects and error terms is

$$Var\left(\begin{bmatrix}\alpha\\ \varepsilon\end{bmatrix}\right)=\begin{bmatrix}G & 0\\ 0 & R\end{bmatrix}\sigma^2 \qquad \text{[Eq. 2]}$$

where $\alpha = G\sigma^2$ is the additive variance–covariance matrix for the lines. G can be calculated as $2Ch^2/(1- h^2)$, where C is the matrix of coancestries among lines and h^2 is the narrow sense heritability of the trait (Pattee *et al*., 2001). Genetic relationships among parents and progeny are expressed in terms of Malécot's (1948) coefficient of coancestry (θ), which is the probability that, at a given locus, two lines have alleles that are identical by descent, *i.e*., mitotic/meiotic copies of the same ancestral allele. Coefficients of coancestry were calculated using pedigree information on the lines obtained from published records and from personal communications with individual breeders records. Rules for calculation of coancestry are well known (Falconer, 1989). Coancestries among lines derived from the same cross were calculated following the modifications described by Cockerham (1983). Lines that could be traced to different F_2 plants were considered to have the same degree of relatedness as full sibs. However, lines tracing to the same F_3 (or

later generation) plant were considered to be more closely related than full sibs. When no information was available on the commonality of two lines derived from the same cross, it was assumed that the lines traced to different F_2 selections. Because peanut is a highly self-pollinated species and most cultivars are highly homozygous inbred lines, it was assumed that each cultivar, line or introduction in the breeding population had an inbreeding coefficient (F) of 1.

The standard BLUP solutions were obtained from the following equation

$$\begin{bmatrix} \hat{\beta} \\ \hat{\alpha} \end{bmatrix} = \begin{bmatrix} X'R^{-1}X & X'R^{-1}Z \\ Z'R^{-1}X & Z'R^{-1}Z + G^{-1} \end{bmatrix}^{-1} \begin{bmatrix} X'R^{-1}Y \\ Z'R^{-1}Y \end{bmatrix} \qquad \text{[Eq. 3]}$$

The interactive matrix language procedure (PROC IML) in SAS (SAS Institute, 2001a) was used to perform all calculations to compute BLUP estimates.

Heritability Estimates

Narrow-sense heritability (h^2) estimates were not available for the overall breeding population for any of the six traits studied. However, it is known from quantitative genetics theory that the broad-sense heritability is the upper boundary for the narrow-sense heritability. Estimates of broad-sense heritability (H) were calculated based on variance estimates obtained by restricted maximum likelihood estimation using PROC MIXED in SAS (SAS Institute, 2001b). BLUPs were calculated for a range of values around our estimates of H to assess the sensitivity of the method to inaccuracy in the estimation of narrow-sense heritability.

Selection Schemes

To select lines with superior breeding values for a combination of traits, independent culling and index selection were used as selection methods. For independent culling, a threshold value was chosen so that only the best 28-43% of the lines would be selected. For index selection, six different weighting schemes based on assigned importance of disease resistance vs. yield vs. agronomic and quality traits were designed (Table 2). Subsequently, BLUPs were scaled as

$$I_{ij} = \frac{\left(\nu_{ij} - \nu_{worst}\right)}{\left(\nu_{best} - \nu_{worst}\right)} \quad \text{[Eq. 4]}$$

and the index was then calculated as the geometric mean of the weighted variables

$$I_i = \left\{\left(I_{i1}\right)^a \left(I_{i2}\right)^b \left(I_{i3}\right)^c \left(I_{i4}\right)^d \left(I_{i5}\right)^e \left(I_{i6}\right)^f\right\}^{\frac{1}{a+b+c+d+e+f}} \quad \text{[Eq. 5]}$$

where *a, b, c, d, e,* and *f* are the weights to be assigned to each trait.

RESULTS AND DISCUSSION

Heritability Estimates and their Effect on BLUP Values

The additive variance-covariance matrix needed for BLUP estimation is based on estimates of narrow-sense heritability (h^2). Only estimates of broad-sense heritability (H) were available for the six traits analyzed in this study. Only the additive variance is accounted for in h^2, while H reflects all genotypic variance. Given that h^2 must be less than or equal to H, BLUPs were calculated for a range of values around our estimates of

H (Table 3). Subsequently, correlations among BLUPs calculated at different values of H were computed in order to investigate the sensitivity of the technique to variation in the heritability estimate. Correlations ranged from high to extremely high depending on the trait ($r = 0.85$ for TSWV incidence, $r > 0.90$ for all other traits). These results suggest that best linear unbiased prediction is relatively insensitive to inaccuracy in the estimation of narrow-sense heritability. Therefore, broad-sense heritability estimates can be used as substitutes without much loss in the estimation precision when estimates of narrow-sense heritability are not available (Pattee *et al*., 2001).

Correlation between BLUP Values and Means

The use of phenotypic values to select parents should be effective in cases where the narrow-sense heritability is high (Falconer, 1989). However, for traits with low narrow-sense heritability values, breeding values (BV) would give a better ranking of the genetic value of the parents than would their genotypic values, and, therefore, selection efficiency would be enhanced (de Souza *et al.*, 2000). In this study, meat content, extra large kernels (ELK), and pod brightness had moderate broad-sense heritabilities of 0.20, 0.42, and 0.18, respectively. The predicted BVs of these three traits were well correlated (0.88, 0.96, and 0.93, respectively) with observed phenotypic values (Table 3). On the other hand, TSWV incidence, yield, and crop value, had very low broad-sense heritabilities (0.05, 0.02, and 0.05, respectively) and showed poor correlations between predicted BVs and observed phenotypic values (0.66, 0.53, and 0.65, respectively). For TSWV incidence, the plot of predicted BVs vs. means supports the low correlation

between these parameters (Fig. 1). Therefore, TSWV seems to be an ideally suited trait for parental selection based on BLUP estimation of BVs.

Variation of BLUP Values

Best linear unbiased prediction was used to predict BVs of parents for TSWV incidence, yield, meat, content, ELK, pod brightness, and crop value (Table 4). The predicted BV ranged from –6.29 to +8.04% for TSWV incidence, -262 to +142 lb A^{-1} for yield, -2.4 to +3.0% for meat content, -20.8 to +10.0% for ELK, -1.4 to +1.6 Hunter L units for pod brightness, and –272 to +166 \$ A^{-1} for crop value (Table 5). Predicted BVs suggest that not only is there genetic potential to develop lines with increased field resistance to spotted wilt, but also that agronomic and quality traits can be improved.

Based on the BLUPs, several lines had superior BVs for TSWV incidence. A group of lines from our leafspot breeding program had negative BVs for TSWV incidence, indicating that progenies from these lines would have reduced incidence of spotted wilt. Of the cultivars included in this study, none had negative BVs for TSWV incidence. Georgia Green and Wilson had the lowest positive values. *Hirsuta* accession PI 576636, a genotype with high field resistance to TSWV that was used as a resistant check in all tests, had the lowest BV for TSWV incidence among all genotypes analyzed. However, BVs for this accession might not be accurate due to its complete lack of genetic relationship to any other line in the data set.

For each agronomic and quality trait, several lines possessed an extremely high BV. However, no line had the best BV for all the traits combined. Predicted BVs for TSWV incidence were plotted against those for yield to select lines that would combine

negative BVs for TSWV incidence and positive BV for yield (Fig. 2). A set of leafspot and TSWV lines was found to possess superior BV for both traits. An important point to highlight is how the BVs for cultivar Gregory for these two traits compare to those of other cultivars. Although its BV for TSWV incidence is slightly inferior to that of resistant cultivar Georgia Green, its BV for yield is considerably higher than that of any other cultivar.

BLUPs of BV for meat content and pod brightness were highly correlated (r = 0.85) (Table 6). Plots of predicted BVs for these two traits against those for TSWV incidence indicate that only two lines, N96076L and N99133CSm, combine desirable BVs for meat content and TSWV incidence (Fig. 3); and only one line, N99027L, combines desirable BVs for pod brightness and TSWV incidence (Fig. 5). For ELK and crop value, several lines combined negative BVs for TSWV incidence and positive BVs for these traits (Figs. 4 and 6).

Independent Culling

To select lines that would combine superior BVs for all traits analyzed, threshold values were selected that would pick the top 28-43% percent of the lines (top 28% for TSWV, top 41% for yield, top 43% for meat content, top 38% for ELK, top 35% for pod brightness, and top 28% for crop value). Subsequently, lines that had been picked for TSWV incidence, yield and at least one of the four other traits were selected. Ten lines were selected including three (N99122CSm, N99132CSm, and N99133CSm) belonging to the Sclerotinia blight-CBR resistance breeding program, two (N99027L, and N00022L) to the early leafspot resistance breeding program, and five (N01009T,

N01010T, N01011T, N01014T, and N01015T) to the TSWV resistance breeding program. These lines should transmit to their progenies not only reduced TSWV incidence, but also increased yields and improved quality traits.

Index selection

Weights of the different index selection schemes were based on assigned importance of disease resistance vs. yield vs. agronomic and quality traits (Table 2). The first scheme considered all traits to be equally important. Schemes II and VI emphasized TSWV and yield. Schemes III and V were reciprocal: III emphasized agronomic and quality traits, while V emphasized disease resistance. Scheme IV gave more importance to yield than to any of the other traits. Lines were ranked based on their index values. Subsequently, lines that had been ranked among the top 18 with at least four of the six weighting schemes were selected. Index values obtained using different weighting schemes were highly correlated ($0.78 < r < 0.97$) with the exception of schemes II and III ($r = 0.68$) and V and III ($r = 0.61$) (Table 7). Rank correlations were also found to be high (Table 7).

Thirteen lines were selected under each of four weighting schemes: N97085, N00033, N00090ol, N00091ol, N00098ol, N00099ol, N99133CSm, N99027L, N01001T, N01011T, N01014T, N01015T and Gregory. Of these, eight were selected under five weighting schemes and only one under all six schemes (Table 8). High oleic lines N00090ol, N00091ol, N00098ol, and N00099ol had excellent BVs for meat content and ELK. Moreover, N00098ol and N00099ol also had extremely high BVs for crop value. Although their BVs for TSWV incidence were positive, they were not large (Table 4).

Likewise, TSWV lines N01001T, N01011T, N01014T, and N01015T had moderate positive BVs for TSWV incidence and good BVs for crop value. Leafspot line N99027L and CBR line N99133CSm had highly desirable BVs for TSWV incidence, but their BVs for agronomic and quality traits were not very high. Therefore, it would be valuable to utilize lines from the first set in crosses with lines from the second one to develop progenies that combine superior values for all traits. In doing so it is important to consider the degree of relationship between the lines to be crossed in order to have enough genetic variability in hybrid populations to allow additional improvement. Coefficients of coancestry among selected lines are presented in Table 8.

Surprisingly, none of the cultivars studied was chosen among the top 18 genotypes with any of the weighting schemes used with the exception of Gregory, which was the only genotype from the 131 analyzed to be selected with all six schemes (Table 8). These results indicate that Gregory would be an excellent choice as a parent for an array of traits. This cultivar has the ability to transfer to its progeny good TSWV incidence, superior yield, and good values for meat content, ELK, pod brightness and crop value.

Application in Breeding Programs

The BLUP approach used in this study is the only procedure proven effective to predict single-cross performance (Bernardo, 1996a, b, c). Therefore, BLUP estimation proves useful in identifying superior single crosses prior to making the actual crosses and evaluating them in disease and/or yield trials. Perhaps the most attractive feature of BLUP estimation is that no special experiments are required to obtain the predictions.

Instead, the predictions are obtained by using data that is routinely generated in a breeder's testing program. Moreover, as more lines are tested in disease and/or yield trials each year, the effectiveness of the predictions will increase due to the larger number of observations that went into their estimation (Bernardo, 1996a).

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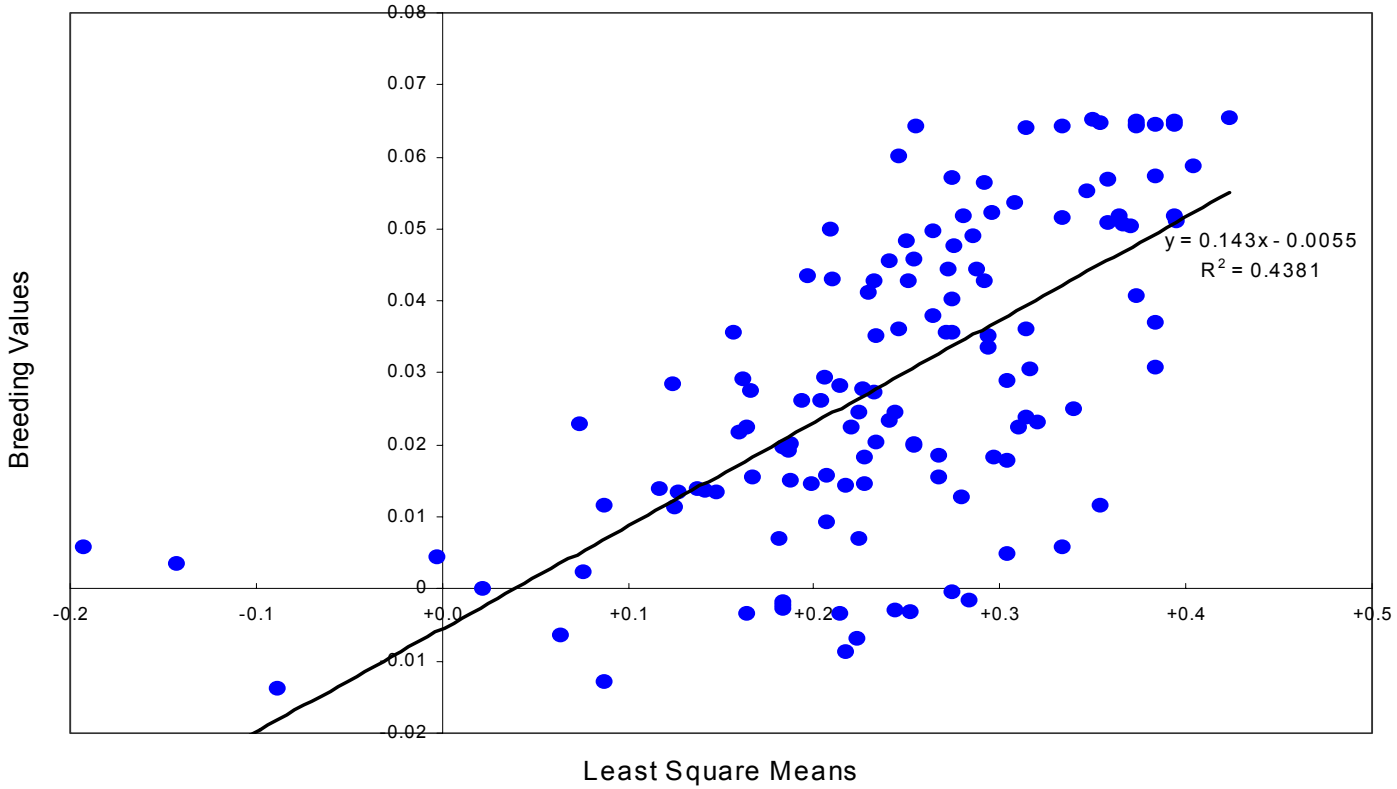

Figure 1. Best linear unbiased predictors (BLUPs) of breeding values vs. least square means for tomato spotted wilt virus (TSWV) incidence in virginia peanuts.

