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Rational Development of *in vitro* Methods for Conservation, Propagation and Characterization of *Caralluma edulis*

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INTRODUCTION

Caralluma edulis belongs to family Asclepiadaceae. There are about ten species of *Caralluma* that occur in India mainly adapted to dry habitats. *Caralluma edulis* and *Caralluma adscendens* are edible. *Caralluma edulis* is confined to drier parts of Rajasthan. *Caralluma edulis* is locally known as '*Paimpa*'. It is gregarious and highly drought resistant plant [1]. It is an erect succulent branched perennial herb; stem is creeping with scars of fallen leaves. The young shoots of this plant are edible and cooked as vegetable, also used in pickles and preserved. *Caralluma edulis* has higher content of Vitamin C, Calcium and Iron. The purpose of this study was to develop *in vitro* techniques for conserving *Caralluma edulis* by mass multiplication for subsequent reintroduction in their natural habitat as this can be grown in degraded wastelands [2]. There is a need to develop micropropagation protocol as plant populations have already disappeared from the region.

MATERIALS AND METHODS

The stem of *Caralluma edulis* was cut into two-three cm long nodal segments (with at least one or two node per segment). Nodal shoot explants were first treated with systemic fungicide Bavistin (0.1%) for five-seven minutes and treated with $HgCl_2$ (0.1%) for three-five minutes for surface sterilization. These were washed thoroughly with autoclaved water for six-seven times. Surface sterilized nodal shoot explants were inoculated on MS medium [3] containing ranges of cytokinins namely BAP (6-Benzyalamino Purine) and Kinetin (1.0-5.0 mg l^{-1} each). Explants placed vertically on culture medium.

Shoots differentiated by axillary bud activation were excised and the mother explants were repeatedly transferred onto fresh MS medium for production of new shoots. In vitro regenerated shoots were cut into shoot segments each with one or two nodes (1.5-2.5 cm) and subcultured on MS-basal medium supplemented with auxin IAA (Indole-3-acetic acid) 0.1 mg l^{-1} and cytokinins BAP, Kin, 2-iP in different concentrations (0.25-0.5 mg l^{-1} each for further multiplication. The micropropagated shoots (5-8 cm long) were pulse-treated with different concentrations of IBA (Indole-3-butyric acid) or NAA (α-Naphthalene acetic acid) (100-500 mg l^{-1} each) for 3 minutes. Shoots treated with root inducing PGRs (Plant growth regulators) were transferred on to bottles containing sterile soil-rite moistened with an aqueous solution of one-fourth strength of MS salts. These bottles were incubated in a greenhouse at 28±2°C. After root initiation the caps of bottles were gradually opened. These were finally removed. The bottles containing plantlets were shifted away from pad section towards fan section. The acclimatized plantlets were transferred to poly-bags containing mixture of garden soil, organic manure and sand (3:2:1). These plantlets were kept in green house for two months and then the hardened and acclimatized plants were shifted to nursery for further evaluation.

RESULTS

Juvenile and thick shoot segments with one-two nodes were found to be appropriate for culture initiation. Ninety percent of explants showed bud break within ten days of inoculation. Three-four shoots per explant differentiated on MS medium from each node, supplemented with 2.0 mg l^{-1} of BAP. On Kinetin containing media only one-two shoot regenerated. Out of various concentrations of cytokinins tested, 2.0 mg l^{-1} of BAP was found to be optimum for initiation of cultures on

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Table 1: Effect of concentrations of cytokinins (BAP and Kin) on bud breaking and multiple shoot induction from nodal explants of *Caralluma edulis*

	Concentration (mg l^{-1})	Response (%)	Shoot number (mean±SD)	Shoot length (cm, mean±SD)
BAP	1.0	50	1.25±0.43	0.67±0.20
	2.0	80	3.25±0.82	2.77±0.17
	3.0	70	2.50±0.50	1.55±0.42
	5.0	65	1.60±0.80	1.42±0.27
Kin	1.0	25	1.20±0.40	0.96±0.30
	2.0	55	1.75±0.43	1.00±0.61
	3.0	40	1.25±0.43	0.55±0.28
	5.0	35	1.40±0.48	0.96±0.35

Table 2: Multiplication of shoots of *Caralluma edulis* as affected by combinations of plant growth regulators

