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BRIEF COMMUNICATION

Regeneration of plants from callus tissue of *Desmodium affine* and *Desmodium uncinatum*

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Abstract

Plants were *in vitro* regenerated from leaf callus of *Desmodium affine* and *D. uncinatum*. Leaf explants were induced to form callus when aseptically cultured on Murashige and Skoog medium (MS) supplemented with 6 mg dm^{-3} 6-benzylaminopurine (BAP) in combination with 1 mg dm^{-3} naphthaleneacetic acid (NAA). Regeneration of shoots was induced when callus was cultured on MS medium supplemented with 6 mg dm^{-3} BAP and 0.01 mg dm^{-3} NAA. Roots regenerated in high frequency when differentiated shoots were subcultured on MS medium supplemented only with 0.01 mg dm^{-3} NAA. The regenerated plantlets were successfully grown in pots. Calli from *D. incanum* failed to regenerate shoots.

Additional key words: *Leguminosae*, growth regulators, tissue culture.

The genus *Desmodium* has a wide distribution and comprises over 300 species, with centres of great diversity in Eastern Asia, Mexico and Brazil (Ohashi *et al.* 1981, Schubert 1980). Some of them (*Desmodium intortum, D. uncinatum, D. affine, D. barbatum*) are of economic importance in tropical and subtropical areas of the world ('t Mannetje 1980, Aragao de Oliveira 1983, Burkart 1952).

Plant species which belong to the *Leguminosae* family in general have proved rather difficult to be manipulated *in vitro*. However, plants have been regenerated from tissue, cell and protoplast cultures of several genera of forage legumes,

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Abbreviations: BAP - 6-benzylaminopurine; MS - nutrient medium (major and minor salts, vitamins and 3 % saccharose) after Murashige and Skoog (1962) with 0.8 % agar; NAA - naphthaleneacetic acid.

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such as *Lotus, Medicago, Trifolium*, and *Stylosanthes* (for reviews see Mroginski and Kartha 1984, Hammat *et al.* 1986). Plant regeneration through either organogenesis or somatic embryogenesis has also been described in *Coronilla varia* (Arcioni *et al.* 1988, Gustine and Moyer 1990), *Galega officinalis* (Našinec and Němcová 1990), *Hedysarum coronarium* (Arcioni *et al.* 1985), *Lotononis bainesii* (Bovo *et al.* 1986), *Lupinus* spp. (Sotar 1990) and *Onobrychis coronaria* (Arcioni *et al.* 1988). More recently plant regeneration from callus tissue has also been accomplished in *Centrosema brasilianum* (Angeloni *et al.* 1992) and *Arachis pintoi* (Rey *et al.* 1996). Two reports describe procedures for inducing plant regeneration in tissue culture of *Desmodium incanum* and *D. affine* (Angeloni *et al.* 1988), and hypocotyl derived-callus of *D. heterocarpum* and *D. ovalifolium* (Wofford *et al.* 1992).

This paper describes the morphogenetic responses of *in vitro* cultured leaf explants from three species of *Desmodium* as affected by a wide range of BAP and NAA concentrations.

Seeds of *Desmodium affine* Schlech., *D. incanum* DC. and *D. uncinatum* (Jacq.) DC, kindly supplied by Royo Pallarés (Estación Experimental Agropecuaria INTA, Mercedes, Corrientes, Argentina), were germinated in a potting mixture of soil and sand under greenhouse conditions. The explants were excised from the first expanded

Table 1. Morphogenetic responses of explants from leaves of *Desmodium incanum, D. affine* and *D. uncinatum* cultured on MS media with BAP and NAA for 60 d

Plants	Growth regulators [mg dm^{-3}]		Morphogenetic responses [%]			
	BAP	NAA	calli only	buds	shoots	roots
D. incanum	0	0 - 0.01 - 0.1 - 1 - 3	0	0	0	0
	0.01	0 - 0.01 - 0.1	0	0	0	0
	0.01	1 - 3	2 - 14	0	0	0
	0.1	0 - 0.01 - 0.1	0	0	0	0
	1 or 3	0 - 0.01 - 0.1 - 1 - 3	61 - 100	0	0	0
	6	0 or 0.01	84 - 96	4 - 16	0	0
	6	0.1 - 1 - 3	100	0	0	0
D. affine	0 or 0.01	0 - 0.01 - 0.1 - 1 or 3	0	0	0	0
	0.1	0 - 0.01 - 0.1 - 1	0	0	0	0
	0.1	0.1	22	0	0	0
	1	0 - 0.01 - 0.1 - 1 or 3	26 - 100	0	0	0
	3	0	79	21	0	0
	3	0.01 - 0.1 or 1	90 - 100	0	0	0
	3	3	72	28	0	0
	6	0 - 0.01 - 0.1	70 - 85	15 - 30	0	0
	6	1 - 3	66 - 79	12 - 20	8 - 22	0
D. uncinatum	0 - 0.01	0 - 0.01- 0.1- 1 - 3	0	0	0	0
	0.1	3	93	0	0	0
	1 - 3	0 - 0.01 - 0.1 - 1 - 3	34 - 96	0	0	0
	6	0	95	0	0	0
	6	0.01 - 0.1 - 1 - 3	34 - 67	5 - 11	9 - 32	0

