**TRANSFORMATION OF TAES COTTON FOR SEEDLING DISEASE RESISTANCE**

**J. Luo[1], J. M. Arriaga[2], H. Li[1], J.K. Hemphill[1], M. Raisor[1], N. Keller[2], T. Isakeit[2] and J.H. Gould[1]**
**Department of Forest Science[1], Department of Pathology and Microbiology[2]**
**Texas A&M University**
**College Station, TX**

## Abstract

Five TAES cotton (*Gossypium hirsutum* L.) cultivars were transformed using two vectors, pXBOP and pJMA4, which contained genes associated with plant pathogenesis (PR genes): osmotin (PR-5) and a basic PR-1 in pXBOP, and *lox*1 in pJMA4. Plants were transformed using the shoot apex/*Agrobacterium* method (US Pat. # 5,164,310; Smith et al., 1992) developed earlier for cotton (Gould et al., 1991a; 1991b). After kanamycin selection, shoots ($T_0$) were grafted onto in vitro grown seedling rootstocks (Luo and Gould, 1999). Grafted/regenerated plants were normal, fertile, and produced normal progeny. The transferred genes were detected in the DNA of regenerated plants ($T_0$) and in progeny ($T_1$) using PCR amplification and Southern DNA analyses. Gene expression was detected by RT-PCR in the progeny.

## Introduction

The primary pathogens involved in the cotton (*Gossypium hirsutum* L.) seedling disease complex are: *Thielaviopsis* (black root rot), *Rhizoctonia solani, Pythium*, and *Phymatorichum*. Loss in cotton production in Texas due to these fungal pathogens and others has been estimated at 367,000 bales or 10% yield potential (Blasingame and Patel, 1999). The objective of our research was to improve resistance to seed and seedling diseases in Texas public-sector cotton.

Selected cotton cultivars, developed by TAMU/TAES, were transformed with the following antifungal, pathogenesis related (PR) genes: tobacco osmotin (PR-5) and a basic PR-1 in pXBOP, and soybean *lox*1 in pJMA4, to increase resistance to fungal pathogens. Osmotin has a lytic activity on fungal cells causing release of intracellular material and rupture of cells (Abad et al., 1996). The PR-1 genes are expressed in response to infection and are believed to play an important role in host-plant protection (Ohashi and Ohshima, 1992). Soybean *lox*1 gene encodes lipoxygenase-1, which is induced when plants are subjected to biotic (i.e., fungal infection) and abiotic (i.e., drought) stress. When induced, lipoxygenase-1 catalyzes the addition of oxygen to linoleic and linolenic acids to produce hydroperoxy fatty acids, which have been shown to inhibit *Aspergillus* growth and Aflatoxin biosynthesis (Burow, et al., 1997).

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## Materials and Method

Cultivars were chosen from the three TAES/TAMU cotton breeding programs and represent improved public sector germplasm: 91D-92 (C. Wayne Smith, College Station, TX); Tamcot HQ95 and Sphinx (K. El-Zik and P. Thaxton, College Station, TX); Stovepipe and CA3076 (J. Gannaway, Lubbock, TX).

The binary vector, pXBOP (provided by P.M. Hasegawa and R. Bressan, Purdue University), contained the genes encoding the tobacco osmotin (Nelson et al., 1992) and a basic PR-1 protein (Payne et al., 1989). The CaMV 35S promoter drove both genes. The binary vector, pJMA4 (developed by Arriaga and Keller) contained the soybean *lox*1 gene (Shibata et al., 1987) encoding lipoxygenase-1 driven by the seed-specific carrot DC3-577 promoter (Seffens et al., 1990; provided by T. Thomas, TAMU). The DC3 promoter, isolated from developing embryos of carrot, has been shown to be active in stressed seedlings and embryo tissues of the tobacco plant. Both vectors contained the *nos-nptII-nos* cassette to confer resistance to kanamycin. The vector, pXBOP, was used in *Agrobacterium tumefaciens* EHA105 (Hood et al., 1993); pJMA4 was used in EHA101 (Hood et al., 1986).

