Effect of Anti-Fungal Transgene(s) on Agronomic Traits of Transgenic Peanut Lines Grown under Field Conditions

K.D. Chenault*, H.A. Melouk, and M.E. Payton

ABSTRACT

Agronomic traits may become adversely affected when field crops are transformed with foreign genes which confer resistance to plant pathogens. Field testing of such transformed plant lines is necessary to determine if desirable yield components have been retained through the transformation and regeneration process. Thirty-four peanut lines, thirty-two of which are transgenic containing anti-fungal genes, were evaluated under field conditions for a 3-year period. Peanut lines were arranged in a complete randomized block design with three replications. Disease incidence was recorded throughout the growing season, the yield components of pod mass, shelling percentage, and seed weight were determined upon harvest, and the data were analyzed for statistical significance. Deterioration of yield components was observed for 5/32 transgenic lines and was not always consistent with increased disease incidence. Yield components measured for the majority of transgenic lines tested were comparable to the parental genotype Okrun, suggesting retention of desirable market traits by these plant lines. Of the plant lines retaining desirable quality traits, 10 demonstrated increased resistance to fungal infection compared to Okrun. These results have identified transgenic peanut lines with potential use in breeding programs for disease resistance coupled with desirable yield components.

Key Words: Peanut, transgenic, hydrolases, agronomic traits.

The use of biotechnology to produce transgenic crops is rapidly becoming a viable extension of conventional plant breeding, particularly since transgenic plants are now being produced to meet broad objectives such as pathogen and herbicide resistance. Genetic engineering promises to provide those involved in agricultural biology with the tools needed to overcome the limited gene pools accessible to conventional breeding programs. The list of crop species being transformed with genes responsible for agronomically beneficial traits is rapidly expanding. Herbicide resistance has been engineered in a wide variety of crops including, but not limited to, soybean (Delannay *et al*., 1995), tobacco (Brandle and Miki, 1993), cotton (Coulombe *et al*., 1994), sorghum (Casas *et al*., 1993), wheat (Vasil *et al*., 1992), sugar beet (D'Halluin *et al*., 1992), and alfalfa (D'Halluin *et al*., 1990). Engineered crop resistance to pathogen attack has also become common place. Virus resistance in squash (Arce-Ochoa *et al*., 1995; Tricoli, *et al*., 1995) and tomato (Fuchs and Provvidenti, 1996), bacterial resistance in potato (Allefs *et al*., 1995), fungal resistance in rice (Lin *et al*.,1995), and insect resistance in cotton (Wilson *et al*., 1994) are only a few examples of these efforts.

Peanut is susceptible to many pathogens, with most damage being caused by fungi (Melouk and Backman, 1995). Soilborne fungi cause diseases that adversely affect peanut health and productivity throughout the peanut growing areas of the United States. Diseases such as pod rot (*Rhizoctonia solani* Kühn, *Pythium myriotylum*), crown rot (*Aspergillus niger* Teigh), southern blight (*Sclerotium rolfsii* Sacc) and root knot (*Meloidogyne arenaria* [Neal] Chitwood) occur in all U.S. peanut-producing areas, while others such as Sclerotinia blight (*Sclerotinia minor* Jagger) are limited to certain geographic regions. Sclerotina blight is of major concern to peanut producers in the Southwest U.S. Traditional breeding and screening practices have resulted in few cultivars resistant to fungal diseases that are suitable for commercial use (Smith *et al*., 1991; Simpson *et al*., 2000), making expensive fungicide applications throughout the growing season required for effective disease management. These obstacles to profitable peanut production along with recent reductions in the U.S. peanut price support system have resulted in the urgent need for effective alternative methods of disease management that will provide disease resistance without pesticide application.

