FIELD EVALUATION OF TRANSGENIC PEANUT LINES FOR RESISTANCE TO SCLEROTINIA BLIGHT AND YIELD

By

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FIELD EVALUATION OF TRANSGENIC PEANUT

LINES FOR RESISTANCE TO SCLEROTINIA

BLIGHT AND YIELD

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NOMENCLATURE

ANOVA: Analysis of Variance

EPA: Environmental Protection Agency

ICRISAT: International Crop for Research Institute for the Semi- Arid Tropics

LASPAU Academic and Professional Programs for the Americas

MP: Main Plot

NAFTA: North American Free Trade Agreement

NASS National Agricultural Statistic Services

OAS Organization of American States

PERCENT TOTSMK: The percentage of total sound mature kernels equals the percent Summation of sound mature kernels (SMK) and sound splits

PERCENT OK: The percentage of other kernels that pass through the minimum screen

PERCENT DK: The percentage of damaged kernels which ride the minimum screen size for SMK's and the defective splits.

g/100 SMK(SDWT100): The weight in grams per 100 sound mature kernels (seeds).

RBD: Randomize Block Design

SWR: Southwest Runner

SP: Sub-Plot

USDA: United States Department of Agriculture

USDA-ARS: United States Department of Agriculture- Agriculture Research Service

CHAPTER I

1.0 INTRODUCTION

1.1 BACKGROUND

1.1.1 ORIGIN OF PEANUT, *Arachis hypogaea* L.,

Peanut, *Arachis hypogaea* L., is a New World crop that originated in South America and was "domesticated in the Gran Chaco area including the valleys of Paraguay and Parana Rivers (Purseglove 1968)." The crop was found to be extensively cultivated in Mesoamerica, South America and the islands of the West Indies by early European explorers in the 1500s. While no one is certain of the exact date of domestication, records from archeological sites in Peru put the crop in agricultural use as early as 800BC. In North America, the crop was first cultivated in Mexico about the time of Christ, but there is no record of the crop in cultivation in the United States of America (USA), prior to Columbian time (Purseglove 1968).

The earliest history of the crop is revealed in arts and artifacts of the early Peruvian civilization, which began along the eastern slopes of the Andes. The discovery of well-preserved groundnut fruits in terracotta jars and funerary vases, which were decorated with well-sculptured groundnut pods, reveals the esteem to which this crop was held by those cultures. As this civilization became more technologically advanced, peanut was then cultivated in small irrigation systems along-side other crops such as maize, manioc, and potatoes (Hammons 1994).

1.1.2 THE TAXONOMY AND GENETICS OF *A. hypogaea* L.

The genus Arachis L., belongs to the family Leguminnosae and comprises a very large and diverse group of diploid and tetraploid taxa. In excess of 50 species exist in this genus; all indigenous to the area east of the Andes and south of the Amazon rainforest (Hammons 1994). The diversity of this genus is evident by the numbers of annuals and perennials it comprises and their varied forms of reproduction. Most of the species reproduce sexually, but some do reproduce a sexually by forming rhizomes (Knauft et al., 1999).

The members of this genus can be identified and characterized by their "alternately attached basal and dorsal anthers, flowers in the terminal or axillary spikes or small heads, pinnate leaves and leaflets few without stipules (Stalker 1985)."

Arachis hypogaea L., was the first species, in this genus, to be described by Linnnaeus. However, over twenty-two species of the genus including *A. villosa* Benth, *A. prostrate* Benth, *A. tuberosa* Benth, *A. glabrata* Benth, *A. repens* H and *A. pusilla*, have now been described and characterized (Stalker 1985). *A. hypogaea* L., the principal genotype, and *A.villosa* are grown for their edible seeds while some of the others were adopted for varying uses, including grazing and ground cover (Hammons1994).

A. hypogaea L., is one of two tetraploid species (2n = 4x = 40) found in the *Arachis* section. The other tetraploid is *A. monticola.* Most of the other species are diploid, hence contain 2n= 20 complement of chromosomes (Stalker 1992). Cytological characterization of *A. hypogaea* L., reveals two distinctive pairs of

chromosomes; the distinctively smaller pair of chromosomes, the A chromosomes, and the pair of B chromosomes, which have a secondary constriction and are satellited. The cytological differentiation between the two genomes of *A. hypogaea* L., is indicated by the presence of only one pair of A chromosomes (Smartt et al., 1978)." *A. hypogaea* L., is thus a segmental allotetraploid, which combines the A and B genomes. During meiosis, chromosomes mostly pair as bivalents. However, quadrivalents can sometimes occur (Stalker 1992).

Although the exact origin of the A and B genomes remain unclear, six A genome species, *A. cardenasii, A. chacoense, A. correntina, A. duranensis nom.nud., A.villosa* and *A. ipaensis nom.nud* and one B genome species, *A. batizocoi,* have been identified as possible progenitors of *A. hypogaea* L., Interspecific hybrids of *A. batizocoi* have been noted to be sterile, but upon doubling of the chromosome complement, such hybrids may be able to produce stable, fertile amphidiploids. Interspecific hybridization involving *A. batizocoi* could explain the origin of A. *hypogaea* L., (Smartt et al., 1978). However, a number of molecular studies including RFLPs and chloroplast analyses indicate that *A. batizocoi* may not be a progenitor of the cultivated species, but A. *duranensis* had the same banding profile as *A. hypogaea* L., and *A. monticola* (Stalker 1992).

A. hypogaea L., is divided into two subspecies, *hypogaea* and *fastigiata* (Table1), each with two botanical varieties. The cultivated lines of Spanish, sub-species *fastigiata* var.*vulgaris,* and Valencia, *fastigiata* var *fastigiata,* are observed in the *fastigiata* subspecies. The *hypogaea* subspecies is divided into the Virginia and Runner type varieties. The subspecie *hypogaea* is characterized by the absence of flowers on

the main stem and has pairs of vegetative and reproductive axes alternating along the lateral branches. The sub-specie *fastigiata* has a sequential arrangement of reproductive axes along lateral branches and has flowers on the main stem (Ramanatha Rao and Murty 1994).

Table 1: The classification of groundnut into varieties and botanical types (*Arachis hypogaea* L.,)

Subspecies	Variety	Botanical type	Branching pattern	Growth habit	Seed/pod
hypogaea	*hypogaea*	Virginia	Alternate	Prostrate to erect	2-3
	hirsuta	Peruvian Runner	Alternate	Prostrate	2-4
fastigiata	*fastigiata*	Valencia	Sequential	Erect	3-5
	vulgaris	Spanish	Sequential	Erect	2

(Ramanatha Rao and Murty 1994)

1.1.3 MORPHOLOGY OF ARACHIS

The plant *Arachis hypogaea* L., is an erect or trailing annual herbaceous legume with three or four leaflets, stipulate leaves, the characteristic papilionate flowers, a tubular hypanthium and subterranean fruits. The peg is unique to the species and is an expanded intercalary meristem at the base of the basal ovule. The intercalary meristem expands into a carpel which contains one to five segments, each containing a single seed. The sub-specific classification is characterized on branching pattern, the presence or absence of reproductive axes (inflorescence) on the main stem and arrangement of vegetative and reproductive axes on primary laterals (Ramanatha Rao and Murty 1994).

ROOTS

A. hypogaea L., has evolved a well-developed taproot with many lateral roots unlike its other wild relatives, that have tuberous roots. Adventitious roots develop from the hypocotyls and aerial branches. Because there is no true epidermis (hence the absence of root hairs) absorption takes place 8-10mm behind the root cap. Nodules can normally be found on roots (Purseglove 1968).

STEM

The terminal bud of the epicotyl gives rise to the main stem. The first two lateral monopodial branches later develop from buds in the axils of the cotyledons. Nodes on the main axis give rise to monopodial vegetative branches. Secondary monopodial branches may be produced from the lateral branches. Reduced reproductive branches are produced from the monopodial branches. The monopodial

vegetative and reduced reproductive branches are of two distinct types (Purseglove 1968).

ALTERNATE BRANCHED FORM

This branching pattern (Figure 1) occurs in the Runner (prostrate) and the spreading branch forms of the Virginia cultivars. There are no reproductive axes on the main stem. Alternating pairs of vegetative and reproductive branches arise from the lateral monopodial branches (Ramanatha Rao and Murty 1994).

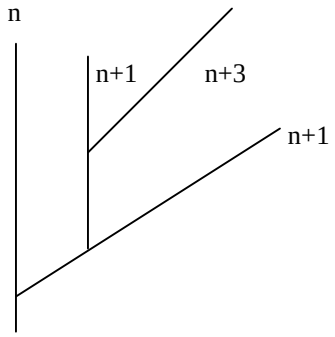

Figure 1: Alternate Branching Pattern of Runner and Virginia Cultivars (Ramanatha Rao and Murty 1994).

n Main Stem

n+1 Primary Lateral Branch

n+2 Secondary Branch

n+3 Tertiary Branch

SEQUENTIAL BRANCHED FORM

This branching pattern (Figure 2) occurs in the true erect branched forms of the Spanish and Valencia cultivars. Reproductive axes are borne on the main stem and are arranged in a continuous series on successive nodes of lateral branches (Ramanatha Rao and Murty 1994).

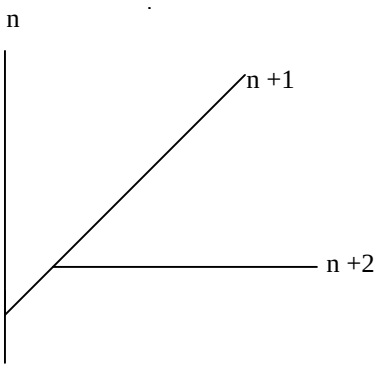

Figure 2: Sequential Branching Pattern of Spanish and Valencia Cultivars (Ramanatha Rao and Murty 1994).

n Main Stem
n+1 Primary Lateral Branch
n+2 Secondary Branch
n+3 Tertiary Branch

LEAVES

The primordia of the seeds give rise to the first leaves. The leaves, except in Trifoliolatae, are borne spirally in a 2/5 phyllotaxy. The leaves are paripinnate; leaflets which are borne on a slender, grooved and jointed rachis, are opposite, subsessile and elliptic. Stipules are present and prominent (Ramanatha Rao and Murty 1994).

