Plant Cell Reports (1989) 8:133–136

Plant Cell Reports

Genotype specificity of the somatic embryogenesis response in cotton

Norma L. Trolinder and Chen Xhixian

Plant Physiologist and Visiting Scientist, USDA-ARS, Lubbock, TX 79401, USA

Received December 14, 1988/Revised version received April 8, 1989 – Communicated by J. M. Widholm

Summary

Thirty eight cultivars, strains, and races of Gossypium were screened for somatic embryogenesis with the protocols developed as a model for G. hirsutum L. cv. Coker 312. Four classes of response were identified; high, moderate, low, and non-embryogenic. Four cultivars were further screened with 13 growth regulator regimes to determine if culture environment could change the classification or induce a higher level of response. The classification or level of response did not change. Screening of individual seedlings within a cultivar indicated that genotypic variation for embryogenesis existed. Highly embryogenic individuals were selected from cvs. Coker 312 and Paymaster 303 for use as germplasm sources for transfer of the embryogenic trait to other cultivars and genetic stocks. Only genetically responsive genotypes are amenable to the model developed for Coker 312.

Key words: Cotton, somatic embryogenesis, cultivar.

Abbreviations: 2,4-D: 2,4-dichlorophenoxyacetic acid (Carolina Biological); 2iP: N_6-(2-iso-pentenyl) adenine (Sigma); NAA: A-naphthaleneacetic acid (Sigma); K: kinetin (Sigma); IE: Index of embryogenesis.

Introduction

The use of in vitro genetic manipulation for selection and gene transfer of many of the major crop species has long been hampered by cultivar-specific regeneration (Tomes 1985, Powell and Caligari 1987, Felsenburg et al. 1987, Chen et al. 1987a). Commercial cotton (Gossypium hirsutum L.) entered the biotechnology arena rather recently (Davidonis and Hamilton 1983, Shoemaker et al. 1986, Trolinder and Goodin 1987, 1988a, 1988b, Umbeck et al. 1987). A survey of the cultivars studied by these researchers reveals that most success has been achieved with Coker cvs. 201, 310, 315, and 312. All are sister lines from a cross between Coker 100 wilt and Delta Pine 15. The data to date, therefore, suggest that the regeneration response in cotton is highly cultivar dependent.

While results for a number of cultivars have been reported, an extensive study which explores both genotypic effects and tissue culture effects has been lacking. We have previously identified optimal explant and growth regulator regimes for induction of somatic embryogenesis in Coker 312, our model system (Trolinder and Goodin, 1988a). The purpose of this research was to determine the applicability of the model system to other cultivars, i.e. genotypic dependency. To this end, 38 cultivars, representing various strains, races, and species (genotypes) were tested for the embryogenesis response with four induction systems previously selected as being optimal for Coker 312 regeneration. From this survey, four entries were selected to represent high, medium, low, and non-responsive types. These were then subjected to increasing and decreasing ratios of growth regulator concentrations to determine if the response of the less embryogenic types could be increased by changing culture conditions. Lastly, the possibility of selecting for highly regenerative germplasm within a specific cultivar was investigated.

Offprint requests to: N. L. Trolinder

Materials and Methods

Seeds of Coker 312, 3131, and 5110 were obtained from Seedco, Lubbock, Texas. Other Coker cultivars were obtained from Coker Pedigreed Seed Co., with the exception of Coker 100S and 100W, which along with Deltapine 15, were obtained from Texas A&M and Stoneville, Ms. Paymaster, Acala, Lankart, genetic marker lines, Deltapine and G. barbadense lines were obtained from germplasm maintained at Lubbock, TX. G. arboreum and G. hirsutum cv. Laxmi were gifts of Dr. Tony Mascarenas of the National Chemical Laboratory, Poona, India. The Lu cultivars were gifts by the Shandong Cotton Research Institute, Peoples Republic of China.

Screening of cultivars, species, strains.