Efforts to engineer fungal resistance in peanut have resulted in the production of transgenic peanut lines that express a chitinase from rice (*Oryza sativa* L.) and or a glucanase from alfalfa (*Medicago sativa* L.) (Chenault *et al*., 2002). Pathogenesis-related (PR) proteins such as chitinases and β-1-3 glucanases that are capable of hydrolyzing the cell walls of many fungi that attack

*Corresponding Author: K.D. Chenault, USDA-ARS Wheat, Peanut, and Other Field Crops Research Unit, Stillwater, OK 74075; kelly.chenault@ars.usda.gov

plants are rational candidates for over-expression to produce disease-resistant crops (Stahl and Bishop, 2000). The cultivar Okrun served as the parent genotype to all transgenic lines produced due to its desirable and marketable agronomic characteristics and its performance under tissue culture conditions. Resulting transgenic peanut lines were analyzed for stable transgene inheritance and expression (Chenault *et al*., 2002), as well as for their response to fungal infection under greenhouse conditions (Chenault *et al*., 2003).

The evaluation of transgenic plants in the laboratory and greenhouse provides valuable and necessary information about the inheritance and expression of the introduced gene(s). However, the introduction of transgene(s) into the plant genome is not always site-specific and endogenous genes that perform essential functions in plant productivity may be disrupted. True assessment of plant phenotype and performance can be determined only in a field environment with inherent environmental variations. Moreover, field testing of transgenic crops is an essential step toward variety release and commercial production. The purpose of this study was to determine whether transgenic peanut lines expressing anti-fungal genes have retained the desirable agronomic characteristics of the parent genotype Okrun under field conditions.

Materials and Methods

Transgenic peanut lines were produced from somatic embryos of the cultivar Okrun and analyzed as previously reported (Yang *et al*., 1998; Chenault *et al*, 2002). Thirty-two transgenic lines with single-copy transgene insertions were chosen for evaluation in field experiments. Field tests were conducted for three growing seasons (2000, 2001 and 2002) at the Oklahoma State University Agricultural Experiment Station in Caddo County, OK. Disease inoculum was assessed for each test by determining the number of *S. minor* sclerotia present in soil samples taken from each plot. Viable sclerotial density was determined in the top 5 cm of field soil by a modified elutriation technique (Porter and Steele, 1983). Each individual plot was 6.1 m $\times$ 7.6 m and consisted of six rows (0.91 m row spacing): four rows of transgenic test lines, one of the resistant cultivar Southwest Runner (Kirby *et al*., 1998), and one of the susceptible cultivar Okrun. Plots were arranged in a randomized complete block design with 8 plots/replication and 3 replications per test. The presence of transgene(s) is transgenic lines was confirmed via PCR as previously reported (Chenault *et al*., 2002) before planting. All kernels were treated with TOPS 90 fungicide (2.5 g/kg kernel) before planting. Transgenic kernels to be planted the first year of evaluation (2000) originated under greenhouse conditions (T_5), but were taken from the field plots for the two subsequent years (T_6 and T_7). Kernels were hand-planted 23 cm apart on the 15th of May, 2000–2002.

All plots were weeded on a weekly basis and assessed for disease symptoms. Disease incidence (#diseased plants/line/replication) was recorded at weekly intervals after initial onset. The percentage of plants infected by Sclerotinia blight was determined by the presence of visible above-ground symptoms. Individual plants were considered infected if symptoms were present, however slight or severe, and marked with a flag at the time of recording. Disease data were tested for statistical significance with SAS v. 8.2 using Least Squared Mean analysis. Plant lines were placed into disease category 1 if their reaction to Sclerotinia blight was not significantly different than the parent genotype Okrun. Alternatively, plant lines were placed into disease category 2 if they demonstrated a significant increase in resistance to Sclerotinia blight when compared to Okrun.

Plant lines were assessed for growth habit type, either spreading (S) or upright (U), as discussed by Smith and Simpson (1995). Any other abnormalities in phenotype, such as stunting, were also noted as part of the plant line growth habit. Plants were harvested individually (October 15 2000–2002) and returned to the laboratory for analysis. Pods (pod mass) were pulled from individual peanut plants and weighed to the nearest gram. Peanuts from individual plant samples were then weighed, counted, and processed through a 16/64 grading screen. Shelling percentage for each sample was calculated by dividing the total kernel weight by the pod mass. Kernels retained on the screen were then weighed and counted. Weight of kernels retained on the screen was calculated by dividing the kernel weight by the kernel count. Statistical analysis was performed using Least Squared Means analysis and Dunnett's test via SAS version 8.2 (Cary, NC).