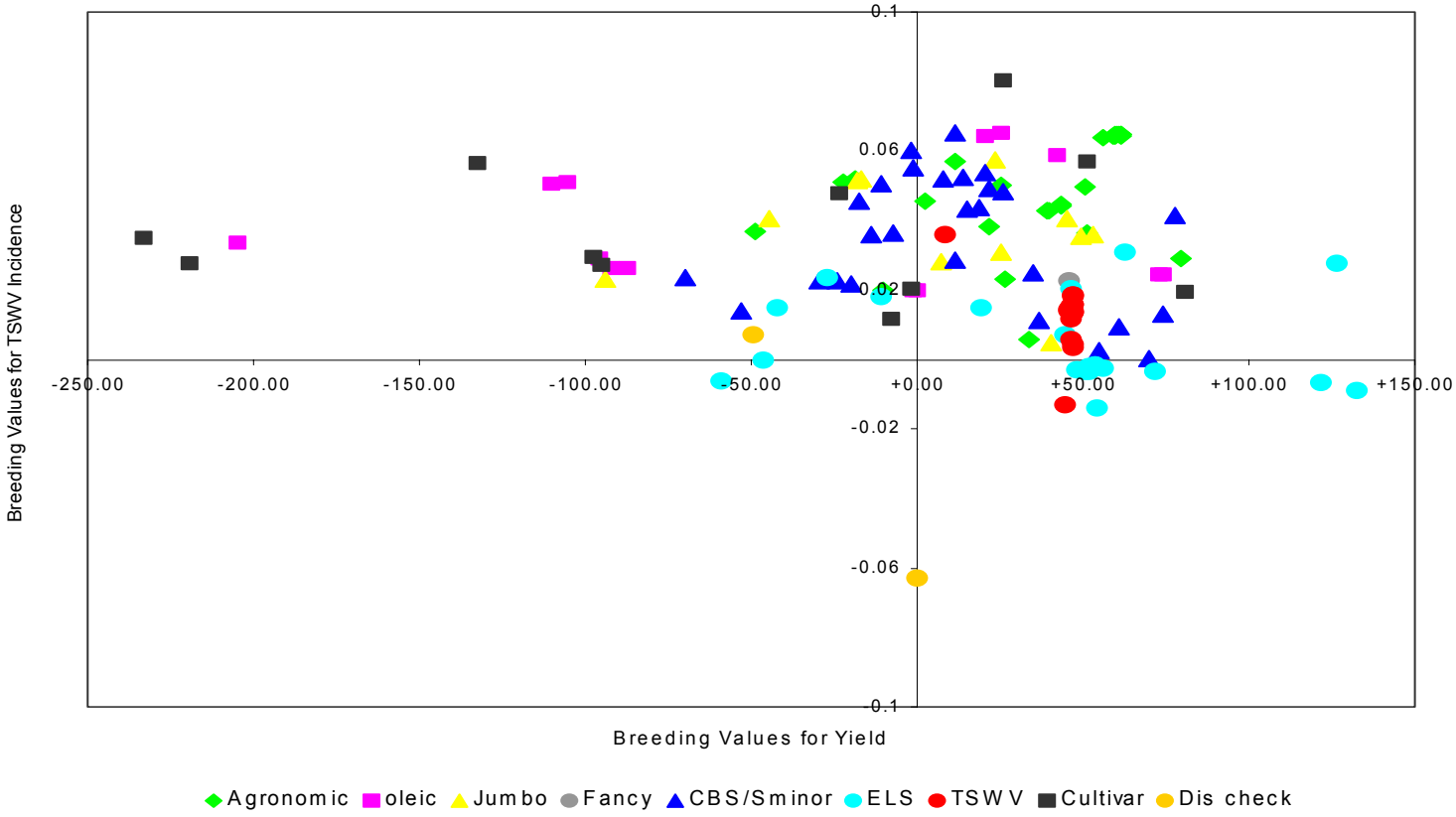

Figure 2. Best linear unbiased predictors (BLUPs) of breeding value for TSWV incidence vs. yield for virginia-type peanuts.

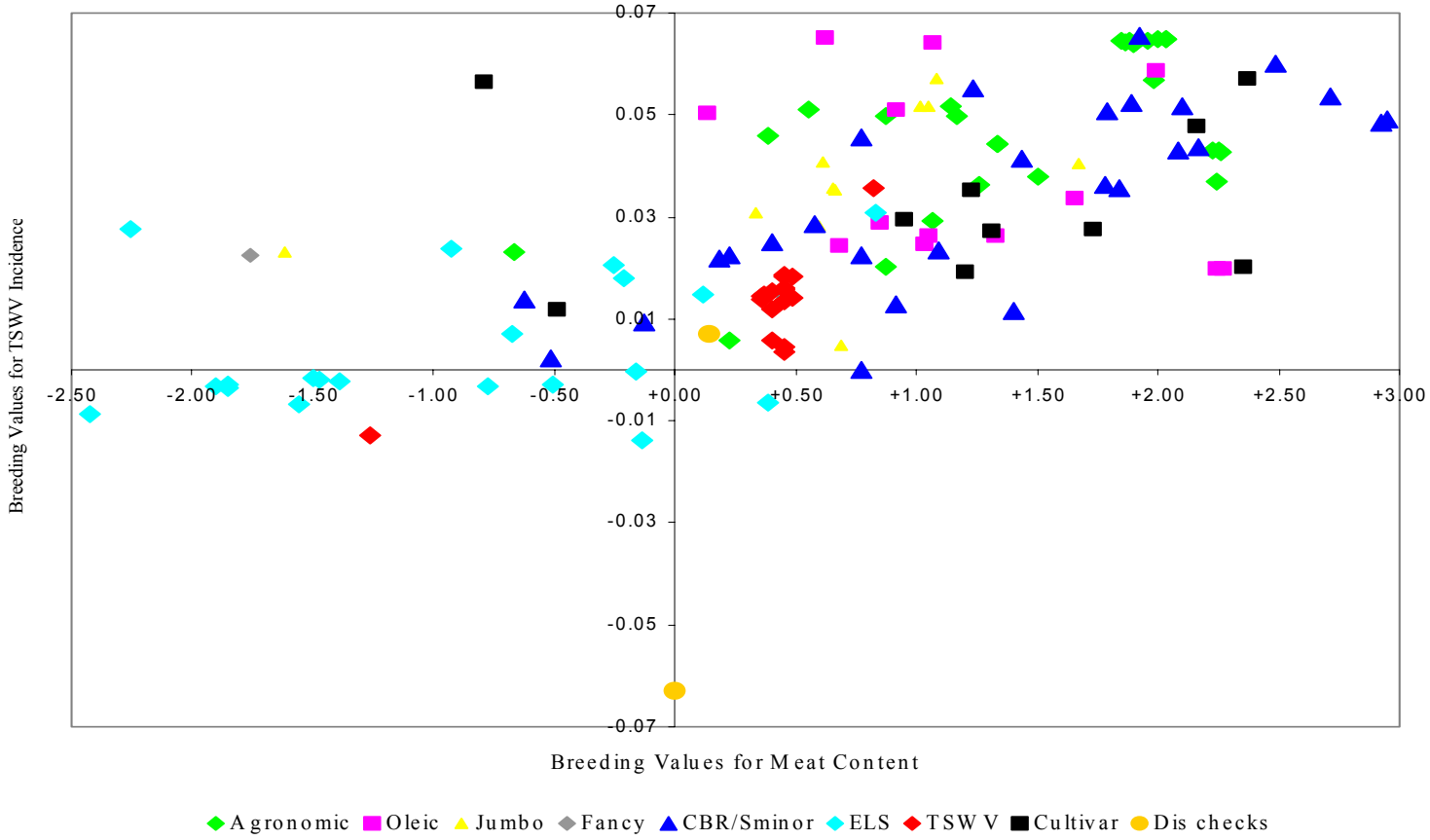

Figure 3. Best linear unbiased predictors (BLUPs) of breeding value for TSWV incidence vs. meat content in virginia-type peanuts.

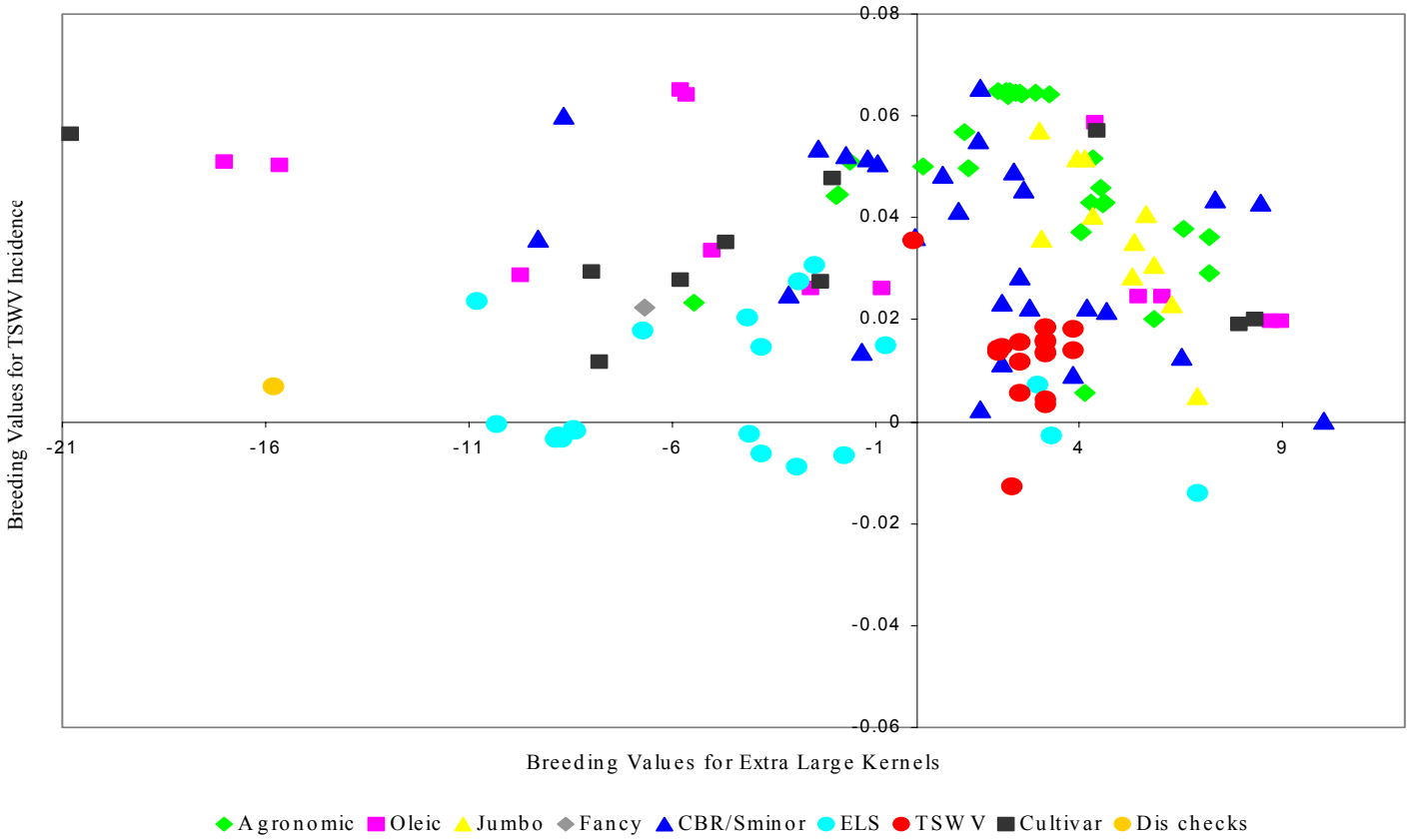

Figure 4. Best linear unbiased predictors (BLUPs) of breeding value for TSWV incidence vs. extra large kernels (ELK) in virginia-type peanuts.

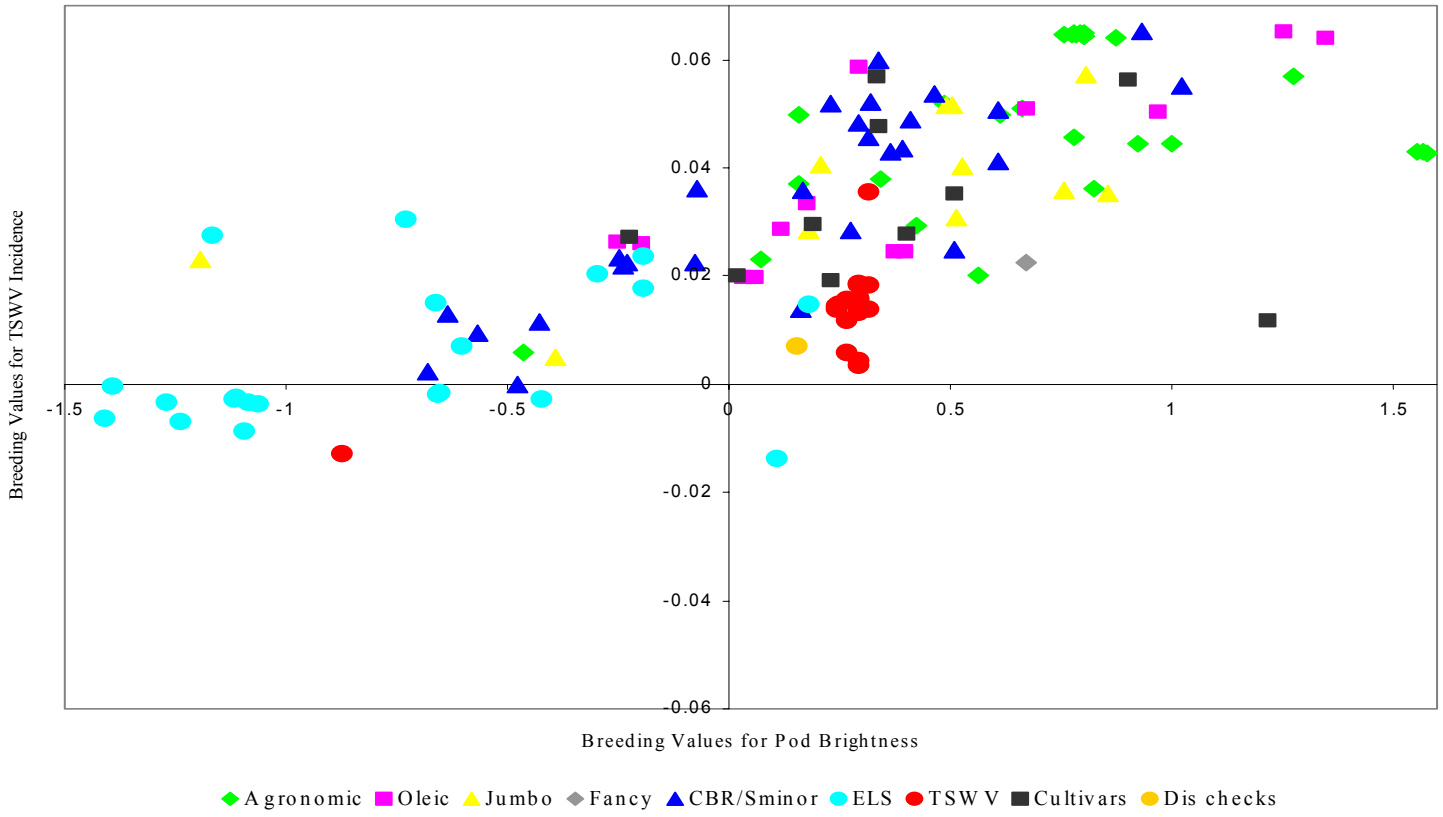

Figure 5. Best linear unbiased predictors (BLUPs) of breeding value for TSWV incidence vs. pod brightness in virginia-ttype peanuts.

