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Regeneration of *Gossypium hirsutum* and *G. barbadense* from shoot apex tissues for transformation *

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Abstract. A method of regenerating cotton plants from the shoot apical meristem of seedlings was developed for use with particle gun and *Agrobacterium*-mediated transformation. This method was developed to circumvent the problems of genotype restriction and chromosomal damage frequently encountered in cotton regeneration in tissue culture through somatic embryogenesis. In this procedure, the cells of the shoot meristem are targeted for transformation. Normal and fertile plants of *Gossypium barbadense* Pima S-6, and 19 cultivars of *G. hirsutum* were regenerated using this method. Shoot regeneration from these tissues was direct and relatively rapid. A MS based, hormone-free medium could be used with all the varieties tested.

Keywords: *Gossypium hirsutum* - *G. barbadense* - apical meristem - plant regeneration - plant transformation

Introduction

Members of the *Gossypium* genus, as well as members of other important crop families, are severely limited in their regeneration *in vitro* from protoplast, callus or leaf tissues. This widespread problem presently restricts improvement, which can be achieved through genetic engineering, to the few genotypes which can be regenerated from callus.

Gossypium hirsutum, and several other *Gossypium* species, can be regenerated from hypocotyl callus by asexual embryogenesis (Davidonis & Hamilton, 1983; Finer, 1988; Lev *et al., 1986*; Mitten, 1985; Rangan & Zavala, 1984; Shoemaker *et al., 1986*; Trolinder & Gooden, 1987; 1988). This regeneration method, used together with the callus-based plant transformation procedure developed by Horsch and colleagues (1985), has been successfully used in *Agrobacterium*-mediated transformation of cotton (Firoozabady *et al., 1987*; Umbeck *et al., 1987*) and in particle bombardment procedures (Finer and McMullen, 1990). These techniques have been successfully applied to the regenerable Coker 312 and 315 cultivars. Bacterial genes encoding herbicide resistance and the Bt endotoxin (Perlak et al., 1990) have permitted studies of cotton transformants of potential agronomic value to proceed to the field trial stage. These advances presently remain restricted to the cultivars which can be regenerated from calli and cell suspensions by somatic embryogenesis.

Almost one hundred cotton cultivars are under cultivation in the United States (G. Jividen, personal communication). Many of these cultivars were selected for yield, fiber quality and resistance to the stresses of the various regions in which they are grown. These and other improved genotypes are typically difficult to regenerate *in vitro* by somatic embryogenesis (Trolinder & Xhixian, 1989). The strategy proposed to move the transferred genes from the transformed regenerable cultivars into the other commercial cultivars, is to cross-breed to the desired cultivars and back-cross to regain the characteristics of the desired cultivar. A more efficient method would be to transform the desired cutlivar directly.

Aside from genotype limitation, many of the plants regenerated from callus as somatic embryos are not normal (Stelly *et al., 1985*). Culture induced genetic damage is commonly observed among plants regenerated through a callus intermediate (Murashige, 1974). Limited analysis of plants regenerated from callus by somatic embryogenesis of two cotton cultivars (Stelly *et al., 1985*; Stelly *et al., 1989*) and of cotton callus cultures (Li *et al., 1989*) revealed extensive phenotypic abnormalities and cytogenetic changes.

In view of the economic importance of cotton and the potential to improve commercial cultivars by genetic transformation, a search was initiated for alternative regeneration methods. This report describes protocols that were developed for the production of shoots and intact plants from pre-existing shoot meristems of isolated seedling plumules of *G. barbadense*, Pima S-6, and 15 commercial and 4 experimental lines of *G. hirsutum*.

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This method differs from other shoot regeneration methods described previously for cotton (Renfroe & Smith, 1986; Bajaj & Gill, 1986). The shoot explant used here is approximately 0.3 to 1 mm in height, consists of an apical meristem plus primordial and 1 or 2 expanding leaves. The larger shoot tip explants described previously were from 1 to 3 cm in height. Preliminary reports of this method have been presented elsewhere (Gould & Smith, 1988; Smith & Gould, 1989; Veluthambi *et al., 1989*).