FLOWERS

Flowers emerge on compressed spikes in the axils of foliage leaves. Flowers are never borne at the same node as vegetative branches. The flower is sessile. The calyx is

composed of five lobes (four fused) and elongates as the bud develops. There are five petals at the top of the calyx tube; 10 stamens (two fused) four stamens with oblong, locular anthers, four stamens with smaller globose anthers; pistil is composed of the ovary of single sessile carpel with 2-6 ovules (Purseglove 1968).

FRUITS

Post fertilization, the ovary begins to grow after the activation of the intercalary meristem below the ovary. The peg is a stalk-like structure that carries the fertilized ovules at its tip. Upon entering the soil, the peg then begins to develop into the fruit (Ramanatha Rao and Murty 1994).

1.1.4 DISTRIBUTION AND DISPERSAL

DISTRIBUTION

In its native habitat, peanut's geographic distribution ranges from near the equator to approximately 34^{o} S in southern Uruguay. From East to West, the plant is distributed from the Atlantic Ocean to the foothills of the Andes. The peanut plant can be found growing from sea level to an elevation of 1450 m. It is found growing in diverse ecological zones ranging from open grasslands to broken forests; from floodplains to semi arid regions (Stalker 1985)."Because of the geocarpic nature of the fruit, species distribution generally follows major river valleys (Stalker 1985)."

As a crop peanut can be found cultivated 40^{o} north and south of the equator. Because it is a warm season crop, peanut is very susceptible to cold weather and will be killed by frost. Peanut is cultivated in areas that normally receive 40 inches or more of annual rainfall. In the growing season there should be a minimum of 20 inches

annually. The crop prefers well drained, loose, friable, sandy loam soils which are well supplied with calcium and moderate amounts of organic matter (Purseglove 1968).

The crop requirements for well-drained sandy soils are biased towards the ecological preference for cultivation. Because of the greater genetic diversity that exists in the wild species, as compared to the cultivated varieties, *Arachis hypogaea* L. is adapted to a wide range of environments. Wild species can be found growing on ill-drained soils, in running water, heavy soils and rock outcrops (Valls et al., 1985).

DISPERSAL

Prior to the colonization of the New World, there is no credible historical record of the cultivation of peanuts outside of the Americas. The Portuguese are credited with the initial dispersal of the crop to Africa and India. Groundnuts from Brazil were first introduced to Africa then to India via the Portuguese trading ships. From Peru to Mexico, then across the Pacific, the Peruvian type *A. hypogaea* var. *hirsuta* was transported to China, Indonesia and Madagascar as an item of trade prior to 1815 (Valls et al., 1985).

The exact introduction into the United States of America remains obscured. It may have been introduced indirectly by European farmers or directly from South American and Central American agricultural societies (Valls et al., 1985). There is also some evidence that the crop may have been brought from Africa to North America by slave traders, who used it as food for the slaves during the middle passage (Woodroof 1966).

Early records from colonial America mention the crop being grown in Virginia and North Carolina. However, it was only after the Civil War in 1865 that extensive

cultivation began. Due to the ravages of the Cotton Boll Weevil in 1920, farmers from Alabama, Florida and Georgia substituted peanuts for cotton (Woodroof 1966). The need for peanut oil, food, and feed during World Wars I and II, and the many food and industrial products generated from peanuts by George Washington Carver, were significant contributing factors for the expansion of acreages and development of the peanut industry in North America (Woodroof 1966).

1.1.5 PRODUCTION SYSTEM IN THE U.S.

HIGH INPUT SYSTEM

The concerted efforts at mechanization in the 1920's resulted in the expansion of acreages under cultivation. By 1964, 90% of concentrated production was mechanized (Woodroof 1966). Cultivation practices such as deep tillage and smooth soil preparation, treating and planting seeds, applying fertilizers and herbicides, digging, windrowing and thrashing as well as drying were all mechanized. The mechanization of cultivation and harvesting has reduced the labor requirement from 43 to 24 man-hours per acre. It is now possible to grow 300-1000 acres of peanut per farm (Woodroof 1966).

In the U.S., peanut is cultivated under commercial conditions of improved varieties, high inputs, irrigation, modern crop management and mechanization. Yields of 2-4 t/ha are achieved under this system (Freeman et al., 1999).

The price support program instituted by the U.S. government in 1934 had a multitude of positive effects on the peanut industry. Under that program, each farm was allocated a poundage quota and peanuts produced within that poundage quota and meeting the quality requirements, were eligible for the price support. It was this price

support that provided the major incentive for the production thus precipitating the expansions in acreages, and the mechanization of production (Isleib et al., 1992).

Because of the commercial nature of production in the U.S., a functional research infrastructure has evolved to support the production, processing and utilization of the peanut crop. New technologies are being developed to resolve the constraints to all phases of the industry. Some of the research efforts include, (1) breeding programs for higher yielding cultivars adapted to mechanical harvesting, (2) development of machinery to completely mechanize production, (3) appropriate use of fertilizers based on soil tests and plant analysis, (4) use of high quality certified seeds, and (6) use of irrigation (Isleib et al., 1992).

1.1.6 PRODUCTION CONSTRAINTS

Improvements in yield, hence productivity, have been constrained by a number of biotic and abiotic factors. High incidences of diseases, insect pests, and adverse environmental factors such as low soil fertility and drought, have challenged peanut breeders to develop cultivars that are resistant to local pests and environmental conditions (Freeman et al., 1999). However, breeding programs for resistance have not been overly successful.

One of the more successful disease-resistant breeding programs resulted in the development of cultivars resistant to rosette virus in Senegal, Nigeria and Malawi (Coffelt 1989). In the U.S., a cultivar resistant to Cylindrocladium black rot (*Cylindrocladium crotalariae)*, and a cultivar resistant to Pod rot (*Pythium spp.*, *Rhizoctonia solani* Kuhn, and *Fusarium spp*) have been released (Coffelt 1989). A

number of cultivars resistant to Sclerotinia blight (*Sclerotinia minor* Jaggar) have also been released (Kirby et al., 1998, Coffelt et al., 1982, Simpson et al., 2000 and Smith et al., 1991). This disease, Sclerotinia blight, is a major limiting factor to the cultivation of peanuts in many peanut producing countries in the world (Akem et al., 1992).

1.2 RATIONALE

Between 1979 and 1996 the global cultivation of peanut, *Arachis hypogaea* L., increased by 1.3% per annum. This increased trend in cultivation was especially pronounced in Asia where China expanded its production area by almost 60 percent. While Africa experienced a decline in production from the mid1970s to the mid 1980s, the expansion of production areas as Sudan, Nigeria and Egypt effected a reversal of this trend in the 1990s. Presently, developing countries account for over 90 percent of peanut production (Freeman et al., 1999).

In the U.S., the expansion in acreages around 1900 was directly related to the invention and innovation of equipment, hence the mechanization of planting, cultivating, harvesting, picking, shelling and cleaning of kernels. By the 1950s some 1,718,000 acres were under cultivation with production of 763,300 tons; an average of 0.44 tons per acre or 880 lbs per acre (Woodroof 1966). Further technological gains in the 1960s and the 1970s did not increase the acreage under cultivation, but more importantly, increased the total yield per acre (1669.7 tons) by 62 % (Freeman et al., 1999). An increase in acreage planted was experienced in the mid 1980s and mid 1990s. However, this trend was followed by a decline. As of 2002, some 1,358,000 acres were under cultivation and the average yield per acre remained high at 2,561 pounds per acre (http://www.usda.gov/nass/pubs/agr03/acro03.htm).

In 2002, (Table 2) the principal states involved in peanut cultivation were Georgia, Texas, Alabama, North Carolina, Florida, Oklahoma, Virginia, New Mexico and South Carolina. The peanut industry (Table 3) generated some 596 million U.S.

dollars, a decline from the excess of 1 billion dollars U.S. generated in 2001 (http://www.usda.gov/nass/pubs/agr03/acro03.htm). This drop in net farm returns reflects not only the changes in the 2002 Farm Act, which restructured the peanut quota and its price support systems, but also competition from imports and the challenges on the export market (http:// www.ers.usda.gov/publica.PDF).

TABLE 2: Peanut production Figures for the nine peanut producing states in the U.S.

STATES	PEANUTS FOR NUTS In 2002			
	AREA PLANTED (1,000ACRES)	AREA HARVESTED (1,000ACRES)	YIELD PER HARVESTED ACRE (POUNDS)	PRODUCTION (1,000POUNDS)
AL	190.0	185.0	2,050	379,250
FL	96.0	86.0	2,300	197,800
GA	510.0	505.0	2,600	1,313,000
NM	18.0	18.0	3,000	54,000
NC	101.0	100.0	2,100	210,000
OK	60.0	57.0	2,800	159,600
SC	10.0	8.7	2,200	19,140
TX	315.0	280.0	3,100	868,000
VA	58.0	57.0	2,100	119,700
US	1,358	1296.7	2,561	3,320,490

NASS, CROPS BRANCH, (202)-720-2127

TABLE 3: Revenue generated from peanut production by the nine peanut producing states in the U.S.

