

PLANT REGENERATION FROM CALLUS TISSUE OF *GOSSYPIMUM HIRSUTUM* L.

GAYLE H. DAVIDONIS^a and ROBERT H. HAMILTON^b

^aDepartments of Entomology and ^bBiology, Pesticide Research Laboratory and Graduate Study Center, Pennsylvania State University, University Park, PA 16802 (U.S.A.)

(Received March 14th, 1983)

(Revision received May 23rd, 1983)

(Accepted May 25th, 1983)

SUMMARY

The regeneration of plants from cotton callus (*Gossypium hirsutum* L. cv. Coker 310) is described. Somatic proembryoids developed spontaneously after two years in culture on a modified Linsmaier and Skoog (LS) medium. The percentage of calli-forming proembryoids was increased to about 30% by prolonged culture without naphthalene acetic acid (NAA) and kinetin. Development of proembryoids was enhanced by transferring proembryoids to media lacking NH_4NO_3 but containing double the standard KNO_3 concentration and gibberellic acid (GA). Root initiation and growth was promoted by lowering the glucose concentration to 5 g/l.

Key words: Cotton tissue culture — Plant regeneration — *Gossypium*

INTRODUCTION

The development of tissue culture techniques for agronomic crops is the initial phase of studies which could lead to crop improvement. Eventually regeneration of plants from tissue culture must be realized. Factors involved in the initiation and maintenance of callus and suspension cultures of *Gossypium* species have been investigated by a number of laboratories [1–6]. Williams [7] described conditions favorable for root development in *G. hirsutum* L. and somatic embryogenesis in suspension cultures of *G. klotzschianum* has been reported [8]. In one isolated case plantlet regeneration was observed from cotyledon callus [4]. This report describes a

Abbreviations: GA, gibberellic acid; LS, Linsmaier and Skoog; NAA, naphthalene acetic acid.

procedure that permits regeneration of cotton plants from a Coker 310 cotyledon derived callus line.

MATERIALS AND METHODS

Cotton callus tissue was derived from cotyledons of *G. hirsutum* L. cv. Coker 310. After delinting, the seeds were surface sterilized for 10 min in a 50% solution of commercial bleach (2.6% sodium hypochlorite) and rinsed with sterile distilled water. The seeds were germinated in sterile petri dishes containing filter paper moistened with distilled water. Tissue pieces were excised from expanding cotyledons and transferred to an LS medium [9] (8.5 g/l agar) containing 30 g/l glucose, 2 mg/l α -naphthalene acetic acid (NAA) and 1 mg/l kinetin. After 3 months cotyledon tissue gave rise to friable slow-growing gray calli. The callus tissue was subcultured on a modified LS agar medium containing 30 g/l glucose, 1 mg/l NAA and, 0.5 mg/l kinetin. Cultures were maintained under continuous low light conditions (0.5 to $1.0 \mu\text{E}/\text{m}^2 \cdot \text{s}$) at 25°C . The slow-growing callus tissue was subcultured every 6 or 7 weeks. Over a 3-year period 3 callus lines were derived: line 1 was a granular light-beige callus, line 2 was a fast-growing green habituated callus and line 3 was a gray callus. All callus lines formed a few proembryoids. Roots were regularly formed in lines 2 and 3. Only line 3 will be discussed in this paper.

Alterations in media composition included adjustments in inorganic salt concentrations, elimination of NAA and kinetin, addition of GA or changes in the glucose concentration. Some proembryoid cultures and plants were grown under continuous light conditions at a higher light intensity ($58 \mu\text{E}/\text{m}^2 \cdot \text{s}$).

One month after transfer to new media callus tissue was scored visually for the appearance of proembryoids. Proembryoids were defined as yellow or green globular structures (1 mm in diameter) produced on the surface of an otherwise gray-green callus. Proembryoids were transferred to new media and their development was monitored over a 1-month period.

RESULTS AND DISCUSSION

When 2-year-old cotton callus tissue was grown for 13 weeks on LS medium with 30 g/l glucose in the absence of NAA and kinetin and then transferred to either modified LS medium without NAA and kinetin or medium with NAA and kinetin, 30% of the callus pieces developed proembryoids in 4 weeks. Callus tissue (about 135 mg pieces) will grow on media lacking NAA and kinetin for at least two transfers. The increased embryogenic potential of callus tissue after 13 weeks without hormones can be maintained for at least three transfers on media containing NAA and kinetin. Callus tissue was normally transferred every 6 or 7 weeks but if the interval between transfers was greater than 15 weeks, subsequent callus growth and embryogenic potential were greatly reduced.



Fig. 1. Proembryoid development, each division = 1 mm. Top row, globular and cylindrical embryos; middle row, cylindrical and coiled embryos; bottom row, leaf-stem embryos.