Results

Classification of plant lines into disease and growth habit categories is listed in Table 1. Most plant lines were grouped into disease category 1, demonstrating no significant difference in reaction to Sclerotinia blight than the cultivar Okrun. Fourteen plant lines were placed in disease category 2 over the 3-year period, consistently demonstrat-

ing a significant increase in resistance to Sclerotinia blight infection as compared to Okrun. While the majority of plant lines tested did not differ from Okrun in their growth habit, 2 lines had an upright growth habit, with a prominent vertical main stem similar to that of Southwest Runner, and 1 line was noticeably stunted.

The majority of transgenic test lines did not differ from the parent genotype Okrun with respect to pod mass throughout the 3-year test period (Table 2). In 2000, of the 32 lines tested, 8 lines had a significantly smaller pod mass as compared to Okrun. All lines with reduced pod mass contained only the chitinase transgene, with the exception of line #654 which contains both the chitinase and glucanase transgenes. Reduced pod mass was not always consistent with increased Sclerotinia blight incidence. For example, line #188 never became diseased, but consistently had a lower pod mass than cultivar Okrun. Similarly, pod mass reduction could not be correlated with transgene type due to the fact that the majority of test lines contain only the chitinase transgene and show no variation with respect to Okrun when considering pod mass.

Similar results were recorded in 2001 with the majority of test lines being indistinguishable from Okrun with respect to pod mass (Table 2). Six of the plant lines with reduced pod mass in 2000 continued to have lower pod masses than Okrun in 2001. All plant lines with lowered pod mass

Table 1. Transgene, disease category, and growth habit of peanut lines grown under field conditions over a 3-year period.

Plant line	Transgene[†]	Disease category[‡]				Growth[§] habit
		2000	2001	2002	3 year avg	
Okrun	sus. cultivar	1	1	1	1	S
SW runner	res. cultivar	2	2	2	2	U
23	C	2	2	2	2	S
24	C	1	1	1	1	S
33	C	2	1	1	1	S
34	C	2	1	2	1	S
35	C	1	1	1	1	S
51	C	1	1	1	1	S
74	C	1	1	1	1	S
81	C	2	2	2	2	S
87	C	2	2	2	2	S
90	C	1	1	1	1	S
133	C	2	2	2	2	S
135	C	1	1	1	1	S
139	C	1	1	1	1	S
145	C	2	2	2	2	S
146	C	1	1	1	1	S
157	C	1	1	1	1	S
188	C	2	2	2	2	U
412	C	2	2	2	2	S
416	C	2	2	2	2	S
423	C	2	2	2	2	S
461	C	2	2	2	2	SS
487	C + G	2	2	2	2	S
505	G	1	1	1	1	S
511	C	1	1	1	1	S
514	C	1	1	1	1	S
517	C	1	1	2	1	S
531	C	1	1	1	1	S
535	C	1	1	1	1	S
540	C + G	2	2	2	2	U
542	G	2	2	2	2	S
561	C	1	1	1	1	S
654	C + G	2	2	2	2	S

[†]C = chitinase ; G = glucanase.

[‡]1 = not significantly more resistant to Sclerotinia blight than Okrun at the p = 0.05 level; 2 = significantly more resistant to Sclerotinia blight than Okrun at the p = 0.05 level.

[§]S = spreading ; SS = spreading and stunted; U = upright.

contained only the chitinase transgene. Again, pod mass among test lines varied independently of disease incidence and transgene type.

Results from 2002 were less consistent than the two previous years, with only 4 test lines demonstrating a reduced pod mass compared to Okrun (Table 2). These lines (#51, #188, #416, #461) also had a lower pod mass value for other years tested. Over the 3-year period, 8 lines (#51, #74, #157, #188, #461, #561, and #654) averaged a significantly lower pod mass than Okrun.

Shelling percentages for all plant lines tested are shown in Table 3. Throughout the 3-year test period, very little deviation from the values reported for the parent genotype Okrun was seen among the transgenic lines tested. The only variation seen was reported in the year 2000, where 3 of the test lines (#157, #188, and #561) had a significantly lower shelling percentage than Okrun. As with pod mass, shelling percentages varied independently of Sclerotinia blight incidence for all lines tested.