STATES	MARKETING YEAR AVEARGE PRICE PER POUND (2002)	VALUE OF PRODUCTION (2002)
	DOLLARS	1,000 DOLLARS
AL	0.168	63,714
FL	0.177	35,011
GA	0.175	229,775
NM	0.190	10,260
NC	0.208	43,680
OK	0.170	27,132
SC	0.166	3,177
TX	0.182	157,976
VA	0.198	23,701
US	0.179	594,426

NASS, CROPS BRANCH, (202)-720-2127

The value of the peanut crop finds basis in its utility. Peanut, more than any other crop, is widely believed to have the highest combined advantages as a food ingredient due to its "pleasing aroma and flavor; crunchy texture; high energy value; high in protein, minerals, and niacin; and its suitability of being made into hundreds of products for serving any time of the day and all occasions (Woodroof 1966)."

Peanut is cultivated for its kernels, the oils and meals that can be extracted from them and for its vegetative residue which can serve both as a vegetable in soups or as a high quality forage for animals (Asiedu 1994). Peanut is considered to be one of the world's principal oilseed crops and from 1994-1997 was ranked fifth in world's production of vegetable oils and oilseed protein meal (Freeman et al., 1999).

The most important commercial product of the crop is the extracted oil, thus slightly over 50 percent of world's production is crushed into oils for human or industrial use (Freeman et al., 1999). For human consumption, the oil can be used for cooking, for margarines and vegetable ghee, for shortening in pastries and in bread. A number of pharmaceutical and cosmetic products, lubricant and emulsion for pesticides,

and fuel for diesel engines are also produced from the oil (Asiedu 1994). As the urbanization of populations in developing countries continues to increase, the opportunity costs associated with convenience foods will thus increase the demand and consumption of peanut oil (Freeman et al., 1999).

It is the consumption of peanut as a direct food source that has, however, engendered the greatest demand for this highly nutritive food crop. The utilization of confectionary peanuts increased by 80 % between 1979-81 and 1994-96. Much of this increase was realized in developing countries where their share of global utilization increased from 75 to 83 %. The pattern of consumption, though, is quite different among developed countries (Freeman et al., 1999).

In Asian countries, where the populations are experiencing a rise in per capital income, the mode of utilization has shifted to processed and packaged foods. In other countries, such as in Africa, the consumption pattern remains primarily in the forms of roasted nuts, boiled or raw groundnut or paste (Freeman et al., 1999).

In developed countries, utilization of peanut increased by 19 % between 1979 and 1994. The U.S has the highest level of utilization and is the largest producer of confectionary peanut. Most of the peanuts produced in the U.S. are used in confectionary products, packaged snacks and peanut candies (Freeman et al., 1999). About half of the U.S. peanut crop is processed into peanut butter. Among developed countries, the U.S. has experienced a decline from 58 % in 1979-81 to 52 % in 1994-96 in the amount of confectionary peanuts utilized. The rise in price that occurred after the 1990-91 season, consumer's preference for low fat foods and the U.S. government's

reduced purchases of peanut for domestic nutrition programs, have contributed to this decline (Freeman et al., 1999).

The determination that peanut is an important global and domestic agricultural crop commodity is merited, hence the concerted efforts across research institutions in understanding and determining the limiting factors and constraints to production. Though government policies such as the 2002 Farm Act and international trade agreements such as NAFTA currently regulate production acreages (http:// www.ers.usda.gov/publica.PDF), it is understanding and mitigating those constraints imposed by abiotic and biotic stresses (insect pests, diseases, drought and low soil fertility) that are central to the research efforts in productivity improvements. Of those biotic stresses, the constraint to productivity improvements imposed by the disease Sclerotinia blight continues to be a major challenge to researchers (Freeman et al., 1999).

The challenge in managing this disease lies in the realization that there exists a lack of established control practices (Akem et al., 1992). This disease is particularly destructive (Figure 3) because it affects not just the yield (50% crop loss in Oklahoma can occur following severe outbreaks) but can also affect the quality of kernels produced (Figure 4). Runner cultivars grown under irrigation are particularly susceptible to infection. It is estimated that over 45 percent of peanut acreages in Oklahoma are currently infested with *Sclerotinia minor* Jaggar (Damicone et al., 2001).

Figure 3: A field showing typical symptoms of *Sclerotinia minor* Jaggar infestation Courtesy H.A. Melouk

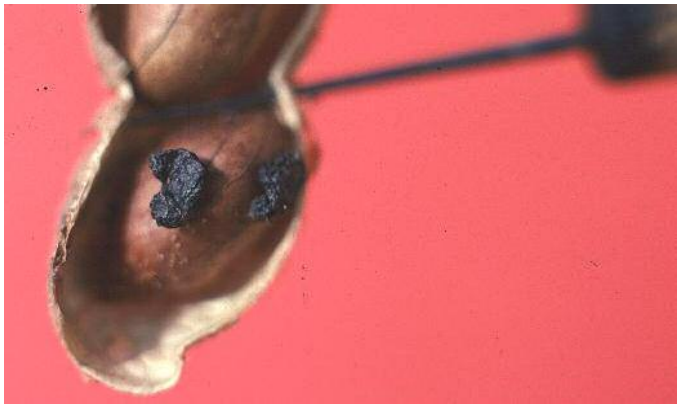

Figure 4: A peanut kernel showing damage caused by *Sclerotinia minor* Jaggar. Courtesy: Texas Agricultural Extension Service

1.3 *Sclerotinia minor* Jaggar and PEANUT CULTIVATION

1.3.1 BIOLOGY of *S minor* Jaggar

TAXONOMY

The fungus *Sclerotinia minor* Jaggar (Figure 5) is one of the most important and damaging pathogens of the peanut plant. This soil-borne fungus has a worldwide distribution and is the causative agent of the disease Sclerotinia blight, which is known to account for 5- 13% annual crop loss in Oklahoma and Virginia (Akem et al., 1997). Since its discovery in Oklahoma in 1972, the pathogen has now rapidly spread throughout the peanut producing counties. This fungus is particularly damaging to Runner genotypes, which account for over 70 % of acreages planted in Oklahoma (Akem et al., 1992).

Figure 5: Symptoms of *Sclerotinia minor* Jaggar infecting peanuts Courtesy North C Carolina State University; Department of Plant Pathology.

The fungus *S. minor* Jaggar is the pathogen of a large number of plant families from the Angiospermae branch. These families include Leguminosae, Solanaceae,

Compositae, Cruciferae, Umbelliferae and Chenopodiaceae. These families host a large group of economically important crop species (Willet et al., 1980). This soil borne pathogen is capable of producing apothecia and sclerotia and is particularly damaging to peanuts because of its ability to spread rapidly within the canopy and its persistence in the soil (Smith 2001). The fungus over winters in the field as sclerotia, then produces apothecia and ascospores in late April. Peanut plants exposed to the ascospores then become infected with the disease (Wadsworth 1976).

INFECTION AND TRANSMISSION

Peanut plant materials can become infected by either the ascospores that are discharged from the apothecia, by mycelium arising from adjacent plants or from mycelium of germinating sclerotia. Under favorable conditions of temperature, humidity and soil pH, the pathogen rapidly invades the plant tissues forming light brown watery rots followed by the growth of a white cotton-like mycelium (Figure 6) on the infected tissue. “Stunting, premature ripening and sudden collapse of the host are common symptoms (Willet et al., 1980).” The pathogen can be transmitted to clean fields by harvest equipment that have been contaminated by infected seeds and plant debris (Akem and Melouk, 1990; Melouk et al., 1991).

Figure 6: MYCELIUM GROWTH OF *S. minor* Jaggar on PEANUT: Courtesy: North C Carolina State University; Department of Plant Pathology.

After several days of infection, sclerotia develop from aggregates of mycelium. These sclerotia can remain dormant for extended periods of time by accumulating in plant debris or in the soil. Upon germination (Figure 7), these vegetative structures can give rise to hyphae or apothecia in which the ascospores, sexual spores of the fungus, are produced (Willet et al., 1980). The management and control of this disease is very difficult. An integrated approach using a combination of cultural practices, fungicides, and partially resistant cultivars is required to reduce disease losses (Chappell et al., 1995).

