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PROPAGATION OF PROSOPIS SPECIES : PROBLEM PERSEVERANCE AND PERSPECTIVES

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ABSTRACT

Prosopis species are predominant components of arid-land vegetation but they are degenerated as biotic pressure has caused large scale deforestation. Plant tissue culture has been proved successful in regeneration of a large number of tree species. However, *Prosopis* species are recalcitrant to regenerate and efforts made by various workers to regenerate and propagate *Prosopis* are discussed in the light of achievements made in the laboratory.

INTRODUCTION

The Indian desert (22°-32°N and 68°-76°E) covering an area of 28600 km^2 represents several specific characteristics. The climate and soil conditions are characterised by high temperature, low and erratic rainfall, evaporation, far exceeding precipitation, low organic matter in the soil and strong desiccating winds. It is also the most densely populated (61 persons/km^2) of the desert areas of the world (Khoshoo and Subrahmanyam 1989). Due to obvious pressure on land, marginal lands are cultivated and available vegetation cover is being over exploited leading to desertification (Paroda 1979). It is in this context that there is an urgent need for development of biotechnology for rapid multiplication of trees for afforestation.

Prosopis cineraria is now available only in protected land areas while *P. juliflora* thrives well in all soils including saline areas of Luni river and Sambhar lake. The other important plants are *Acacia nilotica, Tecomella undulata, Ziziphus mauritiana, Salvadora persica, S. oleoides, Balanites aegyptiaca.* All these species can withstand the long dry and hot periods. In case of monsoon failure they survive without a drop of water for years. It may be due to the fact (Breazeale *et al.* 1950) that certain plants during high moisture regime (e. g. cool night) absorb atmospheric moisture. In case of *P. tamarugo* (recently introduced in India) the unusual epicuticular configuration could be an important system which enhances the foliar absorption of atmospheric moisture (Hull and Bleckmann 1977). It has been pointed out that large amount of mucilage contained in its leaves helps the process of water absorption from atmosphere. Recently (Serrato-Valenti et al. 1989) it has been shown that the stomata together with the anticlinal walls of the epidermal cells are very important sites of water absorption. The histo-chemical tests of leaf section revealed the presence of polyions at these sites (Serrato-Valenti et al. 1989). These

polyions are themselves able to link water in amounts directly related to the intensity of the available negative charges (Modensi and Vanzo 1980).

Origin and importance of Prosopis species : All *Prosopis* species are xerophytic and occur in arid regions of the world. Burkart (1976) described the 44 species of *Prosopis* distributed in tropics and subtropics of both hemispheres. The three species in the section *Prosopis* are natives of Asia and North Africa. Argentina is considered to be the largest centre of diversity for *Prosopis*. The details of botanical description, distribution and biology has been described by Burkart (1976) and Leakey and Last (1980).

Uses : According to Burkart (1976) at least 14 *Prosopis* species are potentially important for afforestation and fodder productions, whereas a few are considered as weed. *P. cineraria* is a multipurpose tree of western Rajasthan. It will not be exaggerated if it is termed as back-bone of rural economy. *Prosopis juliflora* is mainly used as fuel wood and living fence.

Conventional practices and improvement

Prosopis species are propagated through seeds. Being leguminous seeds, they possess hard seed coat and in some cases hard endocarp of fruit wall. Therefore, seeds require pretreatment to enhance success in germination upto 70-80 percent Most species produce abundant seeds (e. g. 1 kg/plant in *P. cineraria*).

Vegetative propagation is possible by suckers in *P. cineraria* and by rooting of stem cuttings in *P. juliflora* (Leakey and Last 1980) and *P. alba* (Felker and Clark 1981). However, the percentage of plantlets obtained through these methods was not very high but they have demonstrated the possibilities of application of the method. Considerable improvement in percentage rooting of *P. alba* stem cutting was obtained by fertilizer application to the stock plants (De-Souza and Felker 1986). Air layering has been used successfully to obtain large number of plants of *P. cineraria* (Solanki et al. 1984). Therefore it is desirable to evolve further these methods before utility at large scale can be established. Simultaneously more species should be tested for these conditions.

Most species of *Prosopis* are diploid (2n = 28) except *P. juliflora* in which tetraploid plants are also known. No serious efforts have been made for hybridisation of *P. cineraria* with other species of *Prosopis*. Little information is available about hybridisation work in *Prosopis* species pertaining to section Algarrobia (See Leakey and Last 1980).