Proembryoid development proceeded in several directions (Fig. 1) and in many cases stopped before leaf production. Proembryoids increased in size and developed into cylindrical embryos or took on a coiled form. GA (0.1 mg/l) enhanced elongation of embryos. In cylindrical embryos and coiled embryos, one pole grew out to produce 'leaves' (abnormal leaf-like structures) with vascular tissue or 'leaves' with vascular tissue, stomates and pigment glands. Some embryos in which only vascular tissue had differentiated later developed into dark green calli. It appeared that as soon as stomates were formed pigment glands would differentiate. Adventitious roots were formed before or after 'leaf' development. More normal leaves appeared after the development of the initial leaf-like structures. After 4 normal-appearing leaves were formed plants were transferred to vermiculite (2.5-inch pots) and watered weekly with one fourth strength LS salts. Then 4–6 weeks later plants were transferred to soil and moved to a greenhouse. Plants grown in the greenhouse appeared normal and one had flowered. The regeneration process from proembryoid development to plant was accomplished in 3 or more months depending on the number of initial leaf-like structures produced before normal leaf development.

When proembryoids were transferred to LS media lacking NH_4NO_3 and containing double the KNO_3 concentration, more of the proembryoids

TABLE I

THE PERCENTAGE OF PROEMBRYOIDS REACHING THE LEAF-STEM STAGE AFTER TRANSFER TO NEW MEDIA

	Media			
	Modified LS ^a	Modified LS ^a -NH ₄ NO ₃ + 2 × KNO ₃	Modified LS ^a -NH ₄ NO ₃ + 2 × KNO ₃ + 0.1 mg/l GA	Modified LS ^a -NH ₄ NO ₃ + 2 × KNO ₃ -NAA -Kinetin
% proembryoids at the leaf-stem stage ^b	6	7	19	12

^a LS = Linsmaier and Skoog medium.

^b Percentages are based on 65, 57, 57, and 52 proembryoids, respectively.

developed into leaf-stem type structures (bottom row of Fig. 1). This degree of development occurred in media containing no hormones or NAA, kinetin and 0.1 mg/l GA (Table I). Embryoid growth was slower in media lacking hormones. Transferring advanced leaf-stem embryoids to media containing 5 g/l glucose enhanced root formation. Of the embryoids that reached the leaf-stem stage about 50% of these continued growing and produced normal looking plants.

Glutamine and asparagine were tested for embryoid formation by Price and Smith [8] since the nitrogen flow in developing and germinating cotton embryos followed the route glutamine → asparagine → storage protein → arginine → asparagine [10,11]. Suspension cultures of *G. klotzschianum* formed somatic embryoids after 3- 4 weeks of culture in media containing glutamine but the development of embryoids into plants was not achieved [8].

A normal requisite for somatic embryogenesis in some plants is a reduced nitrogen supply [12]. Our cotton callus readily formed globular proembryoids without a major adjustment in the reduced nitrogen supply. However, removal of ammonium nitrogen in conjunction with the addition of GA seemed to favor proembryoid development to more advanced stages. Other favorable treatments included omission of NAA and kinetin, and reduction in the glucose to 5 g/l to promote root formation. Various combinations of the above treatments and others are being investigated to increase the regeneration achieved with this system.

ACKNOWLEDGEMENTS

Authorized for publication as Paper No. 6653 in the Journal Series of The Pennsylvania Agricultural Experiment Station and supported in part by the Northeastern Regional Project NE115 and Regional Research Funds. We

wish to thank Dr. R.O. Mumma for his encouragement and assistance during all phases of this project.

REFERENCES

- 1 R.U. Schenk and A.C. Hildebrandt, *Can. J. Bot.*, 50 (1972) 199.
- 2 C.L. Hsu and J. McD. Stewart, *Physiol. Plant.*, 36 (1976) 150.
- 3 H.J. Price, R.H. Smith and R.M. Grumbles, *Plant Sci. Lett.*, 10 (1977) 115.
- 4 R.H. Smith, H.J. Price and J.B. Thaxton, *In Vitro*, 13 (1977) 329.
- 5 D.G. Davis, K.E. Dusbabek and R.H. Hoerauf, *In Vitro*, 9 (1974) 395.
- 6 J. Ryjack, M.R. Downing, J.S. Chang and E.D. Mitchell, Jr., *In Vitro*, 15 (1979) 368.
- 7 M.D. Williams, *Diss. Abstr. Int. B*, 39 (1978) 2041.
- 8 J.H. Price and R.H. Smith, *Planta*, 145 (1979) 305.
- 9 E.M. Linsmaier and F. Skoog, *Physiol. Plant.*, 18 (1965) 100.
- 10 A.M. Capdevila and L. Dure, *Plant Physiol.*, 59 (1977) 268.
- 11 M.F. Dilworth and L. Dure, *Plant Physiol.*, 61 (1978) 698.
- 12 H.W. Kohlenbach, Comparative somatic embryogenesis, in: T.A. Thorpe (Ed.), *Frontiers of Plant Tissue Culture 1978*, University of Calgary Press, Calgary, 1978, p. 59.