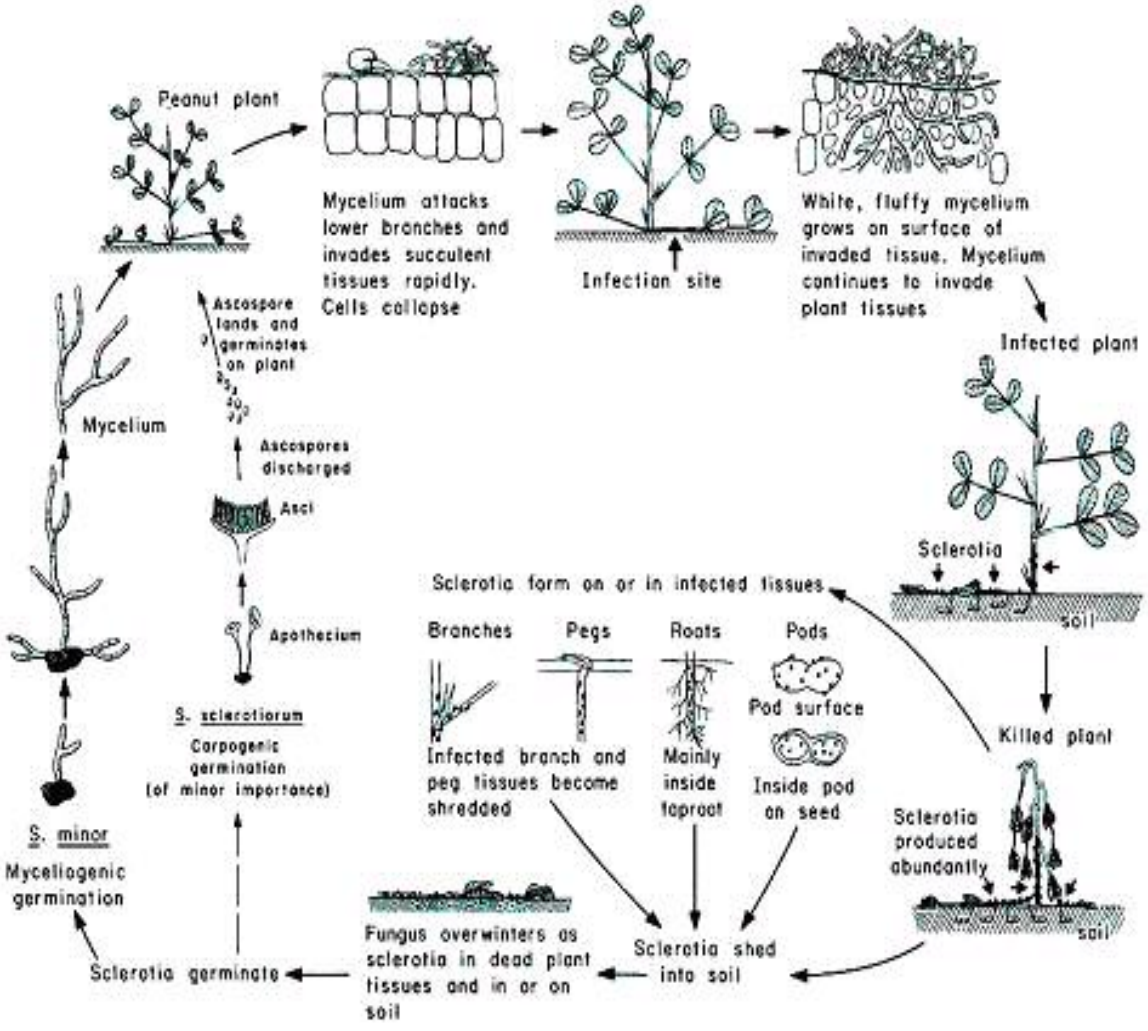

Figure 7: Life cycle and infection of *Sclerotinia minor* Jaggar in peanut field. Photo courtesy North Carolina State University; Department of Plant Pathology.

1.3.2 MANAGEMENT OF SCLEROTINIA BLIGHT

DISEASE RESISTANCE

The identification and use of new sources of disease resistance in a breeding program is a viable strategy in the control of Sclerotinia blight. In general, Spanish and Valencia genotypes, because of their architecture (upright plant canopy), tend to exhibit greater resistance to *Sclerotinia minor* Jaggar than the dense spreading Virginia and Runner genotypes. The increased levels of resistance exhibited by those erect genotypes appear to be a morphological escape mechanism (Akem et al., 1992). This escape mechanism may not only be the result of the upright plant canopy, but also the early maturity habit or result from greater resistance of the plant tissues to the pathogens (Chappell et al., 1995). This resistance is heritable hence the use of open or upright canopy types as a parent in breeding for resistance in Virginia types (Chappell et al., 1995). Southwest Runner (SWR) is a cultivar with moderate resistance that was released by the Oklahoma Agricultural Experimental Station and the USDA-ARS in 1995. This cultivar is the product of a cross between the Spanish cultivar Comet and Florunner, a Runner cultivar (Kirby et al., 1998).

CHEMICAL MANAGEMENT

While the use of resistant cultivars remain a viable option for the management of Sclerotinia blight, the use of fungicides may be necessary in cases of severe infestation. Presently, a number of fungicides have been tested for use in the control of this disease. Two and three applications of Iprodione at the rate of 1.12 kg/ha reduced the disease incidence for Okrun by 56% and 70%, respectively. Yields increased by

547 kg/ha with the two applications and by 801 kg/ha with the three applications. Two applications of Fluazinam applied at a rate of 0.56 kg/ha reduced the disease incidence in the range of 56-80% in Okrun. One application of Fluazinam increased yields in Okrun by 1030 kg/ha. (Damicone et al., 1996). Other fungicides as Pentachloronitrobenzene and dicloran have also been used in fungicidal programs for Sclerotinia blight (Damicone et al., 2001).

BIOTECHNOLOGY IN *Arachis* IMPROVEMENT*:*

Recombinant DNA technology, technologies for genetic analysis and gene transfer, provides additional options for groundnut improvements. Protocols in somatic embryogenesis, plant regeneration and particle bombardment for gene transfer, are being employed to transfer genes of agronomic importance to peanut. Genes from viruses and other plants that may confer some useful properties are being identified and sequenced for improving the peanut crop. Restriction fragment length polymorphism (RFLP) and randomly applied polymorphic DNA (RAPD) have both been applied to cultivated groundnut and its wild relatives to determine the genetic variations among cultivars and accessions so as to produce genetic maps (Weissinger 1992).

The production of chitinases and glucanases hydrolytic enzymes has shown to be effective defense mechanisms to chitin-containing pathogens (Ji et al., 1996). The transformation of peanut cultivars with those hydrolytic enzymes serves as an alternate approach to classical breeding and fungicidal applications for the control of Sclerotinia blight. “Traditional breeding and screening practices have resulted in few cultivars resistant to fungal diseases that are suitable for commercial use (Chenault et al., 2002).” The expense of fungicide applications and concerns of environmental contamination

make genetic engineering an alternate method for Sclerotinia blight management (Chenault et al., 2002).

1.4 RESEARCH OBJECTIVES

The first objective of this research was to evaluate (in the field) the resistance to Sclerotinia blight of two transgenic peanut lines that were transformed with chitinase and glucanase hydrolase genes. These genes were placed into the Okrun genetic background and the transformed plant lines were evaluated for disease resistance, quality factors and return.

The second objective was to evaluate (in the field) the resistance to Sclerotinia blight of the two transgenic peanut lines to commercial cultivars with good to excellent levels of resistance to Sclerotinia blight by evaluating yield, Sclerotinia blight incidence, and grade quality factors under three disease pressure environments.

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CHAPTER II

2.0 MATERIALS AND METHODS

2.1 Objective I

Evaluation of resistance to Sclerotinia blight by comparing Sclerotinia blight incidences of the two transgenic lines and the four Genotypes.

2.1.1 INTRODUCTION

The control of fungal diseases through the repeated applications of fungicides is limited by the associated costs and environmental concerns. Further, the inherent abilities of pathogens to develop resistance to chemicals limit the effectiveness of fungicides (Bliffeld et al., 1999). An integrated pest management strategy, which incorporates the use of cultivars resistant to specific fungal races, serves as an alternative to chemical control (Patterson et al., 1987). Genetic engineering can also be an alternative to enhancing the disease resistance of commercial crops while reducing the need for chemical inputs (Bliffeld et al., 1999).

Plants have evolved a complex defense of mechanisms in response to fungal attacks (Lozovaya et al., 1998). These mechanisms are designed to limit the penetration and development of pathogens and "include structural and biochemical responses like reinforcement of the plant cell wall, accumulation of phytoalexins with microbial toxicity, ribosome-inactivating proteins that inhibit protein synthesis, antimicrobial peptides and the synthesis of other pathogenesis- related proteins (Bliffeld et al.,

1999).”Chitinase and β-1, 3 glucanase are two known pathogenesis- related (PR) plant proteins (Mauch et al., 1988). Chitinase and β-1, 3 glucanase are capable of hydrolyzing the chitin and β-1,3 glucans; the major cell wall component of many fungi (Ji and Kuc., 1996). These two PR proteins can be induced by ethylene, by a pathogen infection or by pathogen derived elicitors (Mauch et al., 1988). In the lab, these two PR proteins are known to act synergistically to restrict the growth of several genera of fungi. (Lozovoya et al.,1998). According to Zhu et al., 1994, the combination of the two genes, in transgenic tobacco, gave substantially greater protection against the fungal pathogen *Cercospora nicotianae*, the causal agent of the frogeye, than either transgene alone.

2.1.2 MATERIALS AND METHOD

From the split plot experiment data were collected over the two years to determine Sclerotinia blight incidences, that is, percentage of peanut plants infected with the disease Sclerotinia blight.

Disease counts were taken once a week over a three-week period, hence a total of three counts per year. Disease symptoms of dead, wilting shredded limbs and mycelial growth were observed and diseased plants were counted and color tagged. Three different color tags were used, one at each count. At each count, only newly and unrecorded infected plants were counted and color tagged. The totals for the three counts per year were then summed to obtain a grand total of disease rating for each sub-plot.

2.2 OBJECTIVE II

Evaluation of resistance to Sclerotinia blight by comparing yields of the two transgenic lines and four commercial cultivars.

2.2.1 INTRODUCTION

The average yield per acre of peanuts in the United States has steadily increased. In the 1930's the average yield was 861 pounds per acre. During the decade of the 1960's the yield increased to 1,735 pounds per acre (Marshall and Schools., 1968). In 2002, the average yield was 2561 lbs/acre http://www.usda.gov/nass/pubs/agr03/acro03.htm). As of 2003, some research plots on the Caddo County Research Station in Oklahoma, have yielded over 5000 pounds per acre (Sholar et al., 2003).

While U.S. farmers have experienced a substantial increase in mean yield per acre, the global mean dry pod yield ranged from 0.7 to 2.8 tons/ha with an average of about 1ton/ha, however yields of over 9 ton/ha have been realized. China has recorded mean dry pod yields of 11.2 tons/ha in 0.1 ha plots and 9.6 ton/ha in 14 ha plots. Zimbabwe has also reported similar yields of 9.6 tons/ha (Johansen and Rao., 1996). According to Johansen and Rao., 1996, the yield potential estimated by radiation use efficiency for short duration and medium duration Spanish cultivars were 11.9 ton/ha and 17.3 ton/ha, respectively.