Prosopis species exhibit a wide range of characters due to heterozygosity. Therefore it is essential to select the plants for their desirable characters viz. leaf protein, absence of protease inhibitors, thorniness, leaves yield, timber quality and

quantity etc. These species show moderate to high salinity resistence. These plants are infested with a variety of insects. After careful selection improvement programme can be implemented by conventional breeding methods or through plant tissue, cell and protoplast culture methodology.

In vitro approaches : Plant tissue culture provides an excellent system to study the factors involved in vegetative multiplication and the possibilities of obtaining improved plants by genetic engineering. Vegetative multiplication through tissue culture has emerged as major applied aspect of plant tissue culture research (Murashige 1974; Bajaj 1986). A large number of research groups are working to develop technology for their regeneration through plant tissue culture as an alternative mean to provide large scale plants for afforestation of arid lands. The results obtained with *Prosopis* species are summarised in Table 1 and discussed in the light of results obtained by other workers on *Prosopis* species.

Table 1. Tissue culture and regeneration studies in various species of *Prosopis*.

S. No.	Plant species	Explant	Medium	Result	Reference
1	2	3	4	5	6
1.	*Prosopis cineraria*	Hypocotyl	MS medium + IAA (0.25 mg/l) Kinetin (4.5 mg/l)	Shoot Bud	Goyal & Arya 1981
		Stem	MS medium IAA (3.0 mg/l) Kinetin (0.05 mg/l)	3-5 shoots formation by growth regulator	Goyal & Arya 1984
		Bud explant	-do-	1-3 shoots, medium factors	Goyal & Arya 1984a
		Stem, Root	MS medium	Shoot formation	CAZRI (Per. Comm.)
		Stem	MS medium + Kin. (1.5 mg/l) + BAP (1.5 mg/l)	Shoot formation	Nandwani & Ramawat 1989
		Stem	MS medium + BAP (10.0 mg/l) NAA (5.0 mg/l)	Shoot development	Batchelor et al. 1989
		Stem	MS medium + BAP (5.0 mg/l)	Shoot regeneration	Nandwani 1990
		Hypocotyl	MS medium +	Plantlets formation	Nandwani, 1990
2.	*Prosopis tamarugo*	Nodal section	MS medium	Shoot regeneration	Jordan et al. 1985
		Shoot tip	MS medium	Shoot-like structure	Jordan, et al. 1987
		Hypocotyl	MS medium + BAP (5.0 mg/l)	Plantlets formation	Nandwani & Ramawat 1989, Nandwani 1990

1	2	3	4	5	6
		Stem	MS medium + NAA (0.2 mg/l)+ BAP (5.0 mg/l)	Shoot buds formation	Nandwani 1990
3.	*P. alba*	Shoot tip	MS medium	Contamination, somatic embryo	Anno. 1987
		Lateral bud explant	MS medium+ BAP (4.4 x 10^{-5} M)+IAA (2.9 x 10^{-5}M)	Shoot multiplication	Tabone et al. 1986
4.	*P. chilensis*	Node	MS medium	Callus	Jordan et al. 1985
		Nodal segment, callus	B5 medium + 2, 4-D (2.0 mg/l)+ cysteine (60 mg/l)	Embryoid formation	Jordan et al. 1987
		Shoot tip	MS medium + NAA (0.3 mg/l), BAP (0.1 mg/l), GA_3 (0.01 mg/l)	Multiple shoots formation	Jordan 1988
		Stem	MS medium + BA (15.0 mg/?), NAA (5.0mg/l)	Shoot proliferation	Batchelor et al. 1989
5.	*P. juliflora*	Stem	MS medium	Not known	TERI, Delhi (per. comm.)
		Meristem/ shoot tip Nodal segment	—	Shoots	Annon. 1987
		Nodal cutting	MS medium, kinetin BAP (0.25 uM)	Shoots development	Wainright and England 1987
		Stem	MS medium IAA (0.1 mg/l) BAP (5.0 mg/l)	Multiple shoots	Nandwani & Ramawat 1989
		Stem	MS medium	Shoot development	Batchelor et al. 1989
		Stem	MS medium + IAA (0.1 mg/l), BAP (5.0 mg/l)	Plantlets formation	Nandwani & Ramawat, 1991
6.	*P. tamarugo*	Meristem, node, callus	—	Microbial contamination, Embryo like structure	Annon. 1987
7.	*Prosopis* sp	Meristem/ shoot cotyledon	—	—	Delgado (Per. comm.) Annon., 1987

Table 2 : Summarized results showing effect of growth hormones, and medium factors on response of callus tissue of *P. tamarugo*. Tissues were grown at 28 ± 2'C under 16 hr illumination (2500 lux) for eight weeks. Average of atleast six samples.