Methods

Cultivars. The cultivars of *G. hirsutum* and *G. barbadense* used in this study were: (a) Commercial cultivars, *G. barbadense*, Pima S6 (courtesy of J. W. Radin Phoenix AZ); *G. hirsutum*, Acala SJ-2, Coker 310, 312, DeltaPine 50, 90, Paymaster 145, 404, Stoneville 213, 453, 506; TAMCOT CAB-CS glaborous (courtesy of C. W. Smith, College Station, TX); (b) Improved cultivars, MAR-CABCHUS-2-86, MAR-CABUCD3H-1-86; glandless MAR-CCNHLEBS-1-51 (courtesy of K. El Zik, College Station, TX) and TAM 2111 (courtesy of C. W. Smith, College Station, TX); (c) Experimental glandless lines, Acala L4999, L3940, L3938, L3716 (courtesy of G. Jividen, Cotton Incorporated, Raleigh NC and J. Dodds, Schafter CA).

Culture Procedures. Acid delinted seeds were rinsed in running water 15 min, soaked 15 min in 20% commercial bleach which included one drop of 'Tween 20' per 100 ml, and rinsed 3x in sterile water. The disinfected seeds were transferred to medium containing 10g/L TC agar, 1X MS (Murashige and Skoog, 1962) halide stock solution to provide calcium, in petri plates chalazal end down at the density of 5 seeds per plate. Plates were incubated in darkness at 28 C, for 3 to 4 d and excised, or then transferred to a 16 h day regime for 0, 1, 2, or 3 d. The rate of uncontaminated seedlings from this treatment varied between seed lot and genotype.

Shoot apices were isolated with the aid of a dissecting microscope. The seedling apex was exposed by pushing down on one cotyledon until it broke away, exposing the seedling shoot apex. The apex was removed just below the attachment of the largest unexpanded leaf. Additional tissue was removed to expose the base of the shoot meristem (Fig. 1 and 2). The unexpanded and primordial leaves were left in place to supply hormones and other growth factors (Smith & Murashige, 1970, 1982; Shabde & Murashige, 1977).

Shoot apices isolated from seedlings after 3 d germination consisted of the meristematic dome and two primordial leaves (Fig. 1). The shoot apex of 5 d old seedlings (Fig. 2) contained more than 2 leaf primordia. Overall outside length of the 3 d apex was 0.5 mm; that of the 5 d apex was approximately 1.0 mm, most of which was expanding leaf tissues. Cultures were maintained at room temperature, 27°C, under a combination of Gro-Lux (GE) and Vita-lite (Duro-test) lighting, 90 $\mu E/m^2s$ at plate level under continuous light.

Media, Shoot Culture. Media for shoot apex culture contained the salt formulation of MS (Murashige and Skoog, 1962) and the following in mg/L: i-inositol, 100; thiamine-HCL, 1; glucose, 20,000; or sucrose, 20,000; TC agar, 8,000 (KC Biologicals). The following antibiotics were tested in mg/L kanamycin (Sigma), 5 to 200 mg/L; Cefotaxime (Calbiochem, La Jolla, CA), 100 to 300 mg/L; carbenicillan (Sigma, St Louis MO), 100 to 500 mg/L; Clavamox (Beecham, TN), 250 to 500 mg/L. Media were sterilized by autoclaving. Plant hormones and antibiotics were filter sterilized (0.2 μm, Schleicher & Schuell, Keene, NH) and added to cooled media. Some of the hormone supplementation regimes tested for shoot development are described in Table 1. Other media additives tested were: fluridone (Lilly), citric acid and activated charcoal (Table 2). Plates were sealed with Parafilm. Tissues were recultured at 14 d intervals.