Average kernel weight among transgenic test lines, shown in Table 4, deviated extensively from that recorded for Okrun in the year 2000, but this variation decreased over the 3-year test period. None of the test lines had a kernel weight greater than Okrun. However, 21 lines averaged a kernel weight less than Okrun with the mean decrease being 17%. The number of test lines with kernels significantly smaller than Okrun dropped in 2001 from 21 to six, and then again in 2002 to five. Three of the transgenic lines (#157, #188, and #416) averaged a reduced kernel weight compared to

Table 2. Pod mass of plant lines grown under field conditions for 3 years.

	Pod mass/plant (g)			
Plant line	2000	2001	2002	3 year avg
Okrun	62	72	64	66
SW runner	66	69	75	70
23	64	71	75	70
24	38*	64	72	58
33	58	61	62	60
34	65	75	68	69
35	53	78	66	66
51	42*	54**	49**	48**
74	46*	59*	61	55*
81	52	62	68	61
87	52	76	61	63
90	61	71	65	66
133	48	68	63	60
135	46	75	64	62
139	43	77	53	57
145	49	65	64	59
146	45	68	65	59
157	18***	57**	67	47***
188	11***	47***	48***	35***
412	35	77	80	64
416	63	48***	36***	49**
423	52	64	83	66
461	22***	33***	36***	30***
487	40	73	82	65
505	59	61	66	62
511	46	55	69	57
514	60	75	58	64
517	54	68	62	61
531	59	59*	63	60
535	63	60	65	63
540	44	63	57	55
542	25	82	75	60
561	18***	56**	60	45***
654	28***	65	61	51*

*, **, and *** denote significant differences as compared to Okrun at the 0.05, 0.01, 0.001 probability levels, respectively.

Table 3. Shelling percentage of plant lines grown under field conditions.

Plant line	Shelling percentage			
	2000	2001	2002	3 year avg
Okrun	69	70	70	70
SW runner	68	67	67	67
23	68	72	72	71
24	57	70	69	65
33	64	70	71	68
34	67	70	69	69
35	66	72	66	68
51	62	67	72	67
74	64	71	72	69
81	61	70	72	68
87	69	73	68	70
90	68	73	71	71
133	78	71	70	73
135	59	75	70	68
139	68	74	70	71
145	61	70	70	67
146	62	74	69	68
157	45**	71	69	62
188	54*	68	72	65
412	72	72	62	69
416	62	67	71	67
423	67	72	63	67
461	62	69	71	67
487	63	72	69	68
505	65	70	70	68
511	68	74	71	71
514	71	72	71	71
517	70	73	67	70
531	71	74	70	72
535	74	74	72	73
540	68	67	70	68
542	62	72	70	68
561	48**	70	60*	59*
654	60	68	70	66

* and ** denote significant differences as compared to Okrun at the 0.05 and 0.01 probability levels, respectively.

Okrun for each of the three years tested, with the mean decrease being 23%. Average kernel weight was not consistent with Sclerotinia blight incidence. However, there was a slight correlation of average kernel weight and pod mass, with 5 of the test lines (#51, #157, #188, #461, and #561) having a reduction in both quality traits compared to the parent genotype Okrun over the 3-year test period.

Discussion

This study examined the agronomic performance of transgenic peanut plants under natural field conditions to determine the effect that genetic engineering and the introduction of foreign genes may or may not have on agronomic traits and/or yield components. The performance of transgenic plants may be affected by several aspects of the transformation process including, but not limited to: (1) Insertion mutagenesis: insertion of the transgene disrupts the genomic DNA causing an obvious change in phenotype or field performance (Feldmann and Marks, 1987; Feldmann *et al.*, 1989), (2) Pleiotrophy: individual genes, apparently unrelated, affect plant phenotype and performance (Dale and McPartlan, 1992), and (3) Somoclonal variation: genetic variation resulting from the tissue culture and regeneration process itself (Larkin and Scowcroft, 1981; Karp and Bright, 1985; Karp 1991). Somoclonal variation and insertion/expression of the anti-fungal genes caused no differences in the agronomic characteristics and yield components of the majority of the transgenic lines tested

Table 4. Weight per 100 kernels for peanut lines grown under field conditions for 3 years.

