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Direct organogenesis in single bud explant of lasoda (*Cordia myxa* Roxb.)

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Abstract

Organogenesis of shoot and root in single bud explant taken from mature tree of Lasoda (*Cordia myxa* Roxb) has been achieved. Organogenesis was obtained by placing nodal explant on shoot induction medium and subsequently on rooting medium. More than 90% culture were responsive for *in-vitro* axillary shoot proliferation on MS media supplemented with BA 2.0 mg and NAA 0.1 mg per litre. After culture period of 6-8 week, the nodal explants with microshoot were rooted on the MS media supplemented with 3g activated charcoal and different level of NAA (0, 1, 3 and 6 mg/ litre). The season and position of explant on the mother plant was also shown to influence *in-vitro* performance of the regeneration in terms of establishment of aseptic culture, percentage of responsive explants and intensity of leaching of growth inhibitory phenolics.

Key words : *Cordia myxa, tissue culture, organogenesis*

Introduction

Lasoda (*Cordia myxa* Roxb) belongs to the family Boraginaceae and is one of the important arid fruit tree having characteristics of better adaptation to arid and semi arid conditions of tropical and subtropical climates and diverse economic and nutritional utility in culinary processing, preservation and value addition of pickles product alone or in combination with others fruits and vegetables. The plant is a small and medium size tree with a short and crooked trunk. Moreover, the fruits have medicinal value and considered as anthilmintic, diuretic, demulcent and expectorant (Chundawat, 1990). Looking into the importance of this hardy fruit tree, the Central Institute for Arid Horticulture, Bikaner has started collection, conservation and characterization of its germplasm for systematic and economic orcharding. The existing plantation of this fruit is heterozygous with great variability in fruit quality and tree morphology because of propagation by seeds and inefficient conventional vegetative methods. Therefore, growers face problems of poor quality of fruits with low productivity and income. Recently some high yielding genotypes with big size fruits have been identified (Anon., 2002). For rapid and large scale clonal propagation of superior genotypes of tree species the regeneration of plantlets from pre existing meristems through shoot and node culture is the most reliable and widely used procedure however, the need for multiple subcultures on different media makes shoot and node culture extremely labour intensive (Kane, 2000). Total labourers cost ranging from 50 to 70% of production cost, limit expansion of the micropropagation industries (Aitken-Christie *et al*, 1995). Therefore, current application of the technology is restricted to the high value ornamental crops. Expansion of this Industry to include fruit tree species depend on development of efficient micropropogation system. Cost reduction strategies including elimination of the production steps and development of low cost hardening facility will facilitate this expansion in developing countries. Recently, Chitra and Padmaya (2005) made an attempt to study direct organogenesis in leaf explant of mulberry fruit tree for successful plantlet production. Looking into these aspects, a study was conducted to explore direct organogenesis from single node explant of superior genotype of *lasoda* (*Cordia myxa*).

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Materials and Methods

Preparation of mother plant and collection of explant

Selected tree of *Cordia myxa* were irrigated and fertilized as per general recommendation. In order to induce juvenility in stock plant and maximize lateral branching for

obtaining explants, the mother plants were pruned during the month of October. Actively growing lateral shoots were collected and subdivided into single node segments for culture initiation.

Explant preparation and sterilization

The lateral shoot of 10 to 15 cm long collected from mother plants were subjected to washing in running tap water. Leaf blades were trimmed from each node leaving only the petiole bases. Shoots were further divided into single nodal explant and rinsed in mild concentration of detergents (1%) followed by rinsing in tap water. Thereafter explants were treated with 0.1% $HgCl_2$ for 6 minutes. The surface sterilized explant were thoroughly washed several times with sterile water and trimmed to a size of 2 to 2.5 cm before inoculation.

Culture media and Conditions for shoot induction

Surface sterilized explants were inoculated vertically in conical flask of 250 ml size containing MS medium (Murashige and Skoog, 1962) supplemented with 30g sucrose, 8.0g agar and various concentration of BA (0,1,2, and 4mg/l) alone or in combination of NAA 0.1mg/l. The pH of the medium was adjusted to 5.7 and sterilized by autoclaving at 121° C and 15 psi for 15 minutes. The culture were incubated at 26 ± 2° C, and 16 hrs photoperiod provided by cool fluorescent tube with 2000 lux light intensity at bench level.

Culture media for rooting

After culture period of 4 to 5 week the nodal explants with microshoots were transferred to MS medium supplemented with 30 g of sucrose, 8.0g agar, 3g activated charcoal and varied levels of NAA (0,1,3 or 6 mg/l) for rooting of explants.

Hardening of in-vitro produced plantlets

For hardening of the plantlets in arid agro ecosystem, a low cost plant hardening facility was devised involving three step hardening procedure such as (i) transfer of plantlets to acclimatization hood inside the culture room (ii) transfer of plantlets in evaporative cool chamber (iii) for further growth and development in poly house or shade house equipped with intermittent fogging device. Different combinations of potting mixture such as cocopeat, vermicompost and vermiculite, were tried for survival and acclimatization of plantlets to pots. The data of experiments was statistically analyzed using WINDOSTAT (INDOSTAT) software. The analysis was performed using one factor ANOVA and CD thus obtained was used to compare the mean values.