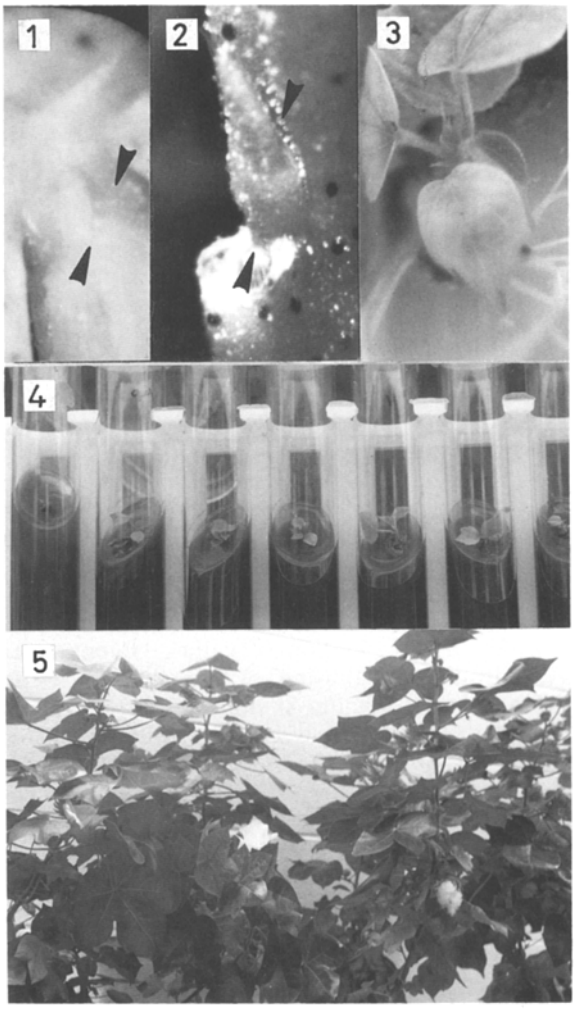

Fig 1. Apex region of a cotton seedling germinated for 3 d. Cotyledons have been trimmed and one has been removed to expose the shoot apex, approximately 0.3-0.5 mm in height, consisting of two primordial leaves and the shoot meristem. The shoot apex is removed from the seedling just below the origin of the leaves.

Fig 2. Magnification of the apex region of a cotton seedling germinated for 5 d. One cotyledon has been removed, exposing the plumule, or shoot apex, approximately 0.5 to 1.0 mm maximum outer dimension of the unexpanded leaves The shoot apex is removed from the seedling just below the attachment of the largest expanding leaf (arrow).

Fig 3. Shoots of Pima S-6 approximately 8 weeks after isolation and a 1 week passage on charcoal containing medium. Note: pale older leaves in most cultures and green new growth.

Fig 4. Spontaneous root formation in vitro following shoot development, Acala L4999.

Fig 5. Plants from shoot apex culture. Plants are: TAM 2111; Acala SJ-2; and Acala L4999.

Media, Rooting. Some of the treatments tested for rooting *in vitro* are described in Table 3. Direct rooting of shoots, 1.0 cm or larger, was attempted using a dry, rooting agent (Rootone, GreenLight, San Antonio, TX). Rooted shoots were transferred to potting soil and hardened off under high light (500-900 $\mu E/m^2S$).

Results

Seedling Age and Stage of Apex Development

The 3 d old shoot apex consisted of a meristematic dome and two, non-green primordial leaves (Fig. 1). At the 5 d stage, the apex consisted of 1 to 2 unexpanded leaves and 2 or more leaf primordia (Fig. 2). Apices from the 5 d stage were used in experiments described in Table 1.

Initiation and Reculture Media

Shoots Isolated from the 5 test cultivars, Coker 312, Paymaster 145, Stoneville 213, TAMCOT and Pima S6, grew well on all media for the first 2 weeks of culture (Table 1). The effects of a medium did not become apparent until 3 to 4 weeks of culture. For the 5 test cultivars, media with or without 0.01 mg/L IAA, were adequate for normal shoot development. Media containing 0.5 mg/L or more IAA enhanced leaf expansion, but also caused loss of apex organization and formation of callus. Higher levels of IAA promoted callusing of the shoot. Media containing 0 to 0.5 mg/L kinetin supported normal development. At 1.0 mg/L and higher levels of kinetin, tissue from some cultivars blackened. In general, cytokinin treatments promoted leaf development at the expense of shoot development. The best reculture media were those which did not deviate substantially from the initial culture medium. The presence of kinetin in the reculture media at 0.01 or 0.1 mg/L was not detrimental to shoot development.