Plant line	Kernel weight (g)			
	2000	2001	2002	3 year avg
Okrun	45	48	47	47
SW runner	42*	42*	42*	42*
23	43	47	45	45
24	34**	46	48	43
33	41*	46	47	45
34	42*	46	46	45
35	42*	49	45	45
51	38**	41*	43	41**
74	41*	45	48	45
81	39*	46	48	44
87	43	45	47	45
90	37**	41*	46	41*
133	41*	46	44	44
135	38*	49	47	45
139	41*	56	45	47
145	39*	46	46	44
146	39*	43	46	43
157	23***	41*	42*	35***
188	32***	37***	36***	35***
412	45	49	49	48
416	33***	42*	41*	39**
423	42*	48	49	46
461	38**	45	42*	42*
487	42*	50	40*	44
505	45	47	49	47
511	43	41*	48	44
514	44	43	46	44
517	44	43	45	44
531	44	44	46	45
535	44	43	46	44
540	44	49	44	46
542	40*	50	52	47
561	27***	43	45	38**
654	40	48	49	46

*, **, and *** denote significant differences as compared to Okrun at the 0.05, 0.01, 0.001 probability levels, respectively.

as compared to non-transformed Okrun. The largest deviation from non-transformed values occurred in the first year of testing which was probably due to the reduced field fitness of greenhouse grown kernels used for seed. This type of result has been reported previously in field tests of other transgenic crops (Arnoldo and Baszczynski, 1991).

The peanut transformation process did result in consistently deteriorated agronomic traits among 5 of the transgenic lines tested: #51, #157, #188, #461, and #561. Although all of these lines contain the rice chitinase transgene, their decrease in field performance appears to be independent of transgene type since many of the unaffected plant lines also contain the same gene. All affected transgenic lines have a spreading growth habit which upon visible inspection is no different than that of Okrun, with the exceptions of line #188 which grows upright and line #461 which is stunted. It was not determined which aspect of the transformation process caused the phenotypic and agronomic abnormalities among the 5 affected lines.

All of the transgenic peanut lines tested here share the common genetic background of the cultivar Okrun, which is susceptible to fungal infection but when unimpeded, produces peanuts with highly marketable agronomic characteristics. The ultimate goal of this research project is to produce a transgenic peanut line, which retains the desirable agronomic traits of Okrun but has an

increased resistance to fungal disease. Several of the peanut lines examined in this study have increased disease resistance and desirable kernel characteristics that warrant further investigation and consideration for commercial release. For example, lines #487 and #540 averaged 50% and 76% less disease, respectively, without loosing any of the yield components characteristic of the Okrun genotype. Assuming 0.91 m row spacing, the average yield for Okrun over the 3-year test period (66 g/plant) can be translated as 3.2 t/ha, with the transgenic plant lines averaging 1.5 to 3.3 t/ha.

Transgenic lines, which have deteriorated agronomic traits may still be of some use in traditional breeding programs. For example, line #188 never became diseased throughout the entire 3-year test period but the kernels from this line are reduced in weight, similar to that of Southwest Runner, which produces kernels of a size undesirable to some shelling companies. Previous studies have shown that morphological resistance exists among lines with upright, bunch growth habits, probably due to the lack of a dense plant canopy which contributes to optimal disease conditions (Melouk and Backman, 1995). The location of transgene insertion for #188 has not yet been determined and thus it is assumed, but unknown, that the insertion event disrupted a gene crucial for a spreading growth habit (mutational interference). Line #188 contains a single copy of the rice chitinase transgene and has been previously shown to have a transgene expression level 22% above background level under non-challenged conditions (Chenault *et al*., 2002). Although the transgene expression level of line #188 is well within the range of hydrolase activity reported elsewhere for plant varieties with heightened fungal resistance (Lozovaya *et al*., 1998), we realize the upright growth habit most likely plays a major role in the total resistance observed. Transfer of the resistance trait of line #188 into other genetic backgrounds could eliminate the problem of small kernel size.