Seeds of all cultivars were sterilized, germinated, and hypocotyles cultured and incubated as previously described (Trolinder and Goodin 1987). Four growth regulator regimes were tested based upon previous responses of Coker 312 (Trolinder and Goodin 1988a). These were (mg/l): (1) 0.1, 2,4-D + 0.1 K; (2) 0.1 2,4-D + 0.5 K; (3) 0.5 NAA + 0.1 K; and (4) 0.5 NAA + 0.1 2iP. Ten 4 mm hypocotyl explants were randomly selected for each treatment from a pool of explants obtained from 30 seedlings. Cultures were evaluated after 10 weeks as previously described (Trolinder and Goodin 1988a). The Index of Embryogenesis (IE), a new concept of comparison introduced by Chen et al. (1987b), was used to compare genotypic response. The IE, which incorporates percentage of embryogenic explants and relative number of embryos per explant, has been modified to include the ability to maintain embryogenic cultures as described by Trolinder and Goodin (1988a).

$$IE = \frac{x(R_1)(M_1) + x(R_2)(M_2) + \ldots x(R_5)(M_5)}{n}$$

Where: x = number of explants with rating R_0, R_1, $R_2 \ldots R_5$.

R = rating = 0-5: 0 = no embryos; 1 = $\geq$ 1 < 3; 2 = $\geq$3 < 5; 3 = $\geq$5 < 25; 4 = $\geq$25 < 50% of culture embryogenic; 5 = $\geq$ 50% of culture embryogenic;

M = % of cultures easily maintained when the rating is 1-5.

n = total number of explants.

Cultivars which formed neomorphic types (Trolinder and Goodin 1987, 1988a) were designated by * (Table 1). The maintenance factor (M) for neomorphic types should be adjusted to a lower value as cultures containing only neomorphic types often require several passages in cell suspension to obtain embryogenic cultures comparable to Coker 312 (Chen et al. 1987b). Values of IE reflect only a measurement of somatic embryogenesis and maintenance of embryogenesis. IE does not reflect the ability of embryos to germinate and give rise to whole plants.

Screening of growth regulator regimes

Four responsive types, highly embryogenic, moderately embryogenic, low embryogenic, and non-embryogeneic, were identified by the above screening. One cultivar of each type, Coker 312, Texas racestock 25, Texas Marker 1 and Paymaster 784, respectively, was selected to determine if culture conditions could enhance the embryogenic response of less responsive types as compared to the most responsive types.

All were cultured, maintained, and evaluated as in the previous section with the exception of the addition of 13 growth regulator regimes as in Table 2.

Selection of Elite Individual Plants

Seeds of Coker 312 and Paymaster 303 were planted in a 1:1 mix of peat and sand. Plants were maintained in a lath house during the summer and a greenhouse during the remainder of the year. A routine regime of fertilization and insect control was practiced. Leaf and petiole explants were taken from the mature plants on two dates. Care was taken to obtain explants from the newest tissues as older tissues were more difficult to surface sterilize. Explants were sterilized and cultured as previously described (Trolinder and Goodin 1988a). The medium was supplemented with 2 mg/l NAA + 0.1 mg/l kinetin. Calli were scored visually after three months for the presence of surface embryos. Calli that did not have visible embryos on the surface were placed in cell suspension as previously described (Trolinder and Goodin 1987) and scored for embryogenesis after 6 weeks. A minimum of 12 explants were cultured for each individual plant on each date. Seeds were harvested from each plant, sterilized, and hypocotyls cultured as for previous screening. Calli were scored for surface embryos as described above after a 10-week culture period.

Results and Discussion

Coker 312 was originally selected from a group of 8 cultivars and strains for use in development of a model regeneration system for cotton (Trolinder and Goodin 1987). Subsequently, Coker 312 was extensively characterized in terms of explant response to growth regulator regimes (Trolinder and Goodin, 1988a). From this work, four growth regulator regimes and hypocotyl explants were identified as superior to other growth regulator regimes and explants tested in terms of embryogenic response. These combinations have been used in this study to determine the extent to which the Coker 312 model could be successfully applied to other cultivars. Screening of 38 genotypes substantiates the genotypic specificity of the embryogenic response in cotton (Table 1). Coker 312 exhibits a higher index of embryogenesis (IE) under these four treatments than any cultivars tested, followed by Coker 304 > Coker 315 > T25 > Coker 310. There is a

Table 1. Effects of genotype/strain on the somatic embryogenesis response as calculated by the Index of Embryogenesis (IE).