Treatment No.	Medium	Growth hormones (mg/l)		Medium factors (mg/l)		Nitrogen	Response
		Auxin	Cytokinin	Ascorbic acid	Sucrose		
1.	MS salts	NAA 0.05-0.3	Kn 1.5-5.0	—	40,000	MS	Callus compact
2.	MS salts	IAA 0.05-0.3	Kn 1.5-5.0	—	40,000	MS	Callus compact
3.	MS salts (3/4)	NAA 0.05-0.2	BAP 2.5-5.0	50	30,000	MS	Loose, white callus
4.	MS salts (3/4)	NAA 0. 2-0.4	2ip 0.5-2.5 BAP 0.5-2.5 Kn 0.5-2.5	—	40,000 + Dextrose 5000	MS + Spermine 5.0, Spermidine 10.0	Callus, slow growth
5.	MS salts (3/4)	—	2ip 1.0 Kn 1.0 BAP 0.5	—	40,000+ Dextrose 5000	MS + Spermine 5.0, Spermidine 10.0	Callus compact green, globular
6.	MS salts (1/6)	NAA 0.05-0.15	2ip 1.0-7.0	15	20,000	half of MS	Callus fragile, soft

Callus culture : Callus induction was observed in all the three species (*P. cineraria* (PC), *P. juliflora* (PJ) and *P. tamarugo* (PT) on several treatments of medium components including growth regulators (Table 2) incorporated in the MS medium using various explants Nandwani and Ramawat 1989).

Except *P. juliflora*, it was possible to maintain callus culture from *P. cineraria* and *P. tamarugo* (Nandwani and Ramawat 1989) and callus and cell suspension cultures from *P. chilensis* and *P. tamarugo* (Jordan et al. 1987; Jordan 1988). However, all attempts to regenerate the isolated and subcultured callus failed. It may be interesting to note that Jordan et al. (1987) observed a single embryo/shoot apex like structure in the cultures grown on B5 medium supplemented with 2, 4-D.

Bud Break : Bud break from nodal explants in different species of *Prosopis* can be obtained on diverse media (Goyal and Arya 1984; Jordan et al. 1987; Wainright and England 1987; Yao et al. 1989; Nandwani 1990). MS medium containing high cytokinin (Nandwani 1990) or high auxin (Goyal and Arya 1984) or high concentration of a cytokinin and an auxin (Batchelo et al. 1989) support shoot regeneration from stems explants of *P. cineraria*. These findings suggest that response is not precisely controlled by concentration and quality of exogenous growth regulators. Perhaps, the requirement is quite different than that provided exogenously. But subsequent changes in metabolic level and endogenous level of growth regulators promote shoot formation. This conclusion is supported by the fact that in most cases only single shoot formation has been achieved. The complete stimulation of explant was lacking, thereby production of adventitious shoots remained suppressed (Table 3).

In *P. cineraria*, we have produced evidence that explants obtained from different genotypes as well as explants of same genotype differ in their regenerative potential on same medium. These explants showed considerable variation in phenotypic expression in relation to delayed regeneration, size, length and vigour of regenerated shoots and leaf expansion (Nandwani 1990). Thus, results obtained by various workers are mostly due to different material (genotype) used, making the reproducibility of results very difficult. Therefore these studies require a detailed account of explant source used in the investigation. Some of these speeies are very recalcitrant to regenerate. In case of *P. juliflora* several hundred permutation and combinations of medium factors could not produce subculturable callus and shoot number could not be enhanced more than seven. Similar results with low shoot number were also obtained with *P. chilensis*, *P. cineraria* and *P. juliflora* nodal explants by Yao et al. (1989).

Adventitious shoot formation

Adventitious shoot formation : In *P. tamarugo* various growth hormones incorporated in MS medium induce single shoot from hypocotyl explants. In one case

Table 3. Summarised results showing shoot formation from stem explants in three species of *Prosopis* on various media. Explants were grown in 16 hrs. light (2500 lux) for four weeks.