Agronomic characteristics of the peanut lines evaluated under field conditions in this study, including pod mass, shelling percentage, and kernel weight, were statistically comparable between the majority of transformed and non-transformed plant lines. These results indicate that peanut can be genetically engineered successfully, and that in this case, plant lines can be regenerated in which the microprojectile bombardment transformation system does not introduce any adverse effects on the intrinsic agronomic and qualitative traits critical to the peanut industry.

Acknowledgements

The authors would like to express their gratitude to the Oklahoma State University Agricultural Experiment Station for their aid in providing land and personnel to conduct these studies. Research assistance provided by Lisa Myers and Doug Glasgow is also deeply appreciated.

Literature Cited

Allefs, S.J.H.M., D.E.A. Florack, C. Hoogendorn, and W.J. Stiekeman. 1995. Erwinia soft rot resistance of potato cultivars transformed with a gene construct coding for antimicrobial peptide cecropin B is not altered. Amer. Pot. J. 72:437-445.

Arce-Ochoa, J.P., F. Daniello, and L.M. Pike. 1995. Field performance comparison of two transgenic summer squash hybrids to their parental hybrid line. HortSci. 30:492-493.

Arnoldo, M., and C.L. Baszczynski. 1991. Evaluation of transgenic canola plants under field conditions. Genome 35:58-63.

Brandle, J.E., and B.L. Miki. 1993. Agronomic performance of sulfonylurea-resistant transgenic flue-cured tobacco grown under field conditions. Crop Sci. 33:847-852.

Casas, A.M., A.K. Kononowicz, U.B. Zehr, D.T. Tomes, J.D. Axtell, L.G. Butler, R.A. Bressan, and P.M. Hasegawa. 1993. Transgenic sorghum plants via microprojectile bombardment. Proc. Nat. Acad. Sci. 90:11212-11216.

Chenault, K.D., J.A. Burns, H.A. Melouk, and M.E. Payton. 2002. Hydrolase activity in transgenic peanut. Peanut Science 29:89-95.

Chenault, K.D., M.E. Payton, and H.A. Melouk. 2003. Greenhouse testing of transgenic peanut for resistance to *Sclerotinia minor*. Peanut Science 30:116-120.

Coulombe, B.A., D.M. Panter, J.J. Stanton, and R.G. Ward. 1994. Yield performance of transgenic cotton strains resistant to Buctril herbicide. Proc. Beltwide Cotton Conf. 2:651.

Delannay, X., T.T. Bauman, D.H. Beighley, M.J. Buettner, H.D. Coble, M.S. DeFelice, C.W. Derting, T.J. Deidrick, J.L. Griffin, E.S. Hagood, F.G. Hancock, S.E. Hart, B.J. LaVallee, M.M. Loux, W.E. Lueschen, K.W. Matson, C.K. Moots, E. Murdock, A.D. Nickell, M.D.K. Owen, E.H. Paschal II, L.M. Prochasks, P.J. Raymond, D.B. Reynolds, W.K. Rhodes, F.W. Roeth, P.L. Sprankle, L.J. Taroshione, C.N. Tinius, R.H. Walker, L.M. Wax, H.D. Weigelt, and S.R. Padgette. 1995. Yield evaluation of a glyphosate-tolerant soybean line after treatment with glyphosate. Crop Sci. 35:1461-1467.

D'Halluin, K., J. Botterman, and W. deGreef. 1990. Engineering of herbicide-resistant alfalfa and evaluation under field conditions. Crop Sci. 30:866-871.

D'Halluin, K., M. Bossut, E. Bonne, B. Mazur, J. Leemans, and J. Botterman. 1992. Transformation of sugarbeet (*Beta vulgaris* L.) and evaluation of herbicide resistance in transgenic plants. Bio/tech. 10:309-314.

Dale, P.J., and H.C. McPartlan. 1992. Field performance of transgenic potato plants compared with controls regenerated from tuber discs and shoot cuttings. Theor. Appl. Gen. 84:585-591.

Feldmann, K.A., and M.D. Marks. 1987. Agrobacterium-mediated transformation of germinating seeds of *Arabidopsis thaliana*, a non-tissue culture approach. Mol. Gen. Genet. 208:1-9.