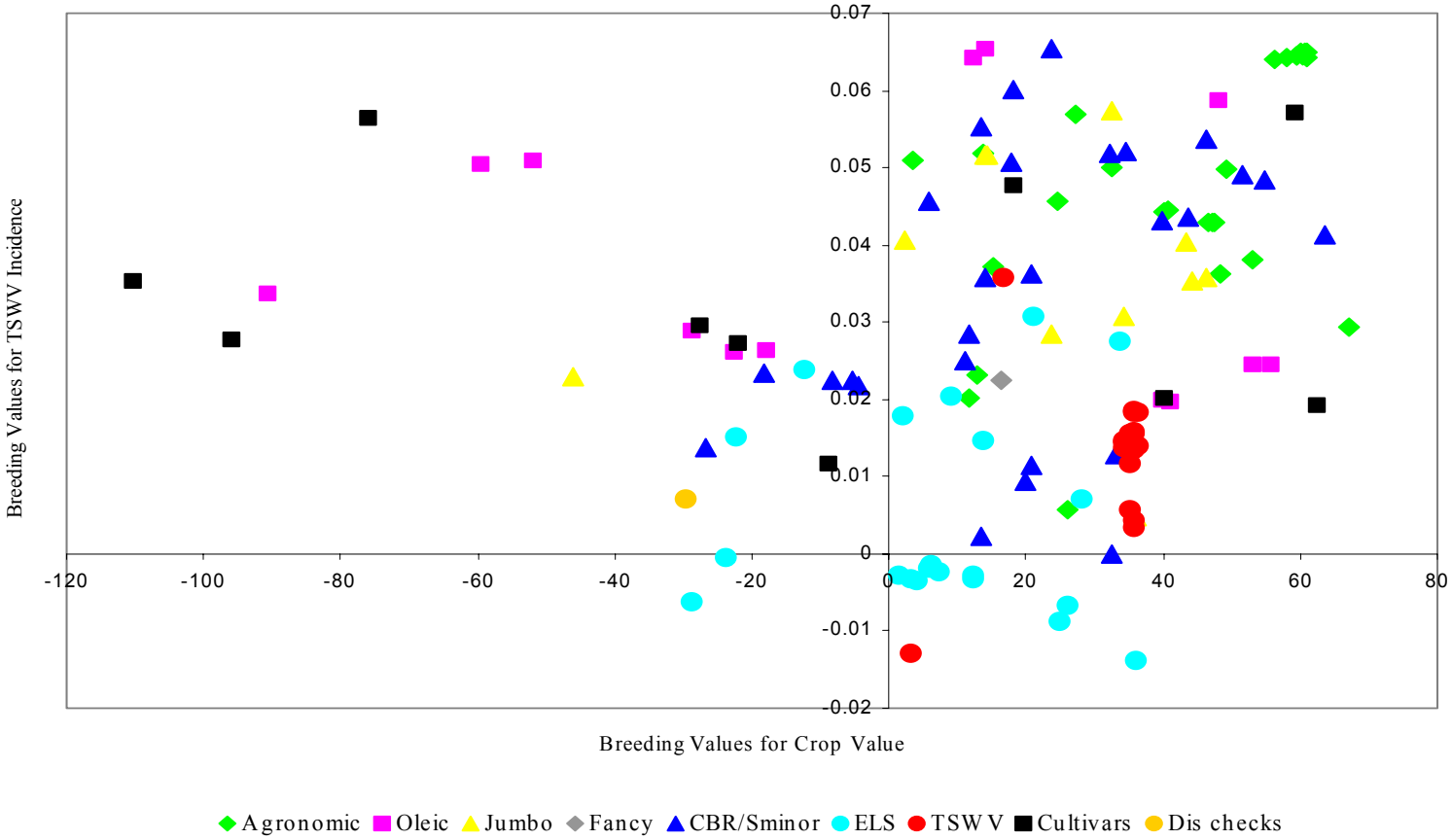

Figure 6. Best linear unbiased predictors (BLUPs) of breeding value for TSWV incidence vs. crop values in virginia-type peanuts.

Table 1. List of entries analyzed, including number of tests and years they were evaluated.

		TSWV incidence		Agronomic traits	
Entry	Program [a]	Tests	Years	Tests	Years
N91003E	Yield and grade	3	3	39	11
N92025	Yield and grade	3	3	26	10
N96029	Yield and grade	5	5	17	6
N97068	Yield and grade	4	4	5	3
N97085	Yield and grade	4	4	18	5
N98001	Yield and grade	4	3	16	5
N98002	Yield and grade	3	3	11	4
N98003	Yield and grade	3	3	11	4
N98022	Yield and grade	3	3	13	4
N98023	Yield and grade	3	3	11	4
N98028	Yield and grade	3	3	11	4
N98032	Yield and grade	3	3	15	4
N98033	Yield and grade	4	3	15	4
N99051	Yield and grade	2	2	8	3
N99073	Yield and grade	1	1	6	3
N00001	Yield and grade	1	1	5	2
N00033	Yield and grade	1	1	6	2
N00049	Yield and grade	1	1	6	2
N00052	Yield and grade	1	1	6	2
N00053	Yield and grade	1	1	6	2
N00054	Yield and grade	1	1	6	2
N00055	Yield and grade	1	1	6	2
N00058	Yield and grade	1	1	6	2
N00060	Yield and grade	1	1	6	2
N00061	Yield and grade	1	1	6	2
N00062	Yield and grade	1	1	6	2
N00064	Yield and grade	1	1	8	2
N99100ol	High oleic acid	2	2	8	3
N99103ol	High oleic acid	2	2	8	3
N99109ol	High oleic acid	2	2	8	3
N99113ol	High oleic acid	2	2	8	3
N00087ol	High oleic acid	1	1	6	2
N00088ol	High oleic acid	1	1	6	2
N00089ol	High oleic acid	1	1	6	2
N00090ol	High oleic acid	1	1	6	2
N00091ol	High oleic acid	1	1	6	2
N00095ol	High oleic acid	1	1	6	2
N00098ol	High oleic acid	1	1	6	2
N00099ol	High oleic acid	1	1	8	2
N00102ol	High oleic acid	1	1	6	2
N97053J	Jumbo pods	1	1	11	6
N99066J	Jumbo pods	1	1	5	3
N99067J	Jumbo pods	1	1	5	3
N99068J	Jumbo pods	1	1	5	3
N99079J	Jumbo pods	1	1	5	3

Table 1 (cont'd).

Entry	Program	TSWV incidence Tests	TSWV incidence Years	Agronomic traits Tests	Agronomic traits Years
N99080J	Jumbo pods	1	1	5	3
N99085J	Jumbo pods	1	1	8	3
N00002J	Jumbo pods	1	1	4	2
N00034J	Jumbo pods	1	1	4	2
N00035J	Jumbo pods	1	1	4	2
N00065J	Jumbo pods	1	1	4	2
N99057F	Jumbo pods	2	2	8	3
N92054C	CBR[b] / SB[c] resistance	6	6	20	10
N94040C	CBR / SB resistance	6	6	19	8
N95025C	CBR / SB resistance	6	6	19	8
N96006C	CBR / SB resistance	5	5	17	6
N96009C	CBR / SB resistance	5	5	17	6
N97122C	CBR / SB resistance	4	4	14	5
N97129C	CBR / SB resistance	4	4	15	5
N97131C	CBR / SB resistance	4	4	10	5
N97135C	CBR / SB resistance	4	4	13	5
N97137C	CBR / SB resistance	4	4	10	5
N97138C	CBR / SB resistance	4	4	13	5
N97140C	CBR / SB resistance	4	4	16	5
N97142C	CBR / SB resistance	4	4	16	5
N98048CSm	CBR / SB resistance	3	3	10	4
N98052C	CBR / SB resistance	3	3	10	4
N99121CSm	CBR / SB resistance	2	2	6	3
N99122CSm	CBR / SB resistance	2	2	7	3
N99128CSm	CBR / SB resistance	2	2	9	3
N99129CSm	CBR / SB resistance	2	2	10	3
N99130CSm	CBR / SB resistance	2	2	6	3
N99131CSm	CBR / SB resistance	2	2	3	3
N99132CSm	CBR / SB resistance	2	2	3	3
N99133CSm	CBR / SB resistance	2	2	6	3
N99137CSm	CBR / SB resistance	2	2	6	3
N99138CSm	CBR / SB resistance	2	2	6	3
N00076CSm	CBR / SB resistance	1	1	2	2
N00077CSm	CBR / SB resistance	1	1	2	2
N92066L	ELS[d] resistance	7	6	25	10
N92068L	ELS resistance	5	5	26	10
N93003L	ELS resistance	5	5	31	10
N93007L	ELS resistance	7	6	24	10
N94015L	ELS resistance	5	5	19	8
N96074L	ELS resistance	5	5	16	6
N96076L	ELS resistance	6	5	16	6
N97104L	ELS resistance	4	4	10	5
N97106L	ELS resistance	4	4	7	5
N97109L	ELS resistance	4	4	12	5
N99027L	ELS resistance	2	2	3	3

Table 1 (cont'd).

Entry	Program	TSWV incidence Tests	TSWV incidence Years	Agronomic traits Tests	Agronomic traits Years
N99034L	ELS resistance	1	1	3	3
N00009L	ELS resistance	1	1	2	2
N00010L	ELS resistance	1	1	2	2
N00011L	ELS resistance	1	1	2	2
N00012L	ELS resistance	1	1	2	2
N00019L	ELS resistance	1	1	2	2
N00020L	ELS resistance	1	1	2	2
N00022L	ELS resistance	1	1	2	2
N00023L	ELS resistance	1	1	2	2
N00024L	ELS resistance	1	1	2	2
N97064NT	TSWV[e] resistance	7	6	5	3
N01001T	TSWV resistance	1	1	6	2
N01002T	TSWV resistance	1	1	6	2
N01003T	TSWV resistance	1	1	6	2
N01004T	TSWV resistance	1	1	6	2
N01005T	TSWV resistance	1	1	6	2
N01006T	TSWV resistance	1	1	6	2
N01007T	TSWV resistance	1	1	6	2
N01008T	TSWV resistance	1	1	6	2
N01009T	TSWV resistance	1	1	6	2
N01010T	TSWV resistance	1	1	6	2
N01011T	TSWV resistance	1	1	6	2
N01012T	TSWV resistance	1	1	6	2
N01013T	TSWV resistance	1	1	6	2
N01014T	TSWV resistance	1	1	6	2
N01015T	TSWV resistance	1	1	6	2
N01016T	TSWV resistance	1	1	6	2
N01017T	TSWV resistance	1	1	6	2
NC 7	Cultivar	13	6	80	13
NC 9	Cultivar	15	6	58	11
NC 10C	Cultivar	7	6	47	11
NC-V 11	Cultivar	14	6	46	11
NC 12C	Cultivar	14	6	58	13
Gregory	Cultivar	15	6	56	11
Perry	Cultivar	13	6	46	11
VA-C 92R	Cultivar	8	6	47	11
VA 93B	Cultivar	5	4	16	8
VA 98R	Cultivar	7	4	22	6
Wilson	Cultivar	1	1	7	3
Georgia Green	TSWV Disease check	8	4	.	.
PI 576636	TSWV Disease check	13	6	.	.

[a] "Program" indicates trait(s) for which lines are being bred.
[b] CBR = Cylindrocladium black rot (*Cylindrocladium parasiticum*)
[c] SB = sclerotinia blight (*Sclerotinia minor*)
[d] ELS = Early leafspot (*Cercospora arachidicola*)
[e] TSWV = tomato spotted wilt virus

Table 2. Weighting schemes utilized for index selection. Assigned weights were based on given importance of disease resistance vs. yield vs. quality traits.

Scheme	TSWV[a]	Yield	Meat content	Extra large kernels	Pod brightness	Crop value
I	1	1	1	1	1	1
II	5	5	1	1	1	1
III	1	5	5	5	5	5
IV	1	5	1	1	1	1
V	5	1	1	1	1	1
VI	2	3	1	1	1	1

[a] TSWV = tomato spotted wilt virus

Table 3. Correlations among BLUPs of breeding values for TSWV incidence, yield, meat content, extra large kernels, pod brightness and crop value estimated at different heritability values.

TSWV Incidence

H	0.01	**0.05**[a]	0.10	0.15	0.20	0.25	Mean
0.01	1.00	0.98	0.94	0.91	0.88	0.85	0.55
0.05	0.98	1.00	0.99	0.97	0.96	0.94	0.66
0.10	0.94	0.99	1.00	1.00	0.99	0.98	0.73
0.15	0.91	0.97	1.00	1.00	1.00	0.99	0.78
0.20	0.88	0.96	0.99	1.00	1.00	1.00	0.81
0.25	0.85	0.94	0.98	0.99	1.00	1.00	0.84
Mean	0.55	0.66	0.73	0.78	0.81	0.84	1.00

Yield

H	0.01	**0.02**	0.03	0.04	0.05	0.06	Mean
0.01	1.00	1.00	0.99	0.97	0.96	0.95	0.48
0.02	1.00	1.00	1.00	0.99	0.98	0.97	0.53
0.03	0.99	1.00	1.00	1.00	0.99	0.99	0.57
0.04	0.97	0.99	1.00	1.00	1.00	1.00	0.60
0.05	0.96	0.98	0.99	1.00	1.00	1.00	0.63
0.06	0.95	0.97	0.99	1.00	1.00	1.00	0.65
Mean	0.48	0.53	0.57	0.60	0.63	0.65	1.00

Meat Content (%)

H	0.10	**0.20**	0.30	0.40	0.50	0.60	Mean
0.10	1.00	0.99	0.97	0.95	0.93	0.91	0.81
0.20	0.99	1.00	1.00	0.99	0.97	0.96	0.88
0.30	0.97	1.00	1.00	1.00	0.99	0.98	0.91
0.40	0.95	0.99	1.00	1.00	1.00	0.99	0.94
0.50	0.93	0.97	0.99	1.00	1.00	1.00	0.96
0.60	0.91	0.96	0.98	0.99	1.00	1.00	0.97
Mean	0.81	0.88	0.91	0.94	0.96	0.97	1.00

Extra Large Kernels (ELK) (%)

H	0.10	0.20	0.30	**0.42**	0.50	0.60	Mean
0.10	1.00	0.99	0.97	0.95	0.94	0.92	0.86
0.20	0.99	1.00	1.00	0.98	0.98	0.96	0.91
0.30	0.97	1.00	1.00	1.00	0.99	0.98	0.94
0.42	0.95	0.98	1.00	1.00	1.00	1.00	0.96
0.50	0.94	0.98	0.99	1.00	1.00	1.00	0.97
0.60	0.92	0.96	0.98	1.00	1.00	1.00	0.98
Mean	0.86	0.91	0.94	0.96	0.97	0.98	1.00

Pod Brightness (Hunter L score)

H	0.05	0.10	0.15	**0.18**	0.25	0.30	Mean
0.05	1.00	0.99	0.98	0.97	0.96	0.95	0.84
0.10	0.99	1.00	1.00	0.99	0.99	0.98	0.89
0.15	0.98	1.00	1.00	1.00	1.00	0.99	0.92
0.18	0.97	0.99	1.00	1.00	1.00	1.00	0.93
0.25	0.96	0.99	1.00	1.00	1.00	1.00	0.94
0.30	0.95	0.98	0.99	1.00	1.00	1.00	0.95
Mean	0.84	0.89	0.92	0.93	0.94	0.95	1.00

Crop Value ($ ha^{-1})

H	0.01	0.02	0.03	0.04	**0.05**	0.06	Mean
0.01	1.00	1.00	0.99	0.98	0.96	0.95	0.53
0.02	1.00	1.00	1.00	0.99	0.98	0.98	0.57
0.03	0.99	1.00	1.00	1.00	0.99	0.99	0.60
0.04	0.98	0.99	1.00	1.00	1.00	1.00	0.63
0.05	0.96	0.98	0.99	1.00	1.00	1.00	0.65
0.06	0.95	0.98	0.99	1.00	1.00	1.00	0.68
Mean	0.53	0.57	0.60	0.63	0.65	0.68	1.00

[a] Bold values indicate estimates of H calculated from out data.

Table 4. Best linear unbiased predictors (BLUPs) of breeding values with standard errors for TSWV incidence, pod yield, meat content, percent extra large kernels, pod brightness, and crop value.

