Plant Cell Reports (1983) 2:308–309

Plant Cell Reports

Production of Haploid Plantlets in Anther Cultures of *Albizzia lebbeck* L.

P. K. Gharyal, A. Rashid, and S. C. Maheshwari

Department of Botany, University of Delhi, Delhi-110007, India

Received May 25, 1983 / October 24, 1983 – Communicated by J. Widholm

ABSTRACT

Anthers of Albizzia lebbeck on B_5 medium (BM) supplemented with kinetin (2 mg/l) and 2, 4-D (0.5 mg/l) showed callus initiation from microspores. Differentiation of embryoids and shoots was obtained on BM + BAP (1 mg/l) + IAA (0.5 mg/l) and of roots on BM. Root tip squashes of the regenerated plantlets showed the haploid chromosome number (n=13), confirming the microspore origin of the regenerants.

INTRODUCTION

Since the first report on induction of haploids in Datura innoxia through aseptic culture of anthers (Guha and Maheshwari 1964), this method has become a standard procedure for obtaining haploid plantlets in many angiosperms (see Maheshwari et al. 1982). This technique is of special relevance for tree improvement because it can help circumvent the lengthy period required to produce a homozygous line by continuous inbreeding (Bonga 1977). In continuation of our earlier research (Gharyal and Maheshwari 1981, 1983), on tissue differentiation in Albizzia lebbeck, known also as the "East Indian Walnut", we report on the induction of haploid plantlets in anther cultures of this important leguminous tree.

MATERIAL AND METHODS

Flower buds were collected from a tree growing at the University Campus in Delhi. Anthers from 0.6 to 0.7 cm long flower buds were employed and in these the microspore development ranged from the late uninucleate to the early bicelled stage. In this plant species, the pollen are released in masullae each of which comprises an aggregate of 16 pollen grains (Fig. 1A).

Anthers were dissected aseptically and implanted on B_5 nutrient medium (BM: Gamborg et al. 1968), supplemented with kinetin or 2,4-dichlorophenoxyacetic acid (2,4-D). To avoid browning, polyvinylpyrrolidone (PVP) or polyvinylpolypyrrolidone (PVPP) was added to some of the media. For all experiments, a minimum of 12 cultures were raised per treatment, each containing about 50-80 anthers per culture. Other cultural conditions were similar to those described earlier (Gharyal et al. 1983).

RESULTS AND DISCUSSION

Anthers cultured on the BM medium senesced within 2 weeks without any apparent change. Addition of kinetin (0.5, 1.0, 2.0 and 4.0 mg/l) or 2,4-D (0.1, 0.5, 1.0 and 2.0 mg/l) resulted in delayed senescence but without any indication of microspore division. However, in the combined presence of kinetin (2 mg/l) and 2,4-D (0.5 mg/l) callus masses emerged from about 20% of the anthers (Fig. 1B). PVP or PVPP, when added together with the hormones at 1,2,4,8 and 10 g/l, did not inhibit browning totally, but delayed it temporarily by about 15 days. PVP also inhibited callusing though PVPP had no such effect. On subculture of young callus masses to BM abundant root production occurred (Fig. 1D) and on medium supplemented with BAP (1 mg/l) + IAA (0.5 mg/l), shoot buds could also be initiated. On the former medium almost 60% of the callus cultures developed shoot buds, whereas on the latter medium such response could be observed in every culture. Frequently, embryoids were also observed arising from the callus (Fig. 1E). Despite the abundance of shoot buds, well developed shoots were rare, but at least two plantlets could be produced upon transfer of shoots (Fig. 1F) to BM for root initiation.

