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Research note

In vitro shoot-tip grafting improves recovery of cotton plants from culture

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Abstract

A rapid *in vitro* shoot-tip grafting (STG) technique was adapted to increase recovery of intact cotton plants from shoots developed in culture. Induction of root organogenesis in cotton shoots is genotype dependent and unreliable. The resulting loss of regeneration potential due to failure to form roots can vary from 30 to 80% according to genotype and represents a significant bottleneck in the overall recovery of plants from culture. If the non-rooting shoots are transgenic, the loss in regenerated plant material can be substantial. *In vitro* grafting of cotton shoots to seedling rootstock proved to be a simple and reliable method allowing 90–100% recovery of non-rooting shoots from culture. Success of any given graft was directly related to scion size (0.8–1.0 cm) and age (14–35 days) of the seedling rootstock. The method appeared to be genotype independent, and varietal differences between rootstock and scion did not effect the rate of plant recovery from culture.

Abbreviations: MS – Murashige & Skoog (1962)

Cotton (*Gossypium hirsutum* L.) plants can be regenerated from callus by somatic embryogenesis (Trolinder & Goodin 1987), protoplasts or pre-existing shoots (Gould et al. 1991; Hemphill et al. 1998). Regardless of the regeneration method employed, all methods depend on root formation for recovery of plants from culture. Rooting in cotton is genotype dependent and highly variable (10–93%) (Gould et al. 1991; Hemphill et al. 1998). In the past, we removed non-rooting shoots from culture and allowed them to root in soil (30–70%) following treatment with rooting hormone (Gould et al. 1991; Hemphill et al. 1998); however, shoots that did not produce roots under these conditions often died. In situations where the shoot tissue is valuable, i.e., transgenic shoots regenerating in culture, inefficient rooting produces a significant loss.

Traditional grafting techniques have been adapted to cultured plants for a variety of applications. The original *in vitro* grafting techniques helped speed clonal production of virus-free *Citrus* plants (Murashige et al. 1972; Navarro et al. 1975). Later, *in vitro* grafting was used to rejuvenate adult-phase woody shoots, to assist in the rooting of woody perennials (Navarro 1988), and to aid in the recovery of transgenic *Citrus* plants from culture (Pena et al. 1995a, b). Although cotton is cultivated as an herbaceous annual in the US, the genus is both perennial and woody and induction of shoot and root organogenesis is characteristically difficult (Gould et al. 1991).

Cultivars were chosen from the three cotton (*Gossypium hirsutum* L.) breeding programs of the Texas Agricultural Experiment Station (TAES), and represent non-model improved public sector germplasm: 91D-92, (C. Wayne Smith, College Station, TX); Tamcot HQ95 & Sphinx, (K. El-Zik and P. Thaxton, College Station TX); Stovepipe and CA3076 (J. Gannaway, Lubbock TX). De-linted seed were surface sterilized by soaking for 30 min in 20% household bleach which contained one drop of detergent (Tween-20) and rinsed 4 times with 500 ml sterile water. Surface sterilized seeds were germinated at the rate of two seeds/Petri-plate in sterile Murashige & Skoog

(MS) medium (Murashige & Skoog 1962; Sigma, St. Louis, #M-9274) which contained sucrose, 30 g l^{-1}; myo-inositol, 100 mg l^{-1}; thiamin, 40 mg l^{-1}, and agar 8 g l^{-1}. Medium was dispensed after autoclaving to sterile plastic-Petri dishes (35 ml/plate). Seeds were germinated under 16-h light (1000 Lux) at 28–30°C. Shoot apex of approximately 0.5 × 1.0 mm were isolated from 7–10 day-old cotton seedlings (Gould et al. 1991). Shoots were cultured in agar-solidified MS medium containing kinetin, 0.1 mg l^{-1}, dispensed into petri-dishes for 1–6 weeks or until shoots were large enough for grafting (0.5–1.0 cm).

A modified cleft-graft developed previously for *Citrus* (Luo 1991; Pena et al. 1995a, b) was used. Rootstock seedlings were germinated as described above and kept in their original culture dish during the entire grafting operation. The native shoot immediately above the cotyledonary node was removed and discarded and the cotyledons retained. After removing the shoot, the node-hypocotyl axis was split vertically to a depth of 2–4 cm until resistance in the stem vascular tissue was encountered. Each cotton shoot tip scion was taken from culture and the base cut to form a deep 'V'. The scion was inserted into the base of the vertically split stem until it fit securely. The scion was secured in the graft union by the close fit in the hypocotyl axis and by compression of the hypocotyl/stem halves. When the graft union area was allowed to come into contact with the culture medium, grafts failed to grow together. This problem was prevented by placing sterile material, such as a detached cotyledon, or leaf from another seedling, between the culture medium and the grafted region.