Breeding for increased yields continues to be a viable objective of most peanut breeding programs (Coffelt, 1989). The objective of those breeding programs then is to narrow the yield gap; "the difference between the yields realized by farmers and potential yields; by identifying and addressing the biotic and abiotic factors responsible

for this gap (Johansen and Rao., 1996).” Abiotic stress factors as drought and high temperature and biotic factors as pest, diseases, nematodes, and viruses can seriously constraint yields and quality of groundnuts (Maiti, 2002). Soil physical characteristics and nutrient availability can also limit yield potential (Johansen and Rao., 1996).

2.2.2 MATERIALS AND METHOD

EXPERIMENT DESIGN

A split plot experiment was conducted at the Caddo County Research Station, near Fort Cobb Oklahoma, to evaluate the resistance of three commercial cultivars, one breeding line and two transgenic lines of peanuts to the disease Sclerotinia blight. In this experiment, three spray treatments of 0, 1 and 2 applications of Omega, (Syngenta, Greensboro, NC) at a rate of 1.5 pts/acre, were the main factors and six genotypes were the sub-factors. These three spray treatments were used to create the three disease pressure environments. There were five replicates; 15 main plots each measuring 25 ft x 18 ft and the experiment was conducted over two years. The soil type was Cobb Sandy Loam.

GENOTYPES

The genotypes chosen for this experiment were all Runner genotypes. The three commercial cultivars were TX 977006 (Tamrun OL 01), Southwest Runner, which are moderately resistant to Sclerotinia blight (Simpson et al., 2003, Kirby et al., 1998), and Okrun, which is susceptible to this disease (Kirby et al., 1989). The breeding line TX 994336, which was developed by Texas A&M and USDA-ARS and has some

resistance to Sclerotinia blight, was also evaluated. Two transgenic lines 654 and 487, that were developed at the USDA-ARS peanut lab in Stillwater, were evaluated (Chenault et al., 2002). These two lines were generated from Okrun somatic embryos by transforming them with chitinase and glucanase gene constructs. These plant lines exhibited higher levels of hydrolase activity than the non-transformed Okrun cultivar when assayed.

PLASMID CONSTRUCTS:

CHITINASE PLASMID CONSTRUCT:

A rice chitinase gene cassette was excised from plasmid pBZ56 by *Bam*HI digestion and sub-cloned into plasmid pRTL2, which contained a duel-enhanced CaMV 35S promoter. *Hind* III was used to isolate this expression cassette and the construct was then ligated with plasmid pTRA141, which contained a hygromycin (*hph)* resistance gene, thus creating plasmid pAB2.5 (Chenault et al., 2002).

GLUCANASE PLASMID CONSTRUCT:

An alfalfa glucanase gene cassette (AGLU1) was excised from plasmid pMU2X by *Eco*RI digestion and inserted into pRTL2. *Hind* III was used to isolate this expression cassette and the construct was then ligated with plasmid pTRA141, thus creating plasmid pAB8 (Chenault et al., 2002).

SEED TREATMENT:

All seeds were given a seed protectant treatment with the fungicide Tops 90 (Gustafson LLC, Plano, TX) and hand planted at a distance of 2.5”apart. Seed germination count was taken 5 weeks after planting to determine plant stand for disease ratings.

AGRONOMIC AND CULTURAL PRACTICES.

HERBICIDE AND INSECTICIDE APPLICATIONS:

Herbicides and insecticides applications were used to control weeds and insects and were similar to the practice employed by peanut farmers in Oklahoma. (Table 4 and 5).

Table 4: Herbicide and insecticide applications used to control weeds and insects (2002)

HERBICIDES		
TYPE	APPLICATION RATE	DATE
SONALAN	2.5pt/acre	5-10 02
CADRE + BUTYRAC 200	1.44 oz + 1pt/acre (resp)	7-10 02
INSECTICIDES		
ORTHENE 90WSP	8oz/acre	6-6-02

Table 5: Herbicide applications used to control weeds (2003)

HERBICIDES		
TYPE	APPLICATION RATE	DATE
PROWL	2.4pt/acre	5-22 03
CADRE +BUTYRAC 200	1.44 oz + 1pt/acre (resp)	7-10 03

*SONALAN and BUTYRAC 200 (Dow AgroSciences LLC, Indianapolis, IN)
*CADRE and PROWL (Basf Corporation, Research Triangle Park, NC)
*ORTHENE 90WSP (Monsanto, San Ramon, CA)

FUNGICIDES

Fungicides were applied to control the varied numbers of fungal diseases of peanuts such as Sclerotinia blight, Southern blight, and Leaf spot. Tables 5 and 6 list the fungicides applied.

Tilt + Bravo (Syngenta, Greensboro, NC) were used to control Leaf Spot, Early and Late blight and Southern blight. Folicur (Bayer, Kansas City, MO) was used to control a range of foliar and soil borne fungal diseases including Rhizoctonia, limb and Pod rot, and Sclerotium stem and Pod rot. Omega, with the active ingredient fluazinam (Syngenta, Greensboro, NC), was the fungicide used in the experiment to create the different disease pressures necessary to evaluate the transgenic lines' resistance to Sclerotinia blight. In 2002, the main plots (MP) receiving one treatment of Omega (Syngenta, Greensboro, NC) were sprayed on 8-05-02. The main plots receiving two treatments were sprayed on 08-05-02 and 09-12-02. In 2003, main plots receiving one treatment were sprayed on 08-13-03, while main plots receiving two treatments were sprayed on 08-13-03 and 09-12-03.

Table 6: Fungicide applications used to control fungal problems in 2002

TYPE	APPLICATION RATE	DATE
TILT + BRAVO	2oz + 1pt/acre (resp)	7-2-02
TILT + BRAVO	2oz + 1pt/acre (resp)	7-23-02
FOLICURE	7.2 oz/acre	8-5-02
OMEGA	1.5pt/acre	8-5-02
FOLICUR	7.2 oz/acre	8-19-02
OMEGA	1.5pt/acre	9-12-02

*TILT, BRAVO and OMEGA (Syngeneta, Greensboro NC)
*FOLICUR (Bayer, Kansas City, MO)

Table 7: Fungicide applications used to control fungal problems in 2003

TYPE	APPLICATION RATE	DATE
TILT + BRAVO	2oz + 1pt/acre (resp)	6-30 03
TILT + BRAVO	2oz + 1pt/acre (resp)	7-14 03
TILT + BRAVO	2oz + 1pt/acre (resp)	7-28 03
TILT + BRAVO	2oz + 1pt/acre (resp)	8-12 03
OMEGA	1.5pts/acre	8-13 03
TILT + BRAVO	2oz + 1pt/acre (resp)	8-21 03
TILT + BRAVO	2oz + 1pt/acre (resp)	9-8-03
OMEGA	1.5pts/acre	9-12 03

*TILT, BRAVO and OMEGA (Syngeneta, Greensboro NC)

IRRIGATION

Figures 8 and 9 represent the total rainfall and irrigation crop received over the two growing seasons.

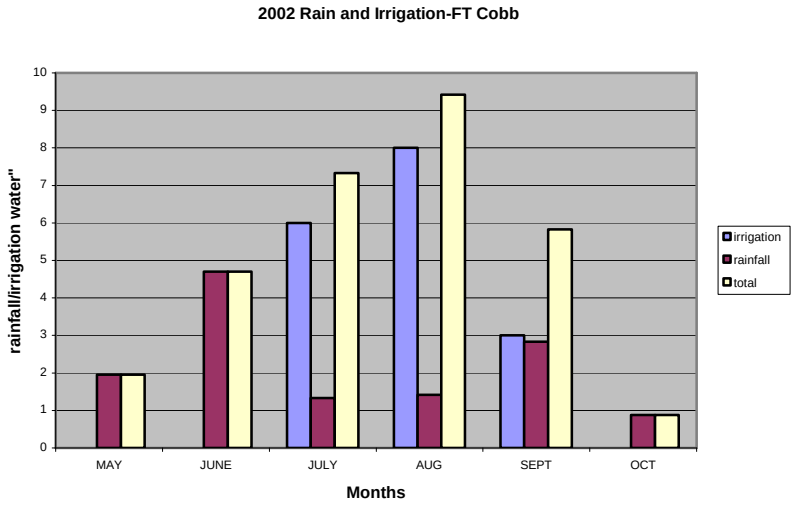

Figure 8: Total rainfall and irrigation the crop received at Ft- Cobb in 2002

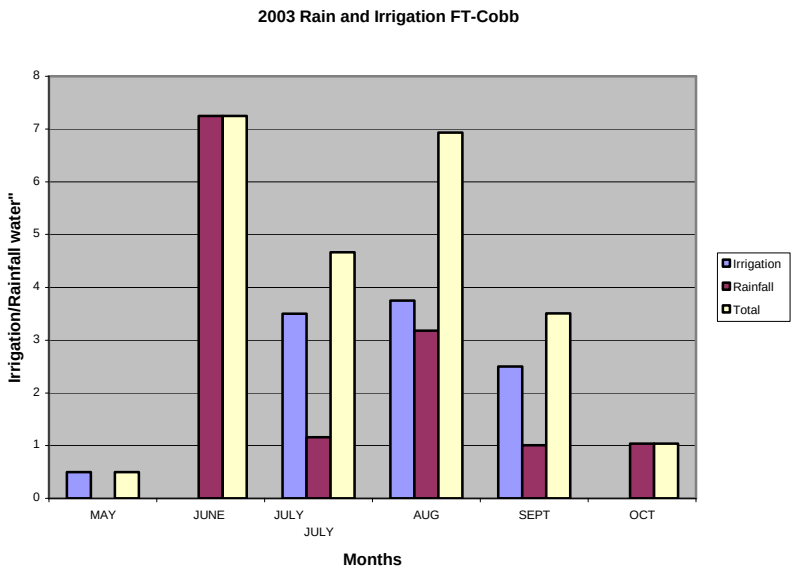

Figure 9: Total rainfall and irrigation the crop received at Ft- Cobb in 2003

HARVESTING

Digging was done with a single row digger and curing was done in wind-rows. After thrashing, all plant materials were gathered and burnt in the field. Pods were dried in an electric drier. All equipment were thoroughly cleaned and the field was re-dug with a hand fork to remove all remaining pods. Weights were taken and all transgenic peanuts were stored at the USDA-ARS facility.