leaf of 6-month-old plants. Leaves were surface sterilized in 70 % ethanol for 40 s followed by immersion in a solution of commercial bleach (0.8 % sodium hypochlorite) for 10 min and were then rinsed several times with water. The laminae were transversally dissected into three pieces and both basal and apical thirds were discarted. The median portion was again cut in squares of approximately 4 mm^2 and placed with the abaxial side down on 4 cm^3 of culture medium in a 11 cm^3 glass tubes. The medium consisted of major and minor salts, as well as vitamins according to Murashige and Skoog (1962), 3 % saccharose and 0.8 % *Sigma* agar. BAP and NAA were added in various combinations and concentrations as indicated. The pH of the media was adjusted to 5.8. The tubes were covered with *Resinite AF - 50*® film and maintained at a constant temperature of 27 ± 2 °C and 14 h photoperiod (white fluorescent lamps, 10 W m^{-2}). Each treatment involved of at least 10 explants (1 explant per tube) and each experiment was repeated 4 times.

Small pieces of callus (30 mg fresh mass) were transferred every 60 d to fresh medium in an attempt to induce shoot regeneration. Two media were tested: *1*) MS lacking plant growth regulators, and *2*) MS + 0.01 mg dm^{-3} NAA + 6 mg dm^{-3} BAP. Shoots regenerated were transferred in a fresh MS medium only with 0.01 mg dm^{-3} NAA. The plants regenerated were potted in a mixture of soil and sand (1:1).

In three species of *Desmodium* most of the media tested induced callus formation, which appeared firstly on the border of leaf explants after 14 d of culture (Table 1). Further growth of the calli was slow and after 60 d of culture, most of the explant surface was covered with brown compact-callus. The observation about the gradual browning of the calli is in agreement with those made on other legumes in which plant regeneration has been accomplished such as *Aeschynomene* spp. (Rey and Mroginski, unpublished), *Stylosanthes guianensis* (Mroginski and Kartha 1981), *Centrosema brasilianum* (Angeloni *et al.* 1992), and *Arachis* spp. (Mroginski and Fernández 1980). The frequency of explants which produced calli was greatly affected by the medium. Specific combinations of BAP and NAA are required for callus proliferation from the explants of tested species of *Desmodium*: only the highest concentrations of BAP tested (3 or 6 mg dm^{-3}) proved to be effective in inducing calli with buds. In addition on MS medium with 6 mg dm^{-3} BAP and 0.01 mg dm^{-3} NAA shoots were also induced to growth in *D. uncinatum* and *D. affine*. On the other hand, under used conditions, calli differentiating roots were not observed. In general, the morphogenetic responses described are in agreement with those obtained with other legumes plants when NAA and BAP have been employed to induce plant regeneration (see reviews by Mroginski and Kartha 1984, Hammat *et al.* 1986).

Like in the case of both *Desmodium heterocarpum* and *D. ovalifolium* (Wofford *et al.* 1992), no structures indicative of somatic embryogenesis were detected in the three plant species involved in this study. Although the origin of bud regeneration will have to be elucidated by histological methods, it appeared that the buds and shoots were differentiated through organogenesis pathway.

When pieces of callus induced from leaf explants of the three plant species were transferred to fresh media containing 6 mg dm^{-3} BAP + 0.01 mg dm^{-3} NAA shoot regeneration was observed in 49 % (range 37 - 64 %) of calli in *D. affine* (Fig. 1*B*)

and 42 % (range 33 - 59 %) of calli in *D. uncinatum* (Fig 1*A*). In most cases multiple shoots were formed (in average 3 shoots per callus).

Shoots were transferred to medium only with NAA (0.01 mg dm^{-3}) in order to induce root formation. As many as 80 % of the shoots of both *D. affine* and

Fig. 1. Plant regeneration in callus derived from leaves of *Desmodium* spp.: *A*) shoot regeneration in callus originated from leaves of *D. uncinatum* cultured on MS + 6 mg dm^{-3} BAP + 1 mg dm^{-3} NAA and subsequently subcultured on MS + 6 mg dm^{-3} BAP + 0.01 mg dm^{-3} NAA; *B*) shoot of *D. affine* regenerated from leaves cultured in the same way; *C*) rooting of shoot of *D. affine* on MS + 0.01 mg. dm^{-3} NAA; *D*) plants of *D. affine* in pots.

D. uncinatum rooted (Fig 1*C*) and the plantlets were successfully potted in a soil and sand (1:1) mixture (Fig 1*D*).

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