For each of the initial five test cultivars, Coker 312, Paymaster 145, Stoneville 213, TAMCOT and Pima S6, an individualized medium was defined. However, excellent results were also obtained with hormone-free MS medium containing 100 mg/L i-inositol, 0.1 mg/L thiamine and 20 g/L glucose or sucrose (Table 1). When tissues were recultured onto this media at 2 week intervals, shoots progressed rapidly to the 4 leaf stage within 5 to 6 weeks.

The protocol which consistently produced the best results from shoot apices of 3 d and 5 d old seedlings from the 20 cultivars tested included the following: excision and culture onto MS medium containing kinetin, 0.1 mg/L, for 1 week; culture on hormone-free MS medium for 1 to 2 weeks (Fig. 3); culture on MS medium containing 0.3% activated charcoal 2 to 3 weeks, alternating with charcoal-free MS medium until roots formed spontaneously (Fig. 4) or until shoots were approximately 3 cm in height, dipped in rootone and transferred to soil.

Auto-inhibition

Shoots tended to miniaturize, discolor, and die after continued and frequent reculture in petri-plates (Table 2). This trend was sometimes reversed by reculture to larger culture vessels. The synthesis and/or build-up of abscisic acid (ABA) in the cultured shoots may have been involved in these instances of shoot stunting and discoloration.

Table 1. Initiation and reculture media: Effect of IAA & kinetin regimes on shoot apex development *in vitro* on MS based medium containing: thiamine, 1 mg/L; i-inositol, 100 mg/L; sucrose, 20 g/L; Clavamox, 250 mg/L or carbenicillin, 500 mg/L; TC agar, 8 g/L. Shoots were isolated from seedlings after 5 d germination. Observations were made after 2-3 culture cycles.

	EXPLANTS DEVELOPING AS SHOOTS, %						
	Hormone Composition mg/L						
Medium:	1 IAA:1.0 Kin:0.05	2 IAA:1.0 Kin:0.5	3 IAA:0.5 Kin:0.5	4 IAA:0.5 Kin:1.0	5 IAA: 0.1 Kin:1.0	6 IAA: 0 Kin:0.1	7 IAA: Kin:
G. hirsutum							
Acala							
SJ-2	-	-	-	-	70	100	100
L4999	-	-	-	-	-	100	99
L3940	-	-	-	-	-	100	100
L3938	-	-	-	-	-	100	97
L3716	-	-	-	-	-	100	100
Coker							
310	-	-	-	-	-	99	99
*312	9	10	70	34	10	99	97
DeltaPine							
50	-	-	-	-	-	100	95
90	-	-	-	-	-	100	99
MAR-CABCHUS-2-86			-	-	-	100	90
MAR-CABUCD3H-1-86			-	-	-	100	96
MAR-CCNHLEBS-1-51 glandless			-	-	-	100	99
Paymaster							
*145	15	25	62	59	45	95	83
404	-	-	-	-	-	95	95
Stoneville							
*145	15	25	62	59	45	95	83
404	-	-	-	-	-	95	95
506	-	-	-	-	-	100	92
TAM							
2111	-	-	-	-	-	98	98
TAMCOT							
*CAB-CS glaborous	14	64	87	95	7	100	100
G. barbadense							
Pima							
*S-6	5	10	29	26	33	85	45

*One of 5 initial test cultivars described in text, test media contained carbenicillin, 500 mg/L.

Table 2. Effects of treatments on auto-inhibition of shoot apex explants of *G. hirsutum*, Stoneville 453, Paymaster and TAMCOT, in vitro. Observations made 2 to 3 weeks after treatment.