2.3 OBJECTIVE III

Evaluation of resistance to Sclerotinia blight by comparing Seed Quality Ratings and Return of the two transgenic lines and the four commercial cultivars.

2.3.1 INTRODUCTION

Defining and establishing standards for peanut quality are challenging exercises because of not only the quantitative standards that are involved, but more importantly, the sensory qualities measured and conferred are subjective in nature. In general, two broad subdivisions of peanut quality are established. The economic qualities, or grade factors, are well defined and determine the economic value of the crop. The sensory qualities, the second subdivision, reflects "all the physical and chemical characteristics of edible peanut seed or their products that influence human senses and bring about acceptability judgments by the consumer (Pattee and Ahmed., 1987)."

The USDA establishes grade factors for the farmers' stock peanuts. Each market type has a slightly different grade requirement developed for price support purposes (Davidson et al., 1982). In determining grade factors the percentages of edible kernels, inedible kernels, split kernels, foreign material, and moisture are measured (Dowell, 1992). The sample for grading is shelled to determine the percentage of sound mature kernels (SMK), sound splits (SS), damaged kernels (DK), small other kernels (OK), and moisture content (MC). It is also required to inspect peanuts for the toxin producing mold, *Aspergillus flavus* (Dowell, 1992). The grading of farmers' stock peanuts determines the suitability of the peanut for food (Davidson et al., 1982).

The importance of sensory qualities in determining peanut quality is secondary to grade factors (Pattee and Ahmed., 1987). The sensory tests and tools for measuring

the desirable characteristics of flavor, texture, odor and appearance are difficult to use or are not known or developed (Matlock, 1969).

Maintaining peanut quality from planting to consumption is a continuous process. Peanut quality, once lost, cannot be restored (Sanders et al., 1987). There are many factors that may cause poor quality, and some of these include the influence of maturity, curing practices and mold infection (Sharon, 1963).

2.3.2 MATERIALS AND METHOD

GRADE SAMPLING

Two hundred grams (200g) were taken from each plot and used for grade sampling. Grading samples were shelled and graded with runner screens; 21/64 of an inch, 18/64 of an inch and 16/64 of an inch, to obtain grade sample data for % TOTSMK, % SS, % DK, % OK and seed weight (SDWT100). All transgenic seeds were handled at the USDA-ARS facility.

Return was calculated by adding the bonuses and subtracting the deductions for the base price to obtain a price per ton. This price was then multiplied by the tonnage to per acre to obtain the return per acre. The base price was obtained from a buying point. Detections include the sound split when > 4% and damaged kernel when ≥ 2%. A bonus is given for every grade above 73% TOTSMK for runners. A price is also given for OK kernel and which is included in the grade per acre.

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CHAPTER III

3.0 RESULTS

Data for the response variables Sclerotinia blight incidence, yield, % Total Sound Mature Kernel (TOTSMK), 100 Seed Weight (SDWT 100) and Return were collected over the two years of the experiment. The SAS software (SAS Institute, 2001) was used for statistical analysis. First, the data were analyzed to determine whether it was possible to combine means over the two years. Through statistical analysis, it was found that the means of certain response variables could be combined, while others had to be analyzed separately over the two years.

3.1 RESULTS FOR OBJECTVE I:

Objective I: Evaluation of resistance to Sclerotinia blight by comparing Sclerotinia blight incidence of two transgenic lines and the four genotypes.

There were no significant effects caused by Years or genotype x Years for Sclerotinia blight incidence (Table 8). There were significant effects caused by Spray Treatments, Genotypes, Spray Treatments x Year and Spray Treatment x Genotype. Because of these significant interaction, it was necessary to look at the test of effect slice to determine which sub factor treatments were significantly different at a fixed level of main factor and which main factor treatments were significantly different at a given sub factor.

At the 0 spray treatment (Figure 10) the transgenic line 654 had significantly less disease than Okrun. This line was also significantly different to line 487 which recorded a mean Disease Score Rating of 40.1%. While line 487 had 7.7% less disease than Okrun, there was no significant difference between their means.

Both lines exhibited lower and significantly different Sclerotinia blight infestation than Okrun at the 1 spray treatment. There was no significant difference between the means of these two lines at this spray treatment level.

At the 2 spray treatment, there was no significant difference between the transgenic line, TX 994336,Tamrun OL 01, and Okrun. Southwest Runner was significantly different to the transgenic lines, TX 994336,Tamrun OL 01, and Okrun at the 2 spray treatment. Only Southwest Runner had no significant difference in disease score ratings across spray treatments.

3.2 RESULTS FOR OBJECTVE II:

Objective II: Evaluation of resistance to Sclerotinia blight by comparing Yield of two transgenic lines and the four genotypes.

The only effect that was not significant for yield was spray treatment x year (Table 8). Because of the significant difference observed between Years, it was necessary to evaluate some of the main effects across years. The test of effect slice was necessary to evaluate the significant interactions.

In 2002, line 654 had a significantly higher yield than Okrun (Table 9). While line 487 had a higher mean yield than Okrun, there was no significant difference between these two means. Though lines 654 and 487 had higher mean yields than Okrun in 2003, there was no significant difference between their means. In both years,

the breeding line TX 994336 had significantly higher yields than the transgenic lines and Okrun.

Southwest Runner had mean yields which were not significantly different across all three spray treatments (Table10). With 2 sprays Okrun out yielded the transgenic lines, but there was no significant difference between their means. The breeding line, TX 994336, had significantly higher yields than both transgenic lines at the 2 spray level. With 1 spray line 654 had a significantly higher yield than Okrun. At 0 spray treatment, while both transgenic lines 654and 487 had higher yields than Okrun, 229lbs/acre and 22lbs/acre respectively, their means were not significantly different.

3.3 RESULTS FOR OBJECTVE III:

Objective III: Evaluation of resistance to Sclerotinia blight by Seed Grade Quality rating and Return of two transgenic lines and the four genotypes.

3.3.1 EVALUATION OF TOTAL SOUND MATURE KERNEL (%)

Spray treatments and genotypes did have a significant effect on %TOTSMK (Table 8). Averaged over all genotypes, the mean %TOTSMK in the 2 spray treatment was significantly higher to the mean %TOTSMK in the 0 and 1 spray treatments (Figure 11). The means for %TOTSMK of line 654 (Figure 12) when averaged over all spray treatments and years, was significantly higher than Okrun and all the other genotypes except line 487. The breeding line, TX 994336, had the lowest % TOTSMK mean at 69.8 % and was significantly different to the mean % TOTSMK of all the other genotypes.

3.3.2 EVALUATION OF 100 SEEDWEIGHT (SDWT100)

For the response variable SDWT100, the ANOVA (Table 8) indicates a significant difference between Genotypes. There were significant interactions between genotype and Year and between Spray Treatments and Genotypes. The test of effect slice was necessary for analysis of these interactions.

Only the breeding line, TX 994336, had significantly different SWDT100 responses across years (Table11). In 2002, the mean SDWT100 for the breeding line, TX 994336, was higher and significantly different to its mean in 2003. The mean SDWT100 for the transgenic lines 654 and 487 were lower and significantly different to Okrun. Line 487 was also significantly different to line 654 in 2002. Tamrun OL 01 registered the highest mean SDWT100 and was significantly different to all other means in both years. The Southwest Runner recorded the lowest mean SDWT100 and was significantly different to all other means in both years.

Only line 654 and Southwest Runner mean SDWT100 were significantly different across spray treatments (Table 12). The 2 spray treatment generated a significantly higher mean SDWT100 than the 0 spray for line 654. For Southwest Runner, the mean SDWT100 in the 0 spray treatment was higher and significantly different to the mean in the 2 spray treatment. Okrun had a significantly larger SDWT100 than 654 and 487 at 0 spray, significantly larger than 654 at 1 spray and significantly larger to both 654 and 487 at 2 sprays.

3.3.3 EVALUATION OF RETURN

In the ANOVA (Table 8) for Return, there were significant differences between spray treatments, genotypes and years. There were significant interactions between genotypes and year and between spray treatment and genotypes. Test of effect slice was necessary to evaluate these interactions.

When the genotypes were analyzed across years for return (Table 13), line 654 mean was U.S.$ 71 more per acre than Okrun in 2002. There was a significant difference between their means. While line 487 mean was higher than Okrun, there was no significant difference. In 2002, Southwest Runner had the highest mean at U.S$ 497 per acre. In 2003, both transgenic lines' means were not significantly different to Okrun. The 2 spray treatment (Table 14) had significantly higher return than the 0 spray for the transgenic lines and the other genotypes, except Southwest Runner. In all three spray treatments, the mean returns for Southwest Runner were not significantly different. The transgenic lines had higher return than Okrun in the 0 spray treatment but, there was no significant difference between their means. In the 1 spray treatment, line 654 return was higher and significantly different to Okrun's. When there were two sprays, TX 994336 had the highest return and it was significantly different to Southwest Runner, line 654 and line 487.

Table 8: ANOVA of Yield, Sclerotinia Blight Incidence, % Total Sound Mature Kernel 100 Seed Weight and Total Return for a Peanut Trial Conducted near Ft Cobb Oklahoma in 2002-2003.