Entry	TSWV	Pod yield	Meat content	Extra large kernels	Pod brightness	Crop value
	%	lb/A	%	%	Hunter L	$/A
N91003E	+3.80±0.14**	+24±55	+1.5±0.4**	+6.6±1.3**	+0.34±0.26	+131±25**
N92025	+4.98±0.14**	+57±56	+0.9±0.4†	+1.3±1.4	+0.16±0.28	+122±26**
N96029	+3.71±0.14**	-54±56	+2.2±0.5**	+4.0±1.5**	+0.16±0.30	+38±27
N97068	+2.01±0.14**	-11±57	+0.9±0.5†	+5.8±1.6**	+0.56±0.32†	+29±27
N97085	+2.93±0.13**	+90±54†	+1.1±0.4*	+7.2±1.4**	+0.42±0.28	+166±25**
N98001	+4.29±0.13**	+44±54	+2.2±0.4**	+4.3±1.4**	+1.56±0.28**	+115±25**
N98002	+4.28±0.13**	+45±54	+2.3±0.4**	+4.6±1.4**	+1.58±0.28**	+117±25**
N98003	+4.29±0.13**	+44±54	+2.3±0.4**	+4.6±1.4**	+1.57±0.28**	+116±25**
N98022	+4.44±0.13**	+49±54	+1.3±0.4**	-2.0±1.4	+0.93±0.28**	+99±25**
N98023	+4.44±0.13**	+49±54	+1.3±0.4**	-1.9±1.4	+1.00±0.28**	+100±25**
N98028	+5.69±0.13**	+13±57	+2.0±0.5**	+1.2±1.6	+1.28±0.32**	+67±27*
N98032	+5.10±0.14**	-25±57	+0.6±0.5	-1.6±1.5	+0.66±0.31*	+9±28
N98033	+4.99±0.13**	+28±58	+1.2±0.6*	+0.2±1.8	+0.61±0.35†	+80±28**
N99051	+2.32±0.15**	+30±64	-0.7±0.6	-5.4±1.7**	+0.07±0.37	+31±32
N99073	+5.18±0.14**	-21±58	+1.1±0.5*	+4.3±1.7*	+0.49±0.34	+34±28
N00001	+0.58±0.15**	+38±65	+0.2±0.6	+4.1±1.9*	-0.46±0.40	+64±33*
N00033	+3.62±0.13**	+58±55	+1.3±0.5*	+7.2±1.6**	+0.83±0.32*	+119±27**
N00049	+6.43±0.13**	+69±55	+1.9±0.5**	+3.3±1.6*	+0.80±0.29**	+151±26**
N00052	+6.45±0.13**	+67±54	+2.0±0.5**	+2.9±1.5†	+0.77±0.29**	+147±25**
N00053	+6.43±0.13**	+66±54	+1.9±0.5**	+2.6±1.5†	+0.80±0.29**	+144±25**
N00054	+6.45±0.13**	+68±54	+1.8±0.5**	+2.4±1.5	+0.76±0.29**	+147±25**
N00055	+6.46±0.13**	+69±54	+1.9±0.5**	+2.5±1.5	+0.78±0.29**	+149±25**
N00058	+6.40±0.13**	+63±55	+1.9±0.5**	+2.2±1.6	+0.88±0.29**	+139±26**
N00060	+6.49±0.13**	+68±54	+2.0±0.4**	+2.2±1.5	+0.79±0.28**	+150±25**
N00061	+6.49±0.13**	+69±54	+2.0±0.4**	+2.0±1.5	+0.78±0.28**	+151±25**
N00062	+6.49±0.13**	+67±54	+2.0±0.4**	+2.3±1.5	+0.80±0.28**	+148±25**
N00064	+4.58±0.14**	+2±59	+0.4±0.5	+4.5±1.7**	+0.78±0.34*	+61±29*
N99100ol	+6.53±0.13**	+29±55	+0.6±0.5	-5.8±1.6**	+1.25±0.31**	+34±26
N99103ol	+6.42±0.13**	+23±55	+1.1±0.5*	-5.7±1.6**	+1.35±0.31**	+30±26
N99109ol	+5.10±0.14**	-118±57*	+0.9±0.5†	-17.0±1.6**	+0.67±0.32*	-128±27**
N99113ol	+5.04±0.14**	-123±57*	+0.1±0.5	-15.7±1.6**	+0.97±0.32**	-147±27**
N00087ol	+2.89±0.13**	-108±54*	+0.8±0.5†	-9.7±1.5**	+0.12±0.28	-71±25**
N00088ol	+2.63±0.13**	-98±54†	+1.3±0.4**	-2.6±1.5†	-0.25±0.28	-44±25†
N00089ol	+2.62±0.13**	-104±54†	+1.0±0.4*	-0.8±1.5	-0.20±0.28	-56±25*
N00090ol	+1.98±0.12**	+0±49	+2.2±0.4**	+8.7±1.4**	+0.06±0.25	+101±22**
N00091ol	+1.99±0.12**	-1±49	+2.3±0.4**	+8.9±1.4**	+0.03±0.25	+98±22**
N00095ol	+5.87±0.13**	+47±53	+2.0±0.5**	+4.4±1.5**	+0.30±0.28	+118±24**
N00098ol	+2.46±0.13**	+83±52	+1.0±0.4*	+5.5±1.5**	+0.37±0.28	+137±24**
N00099ol	+2.45±0.13**	+82±52	+0.7±0.4	+6.0±1.5**	+0.40±0.27	+131±24**
N00102ol	+3.36±0.14**	-230±59**	+1.7±0.5**	-5.1±1.7**	+0.18±0.33	-224±29**
N97053J	+2.30±0.15**	-105±61†	-1.6±0.5**	+6.3±1.7**	-1.19±0.33**	-114±30**
N99066J	+2.83±0.14**	+8±58	+0.6±0.5	+5.3±1.8**	+0.18±0.35	+58±28*
N99067J	+4.03±0.13**	+50±55	+1.7±0.5**	+4.3±1.7*	+0.53±0.33	+107±27**
N99068J	+4.07±0.14**	-50±59	+0.6±0.6	+5.7±1.9**	+0.21±0.36	+6±29
N99079J	+5.16±0.14**	-20±58	+1.1±0.5*	+4.1±1.7*	+0.49±0.34	+34±28

Table 4 (cont'd).

Entry	TSWV	Pod yield	Meat content	Extra large kernels	Pod brightness	Crop value
	%	lb/A	%	%	Hunter L	$/A
N99080J	+5.17±0.14**	-19±58	+1.0±0.5†	+3.9±1.7*	+0.51±0.34	+35±28
N99085J	+3.08±0.14**	+28±57	+0.3±0.5	+5.8±1.6**	+0.51±0.33	+85±28**
N00002J	+0.49±0.15**	+45±65	+0.7±0.7	+6.9±2.0**	-0.39±0.42	+89±33**
N00034J	+3.58±0.13**	+60±56	+0.7±0.5	+3.1±1.8†	+0.76±0.33*	+114±27**
N00035J	+3.53±0.13**	+55±56	+0.7±0.5	+5.4±1.8**	+0.86±0.33*	+110±27**
N00065J	+5.73±0.14**	+27±57	+1.1±0.6†	+3.0±1.9	+0.81±0.36*	+80±28**
N99057F	+2.25±0.15**	+51±64	-1.8±0.6**	-6.7±1.7**	+0.67±0.37†	+40±32
N92054C	+2.34±0.14**	-79±58	+1.1±0.5*	+2.1±1.4	-0.25±0.29	-45±27†
N94040C	+1.38±0.14**	-59±65	-0.6±0.8	-1.3±2.7	+0.16±0.48	-66±34†
N95025C	+4.13±0.14**	+87±58	+1.4±0.5**	+1.0±1.5	+0.61±0.31*	+157±28**
N96006C	+5.07±0.14**	-12±58	+1.8±0.5**	-0.9±1.5	+0.61±0.30*	+44±28
N96009C	+6.00±0.13**	-2±55	+2.5±0.5**	-8.7±1.4**	+0.34±0.29	+45±26†
N97122C	+5.52±0.14**	-2±58	+1.2±0.5*	+1.5±1.5	+1.02±0.31**	+33±28
N97129C	+6.54±0.14**	+13±58	+1.9±0.5**	+1.6±1.5	+0.93±0.31**	+59±28*
N97131C	+4.56±0.14**	-20±59	+0.8±0.5	+2.6±1.6	+0.32±0.34	+14±29
N97135C	+5.22±0.13**	+16±55	+1.9±0.5**	-1.7±1.5	+0.32±0.29	+86±26**
N97137C	+5.18±0.13**	+9±56	+2.1±0.5**	-1.2±1.5	+0.23±0.30	+79±26**
N97138C	+5.37±0.13**	+23±56	+2.7±0.5**	-2.4±1.5	+0.47±0.30	+114±27**
N97140C	+4.84±0.13**	+29±56	+2.9±0.5**	+0.7±1.4	+0.29±0.29	+135±26**
N97142C	+4.91±0.13**	+24±56	+2.9±0.5**	+2.4±1.4†	+0.41±0.29	+128±26**
N98048CSm	+3.58±0.14**	-16±61	+1.8±0.5**	-9.3±1.6**	+0.17±0.35	+35±30
N98052C	+3.62±0.14**	-8±63	+1.8±0.6**	-0.0±2.0	-0.07±0.41	+51±32
N99121CSm	+1.14±0.15**	+42±62	+1.4±0.6*	+2.1±1.8	-0.43±0.37	+52±31†
N99122CSm	+1.29±0.15**	+83±62	+0.9±0.6	+6.5±1.8**	-0.64±0.37†	+82±31**
N99128CSm	+2.49±0.14**	+39±60	+0.4±0.5	-3.1±1.7†	+0.51±0.34	+27±29
N99129CSm	+4.35±0.14**	+21±57	+2.2±0.5**	+7.3±1.6**	+0.39±0.31	+108±27**
N99130CSm	+4.31±0.14**	+17±57	+2.1±0.5**	+8.5±1.7**	+0.36±0.33	+98±28**
N99131CSm	+0.23±0.15	+61±64	-0.5±0.7	+1.6±2.2	-0.68±0.42	+33±33
N99132CSm	+0.93±0.15**	+68±64	-0.1±0.7	+3.8±2.3	-0.57±0.44	+49±33
N99133CSm	+0.01±0.15	+78±63	+0.8±0.6	+10.0±1.8**	-0.48±0.38	+80±31*
N99137CSm	+2.25±0.14**	-27±61	+0.2±0.6	+2.8±1.7	-0.07±0.35	-20±30
N99138CSm	+2.18±0.14**	-22±61	+0.2±0.6	+4.7±1.7**	-0.24±0.35	-11±30
N00076CSm	+2.85±0.14**	+13±62	+0.6±0.7	+2.5±2.3	+0.28±0.42	+29±31
N00077CSm	+2.25±0.15**	-33±63	+0.8±0.7	+4.2±2.2†	-0.23±0.42	-13±32
N92066L	+3.07±0.14**	+70±57	+0.8±0.4†	-2.5±1.4†	-0.73±0.28*	+52±27†
N92068L	-0.33±0.14*	81±56	-0.8±0.4†	-8.9±1.4**	-1.27±0.28**	+30±26
N93003L	-0.67±0.13**	36±53*	-1.6±0.4**	-1.8±1.3	-1.24±0.26**	+64±24**
N93007L	-0.88±0.13**	49±54**	-2.4±0.4**	-3.0±1.4*	-1.09±0.28**	+61±25*
N94015L	+2.75±0.14**	+142±59*	-2.3±0.5**	-2.9±1.4*	-1.17±0.30**	+83±28**
N96074L	-0.04±0.14	-52±58	-0.2±0.5	-10.3±1.4**	-1.39±0.29**	-59±27*
N96076L	-0.63±0.14**	-66±58	+0.4±0.5	-3.8±1.4**	-1.41±0.29**	-71±27**
N97104L	+0.71±0.14**	+50±60	-0.7±0.5	+3.0±1.6†	-0.60±0.34†	+69±29*
N97106L	+1.51±0.14**	-47±63	+0.4±0.6	-0.7±1.8	-0.66±0.38†	-55±31†
N97109L	+1.47±0.14**	+21±61	+0.1±0.5	-3.8±1.6*	+0.18±0.33	+34±30
N99027L	-1.38±0.15**	61±62	-0.1±0.7	+6.9±2.1**	+0.11±0.42	+89±32**

Table 4 (cont'd).

Entry	TSWV	Pod yield	Meat content	Extra large kernels	Pod brightness	Crop value
	%	lb/A	%	%	Hunter L	$/A
N99034L	+2.05±0.15**	+52±64	-0.3±0.7	-4.2±2.2†	-0.30±0.43	+22±33
N00009L	-0.28±0.15†	54±64	-1.8±0.6**	-8.8±2.1**	-1.12±0.41**	+4±33
N00010L	-0.35±0.15*	58±64	-1.9±0.6**	-8.7±2.0**	-1.06±0.41**	+10±32
N00011L	-0.33±0.15*	57±64	-1.9±0.6**	-8.9±2.0**	-1.09±0.41**	+8±32
N00012L	-0.23±0.15	58±65	-1.4±0.7†	-4.1±2.4†	-1.11±0.45*	+18±34
N00019L	+1.79±0.15**	-12±65	-0.2±0.7	-6.8±2.4**	-0.19±0.46	+5±34
N00020L	+2.38±0.15**	-30±65	-0.9±0.7	-10.8±2.4**	-0.19±0.46	-30±34
N00022L	-0.27±0.15†	63±64	-0.5±0.7	+3.3±2.3	-0.42±0.44	+30±33
N00023L	-0.18±0.15	60±64	-1.5±0.7*	-8.4±2.1**	-0.66±0.42	+14±32
N00024L	-0.15±0.15	60±64	-1.5±0.7*	-8.4±2.1**	-0.65±0.42	+15±32
N97064NT	-1.28±0.14**	50±62	-1.3±0.6*	+2.3±1.9	-0.87±0.38*	+8±31
N01001T	+0.58±0.14**	+52±59	+0.4±0.6	+2.6±2.2	+0.27±0.39	+86±29**
N01002T	+1.17±0.14**	+52±59	+0.4±0.6	+2.6±2.2	+0.27±0.39	+86±29**
N01003T	+1.47±0.14**	+52±59	+0.4±0.6	+2.1±2.0	+0.25±0.38	+85±29**
N01004T	+1.38±0.14**	+52±59	+0.4±0.6	+2.0±2.0	+0.24±0.38	+84±29**
N01005T	+1.44±0.14**	+52±59	+0.4±0.6	+2.0±2.0	+0.24±0.38	+84±29**
N01006T	+1.56±0.14**	+52±59	+0.4±0.6	+2.6±2.2	+0.27±0.39	+86±29**
N01007T	+1.86±0.14**	+53±59	+0.5±0.6	+3.2±2.1	+0.29±0.39	+88±30**
N01008T	+1.84±0.14**	+53±59	+0.5±0.6	+3.2±2.1	+0.29±0.39	+88±30**
N01009T	+1.36±0.14**	+53±59	+0.5±0.6	+3.2±2.1	+0.29±0.39	+88±30**
N01010T	+1.34±0.14**	+53±59	+0.5±0.6	+3.2±2.1	+0.29±0.39	+88±30**
N01011T	+1.40±0.14**	+53±59	+0.5±0.6	+3.8±2.2†	+0.31±0.40	+90±30**
N01012T	+1.59±0.14**	+53±59	+0.5±0.6	+3.2±2.1	+0.29±0.39	+88±30**
N01013T	+1.56±0.14**	+53±59	+0.5±0.6	+3.2±2.1	+0.29±0.39	+88±30**
N01014T	+0.34±0.14*	+53±59	+0.5±0.6	+3.2±2.1	+0.29±0.39	+88±30**
N01015T	+0.44±0.14**	+53±59	+0.5±0.6	+3.2±2.1	+0.29±0.39	+88±30**
N01016T	+1.83±0.14**	+53±59	+0.5±0.6	+3.8±2.2†	+0.31±0.40	+90±30**
N01017T	+3.57±0.15**	+10±63	+0.8±0.7	-0.1±2.5	+0.32±0.45	+41±32
NC 7	+2.02±0.12**	-2±47	+2.4±0.4**	+8.3±1.2**	+0.02±0.22	+99±21**
NC 9	+8.04±0.12**	+29±49	+0.3±0.4	-7.0±1.2**	+1.00±0.23**	+18±22
NC 10C	+5.65±0.13**	-149±53**	-0.8±0.4*	-20.8±1.3**	+0.90±0.25**	-188±24**
NC-V 11	+2.95±0.13**	-109±53*	+0.9±0.4*	-8.0±1.3**	+0.19±0.25	-68±24**
NC 12C	+5.71±0.12**	+58±51	+2.4±0.4**	+4.4±1.2**	+0.33±0.23	+146±23**
Gregory	+1.93±0.12**	+90±50†	+1.2±0.4**	+7.9±1.2**	+0.23±0.23	+154±22**
Perry	+4.77±0.12**	-27±53	+2.2±0.4**	-2.1±1.3	+0.34±0.25	+45±24†
VA-C 92R	+2.74±0.13**	-107±52*	+1.3±0.4**	-2.4±1.2†	-0.23±0.24	-54±23*
VA 93B	+3.52±0.14**	-262±58**	+1.2±0.5*	-4.7±1.5**	+0.51±0.30†	-272±28**
VA 98R	+2.77±0.14**	-246±58**	+1.7±0.5**	-5.8±1.4**	+0.40±0.29	-237±27**
Wilson	+1.17±0.16**	-9±64	-0.5±0.6	-7.8±1.8**	+1.22±0.39**	-22±32
Georgia Green	+0.71±0.14**	-55±66	+0.1±0.8	-15.8±2.7**	+0.16±0.49	-73±35*
PI 576636	-6.29±0.13**	+0±67	+0.0±0.8	+0.0±2.7	+0.00±0.51	+0±36

†,*,** Denote significance at the 10%, 5%, and 1% levels of probability, respectively, by t-test.