	EFFECT		
TREATMENT	Tissue Color	Tissue Viability*	Tissue Growth
Control (inhibited shoots)	black	-	miniaturization, death
Citric acid, 10 g/L	brown	+/-	slight benefit
Fluridone, 10 mg/L; 30 mg/L	white	+	vigorous
Activated Charcoal, 0.01, 0.3, 1.0 mg/L	green	+	improved; normal

* + = tissue obviously alive.
- = tissues apparently dead.

A 2 week passage on medium containing 10 mg/L fluridone (Lilly, W. Lafayette IN), an inhibitor of ABA synthesis, (Fong *et al.*, 1983; Smith *et al.*, 1990), was sufficent to release 22 out of 75 apparently dead shoots from this dormant or inhibited state. Five of the 22 shoots emerging after this application were robust but white. Green tissue appeared after reculture on fluridone-free medium.

Addition of 10 g/L citric acid to the culture medium had a beneficial effect on some of the shoots. This compound may interfere in the regulation of secondary metabolites (Apotol *et al.*, 1987). Callus and shoot tissues on media containing citric acid were brown rather than black. Vigorous shoot growth and in one case axillary shoot growth was observed.

Table 3. Effects of rooting treatments on cotton shoots after 6 to 10 weeks of culture, including at least 1 month culture on media containing Clavamox, 250 mg/L or carbenicillin, 500 mg/L.

TREATMENT	CULTIVAR[1] Coker 312	Paymaster 145	Stoneville 213	TAMCOT CAB-CS	Pima S6
MS + IAA: 1, 2, 5 mg/l	0	0	0	0	0
MS + NAA: 1, 2, 3, 5, 5 mg/l	0	0	0	0	0
1/2 MS + sucrose, 0, 10, 15 g/L	0	0	0	0	-
1/3 MS + sucrose 0, 10, 15 g/L	0	0	0	0	-
MS + GA + IAA + kinetin vermiculite	0	0	0	0	0
Activated Charcoal 0.3%	++	++	++	++	++
IBA mg/L dip:					
Vermiculite	+	+	+	+	+
Potting soil	+	+	+	+	+
Fiber glass	0	0	0	0	0
Charcoal containing medium followed by transfer to potting soil + rooting agent	+++	+++	+++	+++	+++

[1]Qualitative evaluation of rooting treatments: 0 = no rooting; + low incidence of rooting; ++ moderate rooting; +++ improved rooting.

The most successful treatment used to prevent and overcome the auto-inhibitory response was culture onto media containing 0.3% activated charcoal (Gould & Murashige, 1985). When growth on this medium began to slow, the shoots were cultured onto charcoal-free medium for 2 to 3 weeks, then re-cultured onto medium containing charcoal.

Rooting Media

All attempts to induce root formation *in vitro* were unsuccessful (Table 3). Hormone treatments tested to induce rooting in culture resulted in tissue mortality. Spontaneous rooting of shoots in culture (Fig. 4), especially in media containing activated charcoal and rooting of 1.0 cm or larger shoots, using rooting agent in vermiculite or potting soil, were the most successful (30%).

Recovery of Plants and Plant Phenotype

The survival of shoots excised after 5 d of germination and cultured on hormone-free media was relatively high (430 to 571 shoots per 1000 excised). Shoots excsied from 3 d old seedlings grew best when first cultured on media containing kinetin 0.1 mg/l. The majority of tissue loss during the first stage of culture was due to seed-borne contamination or accidental contamination of the culture. To date, the overall recovery of rooted shoots from the initial populations of isolated shoots has been approximately 5 to 7%. Recovery of rooted shoots from cultured shoots 1.0 cm or larger was 30% when charcoal was used. Plants recovered from culture were normal in appearance and fertile (Fig. 5). In the cultivar TAMCOT a single abnormality, consisting of two upper branches of varigated leaves, was observed.

Discussion

Shoot Apex Culture

Shoot apex culture was first described by Morel (1955, 1960) for clonal propagation and virus eradication. This method has been used successfully with many monocot and dicot families (Murashige, 1974). Theoretically, the tissues of the apical meristem are best suited for use in plant propagation and regeneration because these tissues are programmed for shoot organogenesis and do not need to differentiate to a meristematic state. In practice, this method has few genotype limitations, and the incidence of somaclonal variation in regenerated plants is low (Murashige, 1974).