EFFECT		RESPONSE VARIABLE				
	DF	SCLEROTINIA BLIGHT INCIDENCE	YIELD	TOTAL SOUND MATURE KERNEL	100 SEED WEIGHT	RETURN
SPRAY TREATMENT	2	***	***	***	NS	***
GENOTYPES	5	***	***	***	***	***
YEAR	1	NS	***	NS	NS	***
SPRAY TREATMENT X YEAR	2	**	NS	**	NS	NS
GENOTYPE X YEAR	5	NS	***	NS	***	***
SPRAY TREATMENT X GENOTYPE	10	*	*	NS	*	*

* ** *** Reflects significance at p= 0.05, 0.01 and 0.001

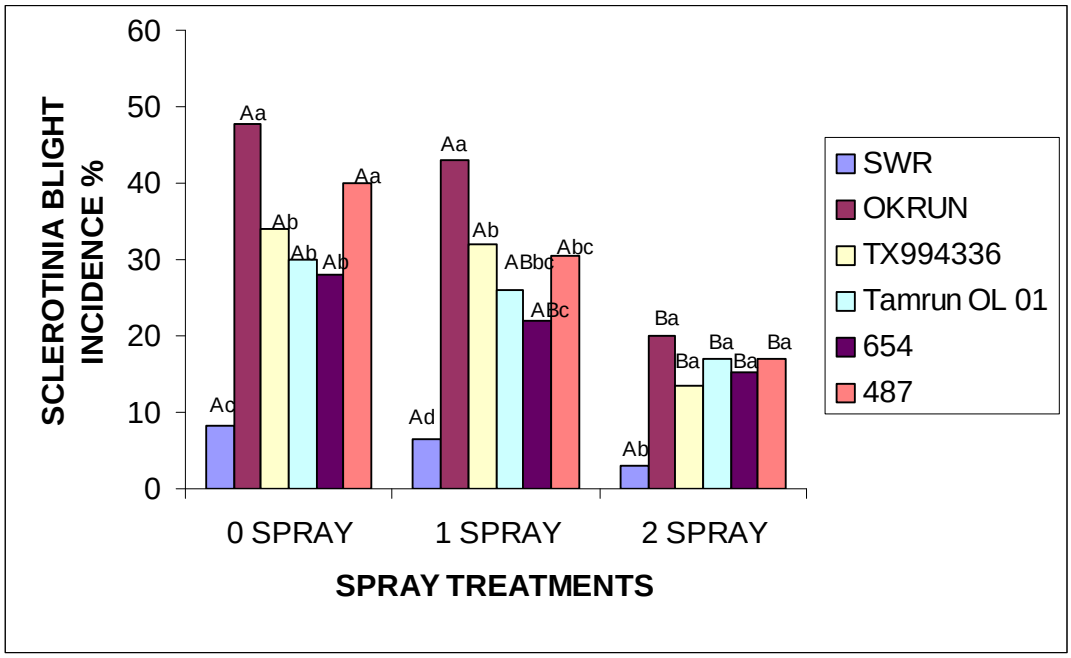

Figure 10: Summary of Sclerotinia blight incidence for six peanut genotypes at three different fungicide treatments conducted near Ft Cobb, Oklahoma during 2002 and 2003.

Upper case letters: Compares a genotype across all spray treatments
Lower case letters: Compares genotypes across a fixed spray treatment.
***Means followed by same case letters are not significantly different at p=0.05**

Table 9: Summary table for interaction for Genotypes by Year for Yield for a Peanut Trial Conducted near Ft Cobb, Oklahoma in 2002-2003.

Genotypes	2002 (Lbs/acre)	2003 (Lbs/acre)
SWR	2819 A a	2982 A c
OKRUN	1850 B c	3114 A bc
TX 994336	2759 B a	3651 A a
TAMRUN OL 01	2719 B a	3561 A a
654	2207 B b	3185 A b
487	1981 B bc	3175 A bc

*Upper case letters: comparison of Years to a given Genotype across rows.
*Lower case letters: comparison of given Genotypes within a Year down a column.
*Means followed by same case letters are not significantly different at p =0.05

Table 10: Summary table of means for Spray Treatment x Genotype interaction for Yield (lbs/acre) for a Peanut Trial Conducted near Ft Cobb, Oklahoma in 2002-2003.

GENOTYPES	SPRAY TREATMENTS		
	O SPRAY	1 SPRAY	2 SPRAYS
SWR	2833 A a	2822 A a	3047 A c
OKRUN	2149 B b	1974 B c	3323 A bc
TX 994336	2868 B a	2924 B a	3821 A a
TAMRUN OL 01	3031 AB a	2869 B a	3520 A ab
654	2378 B b	2628 AB ab	3082 A c
487	2371 B b	2213 B bc	3149 A c

*Upper case letters: comparison of levels of Spray Treatments to a given Genotype across rows.
*Lower case letters: comparison of given Genotypes to a fixed level of Spray Treatments down a column.
*Means followed by same case letters are not significantly different at p =0.05

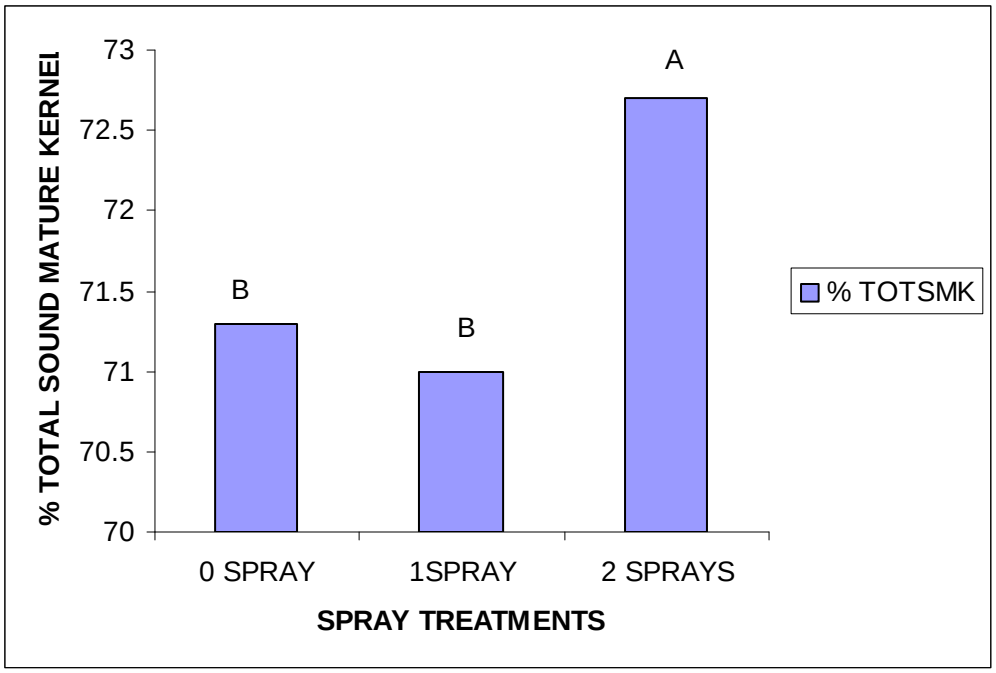

Figure 11: Means of Spray Treatments for %Total Sound Mature Kernel Combined over two years for a Peanut Trial Conducted near Ft Cobb, Oklahoma in 2002-2003.

***Spray Treatment means with same case letters are not significant at p=0.05.**

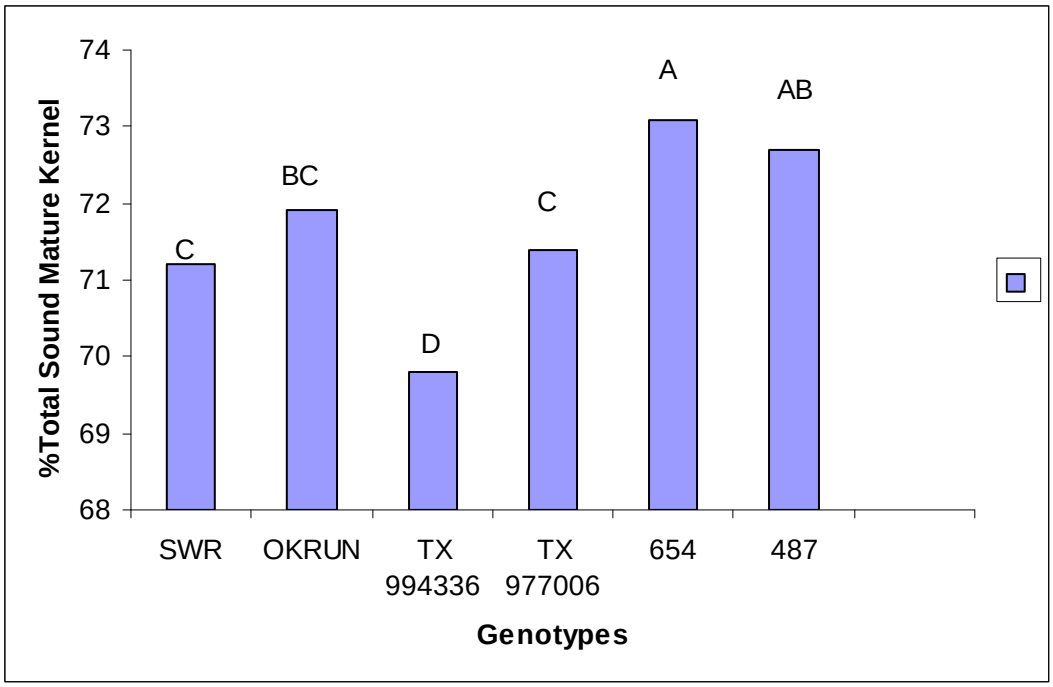

Figure 12: Means of Genotypes for %Total Sound Mature Kernel combined over two years for Peanut Trial Conducted near Ft Cobb, Oklahoma in 2002-2003.