		Growth Regulator (mg/l)			
		0.1 2,4-D	0.1 2,4-D	0.5 NAA	0.5 NAA
Genotype		0.1 K	0.5 K	0.1 K	0.1 2iP
Stoneville	215	0	0	0	0
	453	0	0	0	0
	506	0	0	0	0
Acala	SJ1	8.4	2.8	0.1	0
	SJ2	2.8	3.0	0	0
	SJ5	7.7	9.2	0	0
	44	0	0	0	0
LU (DELTAPINE BACKGROUND)					
	1*	0.3	4.3	0	0
	2*	0.6	3.0	0.1	0
	3*	0.4	24.81	0	0
	4*	0.4	0.1	0	0
Coker	5110	2.9	0.31	1.8	5.1
	3131	0.1	0	0	0
	100S	4.5	2.2	0	0
	304	0.1	32.9	0.1	0
	315	29.7	0.1	0.1	0.1
	310	10.3	5.9	0.1	5.5
	312	39.0	74.9	64.0	63.1
Deltapine	16*	5.3	12.5	0	0
	SR5*	2.8	0.4	0.1	0
	15*	0.2	0.1	NT	NT
Paymaster	303	5.5	0.2	0	0
	784	0	0	0	0
	145*	0.4	2.9	0.1	0.1
G. barbadense		0	0.3	0	0
G. arboreum var. Jyoti		0	0	0	0
G. thurberi		0	0	0	0
RQSX-1 * (*G. barbadense* x *G. hirsutum*)		0.3	0.3	0	0
Texas Marker 1*		6.9	14.3	1.7	0
Texas Marker 1-L*		4.4	2.0	0	0
Texas Marker 586		0.6	0.6	0	0
Texas Marker 582		0	0	0	0
Double haploid 1		0.5	0.3	0	0
Double haploid HL-1	0.4	4.2	0	0	
Lankart 175		0.5	0.2	0	0
Laxmi (India)		0.3	0.4	0	0.1
T25		13.2	11.6	14.8	0.1
T169*		2.7	0.56	0	0.1

*Indicates embryos were neomorphic in appearance.
NT = not tested.

discrepancy between the largest IE values of Coker cultivars 304, 315, and 312 vs other cultivars. The variation across growth regulator treatments for these cultivars supports environmental influence as a factor also. Coker 312 performed well across all treatments but Coker 315 and Coker 304 performed well on only one treatment. Since these cultivars were developed from

sister lines of a cross between Coker 100W and Deltapine 15, we tested both parental lines for the embryogenic response. Coker 100W exhibited a very high embryogenic response whereas Deltapine 15 was essentially non-embryogenic (Tables 1 & 3). This suggests that genetic components rather than culture components are the most critical factor in obtaining efficient regeneration in cotton. However, the four growth regulator regimes used in this screening were chosen based on the response of Coker 312. Therefore, the possibility still exists that other cultivars might respond as favorably as Coker 312 under different growth regulator regimes. Genotype response fell into four classes: highly embryogenic, moderately embryogenic, low embryogenic and non-embryogenic (Table 1). Genotypes representing each class, Coker 312, T25, TM-1, and P784, were cultured with 17 (Table 2) growth regulator combinations. The classification of these genotypes was not influenced by any of the growth regulator regimes tested nor was the IE of any genotype increased (Table 2). It is possible that further culture manipulations might result in less genotype specificity.

Table 2. Effects of growth regulators on the somatic embryogenesis response of cotton as reflected by the Index of Embryogenesis (IE).