Table 5. Summary statistics for BLUPs of breeding value for TSWV incidence, yield, meat content, extra large kernels, pod brightness, and crop value at their estimated heritabilities.

	TSWV h^2=0.05	Yield h^2=0.02	Meat content h^2=0.20	Extra large kernels h^2=0.42	Pod brightness h^2=0.18	Crop value h^2=0.05
	%	lb/A	%	%	Hunter L	$/A
Mean	2.92	15.96	0.73	0.22	0.20	47.48
Minimum	-6.29	-261.64	-2.42	-20.79	-1.41	-272.43
Maximum	8.04	148.60	2.95	10.00	1.58	165.75
Range	14.33	410.24	5.37	30.79	2.98	438.17
Std. dev.	2.31	68.11	1.16	5.79	0.65	81.43

Table 6. Correlations among BLUPs of breeding value for TSWV incidence, yield, meat content, extra large kernels, pod brightness, and crop value at their estimated heritabilities.

	TSWV h^2=0.05	Yield h^2=0.02	Meat content h^2=0.20	Extra large kernels h^2=0.42	Pod brightness h^2=0.18	Crop value h^2=0.05
	%	lb/A	%	%	Hunter L	$/A
TSWV (h^2=0.05)	1.00	-0.12	0.64	0.02	0.69	0.20
Yield (h^2=0.02)	-0.12	1.00	-0.15	0.38	-0.06	0.85
Meat content (h^2=0.20)	0.64	-0.15	1.00	0.37	0.60	0.33
ELK (h^2=0.42)	0.02	0.38	0.37	1.00	0.13	0.61
Pod brightness (h^2=0.18)	0.69	-0.06	0.60	0.13	1.00	0.26
Crop value (h^2=0.05)	0.20	0.85	0.33	0.61	0.26	1.00

Table 7. Correlations and rank correlations among weighting schemes used to select lines with superior breeding values for TSWV incidence in combination with agronomic traits.

Weighting Scheme	Correlation					
	I	II	III	IV	V	VI
I[a]	1.00	0.82	0.97	0.95	0.78	0.96
II[b]	0.82	1.00	0.68	0.87	0.96	0.94
III[c]	0.97	0.68	1.00	0.92	0.61	0.88
IV[d]	0.95	0.87	0.92	1.00	0.76	0.97
V[e]	0.78	0.96	0.61	0.76	1.00	0.88
VI[f]	0.96	0.94	0.88	0.97	0.88	1.00

Weighting Scheme	Rank Correlation					
	I	II	III	IV	V	VI
I[a]	1.00	0.72	0.86	0.93	0.70	0.94
II[b]	0.72	1.00	0.34	0.72	0.96	0.88
III[c]	0.86	0.34	1.00	0.84	0.29	0.70
IV[d]	0.93	0.72	0.84	1.00	0.63	0.93
V[e]	0.70	0.96	0.29	0.63	1.00	0.84
VI[f]	0.94	0.88	0.70	0.93	0.84	1.00

[a] I = 1,1,1,1,1,1= equal weights assigned to all traits.
[b] II = 5,5,1,1,1,1 = stronger weight assigned to TSWV incidence and yield.
[c] III = 1,5,5,5,5,5 = stronger weight assigned to all agronomic traits.
[d] IV = 1,5,1,1,1,1 = stronger weight assigned only to yield.
[e] V = 5,1,1,1,1,1 = stronger weight assigned only to TSWV incidence.
[f] VI = 2,3,1,1,1,1 = slightly stronger weight assigned to TSWV incidence and yield.

Table 8. Coefficients of coancestry among lines identified as superior with at least four of the six selection schemes.

Entry	Gregory	N99027L	N97085	N00033	N99133CSm	N01001T	N01011T	N01014T	N01015T	N00090ol	N00091ol	N00098ol	N00099ol	Mean
Gregory***	1.00	0.50	0.67	0.67	0.23	0.59	0.59	0.59	0.59	0.55	0.55	0.93	0.93	0.64
N99027L**	0.50[a]	1.00	0.34	0.34	0.11	0.29	0.29	0.29	0.29	0.27	0.27	0.47	0.47	0.37
N97085**	0.67	0.34	1.00	0.67	0.28	0.59	0.59	0.59	0.59	0.55	0.55	0.64	0.64	0.59
N00033*	0.67	0.34	0.67	1.00	0.23	0.59	0.59	0.59	0.59	0.55	0.55	0.64	0.64	0.59
N99133CSm**	0.23	0.11	0.28	0.23	1.00	0.17	0.17	0.17	0.17	0.22	0.22	0.22	0.22	0.26
N01001T*	0.59	0.29	0.59	0.59	0.17	1.00	0.56	0.56	0.56	0.41	0.41	0.55	0.55	0.53
N01011T*	0.59	0.29	0.59	0.59	0.17	0.56	1.00	0.50	0.50	0.41	0.41	0.55	0.55	0.52
N01014T**	0.59	0.29	0.59	0.59	0.17	0.56	0.50	1.00	0.88	0.41	0.41	0.55	0.55	0.54
N01015T**	0.59	0.29	0.59	0.59	0.17	0.56	0.50	0.88	1.00	0.41	0.41	0.55	0.55	0.54
N00090ol*	0.55	0.27	0.55	0.55	0.22	0.41	0.41	0.41	0.41	1.00	0.97	0.49	0.49	0.52
N00091ol*	0.55	0.27	0.55	0.55	0.22	0.41	0.41	0.41	0.41	0.97	1.00	0.49	0.49	0.52
N00098ol**	0.93	0.47	0.64	0.64	0.22	0.55	0.55	0.55	0.55	0.49	0.49	1.00	0.93	0.62
N00099ol**	0.93	0.47	0.64	0.64	0.22	0.55	0.55	0.55	0.55	0.49	0.49	0.93	1.00	0.62
Mean	0.65	0.38	0.59	0.59	0.26	0.53	0.52	0.54	0.54	0.52	0.52	0.62	0.62	0.53

*, **, *** denotes lines selected with 4, 5 and 6 weighting schemes, respectively.

[a] Dotted squares identify undesirable combinations due to their degree of relatedness.

APPENDICES

Appendix 1. SAS program for principal component analysis of AFLP data for 108 individuals.

```
data a; infile 'a:\pca data B genome.txt' LRECL=550;
input code $ species $ accession $ m1-m239;
proc sort; by m1-m239;
proc princomp cov out=prin ;
var m1-m239;
proc print;
run;
proc plot;
plot prin2*prin1=accession;
title 'Plot of Principal Components';
run;
```

Appendix 2. Genetic distances between the tetraploid *Arachis* sect. *Arachis* species and 91 accessions representing 24 diploid species of the same section.

			to *monticola*[a]		to *hypogaea*[b]		to both species[c]	
Accession	Species	Genome	distance[d]	rank	distance	rank	distance	rank
35005	*benensis*	A	0.66285	91	0.67248	90	0.66767	90
35006	*benensis*	A	0.65154	89	0.66432	88	0.65793	89
35007	*benensis*	A	0.65625	90	0.68235	91	0.6693	91
860	*benensis*	A	0.61671	84	0.64179	85	0.62925	85
10017	*cardenasii*	A	0.52212	64	0.54924	62	0.53568	64
36015	*cardenasii*	A	0.42672	25	0.46453	25	0.44562	25
36019	*cardenasii*	A	0.44748	28	0.46985	26	0.45866	26
36032	*cardenasii*	A	0.46325	33	0.48063	28	0.47194	32
36035	*cardenasii*	A	0.45144	29	0.48742	33	0.46943	29
19616	*correntina*	A	0.53918	71	0.56532	69	0.55225	70
36000	*correntina*	A	0.49842	54	0.54201	59	0.52022	57
7830	*correntina*	A	0.48337	38	0.49426	36	0.48882	37
7897	*correntina*	A	0.45928	31	0.48064	29	0.46996	30
9530	*correntina*	A	0.48749	42	0.50044	38	0.49396	40
12900	*decora*	A	0.55213	74	0.5937	76	0.57291	75
9953	*decora*	A	0.57483	80	0.62139	82	0.59811	82
9955	*decora*	A	0.52068	62	0.54924	62	0.53496	62
10602	*diogoi*	A	0.46203	32	0.47795	27	0.46999	31
30001	*diogoi*	A	0.48475	40	0.51925	45	0.502	45
30005	*diogoi*	A	0.61993	86	0.66108	87	0.64051	87
30106	*diogoi*	A	0.46718	35	0.51298	43	0.49008	39
10038	*duranensis*	A	0.3964	15	0.43048	15	0.41344	15
15101	*duranensis*	A	0.40264	19	0.43435	21	0.4185	20
21763	*duranensis*	A	0.40264	19	0.43113	19	0.41688	19
21766	*duranensis*	A	0.3964	15	0.43048	15	0.41344	15
21767	*duranensis*	A	0.41523	24	0.44997	24	0.4326	24
30060	*duranensis*	A	0.40264	19	0.43564	22	0.41914	21
30064	*duranensis*	A	0.3865	14	0.41769	13	0.4021	14
30067	*duranensis*	A	0.31635	4	0.32724	3	0.3218	4
30069	*duranensis*	A	0.32319	5	0.34541	5	0.3343	5
30070	*duranensis*	A	0.40134	18	0.43049	18	0.41592	18
30072	*duranensis*	A	0.36703	10	0.38642	8	0.37672	9
30074	*duranensis*	A	0.36211	8	0.40506	11	0.38359	11
30077	*duranensis*	A	0.40638	22	0.43306	20	0.41972	22
36002	*duranensis*	A	0.32784	6	0.34897	6	0.33841	6
36006	*duranensis*	A	0.38644	13	0.41769	13	0.40207	13
36036	*duranensis*	A	0.3964	15	0.43048	15	0.41344	15
7988	*duranensis*	A	0.36816	11	0.39259	10	0.38038	10
30029	*helodes*	A	0.16249	1	0.14666	1	0.15458	1

Appendix 2 (cont.).

			to *monticola*[a]		to *hypogaea*[b]		to both species[c]	
Accession	Species	Genome	distance[d]	rank	distance	rank	distance	rank
30031	*helodes*	A	0.51517	61	0.54057	58	0.52787	60
6331	*helodes*	A	0.51089	58	0.51366	44	0.51227	49
36029	*herzogii*	A	0.51229	59	0.52208	47	0.51719	54
30084	*kempff-merc.*	A	0.44737	27	0.48742	34	0.4674	28
30085	*kempff-merc.*	A	0.48076	37	0.52067	46	0.50072	43
30088	*kempff-merc.*	A	0.46348	34	0.51228	42	0.48788	35
30089	*kempff-merc.*	A	0.5137	60	0.55651	66	0.53511	63
35001	*kempff-merc.*	A	0.49594	53	0.53488	53	0.51541	52
30008	*kuhlmannii*	A	0.52073	63	0.558	67	0.53936	65
30034	*kuhlmannii*	A	0.40648	23	0.43956	23	0.42302	23
7639	*kuhlmannii*	A	0.48628	41	0.52704	48	0.50666	46
8888	*kuhlmannii*	A	0.44356	26	0.48609	32	0.46482	27
8916	*kuhlmannii*	A	0.49433	49	0.50391	40	0.49912	41
9214	*kuhlmannii*	A	0.52937	66	0.58468	74	0.55703	72
9470	*kuhlmannii*	A	0.50529	56	0.53629	54	0.52079	58
13023	*palustris*	A	0.56425	76	0.61517	81	0.58971	78
6536	*palustris*	A	0.57148	78	0.61053	79	0.59101	80
6416	*praecox*	A	0.5773	81	0.61053	79	0.59392	81
36009	*simpsonii*	A	0.27284	3	0.32784	4	0.30034	3
10309	*stenosperma*	A	0.49307	48	0.54634	60	0.51971	56
12575	*stenosperma*	A	0.49161	45	0.53057	49	0.51109	48
13256	*stenosperma*	A	0.4917	46	0.53845	57	0.51507	51
13672	*stenosperma*	A	0.53357	67	0.55359	65	0.54358	66
13796	*stenosperma*	A	0.48891	44	0.53057	49	0.50974	47
408	*stenosperma*	A	0.525	65	0.56678	71	0.54589	68
7377	*stenosperma*	A	0.49574	51	0.53843	56	0.51708	53
7762	*stenosperma*	A	0.49173	47	0.5363	55	0.51401	50
9017	*stenosperma*	A	0.49574	51	0.54637	61	0.52106	59
1117	*trinitensis*	A	0.56979	77	0.60896	78	0.58938	77
22585	*villosa*	A	0.45925	30	0.48606	31	0.47266	33
862	*villosa*	A	0.47255	36	0.50323	39	0.48789	36
30079	*batizocoi*	B	0.62606	87	0.64339	86	0.63473	86
30081	*batizocoi*	B	0.61524	83	0.6339	83	0.62457	83
30082	*batizocoi*	B	0.6467	88	0.66596	89	0.65633	88
30083	*batizocoi*	B	0.61985	85	0.63546	84	0.62766	84
9484	*batizocoi*	B	0.53786	70	0.55216	64	0.54501	67
36024	*cruziana*	B	0.58317	82	0.58538	75	0.58428	76
30006	*hoehnei*	B	0.57306	79	0.60825	77	0.59065	79
9094	*hoehnei*	B	0.5467	73	0.57273	72	0.55971	73
9140	*hoehnei*	B	0.54369	72	0.56534	70	0.55451	71
9146	*hoehnei*	B	0.53378	68	0.53061	52	0.5322	61

Appendix 2 (cont.).

			to *monticola*[a]		to *hypogaea*[b]		to both species[c]	
Accession	Species	Genome	distance[d]	rank	distance	rank	distance	rank
30076	*ipaensis*	B	0.25076	2	0.26046	2	0.25561	2
30092	*magna*	B	0.36351	9	0.38891	9	0.37621	8
30093	*magna*	B	0.37918	12	0.41514	12	0.39716	12
30011	*valida*	B	0.48757	43	0.48198	30	0.48478	34
9153	*valida*	B	0.48353	39	0.49424	35	0.48889	38
9157	*valida*	B	0.50266	55	0.49563	37	0.49914	42
1118	*williamsii*	B	0.34091	7	0.35732	7	0.34911	7
30091	*glandulifera*	D	0.53504	69	0.56531	68	0.55018	69
30098	*glandulifera*	D	0.50816	57	0.53059	51	0.51938	55
30099	*glandulifera*	D	0.56255	75	0.58166	73	0.57211	74
30100	*glandulifera*	D	0.49437	50	0.50948	41	0.50192	44

[a] includes accessions 7264, 21768, 21769, 30062, 30063.

[b] includes accessions PI 339954, NC 4, PI 501296, NM Valencia C, PI 261924, Grif 12518, PI 497615, PI 590455, A1.

[c] includes all accessions in a and b.

[d] = average distance between each accession and the tetraploid accessions as a group.