*** Genotypes means with same case letters are not significant at p=0.05.**

Table 11: Summary table of Means of interaction for Genotypes x Years for SDWT100 for Peanut Trial Conducted near Ft Cobb, Oklahoma in 2002-2003.

GENOTYPES	SDWT100 2002 (g)	SDWT100 2003 (g)
SWR	50.6 A f	51 A d
OKRUN	57.7 A c	58 A b
TX 994336	60.4 A b	56.5 B c
TAMRUN OL 01	70.8 A a	70.7 A a
654	53 A e	55.1 A c
487	55.6 A d	55.7 A c

*Upper case letters: comparison of Years to a given Genotype across rows.
*Lower case letters: comparison of Genotypes to a fixed level of Spray Treatments down a column.
*Means followed by same case letters are not significantly different at p =0.05

Table 12: Summary table of Means of interaction of Spray Treatment x Genotypes for SDWT100 for Peanut Trial Conducted near Ft Cobb, Oklahoma in 2002-2003.

SP	SPRAY TREATMENTS (g)		
(GENOTYPES)	0 SPRAY	1 SPRAY	2 SPRAYS
SWR	52.6 A c	51 AB e	49 B d
OKRUN	58 A b	57 A bc	58.7 A b
TX 994336	58 A b	58.7 A b	58.3 A b
TAMRUN OL 01	70.8 A a	70 A a	71.4 A a
654	53 B c	53.9 AB d	55.6 A c
487	54.8 A c	56.2 A c	55.9 A c

*Upper case letters: comparison of levels of Spray Treatments to given Genotypes across rows.
*Lower case letters: comparison of given Genotypes to a fixed level of Spray Treatments down a column.
*Means followed by same case letters are not significantly different at p =0.05

Table 13: Summary table of Return Means for interaction of Genotype x Year for Peanut Trial Conducted near Ft Cobb, Oklahoma in 2002-2003..

GENOTYPES	U.S.$/ACRE 2002	U.S.$/ACRE 2003
SWR	497 A a	513 A c
OKRUN	324 B c	544 A bc
TX 994336	455 B a	614 A a
TAMRUN OL 01	472 B a	597 A ab
654	395 B b	567 A abc
487	348 B bc	551 A abc

*Upper case letters: comparison of Years a given Genotype across rows.
*Lower case letters: comparison of given Genotypes to a fixed level of Spray Treatment down a column.
*Means followed by same case letters are not significantly different at p =0.05

Table 14: Summary table of means of interaction of Genotype x Spray Treatment for Return for Peanut Trial Conducted near Ft Cobb, Oklahoma in 2002-2003.

GENOTYPES	SPRAY TREATMENTS Means U.S.$/ACRE		
	0 SPRAY	1 SPRAY	2 SPRAYS
SWR	496 A a	486 A a	533 A b
OKRUN	371 B b	336 B c	595 A ab
TX 994336	476 B a	467 B ab	660 A a
TAMRUN OL 01	502 B a	491 B a	610 A ab
654	418 B b	470 AB a	555 A b
487	392 B b	390 B bc	566 A b

*Upper case letters: comparison of levels of Spray Treatments to a given Genotype across rows.
*Lower case letters: comparison of given Genotypes to a fixed level of Spray Treatments down a column.
*Means followed by same case letters are not significantly different at p =0.05

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4.0 CHAPTER IV

DISCUSSION AND CONCLUSION

According to Yang et al.,1998, the levels of transgene expressions tend to be variable and unpredictable among independent transformants. Because of this variability and unpredictability, transformed lines may tend to exhibit different levels of field resistance. Lines 654 and 487 both contained chitinase and glucanase hydrolase genes, but their varying levels of field resistance to Sclerotinia blight, particularly in the 0 spray treatment, can be explained by this variability in transgene expression. Enzymatic expression studies done by Chenault et al., 2002 on the transgenic lines generated, revealed that line 487 had higher enzymatic expression levels than line 654.

Gene silencing could be another possible explanation for the varying levels of expression of the two transgenic lines. Through transcriptional or post-transcriptional gene silencing mechanism, plants have evolved complex mechanisms of inactivating foreign genes (Wassenegger, 2002). Wassenegger, 2002, further states that single copy genes can also be silenced. Fladung 1999, further states that the integration site can influence transgene expression.

The 20% reduction in Sclerotinia blight incidence observed in line 654 as compared to Okrun, in the high disease pressure environment, is an indication of

improved resistance to Sclerotinia blight. Line 487 did not exhibit any significant difference in Sclerotinia blight resistance in comparison to Okrun, however the lower disease incidence observed in this line may indicate some level improvement for resistance to Sclerotinia blight, when compared to Okrun.

The improvement for resistance observed in the transgenic lines, particularly line 654, maybe the result of a genetic difference between these lines and their somatic parent, Okrun. The source of the genetic difference ascribed to the transgenic lines is the presence of the chitinase and glucanase hydrolase genes which were used to transform Okrun somatic embryos. Chitinase and glucanase are anti-fungal hydrolases which break down the chitin component of most filamentous fungi cell wall. Chitinase is capable of degrading the β -1,4 linkage of chitin and glucanase is thought to function in a similar manner (Mauch et al., 1988). In a greenhouse study by Anand et al., 2002, lines of transgenic wheat plants expressing chitinase and glucanase transgenes were effective in delaying the spread of scab infection. However, under field conditions, these plants lacked effective resistance.

The significant interaction observed between the spray treatments and the genotypes would indicate that specific fungicidal treatment programs maybe required for reducing disease pressure and increasing yield in cultivars differing in reaction to Sclerotinia blight. Damicone et al., 1996, support this observation.

The level of resistance that line 654 has for Sclerotinia blight is about the same as the moderately resistant Tamrun OL 01 and the TX 994336. Southwest Runner, having a significantly lower incidence of Sclerotinia blight than all the other genotypes, can be confirmed as a having the best resistance to Sclerotinia blight among the six

genotypes. Hardin 1995 reports similarly that Southwest Runner was able to resist Sclerotinia blight in fields that were heavily infested with the pathogen, *S. minor* Jaggar.

The significant difference in yield observed for the different genotypes, except Southwest Runner, could be the effect of a significant genotype x year interaction. In 2003, the yield for all entries, except Southwest Runner, was significantly higher than in 2002. Damicone et al., 2003, made a similar observation of differences in yield observed in a peanut experiment conducted at the same location between 2002 and 2003. The non- significant increase in yield observed for Southwest Runner could imply that this genotype has reduced genetics potential for yield, as compared to the other genotypes. The improved irrigation system constructed at Ft Cobb in 2003 could have contributed to the increase in yield of five of the genotypes.

Both transgenic lines had higher yield than Okrun in 2002. However, only line 654 yield was significantly different. The increased resistance to Sclerotinia blight observed in line 654 could have resulted in this line having a significantly higher yield than Okrun. Though line 487 yield was not significantly different to Okrun's, the improvement in yield of 131lbs/acre could be the result of its genetic difference.

The higher but not significantly different yields observed for the transgenic lines, compared to Okrun in the high disease pressure environment created by the 0 spray treatment, would further indicate their genetic improvement. Line 654 had a significantly higher yield than Okrun in the 1 spray treatment, thus providing more evidence of the positive change caused by inserting the genes into Okrun.

Line 654 has improved % TOTSMK over all the other genotypes, except line 487. The observed difference in %TOTSMK between 654 and Okrun provides additional evidence that the transformation of Okrun has had positives effects.

There was a significant genotype x spray treatment interaction for SDWT100. As disease pressure increases i.e. spray treatments decrease, the SDWT100 for line 654 decreases, while the SDWT100 for Southwest Runner increases. This may suggest that these two genotypes have different mechanisms for resistance. The SDWT100 for both 654 and 487 are significantly different in the 0 and 1 spray treatments, suggesting the transformations have had an effect on SDWT100.

The higher yields observed for the transgenic lines in 2002, as compared to Okrun, and the higher %TOTSMK for the transgenic lines translated into higher returns/acre in 2002. Line 654 had a significantly higher return than Okrun.

The return figures for lines 654 and 487 in the 0 spray treatment were not significantly different from Okrun. The significantly higher return obtained for line 654, when compared to Okrun, in the 1 spray treatment, further highlight the overall improvement observed in this line.

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CHAPTER V

5.0 SUMMARY

The transgenic lines 654 and 487, which were generated from Okrun somatic embryo and transformed with a chitinase and glucanse gene construct, were evaluated for resistance to Sclerotinia blight by comparing their yields, Sclerotinia blight incidences, seed quality ratings and return. These two lines were evaluated against their somatic parent, Okrun, a susceptible cultivar, Southwest Runner, with good resistance, Tamrun OL 01and TX 994336, which are both moderately resistant.

The significantly lower Sclerotinia blight incidence observed for line 654 when compared to Okrun, is an indication of this line's improved resistance to Sclerotinia blight. The lower disease incidence observed in Southwest Runner at all spray treatments would further confirm this as a cultivar with good resistance.

Line 654 demonstrated improved resistance to Sclerotinia blight during the first year of the experiment as it had a significantly higher yield than Okrun. Though line 654 had a higher yield than Okrun in the 0 spray treatment, that yield increase was not significant. However, at the 1 spray treatment, line 654 yield was significantly higher. At the 2 spray treatment, this line's yield was the same as line 487, Okrun, and Southwest Runner. In comparing seed quality, line 654 showed an improvement over Okrun for total sound mature kernels (TOTSMK).

Line 654 can be very useful as part of a peanut-breeding program for improving resistance to Sclerotinia blight. Line 487 also showed an improvement to Okrun in terms of Sclerotinia blight incidence at the high disease pressures created by the 0 and 1 spray treatments even though the differences were not significant. A similar observation was made for yield where the improvement was not significant. Line 487 showed a significant improvement over Okrun for %TOTSMK, but there was no significant difference in return.

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