Growth Regulators (mg/l)		Genotype C312	P784	T25	TM-1*
				IE	
0.5 2,4-D	.1 K	30.3	0	0	1.2
	.5 K	24.2	0	1.7	0.3
	1.0 K	37.5	0	0	0
0.25 2,4-D	.1 K	64	0	0.3	1.4
	.5 K	5.5	0	2.7	2.7
	1.0 K	16.7	0	0	0.1
0.1 2,4-D	.1 K	39.0	0	13.2	6.9
	.5 K	74.9	0	11.6	14.3
	1.0 K	59.5	0	7.4	0.2
0.05 2,4-D	.1 K	41.2	0	2.7	NT
	.5 K	23.3	0	2.7	0
	1.0 K	24.5	0	NT	0.1
0.025 2,4-D	.1 K	40.1	0	2.2	0.2
	.5 K	40.9	0	2.7	0.4
	1.0 K	63.0	0.1	5.4	0
0.5 NAA	.1 K	64.0	0	14.8	1.7
	.1 2iP	63.0	0	0.1	0

*Data were compiled from cultures with neomorphic embryos.
NT = not tested.

Our previous work with Coker 312 had suggested that specific individuals within a cultivar are more embryogenic than others. Because the two previous screens were carried out on randomly selected explants from a pool representing a number of seedlings, we carried out a third screening in which seedling identity was maintained throughout the experiment. The results observed among seedlings from the Coker lines Therefore, it seemed reasonable that we could select highly embryogenic Coker 312 individuals (elites) for use as a germplasm source to transfer the embryogenic trait to more recalcitrant cultivars as suggested by Koornneef et al.(1986) for tomato. To that end, 79 Coker 312 donor plants from which to select highly embryogenic elites and 25 Paymaster 303 plants from (Table 3) strongly indicate that specific individuals within a cultivar are more embryogenic than others.

Table 3. Effect of genotype within cultivars on the embryogenic response (E = Embryogenic).

Cultivar	Seedlings tested	E seedlings	% E explants (n = 10)
Coker 100W	15	14	52.0
312	28	20	57.0
310	40	4	20.0
315	40	1	100.0
3131	40	0	0
304	40	1	100.0
Paymaster 145	24	0	0
Texas Marker 1	10	0	0
Deltapine 15	40	0	0
Stoneville 506	34	2	10.0
453	38	0	0

Seedlings were scored as embryogenic if at least one explant contained at least one somatic embryo visually detectable on the callus surface.

which to select non-embryogenic recipients were planted in a greenhouse. Each plant was then screened for the embryogenic response. Since we were interested only in selection of an elite Coker 312 donor and verification of non-embryogenic individuals in Paymaster 303, it was sufficient to initially score calli by visual examination of the surface. Calli with no embryos on the surface were transferred to cell suspensions to confirm their non-embryogenic character. A highly responsive C312 plant and a non-responsive P303 have been identified and crossed reciprocally. F_1 and progeny testing are now underway.

We have not attempted to provide data for each individual plant tested, therefore, Table 4 is designed only to illustrate the range of the embryogenic response for Coker 312 genotypes. Two individuals with a very low response were also selected and maintained. Surprisingly, we identified 3 embryogenic Paymaster 303 plants. Two were highly responsive and one was highly responsive only after 2 passages in cell suspension. Not only were elite individuals identified for high embryogenic response within Coker 312, but a number of plants were identified that responded faster than others in induction of somatic embryogenesis and embryo development (data not shown). This is important in terms of efficiency of regeneration and minimization of somaclonal variation when gene transfer is the ultimate goal. Highly responsive individual plants have remained so over repeated testing, both in terms of percent embryogenic progeny, percent embryogenic mature tissue explants, and length of time for induction and development of somatic embryos. Conversely, low responsive types have consistently remained low after numerous tests.