Appendix 3. SAS program for BLUP estimation of breeding values for yield.

```
data a ; infile "d:\1991-2000 Yield trial database.txt" ;
input env $ & 1-8 year 1-2 loc $ 4 test $ 6-8 entry $ & 14-28 fm 29-32
ls 34-37 ok 39-42 ss 44-47 fs 49-52 jumbo 54-57 fancy 59-62 elk 64-67
smk 69-72 meat 74-77 seed 79-83 pod 85-89 price 91-95 yield 97-100
value 102-105 jumbo_l 107-111 jumbo_a 113-117 jumbo_b 119-123 fancy_l
125-129 fancy_a 131-135 fancy_b 137-141 avg_l 143-147 defol 149-152 ;
if year<80 then year=year+2000 ; else year=year+1900 ;
fmpct=0 ;
if fm>4 then fmpct=fm-4 ;
fmpen=0.05*fmpct ;
if fmpen=. then fmpen=0 ;
spva=0.43985 ; sprun=0.43120 ; spelk=0.0175 ; spok=0.07 ; spls=0.07 ;
price=(spva*smk)+(spok*ok)+(spelk*elk)-fmpen ;
if fs<40 then price=(sprun*smk)+(spok*ok)-fmpen ;
lspen=0.01*ls*yield ; if ls=. then lspen=0 ;
value=((price*(yield-lspen))+(spls*lspen))/100 ;
drop fmpct fmpen spva sprun spelk spok spls lspen ;
if test='LAU' or test='LSU' then delete ;
proc sort data=a ; by entry ;
run ;

data b ; infile "d:\sas\Name list.txt" ;
input entry $ & 1-14 xlcode 16-18 procode 20-22 ;
proc sort data=b ; by entry ;
data a ; merge a b ; by entry ; if procode=. then delete ;
y=meat ;
mu=1 ;  if y=. then do ; y=0 ; mu=0 ; end ;
t1=0 ;  if env='89 L EAE' then t1=1 ;  if env='01 W AYT' then t1=-1 ;
t2=0 ;  if env='90 L EAE' then t2=1 ;  if env='01 W AYT' then t2=-1 ;
t3=0 ;  if env='91 L AYT' then t3=1 ;  if env='01 W AYT' then t3=-1 ;
t4=0 ;  if env='91 L EAE' then t4=1 ;  if env='01 W AYT' then t4=-1 ;
t5=0 ;  if env='91 L EAL' then t5=1 ;  if env='01 W AYT' then t5=-1 ;
t6=0 ;  if env='91 L LSS' then t6=1 ;  if env='01 W AYT' then t6=-1 ;
t7=0 ;  if env='91 L UPT' then t7=1 ;  if env='01 W AYT' then t7=-1 ;
t8=0 ;  if env='91 W AYT' then t8=1 ;  if env='01 W AYT' then t8=-1 ;
t9=0 ;  if env='92 L AYT' then t9=1 ;  if env='01 W AYT' then t9=-1 ;
t10=0 ; if env='92 L CAT' then t10=1 ; if env='01 W AYT' then t10=-1 ;
t11=0 ; if env='92 L EAE' then t11=1 ; if env='01 W AYT' then t11=-1 ;
t12=0 ; if env='92 L EAL' then t12=1 ; if env='01 W AYT' then t12=-1 ;
t13=0 ; if env='92 L LSS' then t13=1 ; if env='01 W AYT' then t13=-1 ;
t14=0 ; if env='92 L UPT' then t14=1 ; if env='01 W AYT' then t14=-1 ;
t15=0 ; if env='92 W AYT' then t15=1 ; if env='01 W AYT' then t15=-1 ;
t16=0 ; if env='93 L AYT' then t16=1 ; if env='01 W AYT' then t16=-1 ;
```

```
t17=0 ; if env='93 L CAT' then t17=1 ; if env='01 W AYT' then t17=-1 ;
t18=0 ; if env='93 L EAE' then t18=1 ; if env='01 W AYT' then t18=-1 ;
t19=0 ; if env='93 L EAL' then t19=1 ; if env='01 W AYT' then t19=-1 ;
t20=0 ; if env='93 L LSS' then t20=1 ; if env='01 W AYT' then t20=-1 ;
t21=0 ; if env='93 L UPT' then t21=1 ; if env='01 W AYT' then t21=-1 ;
t22=0 ; if env='93 W AYT' then t22=1 ; if env='01 W AYT' then t22=-1 ;
t23=0 ; if env='94 L AYT' then t23=1 ; if env='01 W AYT' then t23=-1 ;
t24=0 ; if env='94 L CAT' then t24=1 ; if env='01 W AYT' then t24=-1 ;
t25=0 ; if env='94 L EAE' then t25=1 ; if env='01 W AYT' then t25=-1 ;
t26=0 ; if env='94 L EAL' then t26=1 ; if env='01 W AYT' then t26=-1 ;
t27=0 ; if env='94 L LSS' then t27=1 ; if env='01 W AYT' then t27=-1 ;
t28=0 ; if env='94 L UPT' then t28=1 ; if env='01 W AYT' then t28=-1 ;
t29=0 ; if env='94 W AYT' then t29=1 ; if env='01 W AYT' then t29=-1 ;
t30=0 ; if env='95 L AYT' then t30=1 ; if env='01 W AYT' then t30=-1 ;
t31=0 ; if env='95 L CAT' then t31=1 ; if env='01 W AYT' then t31=-1 ;
t32=0 ; if env='95 L EAE' then t32=1 ; if env='01 W AYT' then t32=-1 ;
t33=0 ; if env='95 L EAL' then t33=1 ; if env='01 W AYT' then t33=-1 ;
t34=0 ; if env='95 L LSS' then t34=1 ; if env='01 W AYT' then t34=-1 ;
t35=0 ; if env='95 L UPT' then t35=1 ; if env='01 W AYT' then t35=-1 ;
t36=0 ; if env='95 R AYT' then t36=1 ; if env='01 W AYT' then t36=-1 ;
t37=0 ; if env='95 W AYT' then t37=1 ; if env='01 W AYT' then t37=-1 ;
t38=0 ; if env='96 L AYT' then t38=1 ; if env='01 W AYT' then t38=-1 ;
t39=0 ; if env='96 L CAT' then t39=1 ; if env='01 W AYT' then t39=-1 ;
t40=0 ; if env='96 L EAE' then t40=1 ; if env='01 W AYT' then t40=-1 ;
t41=0 ; if env='96 L EAL' then t41=1 ; if env='01 W AYT' then t41=-1 ;
t42=0 ; if env='96 L JST' then t42=1 ; if env='01 W AYT' then t42=-1 ;
t43=0 ; if env='96 L LSS' then t43=1 ; if env='01 W AYT' then t43=-1 ;
t44=0 ; if env='96 L UPE' then t44=1 ; if env='01 W AYT' then t44=-1 ;
t45=0 ; if env='96 L UPL' then t45=1 ; if env='01 W AYT' then t45=-1 ;
t46=0 ; if env='96 R AYT' then t46=1 ; if env='01 W AYT' then t46=-1 ;
t47=0 ; if env='96 R JST' then t47=1 ; if env='01 W AYT' then t47=-1 ;
t48=0 ; if env='96 W AYT' then t48=1 ; if env='01 W AYT' then t48=-1 ;
t49=0 ; if env='97 L AYT' then t49=1 ; if env='01 W AYT' then t49=-1 ;
t50=0 ; if env='97 L CAT' then t50=1 ; if env='01 W AYT' then t50=-1 ;
t51=0 ; if env='97 L JST' then t51=1 ; if env='01 W AYT' then t51=-1 ;
t52=0 ; if env='97 L LSS' then t52=1 ; if env='01 W AYT' then t52=-1 ;
t53=0 ; if env='97 L UPE' then t53=1 ; if env='01 W AYT' then t53=-1 ;
t54=0 ; if env='97 L UPL' then t54=1 ; if env='01 W AYT' then t54=-1 ;
t55=0 ; if env='97 R AYT' then t55=1 ; if env='01 W AYT' then t55=-1 ;
t56=0 ; if env='97 R JST' then t56=1 ; if env='01 W AYT' then t56=-1 ;
t57=0 ; if env='97 W AYT' then t57=1 ; if env='01 W AYT' then t57=-1 ;
t58=0 ; if env='98 L AYT' then t58=1 ; if env='01 W AYT' then t58=-1 ;
t59=0 ; if env='98 L JST' then t59=1 ; if env='01 W AYT' then t59=-1 ;
t60=0 ; if env='98 L LAS' then t60=1 ; if env='01 W AYT' then t60=-1 ;
```

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t61=0 ; if env='98 L SAT' then t61=1 ; if env='01 W AYT' then t61=-1 ;
t62=0 ; if env='98 R AYT' then t62=1 ; if env='01 W AYT' then t62=-1 ;
t63=0 ; if env='98 R JST' then t63=1 ; if env='01 W AYT' then t63=-1 ;
t64=0 ; if env='98 W AYT' then t64=1 ; if env='01 W AYT' then t64=-1 ;
t65=0 ; if env='99 L AYT' then t65=1 ; if env='01 W AYT' then t65=-1 ;
t66=0 ; if env='99 L LAS' then t66=1 ; if env='01 W AYT' then t66=-1 ;
t67=0 ; if env='99 L SAT' then t67=1 ; if env='01 W AYT' then t67=-1 ;
t68=0 ; if env='99 L UPE' then t68=1 ; if env='01 W AYT' then t68=-1 ;
t69=0 ; if env='99 R AYT' then t69=1 ; if env='01 W AYT' then t69=-1 ;
t70=0 ; if env='99 R JAT' then t70=1 ; if env='01 W AYT' then t70=-1 ;
t71=0 ; if env='00 L AYT' then t71=1 ; if env='01 W AYT' then t71=-1 ;
t72=0 ; if env='00 L JAT' then t72=1 ; if env='01 W AYT' then t72=-1 ;
t73=0 ; if env='00 L LAS' then t73=1 ; if env='01 W AYT' then t73=-1 ;
t74=0 ; if env='00 L SAT' then t74=1 ; if env='01 W AYT' then t74=-1 ;
t75=0 ; if env='00 R AYT' then t75=1 ; if env='01 W AYT' then t75=-1 ;
t76=0 ; if env='00 R JAT' then t76=1 ; if env='01 W AYT' then t76=-1 ;
t77=0 ; if env='00 W AYT' then t77=1 ; if env='01 W AYT' then t77=-1 ;
t78=0 ; if env='01 L AYT' then t78=1 ; if env='01 W AYT' then t78=-1 ;
t79=0 ; if env='01 L JAT' then t79=1 ; if env='01 W AYT' then t79=-1 ;
t80=0 ; if env='01 L LAS' then t80=1 ; if env='01 W AYT' then t80=-1 ;
t81=0 ; if env='01 L SAT' then t81=1 ; if env='01 W AYT' then t81=-1 ;
t82=0 ; if env='01 R AYT' then t82=1 ; if env='01 W AYT' then t82=-1 ;
t83=0 ; if env='01 R JAT' then t83=1 ; if env='01 W AYT' then t83=-1 ;
array z (i) z1-z132 ;
do over z ; z=0 ; if i=procode then z=1 ; end ;
proc sort data=a ; by entry loc year test ;
proc print data=a ; var env t1-t83 ;
run ;

proc mixed data=a covtest ; class env entry ;
model yield = ;
random env entry ;
run ;

proc mixed data=a covtest ; class entry ;
model yield = ;
random entry ;
run ;

data d ; infile "d:\sas\Coancestry matrix.txt" lrecl=13100 ;
input v1-v131 ;
proc iml ;
use a ;
use d ;
```

```
setin a ;
read all var {y} into y ;
read all var {mu
              t1  t2  t3  t4  t5  t6  t7  t8  t9  t10
              t11 t12 t13 t14 t15 t16 t17 t18 t19 t20
              t21 t22 t23 t24 t25 t26 t27 t28 t29 t30
              t31 t32 t33 t34 t35 t36 t37 t38 t39 t40
              t41 t42 t43 t44 t45 t46 t47 t48 t49 t50
              t51 t52 t53 t54 t55 t56 t57 t58 t59 t60
              t61 t62 t63 t64 t65 t66 t67 t68 t69 t70
              t71 t72 t73 t74 t75 t76 t77 t78 t79 t80
              t81 t82 t83} into x ;
read all
     var {z1   z2   z3   z4   z5   z6   z7   z8   z9   z10
          z11  z12  z13  z14  z15  z16  z17  z18  z19  z20
          z21  z22  z23  z24  z25  z26  z27  z28  z29  z30
          z31  z32  z33  z34  z35  z36  z37  z38  z39  z40
          z41  z42  z43  z44  z45  z46  z47  z48  z49  z50
          z51  z52  z53  z54  z55  z56  z57  z58  z59  z60
          z61  z62  z63  z64  z65  z66  z67  z68  z69  z70
          z71  z72  z73  z74  z75  z76  z77  z78  z79  z80
          z81  z82  z83  z84  z85  z86  z87  z88  z89  z90
          z91  z92  z93  z94  z95  z96  z97  z98  z99  z100
          z101 z102 z103 z104 z105 z106 z107 z108 z109 z110
          z111 z112 z113 z114 z115 z116 z117 z118 z119 z120
          z121 z122 z123 z124 z125 z126 z127 z128 z129 z130
          z131} into z ;
setin  d ;
read all
 var {v1   v2   v3   v4   v5   v6   v7   v8   v9   v10  v11  v12  v13
      v14  v15  v16  v17  v18  v19  v20  v21  v22  v23  v24  v25  v26
      v27  v28  v29  v30  v31  v32  v33  v34  v35  v36  v37  v38  v39
      v40  v41  v42  v43  v44  v45  v46  v47  v48  v49  v50  v51  v52
      v53  v54  v55  v56  v57  v58  v59  v60  v61  v62  v63  v64  v65
      v66  v67  v68  v69  v70  v71  v72  v73  v74  v75  v76  v77  v78
      v79  v80  v81  v82  v83  v84  v85  v86  v87  v88  v89  v90  v91
      v92  v93  v94  v95  v96  v97  v98  v99  v100 v101 v102 v103 v104
      v105 v106 v107 v108 v109 v110 v111 v112 v113 v114 v115 v116 v117
      v118 v119 v120 v121 v122 v123 v124 v125 v126 v127 v128 v129 v130
      v131} into c ;
cn={"BLUP" "SE"} ;

h=0.0195397182639442 ; print "Solutions for H=0.0195397182639442" ;
```

```
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.01 ; print "Solutions for H=0.01" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.02 ; print "Solutions for H=0.02" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.03 ; print "Solutions for H=0.03" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.04 ; print "Solutions for H=0.04" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.05 ; print "Solutions for H=0.05" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.06 ; print "Solutions for H=0.06" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
```

```
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.9999999 ; print "Solutions for H=1.00" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

run ;
```

Appendix 4. SAS program for BLUP estimation of breeding values for TSWV incidence.