Combinations of PGR's mg l^{-1}	Shoot number (mean±SD)	Shoot length (cm, mean±SD)
BAP 0.25+Kin 0.5	10.75±1.40	4.10±0.70
Kin 0.5+2-iP 0.5	11.70±2.90	4.20±1.60
BAP 0.25+Kin 0.25+IAA 0.1	15.75±2.58	8.50±1.10
BAP 0.5+Kin 0.5+IAA 0.1	09.50±1.11	5.70±1.20

Table 3: Effect of concentrations of auxins (IBA and NAA) on *ex vitro* rooting of micropropagated shoots of *Caralluma edulis*

Concentration in mgl^{-1}		Response (%)	Root number (mean±SD)	Rhoot length (cm, mean±SD)
IBA	100	90	4.25±0.82	0.87±0.21
	200	100	6.25±0.82	2.55±0.36
	500	100	8.00±0.70	3.25±0.82
NAA	100	50	1.90±0.83	1.10±0.20
	200	65	2.95±0.84	2.25±0.82
	500	70	3.40±0.41	2.90±0.30

the MS medium (Table 1). The number of shoots increased up to only 2 passages (5-6 shoots), after this on transfer to fresh medium, the shoot number started declining (Fig. 1). *In vitro* regenerated shoots were cultured on MS medium supplemented with different cytokinins (BAP, Kin and 2-iP) in different concentrations. Maximum number of shoots (11 to 15 shoots) were produced on MS+0.25 mg l^{-1} BAP+0.25 mg l^{-1} Kin+0.1 mg l^{-1} IAA+additives, within three-four weeks (Fig. 2). About eight fold rate of shoot multiplication was achieved on 0.25 mg l^{-1} BAP+0.5 mg l^{-1} Kin and 0.5 mg l^{-1} Kin+0.5 mg l^{-1} 2-iP proved less effective for shoot multiplication as compared to BAP, Kin and IAA. Shoot length and shoot number decreased on MS medium supplemented with 0.5 mg l^{-1} BAP+0.5 mg l^{-1} Kin+0.1 mg l^{-1} IAA+additives (Table 2).

It was observed that IBA (500 mg L^{-1}) was best for root induction. Cent percent rooting was observed when shoots were treated with 500.0 mg l^{-1} IBA for 3.0 min. The same percentage of rooting could be achieved by treatment of 200 ppm IBA but, the number and length of roots declined. Shoots treated with 100.0 mg l^{-1} treatment could induce rooting only in 90% of shoots (Table 3).

The rooted plantlets were hardened in the green house (Fig. 3). The hardened plantlets were finally transferred to poly-bags. These plantlets were kept in greenhouse for 50-60 days, then shifted to nursery and finally transferred to the field for evaluation. Thousands of plants were hardened and field transfer.

DISCUSSION

Caralluma edulis is an important desert plant which possesses high antioxidant properties [2]. It has been realized that the conventional method of propagation by rooting of cutting is slow and have limitations, some researchers [4, 5] had reported on tissue culture methods for micropropagation of this plant. Studies were conducted to further refine and simply the protocol for cloning and evaluation of micropropagated plants in the field. Young nodal shoots produced by plants maintained in green house/field were used as explants. These were cultured aseptically to induce axillary bud break. Multiple shoot were produced by activation of axillary meristem and bud proliferation on MS medium containing 2.0 mg l^{-1} BAP. Repeated transfer or subculture further multiplied shoots. A very high rate of shoot multiplication was achieved on by subculture on MS+0.25 mg l^{-1} each BAP and Kinetin+0.1 mg l^{-1} IAA and additives. Subculturing was done every 3rd week. For activation of meristem/axillary bud of explants high concentration of BAP is required. It is suggested that the field-grown plant accumulate anti-metabolites that are required for tolerating recurring stresses. Such anti-metabolites prevent bud proliferation.