Results and Discussion

Morphogenic responsive culture

In the present study nodal explants were assessed for *in-vitro* morphogenic response, both factors such as position of explant on the stock plant (Table 1) and season of explantation greatly influenced the responsive explant in terms of induction of axillary shoots and phenolic leaching or media browning. The highest percentage (92%) of responsive explants were observed from mid portion of the lateral shoots as compared to apical and basal portion being observed, 32% and 20% respectively. The phenolic leaching from the explant and subsequent browning of the media was maximum during winter season (December-January) however, newly emerged lateral shoots during the period of spring summer (March-April) and rainy season (July-August) were found completely free from leaching of phenolic compounds. Similar observations on the effect of season and source on the morphogenic competences of mature trees have been noticed by several other workers (Deora and Sekhawat 1995, Purohit and Kukda 2004, Rathore *et al.*, 2004 and Read, 1988) with important fruit tree species.

Table 1: Percentage of responsive culture as influenced by position of explant on lateral shoot on the stock plant.

Position of explant	% Responsive explants in terms of induction of shoots
Explant taken from apical portion of lateral shoots	32
Explant taken from mid portion	92
Explant taken from basal portion of lateral shoots	20

Induction of axillary shoot and callus in nodal explants

Morphogenic response in terms of single axillary shoot induction and formation of callus at the base of nodal explant was observed in all concentration of BA in combination of NAA (Table 2). The maximum mean length of shoots (26 mm was recorded with 2mg / litre BA in combination of 0.1mg/litre NAA. At this stage we have completely eliminated the need of repeated sub culturing which is generally adopted by several workers for multiple shoot induction and only two or three microshoots were obtained particularly with tree species. It is well established research finding that the ratio of cytokinin and auxin controls the various morphogenic responses *in-vitro* culture and relatively low ratio of cytokinin to auxin may induce both shoot and callus or rooting in the culture. The higher concentration of BA at 4.0 mg/litre found to induce callus at the base at explant. However, the induction and

Table 2: Effect of BA and NAA on the growth and development of callus and axillary shoot induction in nodal explant

MS + growth regulators (mg/litre)		Formation of callus at the basal end of explant	Induction of axillary shoot (length in mm)
BA	NAA		
			(±SD)
0	0	No Callus	0 ± 0.00
1.0	0	No Callus	8 ± 1.15
2.0	0	No Callus	16 ± 1.58
4.0	0	Callus	5 ± 0..94
0	0.1	Callus	0 ± 0.00
1.0	0.1	Callus	21 ± 0.88
2.0	0.1	Callus	26 ± 0.81
4.0	0.1	Callus	6 ± 0.81
CD at 5%	—	—	0. 84

growth of axillary shoots was very poor. The studies conducted by the workers (Deora, and Sekhawat 1995, Purohit and Kukda, 2004 and Rathore *etal.*, 2004) also favours with tree species either cytokinin alone or in combination of very low concentration of NAA for only shoot organogenesis.

Rooting of the explants

The in-vitro cultured original explants having single axillary microshoot and callus at their base were directly used for root induction experiment. At this stage we have eliminated the step of excision of micro shoot and subsequent culturing in rooting media. Under different concentration of NAA treatments rooting response in

Table 3: Root induction in original nodal explant of *Cordia myxa* on MS medium + 30g sucrose +8g agar + 3g activated charcoal and different concentration of NAA after culture period of 6 to 8 weeks

Concentration of NAA (mg/litre)	Rooting response (%)	Average No. of roots (±SD)	Average root length (cm) (±SD)
0	00	0.0 ±0.00	0.0 ±0.00
1	20	2.1 ±0.56	5.4 ±0.41
3	60	2.7 ±o.48	4.6 ±0.40
6	00	0.0 ±0.00	0.0±0.00
CD 5%		0.34	0.24

explants was found to be significant (Table 3). The maximum 60% explants with roots were noticed in the media composition MS + 30g sucrose + 8.0g agar + 3.0g activated charcoal and 3.0mg NAA/litre. The medium without NAA or at higher concentration of NAA (6.0 mg) failed to induce root. The few cultures i.e. 20% rooted at the concentration of NAA 1.0mg/litre. The higher concentration of NAA found to be induced excessive callusing in the explant.

Hardening of plantlets

Under hot arid agro-ecosystem, hardening and acclimatization of plantlets is very challenging component of micropropagation. In the present study a three step hardening facility (Table 4) was devised and maximum percentage (70%) of plantlets were acclimatized successfully as compared to low survival rate (40%) under the two step procedure. This could be achieved probably due to integration of evaporative cool chamber as one of the important hardening steps which favourably supported the process and facilitated desired temperature and humidity parameters suited to hardening i.e. 80 to 90% relative humidity and 25 to 30° C temperature under hot

Table 4: Influence of hardening steps on survival percentage of plant lets

Hardening step	Survival % of plantlets	
	Cocopeat Vermiculite (3:2)	Vermicompost: Vermiculite (3:2)
Two step procedure: Primary hardening in acclimatization hood kept in culture room for 7-10 days and subsequent transfer in shade house equipped with intermittent fogging.	40	30
Three step procedure: Primary hardening in acclimatization hood kept in culture room for 7-10 days and subsequent transfer to evaporative cool chamber for 7-10 days, thereafter to shade house equipped with intermittent fogging	70	60

and dry weather condition. Similarly, Preece and Sutter (1991) also emphasized certain environmental condition necessary for acclimatization of plantlets using controlled environmental facilities and Singh *et al.* (2005) obtained conducive environmental conditions under three step hardening technique for acclimatization of in vitro raised plantlets of *Capparis deciduas* and cactus pear under hot arid agro ecosystem.

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