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In vitro propagation of *Prosopis* species (*P. chilensis, P. cineraria* and *P. juliflora*)

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Introduction

The genus *Prosopis* has attracted considerable interest for forestry in arid areas. although normally propagated by seed, vegetative propagation of selected *Prosopis* plants from variable populations and from natural hybrids may also be desirable. Propagation by cuttings has been reported (*e.g.,* Felker and Clark, 1981). The use of tissue culture techniques to regenerate plants from nodal explants has also been reported for *P. cineraria* (Goyal and Arya, 1984), *P. tamarugo* and *P. chilensis* (Jordan and Balbao, 1985), and *P. alba* (Tabone *et al.,* 1986). In this study, tissue culture media were evaluated for root initiation, shoot proliferation and shoot growth of *P. chilensis, P. cineraria* and *P. juliflora.*

Materials and Methods

Explants with 1 or 2 nodes were taken from the youngest 6 nodes of the main stem and branches of 3–12 mo old, greenhouse-grown stock plants. Explants were surface sterilized in 70% industrial methylated spirit for 1 min and 5% (v/v) sodium hypochlorite for 5–10 min, and immersed in an anti-oxidant solution (100 mg/l citric acid, 50 mg/l ascorbic acid, 100 mg/l polyvinyl pyrrolidone) for 15 min. Murashige and Skoog medium was used with 8 g/l agar, 30 g/l sucrose, 1.6 g/l glutamine and 81 combinations of plant hormones, including kinetin (K) (0.05–15 mg/l), benzylamino purine (BA) (0.05–15 mg/l), indole acetic acid (IAA) (1–10 mg/l), indole butyric acid (IBA) (1–15 mg/l) and naphthalene acetic acid (NAA) (1–15 mg/l). Between 4 and 16 explants were placed on each medium and incubated at 25°C with a 16 h photoperiod and a photon flux density of 65–200 $\mu E/m^2$ for 51 d.

Results

Without hormones, results for rooting percentage, mean number of shoots/explant node and mean number of nodes/regenerated shoot were, *P. chilensis,* 0%, 0.8, 1.2, *P. cineraria,* 13%, 0.8, 1.0, and *P. juliflora,* 6%, 0.4, 1.0. A summary of the most successful hormone treatments is given in Table I. High levels of BA induced shoot proliferation of *P. chilensis* with 15 mg/l BA, 5 mg/l NAA giving the highest mean of 4.3 shoots/node. Similar levels of K were much less effective for shoot proliferation. Shoot growth of *P. chilensis* was greatest (5 nodes/shoot) with 0.05 mg/l K,

Table I. Summary of the most successful hormone combinations for root initiation, shoot proliferation and shoot growth of *Prosopis chilensis, P. cineraria* and *P. juliflora.*

		K (mg/l)						*BA (mg/l)*					
		0.05	*1*	*3*	*5*	*10*	*15*	*0.05*	*1*	*3*	*5*	*10*	*15*
P. chilensis													
IBA	1	–	–	–				–	–	M			
(mg/l)	3	RS	–	–				RS	–	–			
	15	–	R	–				–	S	–			
NAA	1	–	–	–	–	–	–	–	S		–	M	M
(mg/l)	3	–	–	–				–		S			
	5				S	–	–				M	M	M
	10				S	–	–				M	M	–
	15	–	–	–	–	–	–	–	–		–	–	–
IAA	1				–	–	–				–	–	M
(mg/l)	5				–	–	–				–	M	–
	15				–	–	–				S	–	M
P. cineraria													
IBA	1	R	R	–						–			
(mg/l)	3	R	–	–					S	–			
	15	R	R					–	–	–			
NAA	1		–	–	–	–	–	–	–	–	S	–	–
(mg/l)	3		–	S					–	–			
	5				–	–	–				–	M	–
	10				–	–	–				–	–	–
	15	–	–	–	–	–	–	–	–	–	–	–	–
IAA	1				–	–	–					–	–
(mg/l)	5				–	–	–				–	–	–
	15				–	–	–				–		M
P. juliflora													
IBA	1	R	–	–					–	–			
(mg/l)	3	R	–	–				–	–	–			
	15	RS	–	–				–	–	–			
NAA	1	–		–	–	–	–	–	–	–	–	–	–
(mg/l)	3	–	–	–				–	–	–			
	5				–	–	–				–	–	–
	10				–	–	–				–	–	–
	15	–	–	–	–	–	–	–	–	–	–	–	–
IAA	1				–	–	–				–	–	–
(mg/l)	5				–	–	–				–		–
	15				–	–	–				–	–	

R = >50% of explants rooted; M = mean of >1.5 shoots per explant node; S = mean of >2 nodes per regenerated shoot: – = combinations tested but giving lower values for the above parameters.

3 mg/l IBA. 0.05 mg/l BA with 3 mg/l IBA, or 0.05 or 1 mg/l K, with 3 or 15 mg/l IBA induced rooting. 0.05 mg/l K, 3 mg/l IBA gave the greatest number (2.5) of roots/explant and 1 mg/l K, 15 mg/l IBA the greatest (75%) percentage of rooted explants.

Shoot proliferation of *P. cineraria* was also promoted by high levels of BA, 10 mg/l BA, 5 mg/l NAA resulting in 3

shoots/node. K failed to induce multiple shoot production in *P. cineraria.* Shoot growth was greatest (3 nodes/shoot) with 3 mg/l K, 23 mg/l NAA but 1 mg/l BA, 3 mg/l IBA gave reduced leaf abscission. Rooting of *P. cineraria* was obtained only with combinations of K and IBA (0.05 mg/l K, 1–15 mg/l IBA, and 1 mg/l K, 15 mg/l IBA), the greatest number of roots (2.8/explant) being initiated with 1 mg/l K, 15 mg/l IBA and the highest rooting percentage (75%) being with 0.05 mg/l K, 15 mg/l IBA.

Results for *P. juliflora* were less conclusive. In very few cases were multiple shoots obtained and no treatment gave a mean of >1.5 shoots/node. Shoot growth of *P. juliflora* was greatest (3 nodes/shoot) with 0.05 mg/l K, 15 mg/l IBA. 10 mg/l K, 1 mg/l IAA gave the greatest leaf retention. Root initiation by *P. juliflora* was most successful with K in combination with IBA. 0.05 mg/l K, 15 mg/l IBA was the best treatment (75% rooted explants, 5.6 roots/explant). Rooted plantlets of each species were transferred to compost in a greenhouse with 100% survival after 3 months.

Discussion and Conclusion

The relative ease of micropropagation in this study was *P. chilensis* > *P. cineraria* > *P. juliflora.* The results for all 3 species show a similar pattern. In general, IBA promotes rooting and K is less inhibitory to root production than is BA. High concentrations of BA, but not of K in combination with auxins, promote shoot proliferation probably by stimulating axillary bud growth. Medium to high concentrations of auxin in combination with low to medium cytokinin concentrations promote shoot growth. The results for *P. chilensis* and *P. cineraria* provide the basis for a possible micropropagation system consisting of shoot proliferation, shoot growth and rooting stages, and this system is being evaluated. Further work with *P. juliflora* is required to optimize culture conditions for each stage.

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