Shoots from germinating seedlings were isolated and inoculated with *Agrobacterium* and regenerated as described earlier (Gould, et al., 1991a; 1991b; Gould & Magallanes-Cedeno, 1998). Shoots were grafted to seedling rootstock in vitro (Luo & Gould, 1999).

Total DNA was extracted from immature cotton leaves (2-3 cm, dia.) according to Doyle and Doyle (1990) or using a kit (Phytopure, Nucleon Biosciences, distributed by Vector Labs, Burlingame CA). PCR was performed using a kit (PCR Master Kit, Boehringer Mannheim) according to the manufacturer's recommendations. Primers used for the PCR amplification of the transferred *nos-nptII* sequence were: 5' – CCC CTC GGT ATC CAA TTA GAG (forward) and 5' - GTG GGC GAA GAA CTC CAG (reverse). Sequences for *lox*1 primers were: 5' – TCT GAT AGA GGA CTA TCC TTA TGC (forward) and 5' – GAG TAA TTA GAG TAA ACC ACA GGC (reverse). For Southern blotting, DNA (10 ug) was analyzed by a modified procedure of Sambrook et al. (1989). Blots were probed with *nos-nptII* and PR-1 probes. Total RNA was extracted from the $T_1$ progeny using a kit (RNeasy Plant Mini-Kit, Qiagen), and RT-PCR was performed using the primers for *nos-nptII* and the Access RT-

PCR kit (Promega) according to the manufacturer's recommendations

## Results and Discussion

Grafted/regenerated plants were normal, fertile, and produced normal progeny. A total of 184 grafted, putative transgenic plants were recovered at 95% grafting efficiency (Table 1). The grafting method was genotype-independent and varietal differences between rootstock and scion did not affect the rate of plant recovery from culture. Overall, 21 $T_0$ plants were PCR positive (21/184 = 11%) (Table 2) in which 2 were detected as positive by Southern blot. A partial analysis of the $T_1$ progeny by Southern (DNA) analysis showed presence of the transferred *nos-nptII* and PR-1 genes in high molecular weight DNA, which is characteristic of stable transformation and inheritance. RT-PCR demonstrated expression of *nptII* in the progeny.

The shoot apex/*Agrobacterium*-mediated transformation system is a rapid and a reasonably efficient means to recover transgenic plants of any given cotton genotype. This method allows for genetic transformation of elite cultivars and may circumvent incidence of the permanent genetic mutations known as 'somaclonal variation' that are prevalent in plants regenerated from callus and by somatic embryogenesis (Hirochika, 1993). Addition of in vitro shoot tip grafting was particularly helpful in recovering shoots from culture following kanamycin selection, since the incidence of rooting in surviving shoots was severely depressed. The degree of expression of the transferred genes and their effects on the pathogens of Texas cotton are being studied.

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Table 1. Survival of Grafted Shoots and Recovery of Intact Plants.

| Scion/ Shoots | Rootstock | Shoots Grafted | Surviving Grafts | Plants Recovered |
|---|---|---|---|---|
| Sphinx | Stovepipe | 47 | 43 | 91% |
| Sphinx | HQ95 | 22 | 20 | 90% |
| Sphinx | 91D-92 | 21 | 19 | 90% |
| HQ95 | HQ95 | 31 | 30 | 96% |
| 91D-92 | 91D-92 | 25 | 25 | 100% |
| Stovepipe | Stovepipe | 30 | 27 | 90% |
| CA3076 | CA3076 | 20 | 20 | 100% |

Table 2. Molecular Characterization of Transgenic Plants ($T_0$).

| Variety | Plasmid | Putative Transgenic | PCR | Southern NPTII | Analyses |
|---|---|---|---|---|---|
| Sphinx | pXBOP | 80 | 10/80 | ND* | |
| HQ95 | pXBOP | 30 | 2/30 | ND | |
| 91D-92 | pXBOP | 25 | 3/25 | 1/3 | |
| Stovepipe | pXBOP | 27 | 3/27 | 1/3 | |
| CA3076 | pXBOP | 14 | 1/14 | ND | |
| Sphinx | pJMA4 | 2 | 1/2 | ND | |
| CA3076 | pJMA4 | 6 | 1/6 | ND | |

* Note: ND = not determined.