In the present study, isolated shoot apices of 0.3 to 1.0 mm containing the meristem, unexpanded leaves, and leaf primordia developed successfully on media which did not contain plant hormones. Media parameters reported in an earlier study of cotton meristem culture (Chappell & Mauney, 1967), and culture of the larger shoot tips (Renfroe & Smith, 1986; Bajaj & Gill, 1986) were tested. Studies with coleus (Smith & Murashige, 1970; 1982) and carnation (Shabde & Murashige, 1979) reported similar hormone-autonomous growth of the shoot meristem when leaf primordia were included in the explant.

Recovery of Plants

Regeneration from the shoot apex was direct and simple. Theoretically, each excised apex will develop into a rooted plant; however, the yield of shoots *in vitro* from isolated apices depended on the incidence of contamination and rooting efficiency. At the time of this writing, the recovery of intact plants from isolated shoot apices was limited by an apparent auto-inhibitory response and the difficulty in manipulating root organogenesis. Both phenomena could be corrected by the addition of activated charcoal to the media which suggests these two problems may be manifestations of a common regulatory mechanism.

By allowing shoots to root spontaneously as the shoots enlarged in culture, the frequency of rooting improved. However, the most successful procedure was the direct application of a dry rooting agent to shoots of at least 1.0 cm and transfer to soil. The frequency of rooting may be cultivar dependent. Cultivars giving the best response to this rooting treatment were Pima S6 and the experimental Acala glandless lines L4999, L3940, L3938, and L3716. These rooted readily when treated with rooting agent and transferred from charcoal containing medium to soil (70 to 90%) representing an overall recovery of 30% of shoots excised recovered as rooted plants. Other investigators have reported the rooting of larger 2 to 3 cm shoot tips to be fairly common (Bajaj & Gill, 1986; Renfroe & Smith, 1986; Trolinder, personal communication).

A similar problem with rooting was encountered in the germination of somatic embryos (Trolinder, personal communication); in this case, root primordia may have been present in many of the embryos.

Plant Phenotype

All of the plants regenerated from *G. barbadense*, Pima S6 and of the 19 *G. hirsutum* cultivars tested exhibited normal phenotype, flowered and set seed. The only exception to normal phenotype was a partially variegated individual of the TAMCOT cultivar. Normal phenotype should be expected of plants regenerated from shoot apices (Murashige, 1974). In comparison, cotton plants regenerated by somatic embryogenesis, produced many abnormal phenotypes and cytogenetically aberrant plants. Upon cytogenetic analysis, Stelly and colleagues (Stelly *et al.*, 1989) found that the loss of chromosomes was common.

Applicability to Plant Transformation

The shoot meristem-based method can be applied to plant transformation, either by particle bombardment (Christou et al., 1989) or *Agrobacterium*-mediated gene transfer. Compared to the callus-based regeneration by somatic embryogenesis, the advantages to using a meristem-based method are: a low incidence of culture induced genetic change, and a simple and direct development of transformed plants.

Despite a popular belief that meristematic tissues of plants cannot be used in *Agrobacterium*-mediated transformation, there is no direct evidence to support this view. To date, meristem-based methods have been used successfully in *Agrobacterium-mediated transformation of* petunia (Ulian *et al.*, 1988), pea (Hussey *et al.*, 1989), sunflower (Schrammeijer et al. 1990), and corn (Gould et al. 1991).

Of the plant genera we have regenerated *in vitro* from shoot apices, cotton has been the most difficult. Despite the recalcitrance of this genus, use of the shoot apical meristem can make a wider range of cotton germplasm accessible to improvement by the currently available transformation methods.