The identification of highly responsive individuals within Paymaster 303 suggests that it may be possible to select responsive individuals within other cultivars. Given the history of breeding of the various cotton cultivars one might postulate that those cultivars of more heterogeneous background and/or with Coker 100W in their background would be more amenable to elite selection than those of homogenous background. Cotton is usually inbreeding; however, outcrossing of 5 to 30% can occur. Commerical breeders

may or may not control for such outcrossing. Blends of more than one breeding line may be used as commerical lines, therefore, commercial cultivars may not be homozygous inbred material. It is possible that while selecting breeding lines from the original DP15 x C100W cross the embryogenic trait was also selected. We have crossed Coker 312 with the low-responsive T586 multiple morphological marker line and obtained highly embryogenic F_1 plants (unpublished data). Further studies utilizing genetic marker lines T586 (multiple homozygous dominant morphological markers), TM-1 (highly inbred standard), T582 (multiple homozygous recessive morphological markers) and monosomics should help elucidate the genetic mechanisms governing the somatic embryogenesis response in cotton. For the purposes of this study, we must conclude that the model system developed for Coker 312 is applicable only to genotypes that are inherently responsive. Responsiveness may, however, differ significantly between individuals of the same cultivar.

Table 4. Response (% embryogenesis) of 10 parental Coker 312 plants and their progeny.

Plant No.	Parental Explants	Progeny	Explants/ Progeny
1	100.0	100.0	58.7
2	100.0	100.0	61.1
3	100.0	95.6	73.3
4	100.0	88.2	59.1
5	100.0	70.0	53.5
6	88.9	75.0	41.4
7	86.7	100.0	69.7
8	74.2	40.0	14.6
9	57.2	83.3	44.0
10	0.0	5.5	1.3

Parental Explants - leaf and petiole sections were cultured on MS + 2 mg/l NAA + 0.1 mg/l K.

Progeny - hypocotyl explants were cultured on MS + 0.1 mg/l 2,4-D + 0.5 mg/l K.

References

Chen Z, Tony HH, Morowitch J (1987a) Screening of Medicago falcato Germplasm for in vitro Regeneration. J Plant Physiol 128:271-277.

Chen Z, Zhixian L, Trolinder NL, Goodin JR (1987b) Some Characteristics of Somatic Embryogenesis and Plant Regeneration in Cotton Cell Suspension Culture. Scientia Agricultura Sinica 20(5):6-11.

Davidonis GH, Hamilton RH (1983) Plant Regeneration from Callus Tissue of Gossypium hirsutum L. Plant Sci 32:89-93.

Felsenburg T, Feldman M, Galun E (1987) Aneuploid and alloplasmic lines as tools for the study of nuclear and cytoplasmic control of culture ability and regeneration of scutellar calli from common wheat. Theor Appl Genet 74:802-810.

Koornneef M, Hanart C, Jorgsma M, Toma I, Weide R, Zabel P, Hille J (1986) Breeding of Tomato Genotype Readily Accessible to Genetic Manipulation. Plant Sci 45:201-208.

Powell W, Caligari PDS (1987) The in vitro genetics of barley (Hordeum vulgare L.): detection and analysis of reciprocal differences for culture response to 2,4-dichlorophenoxyacetic acid. Heredity 59:293-299.

Shoemaker RC, Couche LJ, Galbraith DW (1986) Characterization of somatic embryogenesis and plant regeneration in cotton. Plant Cell Rep 3:178-181.

Tomes DL (1985) Revlevance to Agriculture in the Eighties. In: Zaitlin M, Day P, Hollandew (eds) Biotechnology in Plant Science. Academic Press, Inc. N.Y. pp. 3-14.

Trolinder NL, Goodin JR (1987) Somatic Embryogenesis and Plant Regeneration in Gossypium hirsutum L. Plant Cell Rep 6:231-234.

Trolinder NL, Goodin JR (1988a) Somatic Embryogenesis in Cotton (Gossypium): I. Effects of Source of Explant and Hormone Regime. Plant Cell, Tissue and Organ Culture 12:31-42.

Trolinder NL, Goodin JR (1988b) Somatic Embryogenesis in Cotton (Gossypium): II. Requirements for Embryo Development and Plant Regeneration. Plant Cell, Tissue and Organ Culture. 12:43-53.

Umbeck P, Johnson G, Barton K, Swain W (1987) Genetically transformed cotton (Gossypium hirsutum L.) plants. Biotech 5:263-266.

Acknowledgement

This research was partially funded by Cotton Incorporated.