The origin of callus could be traced to the

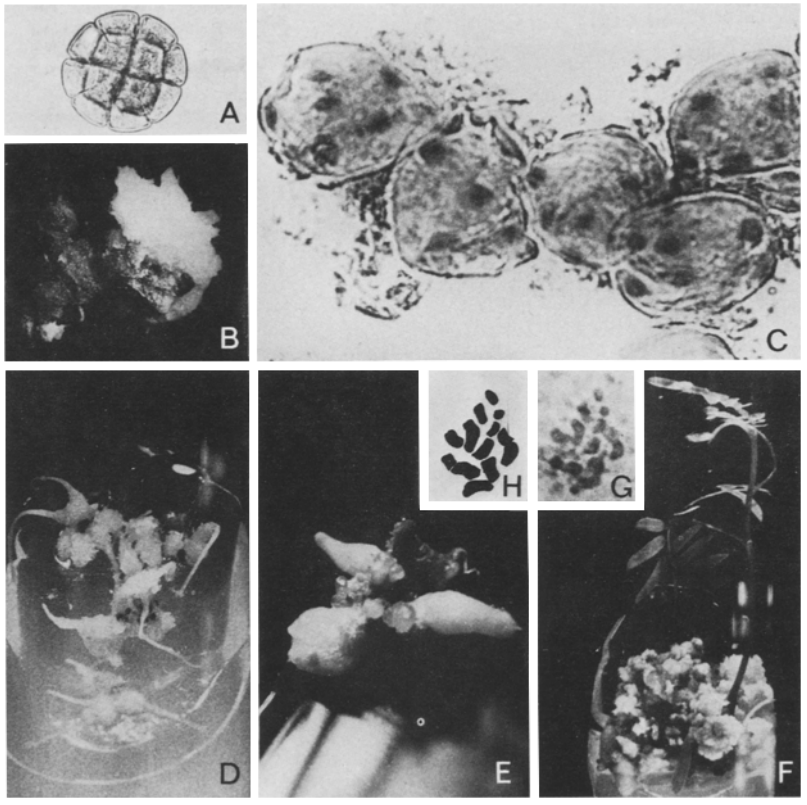

Figure 1. A-H A. A masulla, an aggregate of 16 pollen. X 60. B. Emergence of callus masses from an anther cultured for 2 weeks on BM + Kinetin (2 mg/l) + 2,4-D (0.5 mg/l). C. Multinucleate pollen from cultured anthers. X 630. D-F. Formation of roots, embryoids, and shoot, respectively, from callus masses on different media. G. Haploid karyotype (n=13) from root tip of a regenerated plantlet. X 1650. H. Idiotype of the same.

multinucleate microspores (Fig IC) in the masullae. Root tip squash preparations from the regenerated plantlets showed these to be haploid (n=13; Fig. G,H), thus confirming its microspore origin.

There are only a few reports of haploid plantlet production in the legumes, namely, Pisum sativum (Gupta 1976), Trifolium alexandrinum (Mokhtarzadeh and Constantin 1978), and Arachis hypogaea and A. villosa (Bajaj et al. 1981). In these instances, a direct response i.e. formation of pollen embryoids has been possible only in Trifolium, whereas in the rest -- as in Albizzia lebbeck -- embryogenesis or organogenesis was observed only through a callus phase. Of these species only Albizzia is a tree. Another leguminous tree investigated is Cassia siamea (Gharyal at al. 1983) where, unfortunately, differentiation of the haploid callus was hampered by the quick browning of the callus tissue. In Albizzia, browning could be prevented by use of PVP. Earlier, PVP and PVPP have been employed with success in anther culture experiments and shown to promote pollen embryogenesis probably by adsorption of inhibitory compounds or phenol complexes (Tyagi et al. 1981; Babbar and Gupta 1982).

ACKNOWLEDGEMENTS

This study was aided by a grant from the Department of Science and Technology, Government of India.

REFERENCES

Babbar SB, Gupta SC (1982) Zeitschrift Pflanzenphysiol. 106: 459-464

Bajaj YPS, Ram AK, Labana KS, Singh H (1981) Pl. Sci. Lett. 23: 35-39

Bonga JM (1977)In: Reinert J, Bajaj YPS (eds) Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture, Springer, Berlin Heidelberg New York, pp 93-108

Gamborg OL, Miller RA, Ojima K (1968) Exp. Cell Res. 50: 151-158

Gharyal PK, Maheshwari SC (1981) Naturwissenschaften 68: 379-380

Gharyal PK, Maheshwari SC (1983) Pl. Cell Tissue and Organ Culture 2: 49-53

Gharyal PK, Rashid A, Maheshwari SC (1983) Protoplasma (In press)

Guha S, Maheshwari SC (1964) Nature 212: 97-98

Gupta S (1976) Ind. Agric. 20: 11-21

Maheshwari SC, Rashid A, Tyagi AK (1982) Amer. J. Bot. 69: 865-869

Mokhtarzadeh A, Constantin MJ (1978) Crop Sci. 18: 567-572

Tyagi AK, Rashid A, Maheshwari SC (1981) Physiol. Plant. 53: 405-406