Feldmann, K.A., M.D. Marks, M.L. Christianson, and R.S. Quatrano. 1989. A dwarf mutant of Arabidopsis generated by T-DNA insertion mutagenesis. Science 243-1351-1354.

Fuchs, M., and R. Provvidenti. 1996. Evaluation of transgenic tomato plants expressing the coat protein gene of cucumber mosaic virus strain WL under field conditions. Plant Dis. 80:270-275.

Karp, A. 1991. On the current understanding of somoclonal variation. Oxford Surveys Plant Mol. Cell Biol. 7:1-58.

Karp, A., and S.W.J. Bright. 1985. On the causes and origins of somoclonal variation. Oxford Surveys Plant Mol. Biol. 2: 199-234.

Kirby, J.S., H.A. Melouk, T.E. Stevens, Jr., D.J. Banks, J.R. Sholar, J.P. Damicone, and K.E. Jackson. 1998. Registration of 'Southwest Runner' peanut. Crop Sci. 38:545-546.

Larkin, P.J., and W.R. Scowcroft. 1981. Somoclonal variation- a novel source of variation from cell cultures for plant improvement. Theor. Appl. Gen. 60:197-214.

Lin, W., C.S. Anuratha, K. Datta, I. Potrykus, S. Muthukrishnan, and S.K. Datta. 1995. Genetic engineering of rice for resistance to sheath blight. Bio/tech. 13:686-691.

Lozovaya, V.V., A. Waranyuwat, and J.M. Widholm. 1998. β-1,3 Glucanase and resistance to *Aspergillus flavus* infection in maize. Crop Sci. 38:1255-1260.

Melouk, H.A., and P.A. Backman. 1995. Management of soil-borne fungal pathogens, pp. 75-85. *In* H.A. Melouk and F.M. Shokes, (eds.) Peanut Health Management. APS Press, St. Paul, MN.

Porter, D.M., and J.L. Steele. 1983. Quantitative assay by elutriation of peanut field soil for sclerotia of *Sclerotinia minor*. Phytopath. 73:636-640.

SAS Inst. 1985. SAS/STAT Guide for personal computers, Ver.6 Ed. SAS Int. Cary, N.C.

Simpson, C.E., O.D. Smith, and H.A. Melouk. 2000. Registration of 'Tamrun 98' peanut. Crop Sci. 40:859.

Smith, O.D., C.E. Simpson, W.J. Grichar, and H.A. Melouk. 1991. Registration of 'Tamspan 90' peanut. Crop Sci. 31:1711.

Smith, O.D., and C.E. Simpson. 1995. Selection of peanut cultivars, pp. 19-22. *In* H.A. Melouk and F.M. Shokes, (eds.) Peanut Health Management. APS Press, St. Paul, MN.

Stahl, E.A., and J.G. Bishop. 2000. Plant-pathogen arms races at the molecular level. Current Opinion in Plant Biology 2000 3: 299-304.

Tricoli, D.M., K.J. Carney, P.F. Russell, J.R. McMaster, D.W. Groff, K.C. Hadden, P.T. Himmel, J.P. Hubbard, M.L. Boeshore, and H.D. Quemada. 1995. Field evaluation of transgenic squash containing single or multiple virus coat protein gene constructs for resistance to cucumber mosaic virus, watermelon mosaic virus2, and zucchini yellow mosaic virus. Bio/tech. 13: 1458-1465.

Vasil, V., A.M. Castillo, M.E. Fromm, and I.K. Vasil. 1992. Herbicide resistant fertile transgenic wheat obtained by microprojectile bombardment of regenerable embryonic callus. Bio/tech. 10: 662-674.

Wilson, F.D., H.M. Flint, W.R. Deaton, and R.E. Buehler. 1994. Yield, yield components, and fiber properties of insect-resistant cotton lines containing a *Bacillus thuringiensis* toxin gene. Crop Sci. 34:38-41.

Yang, H., C. Singsit, A. Wang, D. Gonsalves, and P. Ozais-Akins. 1998. Transgenic peanut plants containing a nucleocapsid protein gene of tomato spotted wilt virus show divergent levels of gene expression. Plant Cell Rep. 17:693-699.