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References

Apostol I, Low PS, Heinstein P, Stipanovic R, Altman D (1987) Plant Physiol 84:1276-1280
Bajaj YPS, Gill MS (1986) Ind J Exp Bot 24:581-583
Chappell J, Mauney JR (1967) Phyton 24:93-100
Christou P, Swain WF, Yang NS, McCabe DE (1989) Proc Natl Acad Sci USA 86:7500-7504
Davidonis GH, Hamilton RH (1983) Plant Sci Lett 32:89-93
Finer J (1988) Plant Cell Rep 7:399-402
Finer J, McMullen M (1990) Plant Cell Rep 8:586-589
Firoozabady E, DeBoer DL, Merlo D, Halk EL, Amerson LN, Rashka KE, Murray EE (1987) Plant Molec Biol 10:105-116
Fong F, Smith JD, Koehler DE (1983) Plant Physiol 73:899-901
Gould J, Devey M, Ulian EC, Hasegawa O, Peterson G, Smith RH (1991) Plant Physiol 95:426-434
Gould J, Murashige T (1985) Plant Tissue Cell & Organ Culture 4:29-42
Gould JH, Smith RH (1988) Proc. Beltwide Cotton Production Conferences, pp 91, National Cotton Council, Memphis, TN
Horsch RB, Fry JS, Hoffman NL, Eichholtz D, Rogers SG, Fraley RT (1985) Science 227:1229-1231
Hussey G, Johnson RD, Warren S (1989) Protoplasma 148:101-105
Lev SV, Kohdzhaeva GA, Deisadze LA, Bykova EB, Kosykh TV, Musaru DA (1986) Dokl Akad Nawk SSSR 288:766-768
Li R, Stelly DM, Trolinder NL (1989) Genome 32:1128-1134
Mitten DH (1985) Proc Beltwide Cotton Production Conferences, pp 57-59, National Cotton Council, Memphis, TN
Morel G (1960) Am Orch Society Bull July:495-497
Morel G, Martin C (1955) Report of XIVth Int Hort Cong, pp 303-310, Netherlands
Murashige T (1974) Ann Rev Plant Physiol 25:135-166
Murashige T, Skoog F (1962) Physiol Plant 15:473-497
Perlak FJ, Deaton R, Armstrong T, Fuchs R, Sims S, Greenplate J, Fischhoff D (1990) Bio/Technol 8:939-944
Rangan TS, Zavala T, Ip A (1984) In Vitro 20:256, abstract
Renfroe M, Smith RH (1986) Proc Beltwide Cotton Production Conferences, pp 78-79, National Cotton Council of America, Memphis , TN
Shabde M, Murashige T (1977) Am J Bot 64:443-448
Schrammeijer B, Sijmons PC, vanden Elzan JM, Hoekema (1990) Plant Cell Reports 9:55-60
Shoemaker RC, Couche LJ, Galbraith DW (1986) Plant Cell Reports 3:178-181
Smith JD, Fong F, Magill CW, Cobb BG, Bai DG (1990) In: Recent Advances in Development and Germination of Seeds, R A Taylorson (ed) Plenum Press, NY (In press)
Smith RH, Gould JH (1989) Proc. Beltwide Cotton Production Conferences, National Cotton Council, Memphis, TN pp 15-16
Smith, RH, Murashige, T (1970) *In vitro* development of isolated shoot apical meristems of angiosperms Am J Bot 57,562-568
Smith RH, Murashige T (1982) Am J Bot 69:1334-1339
Stelly DM, Altman DW, Kohel RJ, Rangan TS, Commiskey E (1985) Agronomy Abstracts American Society of Agronomy Madison, WI p 135
Stelly DM, Altman DW, Kohel RJ, Rangan TS, Commiskey E (1989) Genome 32:762-770
Trolinder NL, Goodin JR (1987) Plant Cell Rep 6:231-234
Trolinder NL, Goodin JR (1988) Plant Tissue Cell and Organ Culture 12:43-53
Trolinder NL, Xhixian C (1989) Plant Cell Reports 8:133-136
Ulian EC, Smith RH, Gould JH, McKnight TD (1988) In Vitro Cell & Devel Bio 24:951-954
Umbeck P, Johnson G, Barton D, Swain W (1987) Bio/Technol 5:263-266
Veluthambi K, Krishnan M, Gould J, Smith RH, Gelvin SB (1989) J Bacteriol 171:3696-3703