In order to release the bud from dormancy, the accumulated anti-metabolites must be diluted or eliminated. In nature this occurs during spring or rainy

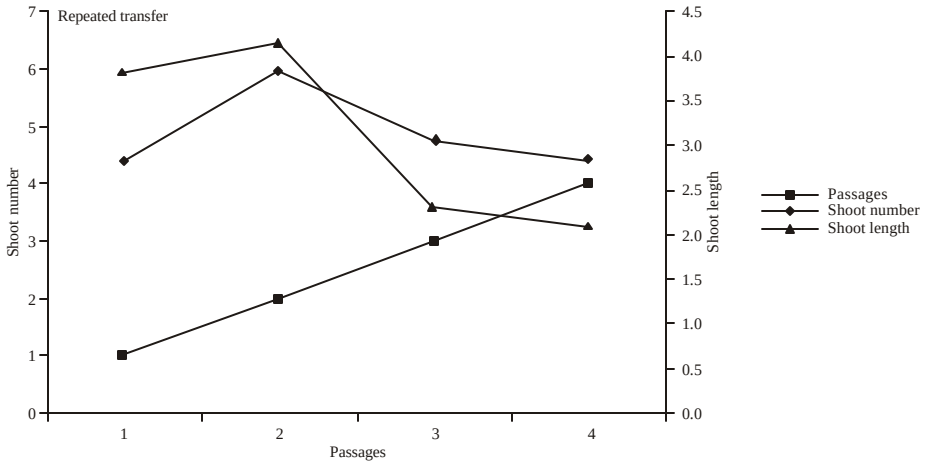

Fig. 1: Shoot production by repeated transfers of mother explant of *Caralluma edulis* on MS+1.0 mg l^{-1} of BAP+0.5 mg l^{-1} of Kinetin

Fig. 2: Multiple shoot induction in *Caralluma edulis* on MS medium+BAP 0.25+Kin 0.25+IAA 0.1 mg l^{-1}

Fig. 3: Hardened and acclimatized plants of *Caralluma edulis* ready for field trials

seasons. This can also be achieved by pruning l^{-1}opping of woody plants. Such treatments of rejuvenation yield shoots/stems that can be used to establish culture [6]. *In vitro* BAP or cytokinins cause activation of meristem by removing constraints posed by anti-metabolites. Once this is done the meristems are conditioned, the anti-metabolites are diluted and shoots are amplified unabated even in presence of low or no cytokinins. After defining the conditioning for cyclo-clonal propagation of shoots amendments were done to replace sugar with sugar cubes (cheaper source) and by adding commercial agar-agar. *In vitro* multiplied shoots pulse-treated with root hormones and directly placed in bottles containing moistened soil-rite in green house. More than 90% of the shoots rooted in the green house. The rooted plants were hardened with ease and transplanted in garden soil+sand (50:50). In order to accelerate the growth, vermin-compost was added to poly-bags or vermin-wash was incorporated in soil and sand mixture. Application of mycorrhizas for establishment of micropropagated plants have been suggested by experts [7]. The biotechnology group of JNV University, Jodhpur found that vermin-compost and vermin-wash helps survival of micropropagated plants of several species including *Chlorophytum borivillianum*.

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REFERENCES

1. Bhandari, M.M., 1990. Flora of the Indian Desert. MPS Repros, Jodhpur, India.
2. Ansari, N.M., L. Houlihan, B. Hussain and A. Pieroni, 2005. Antioxidant Activity of Five Vegetables Traditionally Consumed by South-Asian Migrants in Bradford, Yorkshire, UK, Phytotherapy Research, 19: 907-911.
3. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.
4. Nair, L.G. and S. Seeni 2001. Rapid *in vitro* multiplication and restoration of *Celastrus paniculatus* Wild. Sub sp. *paniculatus* (Celastraceae), a medicinal woody climber. Ind. J. Exp. Biol., 39: 697-704.
5. Arya, V., R.P. Singh and N.S. Shekhawat, 2002. A micropropagation protocol for mass multiplication and off-site conservation of *Celastrus paniculatus*-a valuable medicinal plant of India. J. Sustainable For., 14: 107-120.
6. Rathore, J.S., M.S. Rathore, M. Singh, R.P. Singh and N.S. Shekhawat, 2007. Micropropagation of mature tree of *Citrus limon*. Ind. J. Biotechnol., 6: 239-244.
7. Lavato, P.E., V.G. Pearson, A. Trouvelot and S. Gianinazzi, 1996. The state of art of mycorrhizas and micropropagation. Adv. Hortic. Sci., 10: 46-52.