Grafts were exposed to a 16-h illumination of 1000 Lux at 28–30°C. Shoots arising from the cotyledonary node of the rootstock were removed. Grafted shoot-tips began to grow approximately 2 weeks after grafting. At that time, cubes of fresh medium (MS+kinetin, 0.1 mg l^{-1}) were added to the existing culture to stimulate growth for another 1 or 2 weeks. When grafted shoots produced two new leaves (2–4 weeks), plants were transplanted into small (10 cm) pots containing an artificial soil mixture. Pots were enclosed in polyethylene zip-lock bags and the bags gradually opened 1 week later. Plants were transferred to large pots (5 gal.) after 4 weeks (Figure 1) and moved to the greenhouse. Total time from germination of rootstock to flowering of grafted plants in greenhouse was 3–4 months.

In preliminary tests, we compared side-graft and cleft-graft, removal or retention of rootstock cotyledons, and retention of the rootstock seedling in culture during the grafting operation. We found a modified cleft-graft technique to be the most efficient method. Side-graft and cleft-graft had similar rates of success; however, cleft-grafting was easier in operation and grafted plants grew better. Removal of the cotyledons from the rootstock seedling did not effect graft survival, but severely affected growth of grafted plants and decreased overall survival after transfer to soil. Retention of the seedling rootstock in the original germination culture improved recovery and simplified the procedure.

Figure 1. G. hirsutum cv. '91D-92' shoot grafted onto 'HQ95' rootstock 6 weeks after grafting, approximately 3 weeks after transfer to soil.

Survival of the scion was directly related to scion size. Scions of 0.1–0.2 cm, had a lower survival rate (37.5–40%) than larger scions, with the scions of 0.8-1.0 cm having the highest survival rates (95%) (Table 1). Although successful grafts of Sphinx/Stovepipe unions were obtained following 7, 14, 21, 28, 35, 45 days germination, graft survival was best in seedlings 14–35 days old (Table 2) due to lignification in the hypocotyl region which served to anchor the scion firmly in place. Seedlings older than 35 days, exhibited a reduced rate of graft union formation. Overall, scion and rootstock varietal differences (data not shown) were not statistically significant at the $\alpha = 0.05$ level and had little or no effect on plant recovery. Grafted plants of all TAES lines tested were recovered, grew normally,

Table 1. Successful grafts versus scion size

Scion var.	Shoot size	Rootstock[1] cultivar	Shoots grafted	Graft survival	%Successful grafts
Sphinx	0.1–0.2 cm	Stovepipe	40	15	37.5[b]
	0.5–0.7		40	35	87.5[a]
	0.8–1.0		40	38	95.0[a]
Stove-pipe	0.1–0.2 cm	Stovepipe	40	16	40.0[b]
	0.5–0.7		40	34	85.0[a]
	0.8–1.0		40	38	95.0[a]

Note: [1]Rootstock age 28 days. [a,b]Percentages in columns followed by the same letter are not significantly different at $\alpha = 0.05$ level using the multiple comparison test for proportions based on the arcsine transformation (Marascuilo & McSweeney 1977).

Table 2. Successful grafts versus rootstock age

Rootstock age (days)	Shoot[1] cultivar	Rootstock cultivar	Shoots grafted	Graft survival	%Successful grafts
7	Sphinx	Stovepipe	40	26	65.0[b]
14	Sphinx	Stovepipe	40	37	92.5
21	Sphinx	Stovepipe	40	39	97.5[a]
28	Sphinx	Stovepipe	40	39	97.5[a]
35	Sphinx	Stovepipe	40	38	95.0[a]
45	Sphinx	Stovepipe	40	34	85.0

Note: [1]Scion size 0.5–1.0 cm. [a,b]Percentages in columns followed by the same letter are not significantly different at $\alpha = 0.05$ level using the multiple comparison test for proportions based on the arcsine transformation (Marascuilo & McSweeney 1977).

flowered and produced viable seed. No abnormalities indicating graft incompatibility have been observed. Similar observations were reported in *Citrus* shoot-tip grafting (Luo 1991; Pena et al. 1995a, b).

Shoot-tip grafting is a fast and completely reliable (90–100% effective) means of recovering non-rooting cotton shoots from culture. The procedure was more effective than hormonal or other *in vitro* root induction procedures (10–30% successful), and more effective than the soil rooting method (30–70% successful) used previously (Gould et al. 1991). We found that *in vitro* grafting was particularly helpful in recovering non-rooting putative transgenic shoots from culture following *Agrobacterium* inoculation and kanamycin selection since the incidence of rooting in these tissues was severely depressed (data not shown). As of this writing, over 1710 cotton plants have been recovered from 1800 non-rooted shoots regenerating in culture, which represents a significant improvement over currently available methods for rooting cotton shoots.

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