S. No.	MS medium+Growth regulators (mg/1)	*P. cineraria*			*P. juliflora*			*P. tamarugo*		
		No. of shoots producd	% explants differen-tiated	Shoot length (cm)	No. of shoots produced	% explants differen-tiated	Shoot length (cm)	No. of shoots produced	% explants differen-tiated	Shoot length (cm)
1.	Devoid of growth regulators	2.78±0.92	30	1.52±0.72	1.16±0.81	40	1.07±0.47	—	—	—
2.	BAP (5.0)	8.03±2.96	60	2.91±1.17	5.83±2.65	50	1.71±1.68	3.23±0.92	40	1.08±0.67
3.	NAA (0.1)+BAP (5.0)	5.24±1.43	40	2.95±1.81	5.61±1.03	40	1.86±1.12	3.12±1.12	40	1.01±0.48
4.	BAP (2.5)+Kin. (2.5)	7.28±2.36	50	3.35±1.28	6.34±2.89	50	1.93±1.23	—	—	—
5.	IAA (0.1)+BAP (5.0)	7.21±1.42	60	3.08±1.13	7.39±3.86	60	1.72±1.02	2.78±1.86	60	0.98±0.72

— = Nil

it was observed that when a few hypocotyls grown on high cytokinin (HC) containing medium are transferred to MS medium devoid of growth hormone, it produced multiple shoot buds at the base of developing single shoot. Therefore, a sequence of experiments were designed to evaluate the precise role of growth hormones, particularly the cytokinin. This has resulted in formation of multiple shoot buds from hypocotyles in successive passage on hormone free (HF) and cytokinin supplemented media. Embryonic explants grown on HF or HC medium for a passage and then transferred vice versa, produced multiple shoots. It was observed that number and proliferation in shoot buds increased when tissues grown for second passage on HC medium. Reduction in cytokinin concentration in the medium resulted in elongation of a few shoots from the clump of organogenetic mass. Elongated shoots produced roots on rooting medium containing an auxin.

It has also been observed that if both cotyledons are allowed to remain attached with the embryonic axis, the presence of cotyledons enhanced the multiple shoot formation in the explants. In absence of attached cotyledons not only the number of shoot buds per explant remained low, but the process was also delayed with the

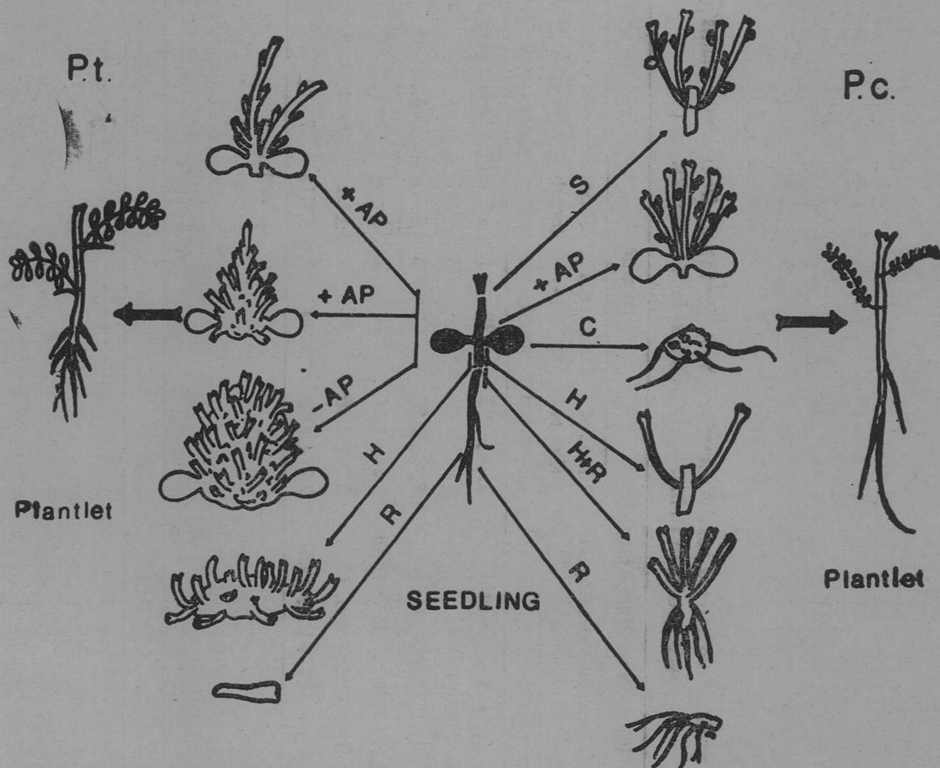

Fig. 1. Differential morphogenetic response of various explants from embryonic axis of *Prosopis cineraria (Pc)* and *P. tamarugo (Pt)* (AP : with (+) or without (—) apical meristem· H: hypocoty,l R : root, C : cotyledon, S:stem).

growth of explant, the number of shoot buds increased by formation of new adventitious buds. It appears that these buds at a later stage develop adventitiously and from newly proliferating callus cells, while earlier were produced from axillary buds. In certain cases, apical meristems in axillary position proliferate to produce a phylody like structure.