```
data a; infile "d:\sas\tswv without x lines.txt";
input env $ 1-4 test $ 6-8 genotype $ 14-30 dis 6.4;

data b ; infile "d:\sas\Name list.txt" ;
input genotype $ & 1-14 xlcode 16-18 procode 20-22 ;
proc sort data=a ; by genotype ;
proc sort data=b ; by genotype ;
data c ; merge a b ; by genotype ;
if dis=. or procode=. then delete ;
mu=1 ;
t1=0 ;  if env='96 G' and test='SMS' then t1=1 ;  if env='01 L' and
test='DST' then t1=-1 ;
t2=0 ;  if env='96 L' and test='CIR' then t2=1 ;  if env='01 L' and
test='DST' then t2=-1 ;
t3=0 ;  if env='97 L' and test='ALT' then t3=1 ;  if env='01 L' and
test='DST' then t3=-1 ;
t4=0 ;  if env='97 L' and test='CIR' then t4=1 ;  if env='01 L' and
test='DST' then t4=-1 ;
t5=0 ;  if env='97 L' and test='TAT' then t5=1 ;  if env='01 L' and
test='DST' then t5=-1 ;
t6=0 ;  if env='97 L' and test='TWT' then t6=1 ;  if env='01 L' and
test='DST' then t6=-1 ;
t7=0 ;  if env='98 L' and test='ALT' then t7=1 ;  if env='01 L' and
test='DST' then t7=-1 ;
t8=0 ;  if env='98 L' and test='TAT' then t8=1 ;  if env='01 L' and
test='DST' then t8=-1 ;
t9=0 ;  if env='98 L' and test='TWT' then t9=1 ;  if env='01 L' and
test='DST' then t9=-1 ;
t10=0 ; if env='99 L' and test='ALT' then t10=1 ; if env='01 L' and
test='DST' then t10=-1 ;
t11=0 ; if env='99 L' and test='TAT' then t11=1 ; if env='01 L' and
test='DST' then t11=-1 ;
t12=0 ; if env='00 L' and test='ALT' then t12=1 ; if env='01 L' and
test='DST' then t12=-1 ;
t13=0 ; if env='00 L' and test='TAT' then t13=1 ; if env='01 L' and
test='DST' then t13=-1 ;
t14=0 ; if env='00 L' and test='TWT' then t14=1 ; if env='01 L' and
test='DST' then t14=-1 ;
t15=0 ; if env='01 L' and test='ALT' then t15=1 ; if env='01 L' and
test='DST' then t15=-1 ;
t16=0 ; if env='01 L' and test='TAT' then t16=1 ; if env='01 L' and
test='DST' then t16=-1 ;
```

```
t17=0 ; if env='01 L' and test='TWT' then t17=1 ; if env='01 L' and
test='DST' then t17=-1 ;
array z (i) z1-z132 ;
do over z ; z=0 ; if i=procode then z=1 ; end ;
proc sort data=c ; by env test genotype ;
proc print data=c ; var env test t1-t17 ;
run ;
proc glm data=c ; class env test procode ;
model dis=test(env) procode ;
lsmeans procode / stderr ;
run ;

proc mixed data=c covtest ;
class env test genotype ;
model dis= ;
random env test(env) genotype genotype*env ;
run ;

proc glm data=c ; class env test genotype ;
model dis= env test(env) genotype ;
lsmeans genotype / stderr ;
run ;

data d ; infile "d:\sas\Coancestry matrix.txt" lrecl=13100 ;
input v1-v131 ;
proc iml ;
use c ;
use d ;
setin c ;
read all var {dis} into y ;
read all var {mu t1 t2 t3 t4 t5 t6 t7 t8 t9 t10 t11 t12 t13 t14 t15 t16
t17} into x ;
read all
 var {z1   z2   z3   z4   z5   z6   z7   z8   z9   z10  z11  z12  z13
      z14  z15  z16  z17  z18  z19  z20  z21  z22  z23  z24  z25  z26
      z27  z28  z29  z30  z31  z32  z33  z34  z35  z36  z37  z38  z39
      z40  z41  z42  z43  z44  z45  z46  z47  z48  z49  z50  z51  z52
      z53  z54  z55  z56  z57  z58  z59  z60  z61  z62  z63  z64  z65
      z66  z67  z68  z69  z70  z71  z72  z73  z74  z75  z76  z77  z78
      z79  z80  z81  z82  z83  z84  z85  z86  z87  z88  z89  z90  z91
      z92  z93  z94  z95  z96  z97  z98  z99  z100 z101 z102 z103 z104
      z105 z106 z107 z108 z109 z110 z111 z112 z113 z114 z115 z116 z117
      z118 z119 z120 z121 z122 z123 z124 z125 z126 z127 z128 z129 z130
      z131}
```

```
     into z ;
setin  d ;
read all
 var {v1   v2   v3   v4   v5   v6   v7   v8   v9   v10  v11  v12  v13
      v14  v15  v16  v17  v18  v19  v20  v21  v22  v23  v24  v25  v26
      v27  v28  v29  v30  v31  v32  v33  v34  v35  v36  v37  v38  v39
      v40  v41  v42  v43  v44  v45  v46  v47  v48  v49  v50  v51  v52
      v53  v54  v55  v56  v57  v58  v59  v60  v61  v62  v63  v64  v65
      v66  v67  v68  v69  v70  v71  v72  v73  v74  v75  v76  v77  v78
      v79  v80  v81  v82  v83  v84  v85  v86  v87  v88  v89  v90  v91
      v92  v93  v94  v95  v96  v97  v98  v99  v100 v101 v102 v103 v104
      v105 v106 v107 v108 v109 v110 v111 v112 v113 v114 v115 v116 v117
      v118 v119 v120 v121 v122 v123 v124 v125 v126 v127 v128 v129 v130
      v131} into c ;
cn={"BLUP" "SE"} ;

h=0.051764257104711 ; print "Solutions for H=0.051764257104711" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.01 ; print "Solutions for H=0.01" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;


h=0.1 ; print "Solutions for H=0.10" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.15 ; print "Solutions for H=0.15" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;
```

```
h=0.2 ; print "Solutions for H=0.20" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.25 ; print "Solutions for H=0.25" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h)))))))) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.3 ; print "Solutions for H=0.30" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*b)) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;