As far as our information goes, adventitious shoot formation from mature or juvenile explants of *Prosopis* species has not been reported. On the basis of results obtained with *P. tamarugo* successful regeneration of adventitious shoots from juvenile explants of *P. cineraria* has also been obtained. However, the number of shoots per explant remained low in *P. cineraria* as compared to *P. tamarugo*. Goyal and Arya (1981) obtained single shoot from juvenile explant, whose exact nature was uncertain.

Protoplast culture

The single report (Shekhawat and Kackar 1987) described the method for isolation of protoplast from leaves of *P. cineraria*.

Rooting

It has been observed that rooting can be induced in *P. tamarugo* and *P. juliflora* with relative ease as compared to *P. cineraria* (Nandwani 1990; Goyal and Arya 1984). These system require more refinement and increased frequency and subsequent viability in field conditions. However, in case of *P. cineraria* the age of explant, culture medium, genotype and age of shoots markedly affect the rooting behaviour of *in vitro* regenerated shoots. Therefore, to develop a reproducible rooting system, all factors require consideration. Once rooted, establishment of these shoots does not pose difficulties. Howeve, low watering is required.

Evidence for callus morphogenesis

It is mentioned earlier that callus once isolated from explants and maintained separately than it never regenerated. But there were cases when highly regenerative tissues were obtained from juvenile explants of *P. tamarugo*. It was suspected that such a high regeneration cannot be obtained without callus morphogenesis. Histological observations of regenerative mass obtained in successive passages from juvenile explants grown on high cytokinin containing medium showed shoot buds development from callus produced by proliferating tissues. This is first evidence of callus regeneration in any of the *Prosopis* species (Table 4). Increased nitrogen content enhanced shoot bud proliferation while added inhibitors of auxin synthesis and auxin transport enhanced callus formation in the regenerative tissues (Nandwani 1990).

Conclusion and Prospects

The *Prosopis* species are most valued trees of arid regions. In case of tree species, which are primarily required for biomass production, regeneration through callus culture is an ideal system to produce large number of plants though they may

Table 4 : Effect of various factors on regeneration of multiple shoot buds of *P. tamarugo*.

S. No.	MS Medium + BAP (5.0 mg/1) Medium factors (mg/1)	Shoot buds produced	Growth of shoot buds	Remarks
1.	Nitrogen KNO_3 $(NH_4)_2SO_4$			
i.	500 4000	∝	+	Fleshy thick leaves,
ii.	300 1000	∝	+++	Green, proliferating shoot buds
iii.	4000 500	∝	++	
	NH_4NO_3			
iv.	1900 1650	∝	+	Poor growth, No callus
v.	3800 —	∝	++	Healthy shoot buds, Shoots 2.0 cm elongated
2.	Adenine sulphate			
i.	25.0	∝	+	Shoot buds and growth of inoculum
ii.	100.0	∝	++	Green, fleshy shoots. Elongation in 3 shoots.
3.	Gibberellic acid			
i.	0.1	∝	+	—
ii.	1.0	∝	+++	—
iii.	5.0	—	+	
4.	Calcium chloride			
i.	220	∝	+	Healthy growth of shoot buds, elongation in 2 shoots
ii.	440	∝	++	Vigour increased.
iii.	880	∝	+	Reduced growth of proliferating mass

Relative value of shoot buds formation:- —Nil; + low, ++ Moderate, +++ High

differ slightly from each other. Clonal propagation leading to monoclonal population is not required in such cases.

The present state of knowledge about regeneration in *Prosopis* suggested that still there is wide gap between laboratory feasibility of regeneration and practical field level application of technology. For afforestation programmes hundred thousand

plants are required regularly. Considering mortality at all steps and frequency of regeneration this cannot be achieved with nodal explants. In case of *P. tamarugo* callus regeneration have been achieved but frequency of shoot e'ongation is still low. Therefore, still the conventional methods of propagation (seeds) have to be used till these barriers are removed.

After obtaining a highly regenerative system in *P. tamarugo* the work on following lines will be useful for large scale multiplication of this and related *Prosopis* species.

1. Precise control of elongation of shoot buds.
2. Details of factors affecting transplantation.
3. Isolation, culture and fusion of protoplasts of *P. tamarugo* with other species of *Prosopis*. Since one system is regenerative, chances of obtaining regenerative hybrids are good.
4. Development of regenerative system in other species of *Prosopis* on the same lines.

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