h=0.9999999 ; print "Solutions for H=1.00" ;
b=inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*((x`*y)//(z`*y)) ;
se=sqrt(vecdiag(inv(((x`*x)||(x`*z))//((z`*x)||((z`*z)+inv(c#(h/(1-
h))))))*b)) ;
b_and_se=b||se ; print b_and_se [colname=cn] ;
run ;
```

Appendix 5. Ranking of lines based on their index values in each of the six weighting schemes.

	Index						Rank						Top 18 lines					
Entry	I	II	III	IV	V	VI	I	II	III	IV	V	VI	I	II	III	IV	V	VI
N91003E	0.57	0.50	0.63	0.62	0.44	0.55	21	51	20	35	50	41						
N92025	0.52	0.45	0.60	0.61	0.37	0.52	50	70	35	38	82	53						
N96029	0.50	0.43	0.54	0.50	0.41	0.47	74	81	76	95	65	79						
N97068	0.60	0.54	0.63	0.60	0.52	0.58	8	36	21	51	16	31	*				*	
N97085	0.62	0.58	0.68	0.71	0.50	0.63	2	16	1	2	33	4	*	*	*	*		*
N98001	0.58	0.50	0.66	0.64	0.42	0.56	15	54	7	16	58	37	*		*	*		
N98002	0.59	0.50	0.67	0.65	0.43	0.57	13	52	3	13	54	34	*		*	*		
N98003	0.59	0.50	0.66	0.64	0.42	0.56	14	53	5	15	57	36	*		*	*		
N98022	0.50	0.46	0.55	0.59	0.38	0.50	76	66	72	63	77	61						
N98023	0.50	0.46	0.55	0.59	0.38	0.51	73	65	71	61	76	59						
N98028	0.50	0.40	0.60	0.56	0.32	0.47	68	103	37	71	103	76						
N98032	0.49	0.40	0.56	0.52	0.35	0.46	81	100	69	87	96	90						
N98033	0.51	0.44	0.59	0.58	0.36	0.50	53	79	50	64	86	62						
N99051	0.41	0.48	0.41	0.51	0.41	0.46	100	59	102	90	66	88						
N99073	0.50	0.40	0.58	0.54	0.35	0.47	65	95	56	82	95	82						
N00001	0.52	0.57	0.52	0.59	0.52	0.56	51	26	81	58	17	40					*	
N00033	0.61	0.54	0.68	0.67	0.46	0.60	5	37	2	6	44	13	*		*	*		*
N00049	0.50	0.38	0.63	0.61	0.28	0.47	64	106	19	43	112	75						
N00052	0.50	0.37	0.64	0.61	0.28	0.47	62	107	17	45	114	77			*			
N00053	0.50	0.37	0.63	0.60	0.28	0.47	69	109	22	49	113	81						
N00054	0.50	0.37	0.63	0.60	0.27	0.47	72	110	24	47	116	83						
N00055	0.50	0.37	0.63	0.61	0.27	0.47	67	108	18	44	115	78			*			
N00058	0.51	0.38	0.64	0.61	0.28	0.47	60	105	16	46	110	73			*			
N00060	0.49	0.37	0.63	0.60	0.27	0.47	77	112	25	52	119	85						
N00061	0.49	0.37	0.62	0.60	0.27	0.46	80	113	27	54	121	87						
N00062	0.49	0.37	0.62	0.60	0.27	0.47	78	111	26	53	118	86						
N00064	0.53	0.45	0.59	0.57	0.39	0.50	48	76	40	67	73	60						
N99100ol	0.41	0.32	0.50	0.51	0.24	0.40	104	121	88	92	124	108						
N99103ol	0.41	0.33	0.50	0.51	0.25	0.40	102	119	86	93	123	107						
N99109ol	0.23	0.25	0.24	0.27	0.22	0.25	124	123	121	122	125	122						
N99113ol	0.30	0.28	0.32	0.31	0.26	0.30	116	122	111	121	122	121						
N00087ol	0.34	0.35	0.33	0.35	0.35	0.35	110	116	109	120	97	119						
N00088ol	0.44	0.41	0.45	0.42	0.42	0.42	95	88	96	110	61	102						
N00089ol	0.46	0.41	0.47	0.43	0.42	0.43	89	87	90	109	56	98						
N00090ol	0.61	0.56	0.65	0.62	0.53	0.59	4	31	11	33	10	16	*		*		*	*
N00091ol	0.61	0.56	0.65	0.62	0.53	0.59	3	32	9	34	9	17	*		*		*	*
N00095ol	0.51	0.40	0.61	0.59	0.31	0.48	58	99	30	56	107	67						
N00098ol	0.61	0.59	0.65	0.69	0.51	0.62	7	11	8	3	27	5	*	*	*	*		*
N00099ol	0.60	0.58	0.64	0.68	0.50	0.61	9	15	14	4	31	6	*	*	*	*		*
N00102ol	0.32	0.21	0.31	0.18	0.32	0.23	114	124	113	124	104	124						
N97053J	0.34	0.37	0.33	0.35	0.36	0.35	109	114	110	119	84	118						
N99066J	0.55	0.51	0.59	0.59	0.47	0.55	38	44	45	60	40	44						
N99067J	0.59	0.51	0.66	0.65	0.44	0.57	11	46	6	9	51	32	*		*	*		
N99068J	0.52	0.43	0.57	0.52	0.40	0.48	52	82	62	88	69	68						

Appendix 5 (cont.).

	Index						Rank						Top 18 lines					
Entry	I	II	III	IV	V	VI	I	II	III	IV	V	VI	I	II	III	IV	V	VI
N99079J	0.51	0.41	0.59	0.54	0.35	0.48	54	92	42	79	92	72						
N99080J	0.51	0.41	0.59	0.54	0.35	0.47	59	93	48	80	93	74						
N99085J	0.57	0.52	0.61	0.62	0.46	0.56	29	41	31	36	42	38						
N00002J	0.57	0.60	0.58	0.64	0.55	0.60	20	8	57	25	6	11		*			*	*
N00034J	0.56	0.52	0.61	0.64	0.44	0.57	33	42	29	22	49	35						
N00035J	0.59	0.53	0.65	0.66	0.46	0.58	12	39	10	7	47	25	*		*	*		
N00065J	0.50	0.40	0.59	0.57	0.32	0.48	70	101	38	66	105	71						
N99057F	0.32	0.44	0.31	0.45	0.35	0.40	113	80	114	106	94	109						
N92054C	0.46	0.44	0.47	0.45	0.43	0.45	87	78	91	105	52	95						
N94040C	0.45	0.47	0.45	0.47	0.46	0.46	92	64	97	101	48	89						
N95025C	0.53	0.50	0.59	0.64	0.41	0.55	45	48	41	17	64	43				*		
N96006C	0.41	0.38	0.46	0.48	0.31	0.42	99	104	92	99	106	105						
N96009C	0.42	0.35	0.50	0.50	0.27	0.41	97	118	89	97	117	106						
N97122C	0.50	0.40	0.59	0.55	0.33	0.47	71	102	46	76	100	84						
N97129C	0.50	0.35	0.64	0.56	0.27	0.45	63	117	13	70	120	94			*			
N97131C	0.46	0.41	0.50	0.51	0.36	0.45	90	89	85	94	89	93						
N97135C	0.46	0.40	0.52	0.54	0.33	0.46	88	98	79	81	101	91						
N97137C	0.49	0.41	0.57	0.55	0.34	0.48	79	86	63	75	98	70						
N97138C	0.48	0.41	0.56	0.56	0.33	0.47	82	91	68	74	99	80						
N97140C	0.53	0.45	0.60	0.59	0.37	0.51	47	72	33	57	79	55						
N97142C	0.54	0.45	0.62	0.60	0.38	0.52	43	75	28	55	78	54						
N98048CSm	0.41	0.42	0.43	0.48	0.37	0.43	101	84	101	100	80	97						
N98052C	0.48	0.45	0.51	0.53	0.40	0.48	83	69	83	84	71	69						
N99121CSm	0.54	0.57	0.55	0.61	0.51	0.57	44	27	73	40	22	33						
N99122CSm	0.55	0.59	0.56	0.65	0.52	0.59	41	9	67	10	20	18		*		*		*
N99128CSm	0.53	0.53	0.56	0.60	0.47	0.55	46	40	70	48	39	42						
N99129CSm	0.56	0.48	0.63	0.61	0.41	0.54	34	60	23	42	63	47						
N99130CSm	0.57	0.48	0.64	0.61	0.42	0.54	26	58	15	41	59	46			*			
N99131CSm	0.45	0.56	0.44	0.56	0.49	0.52	93	30	99	72	35	52						
N99132CSm	0.51	0.58	0.51	0.61	0.50	0.56	57	20	82	39	32	39						
N99133CSm	0.60	0.64	0.60	0.68	0.58	0.64	10	3	34	5	3	2	*	*		*	*	*
N99137CSm	0.50	0.49	0.52	0.53	0.46	0.51	61	55	80	83	46	58						
N99138CSm	0.52	0.50	0.54	0.55	0.47	0.52	49	50	74	77	38	51						
N00076CSm	0.55	0.51	0.58	0.59	0.46	0.55	42	45	55	59	45	45						
N00077CSm	0.51	0.49	0.53	0.53	0.46	0.51	55	56	77	85	43	57						
N92066L	0.36	0.45	0.36	0.50	0.35	0.43	107	74	107	96	90	100						
N92068L	0.00	0.00	0.00	0.00	0.00	0.00	126	126	126	126	126	126						
N93003L	0.31	0.52	0.28	0.49	0.41	0.43	115	43	116	98	68	99						
N93007L	0.00	0.00	0.00	0.00	0.00	0.00	126	126	126	126	126	126						
N94015L	0.24	0.40	0.22	0.42	0.28	0.34	123	96	124	111	109	120						
N96074L	0.17	0.33	0.14	0.27	0.28	0.25	125	120	125	123	111	123						
N96076L	0.00	0.00	0.00	0.00	0.00	0.00	126	126	126	126	126	126						
N97104L	0.47	0.55	0.46	0.57	0.48	0.52	85	35	93	68	36	50						

Appendix 5 (cont.).

	Index						Rank						Top 18 lines					
Entry	I	II	III	IV	V	VI	I	II	III	IV	V	VI	I	II	III	IV	V	VI
N97106L	0.42	0.46	0.41	0.46	0.43	0.44	98	67	103	104	53	96						
N97109L	0.50	0.53	0.50	0.57	0.48	0.53	75	38	87	69	37	49						
N99027L	0.58	0.66	0.57	0.66	0.61	0.63	16	2	61	8	2	3	*	*		*	*	*
N99034L	0.43	0.50	0.43	0.54	0.43	0.49	96	49	100	78	55	66						
N00009L	0.26	0.44	0.23	0.40	0.36	0.36	122	77	123	116	88	117						
N00010L	0.27	0.46	0.24	0.41	0.37	0.37	120	68	120	114	81	114						
N00011L	0.26	0.45	0.23	0.40	0.36	0.36	121	73	122	115	85	116						
N00012L	0.32	0.49	0.29	0.46	0.41	0.42	112	57	115	102	67	104						
N00019L	0.38	0.45	0.37	0.46	0.40	0.43	105	71	105	103	70	101						
N00020L	0.34	0.41	0.33	0.42	0.36	0.39	108	90	108	113	83	112						
N00022L	0.46	0.58	0.45	0.57	0.51	0.53	86	21	98	65	28	48						
N00023L	0.29	0.47	0.26	0.43	0.38	0.39	117	62	118	107	74	110						
N00024L	0.29	0.47	0.26	0.43	0.38	0.39	118	63	119	108	75	111						
N97064NT	0.41	0.56	0.38	0.52	0.49	0.49	103	33	104	86	34	63						
N01001T	0.57	0.60	0.58	0.64	0.55	0.60	23	7	58	19	7	10		*			*	*
N01002T	0.56	0.59	0.58	0.64	0.53	0.59	30	12	59	27	12	19		*			*	
N01003T	0.55	0.57	0.57	0.63	0.51	0.58	37	24	64	30	25	29						
N01004T	0.55	0.58	0.57	0.63	0.51	0.58	39	19	65	31	21	28						
N01005T	0.55	0.57	0.57	0.63	0.51	0.58	40	23	66	32	24	30						
N01006T	0.56	0.57	0.57	0.63	0.51	0.58	35	22	60	29	23	27						
N01007T	0.56	0.57	0.58	0.64	0.50	0.58	32	29	54	28	30	26						
N01008T	0.56	0.57	0.58	0.64	0.51	0.58	31	28	53	26	29	24						
N01009T	0.57	0.59	0.59	0.64	0.53	0.59	25	14	49	21	14	15		*			*	*
N01010T	0.57	0.59	0.59	0.64	0.53	0.59	24	13	47	20	13	14		*			*	*
N01011T	0.58	0.59	0.60	0.65	0.53	0.60	19	10	36	14	11	12		*		*	*	*
N01012T	0.57	0.58	0.59	0.64	0.52	0.59	28	18	52	24	19	22		*				
N01013T	0.57	0.58	0.59	0.64	0.52	0.59	27	17	51	23	18	21		*			*	
N01014T	0.58	0.62	0.59	0.65	0.56	0.61	17	4	43	11	4	7	*	*		*	*	*
N01015T	0.58	0.61	0.59	0.65	0.56	0.61	18	6	44	12	5	9	*	*		*	*	*
N01016T	0.57	0.57	0.59	0.64	0.51	0.59	22	25	39	18	26	20				*		
N01017T	0.50	0.47	0.54	0.56	0.42	0.51	66	61	75	73	60	56						
NC 7	0.61	0.55	0.64	0.62	0.52	0.59	6	34	12	37	15	23	*		*		*	
NC 9	0.00	0.00	0.00	0.00	0.00	0.00	126	126	126	126	126	126						
NC 10C	0.00	0.00	0.00	0.00	0.00	0.00	126	126	126	126	126	126						
NC-V 11	0.36	0.36	0.37	0.37	0.36	0.36	106	115	106	118	87	115						
NC 12C	0.51	0.42	0.61	0.60	0.32	0.49	56	85	32	50	102	64						
Gregory	0.63	0.61	0.66	0.71	0.54	0.64	1	5	4	1	8	1	*	*	*	*	*	*
Perry	0.47	0.41	0.53	0.51	0.35	0.45	84	94	78	91	91	92						
VA-C 92R	0.44	0.40	0.46	0.42	0.41	0.42	94	97	94	112	62	103						
VA 93B	0.00	0.00	0.00	0.00	0.00	0.00	126	126	126	126	126	126						
VA 98R	0.28	0.17	0.26	0.13	0.31	0.18	119	125	117	125	108	125						
Wilson	0.46	0.50	0.45	0.52	0.47	0.49	91	47	95	89	41	65						
Georg. Green	0.34	0.42	0.31	0.39	0.40	0.38	111	83	112	117	72	113						
PI 576636	0.56	0.68	0.51	0.59	0.70	0.61	36	1	84	62	1	8		*			*	*

Appendix 6. Tests of independence for evaluating the null hypothesis that there were no significant differences in TSWV infection (S, R) between the two genotypes (0, 1) at each marker locus.

Marker	n_{00}	n_{01}	n_{10}	n_{11}	$n_{0\cdot}$	$n_{1\cdot}$	$n_{\cdot 0}$	$n_{\cdot 1}$	$n_{\cdot\cdot}$	e_{00}	e_{01}	e_{10}	e_{11}	X^2	Prob	
E32M61-1	22	38	46	70	60	116	68	108	176	23.2	36.8	44.8	71.2	0.05	0.82	
E32M61-2	45	75	23	33	120	56	68	108	176	46.4	73.6	21.6	34.4	0.08	0.77	
E32M61-3	23	36	45	72	59	117	68	108	176	22.8	36.2	45.2	71.8	0.01	0.92	
E32M61-4	38	43	25	63	81	88	63	106	169	30.2	50.8	32.8	55.2	5.41	0.02	*
E32M61-5	46	44	22	64	90	86	68	108	176	34.8	55.2	33.2	52.8	11.04	0.00	**
E33M56-1	60	79	8	29	139	37	68	108	176	53.7	85.3	14.3	22.7	4.85	0.03	*
E33M56-2	15	23	53	85	38	138	68	108	176	14.7	23.3	53.3	84.7	0.00	0.95	
E33M56-3	9	7	59	100	16	159	68	107	175	6.22	9.78	61.8	97.2	1.51	0.22	
E33M56-4	17	28	50	80	45	130	67	108	175	17.2	27.8	49.8	80.2	0.01	0.92	
E33M56-5	24	29	44	76	53	120	68	105	173	20.8	32.2	47.2	72.8	0.81	0.37	
E33M56-6	19	44	49	64	63	113	68	108	176	24.3	38.7	43.7	69.3	2.44	0.12	
E33M56-7	56	65	11	43	121	54	67	108	175	46.3	74.7	20.7	33.3	9.54	0.00	**
E33M56-8	35	39	33	69	74	102	68	108	176	28.6	45.4	39.4	62.6	3.43	0.06	†
E33M56-9	59	73	9	35	132	44	68	108	176	51	81	17	27	7.19	0.01	**
E33M56-10	33	44	35	64	77	99	68	108	176	29.8	47.3	38.3	60.8	0.74	0.39	
E33M57-1	17	38	50	69	55	119	67	107	174	21.2	33.8	45.8	73.2	1.52	0.22	
E33M57-2	30	44	38	64	74	102	68	108	176	28.6	45.4	39.4	62.6	0.08	0.78	
E33M57-3	28	38	40	69	66	109	68	107	175	25.6	40.4	42.4	66.6	0.35	0.55	
E33M57-4	26	34	41	73	60	114	67	107	174	23.1	36.9	43.9	70.1	0.62	0.43	
E33M57-5	14	27	53	81	41	134	67	108	175	15.7	25.3	51.3	82.7	0.19	0.66	
E33M57-6	27	40	41	68	67	109	68	108	176	25.9	41.1	42.1	66.9	0.04	0.84	
E33M57-7	20	30	48	78	50	126	68	108	176	19.3	30.7	48.7	77.3	0.00	0.95	
E33M57-8	29	39	37	69	68	106	66	108	174	25.8	42.2	40.2	65.8	0.75	0.39	
E36M51-1	40	45	28	63	85	91	68	108	176	32.8	52.2	35.2	55.8	4.26	0.04	*
E36M51-2	38	41	30	67	79	97	68	108	176	30.5	48.5	37.5	59.5	4.72	0.03	*
E36M51-3	30	44	38	64	74	102	68	108	176	28.6	45.4	39.4	62.6	0.08	0.78	
E36M51-4	40	62	28	46	102	74	68	108	176	39.4	62.6	28.6	45.4	0.00	0.98	
E36M51-5	22	32	45	75	54	120	67	107	174	20.8	33.2	46.2	73.8	0.06	0.81	
E36M51-6	22	23	46	85	45	131	68	108	176	17.4	27.6	50.6	80.4	2.13	0.14	
E36M54-1	27	42	40	66	69	106	67	108	175	26.4	42.6	40.6	65.4	0.00	0.98	
E36M54-2	28	39	40	69	67	109	68	108	176	25.9	41.1	42.1	66.9	0.26	0.61	
E36M54-3	24	19	43	89	43	132	67	108	175	16.5	26.5	50.5	81.5	6.46	0.01	*
E36M54-4	26	42	39	66	68	105	65	108	173	25.5	42.5	39.5	65.5	0.00	0.99	
E36M54-5	18	42	50	66	60	116	68	108	176	23.2	36.8	44.8	71.2	2.34	0.13	
E36M54-6	18	40	50	68	58	118	68	108	176	22.4	35.6	45.6	72.4	1.66	0.20	
E36M54-7	31	41	37	67	72	104	68	108	176	27.8	44.2	40.2	63.8	0.71	0.40	
E36M54-8	64	108	4	0	172	4	68	108	176	66.5	106	1.55	2.45	4.12	0.04	*
E37M52-1	22	32	45	76	54	121	67	108	175	20.7	33.3	46.3	74.7	0.08	0.78	
E37M52-2	19	29	49	79	48	128	68	108	176	18.5	29.5	49.5	78.5	0.00	0.99	
E37M52-3	26	43	42	65	69	107	68	108	176	26.7	42.3	41.3	65.7	0.00	0.96	

Appendix 6 (cont.)

Marker	n_{00}	n_{01}	n_{10}	n_{11}	$n_{0\cdot}$	$n_{1\cdot}$	$n_{\cdot 0}$	$n_{\cdot 1}$	$n_{\cdot\cdot}$	e_{00}	e_{01}	e_{10}	e_{11}	X^2	Prob	
E37M52-4	15	30	51	77	45	128	66	107	173	17.2	27.8	48.8	79.2	0.35	0.55	
E38M53-1	32	43	35	64	75	99	67	107	174	28.9	46.1	38.1	60.9	0.68	0.41	
E38M53-2	21	45	47	63	66	110	68	108	176	25.5	40.5	42.5	67.5	1.64	0.20	
E38M53-3	18	21	50	85	39	135	68	106	174	15.2	23.8	52.8	82.2	0.71	0.40	
E38M53-4	19	26	49	82	45	131	68	108	176	17.4	27.6	50.6	80.4	0.16	0.69	
E38M53-5	24	50	44	58	74	102	68	108	176	28.6	45.4	39.4	62.6	1.65	0.20	
E38M59-1	30	46	38	62	76	100	68	108	176	29.4	46.6	38.6	61.4	0.00	0.97	
E38M59-2	27	51	41	57	78	98	68	108	176	30.1	47.9	37.9	60.1	0.67	0.41	
E38M59-3	20	35	48	72	55	120	68	107	175	21.4	33.6	46.6	73.4	0.08	0.77	
E38M59-4	20	24	47	84	44	131	67	108	175	16.8	27.2	50.2	80.8	0.91	0.34	
E38M59-5	13	21	55	84	34	139	68	105	173	13.4	20.6	54.6	84.4	0.00	0.96	
E38M59-6	30	38	38	70	68	108	68	108	176	26.3	41.7	41.7	66.3	1.05	0.30	
E38M59-7	21	16	47	92	37	139	68	108	176	14.3	22.7	53.7	85.3	5.56	0.02	*
E38M59-8	27	49	40	59	76	99	67	108	175	29.1	46.9	37.9	61.1	0.25	0.62	
E38M59-9	22	39	45	65	61	110	67	104	171	23.9	37.1	43.1	66.9	0.21	0.65	
E38M59-10	12	19	56	89	31	145	68	108	176	12	19	56	89	0.04	0.85	
E41M49-1	26	33	39	70	59	109	65	103	168	22.8	36.2	42.2	66.8	0.79	0.38	
E41M49-2	10	9	56	95	19	151	66	104	170	7.38	11.6	58.6	92.4	1.13	0.29	
E41M49-3	11	16	55	85	27	140	66	101	167	10.7	16.3	55.3	84.7	0.01	0.94	
E41M49-4	20	42	46	62	62	108	66	104	170	24.1	37.9	41.9	66.1	1.36	0.24	
E41M52-1	27	39	41	68	66	109	68	107	175	25.6	40.4	42.4	66.6	0.07	0.78	
E41M52-2	27	32	41	76	59	117	68	108	176	22.8	36.2	45.2	71.8	1.48	0.22	
E41M52-3	28	45	40	63	73	103	68	108	176	28.2	44.8	39.8	63.2	0.01	0.93	
E41M52-4	17	36	51	71	53	122	68	107	175	20.6	32.4	47.4	74.6	1.09	0.30	
E41M52-5	17	40	50	68	57	118	67	108	175	21.8	35.2	45.2	72.8	2.06	0.15	
E41M52-6	10	17	58	89	27	147	68	106	174	10.6	16.4	57.4	89.6	0.00	0.98	
E45M48-1	23	30	45	75	53	120	68	105	173	20.8	32.2	47.2	72.8	0.32	0.57	
E45M48-2	20	23	48	85	43	133	68	108	176	16.6	26.4	51.4	81.6	1.08	0.30	
E45M48-3	10	13	58	94	23	152	68	107	175	8.94	14.1	59.1	92.9	0.07	0.80	
E45M48-4	12	14	56	94	26	150	68	108	176	10	16	58	92	0.40	0.53	
E45M48-5	20	21	46	87	41	133	66	108	174	15.6	25.4	50.4	82.6	2.11	0.15	
E45M48-6	27	32	41	76	59	117	68	108	176	22.8	36.2	45.2	71.8	1.48	0.22	
E45M48-7	11	10	57	98	21	155	68	108	176	8.11	12.9	59.9	95.1	1.30	0.25	
E45M48-8	19	45	49	63	64	112	68	108	176	24.7	39.3	43.3	68.7	2.83	0.09	†
E45M48-9	19	29	48	77	48	125	67	106	173	18.6	29.4	48.4	76.6	0.00	0.98	
E45M48-10	24	24	43	84	48	127	67	108	175	18.4	29.6	48.6	78.4	3.19	0.07	†
E45M48-11	10	6	58	101	16	159	68	107	175	6.22	9.78	61.8	97.2	3.12	0.08	†
E45M48-12	16	23	52	85	39	137	68	108	176	15.1	23.9	52.9	84.1	0.03	0.87	
E45M48-13	13	19	55	89	32	144	68	108	176	12.4	19.6	55.6	88.4	0.00	0.96	
E45M48-14	16	31	52	75	47	127	68	106	174	18.4	28.6	49.6	77.4	0.43	0.51	
E45M48-15	25	29	43	79	54	122	68	108	176	20.9	33.1	47.1	74.9	1.49	0.22	

Appendix 6 (cont.)

Marker	n_{00}	n_{01}	n_{10}	n_{11}	$n_{0\cdot}$	$n_{1\cdot}$	$n_{\cdot 0}$	$n_{\cdot 1}$	$n_{\cdot\cdot}$	e_{00}	e_{01}	e_{10}	e_{11}	X^2	Prob	
E45M48-16	14	20	54	88	34	142	68	108	176	13.1	20.9	54.9	87.1	0.02	0.89	
E46M47-1	18	22	46	80	40	126	64	102	166	15.4	24.6	48.6	77.4	0.60	0.44	
E46M47-2	19	28	46	77	47	123	65	105	170	18	29	47	76	0.03	0.85	
E46M47-3	40	45	25	60	85	85	65	105	170	32.5	52.5	32.5	52.5	4.88	0.03	*
E46M47-4	22	47	43	58	69	101	65	105	170	26.4	42.6	38.6	62.4	1.56	0.21	
E46M47-5	26	25	39	80	51	119	65	105	170	19.5	31.5	45.5	73.5	4.27	0.04	*
E46M49-1	19	30	40	76	49	116	59	106	165	17.5	31.5	41.5	74.5	0.12	0.73	
E46M49-2	12	26	48	80	38	128	60	106	166	13.7	24.3	46.3	81.7	0.23	0.63	
E46M49-3	60	97	0	9	157	9	60	106	166	56.7	100	3.25	5.75	3.86	0.05	*
E46M49-4	21	28	39	78	49	117	60	106	166	17.7	31.3	42.3	74.7	0.98	0.32	
E46M49-5	20	45	40	61	65	101	60	106	166	23.5	41.5	36.5	64.5	0.98	0.32	
E46M49-6	21	41	38	63	62	101	59	104	163	22.4	39.6	36.6	64.4	0.10	0.75	
E46M49-7	12	32	48	74	44	122	60	106	166	15.9	28.1	44.1	77.9	1.55	0.21	
E46M49-8	49	56	14	50	105	64	63	106	169	39.1	65.9	23.9	40.1	9.42	0.00	**
E46M49-9	13	28	50	78	41	128	63	106	169	15.3	25.7	47.7	80.3	0.44	0.51	
E46M49-10	27	44	36	62	71	98	63	106	169	26.5	44.5	36.5	61.5	0.00	0.99	
E46M49-11	13	20	51	86	33	137	64	106	170	12.4	20.6	51.6	85.4	0.00	0.98	
E46M49-12	22	42	42	65	64	107	64	107	171	24	40	40	67	0.23	0.64	
E46M49-13	6	11	59	96	17	155	65	107	172	6.42	10.6	58.6	96.4	0.00	0.97	
E46M49-14	29	42	36	65	71	101	65	107	172	26.8	44.2	38.2	62.8	0.28	0.59	
E46M49-15	53	61	12	46	114	58	65	107	172	43.1	70.9	21.9	36.1	9.82	0.00	**

†, *, ** significant at the 0.10, 0.05, and 0.01 levels, respectively.