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IN VITRO MICROPROPAGATION OF *CARALLUMA EDULIS* (Edgew.) Benth. & Hook. f. — A RARE EDIBLE PLANT SPECIES OF INDIAN DESERT

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An in vitro *micropropagation protocol was developed using shoot segment explant of* Caralluma edulis *– a very rare and endangered edible plant of the* Thar *desert. Four to six multiple shoots were initiated from nodal explant on MS medium containing 0.25 mg l^{-1} IAA (Indole-3-acetic acid) and 2.5 mg l^{-1} BAP (benzylamino purine). The shoots elongated and multiplied on a medium supplemented with 0.25 mg l^{-1} IAA + 2.5 mg l^{-1} BAP and 2.5 mg l^{-1} GA_3 (Gibberellic acid).* In vitro *produced shoots rooted on half strength MS medium containing 1.0 mg l^{-1} IBA (Indolebutyric acid) within three weeks. Plantlets were transplanted to pots and grown to maturity.*

Increasing human activities and various types of stresses have caused widespread disturbances in the habitats of plants. This has resulted in the gradual reduction of plant resources and the genepools of valuable plants. There has been increasing concern and emphasis on conservation of germplasm of threatened plants (Harry and Thorpe, 1991). Multiplication and conservation of rare, threatened and endangered taxa can be carried out efficiently through techniques (Arora and Bhojwani, 1989; Bhojwani *et al.*, 1989; Upadhyay *et al.*, 1989; Arumugam and Bhojwani, 1990 and Rathore *et al.*, 1991).

Caralluma edulis (Edgew.) Benth. & Hook. f. (Asclepiadaceae) is a very rare herb of the extremely arid regions of the *Thar* desert. It is locally known as **Pimpa**. The tender shoots of plants are used as vegetable in Jaisalmer. Natural propagation of this plant is extremely poor under the xeric conditions. There is an urgent need

to conserve *C. edulis* germplasm. Establishment of *in vitro* cultures of *C. edulis* and its micropropagation using tissue culture technology has been discussed in this paper.

MATERIALS AND METHODS

In vitro cultures of *C. edulis* were established from shoots of plants collected from natural habitat from nearby areas in Jaisalmer. Apical and axillary shoots (each with a single node) and inflorescence segments were taken as explants. These were washed thoroughly with water containing a few drops of Tween-80 and surface sterilized with 0.1% mercuric chloride for 4 min, and washed 5-6 times with autoclaved distilled water. Explants were placed vertically or horizontally on MS (Murashige and Skoog, 1962) medium for shoot induction and multiplication. The pH of the media was adjusted to 5.8 prior to autoclaving. Various concentrations and combinations of auxins and cytokinins were incorporated. Differentiated shoots were sub-cultured after intervals of 3-4 weeks on fresh medium for further multiplication. These were kept under 12 h photoperiod (30 $\mu Em^{-2}s^{-1}$ photon flux density) at 28 ± 2°C and 60 per cent relative humidity. The differentiated shoots were excised and transferred to various rooting media containing different concentrations of auxins and kept in the dark for a week and later under light conditions. Plantlets were carefully removed from the agar medium without damage to the root system and were kept in distilled water for 15 min to avoid dehydration and to remove traces of agar and transferred to pots containing soil and vermiculite in the ratio of 4:1 (Fig. 4). These plants were first acclimatized in a culture room for two weeks and then to the field. Plantlets of 8-12 cm in length were later transferred in pots containing field soil. The root tips of *in vitro* produced plantlets were analysed for chromosome number using the method described by *Rao et al.* (1991).

RESULTS AND DISCUSSION

Tissue cultures were established from apical and axillary shoot explants on MS medium containing 0.25 mg l^{-1} of IAA and 2.5 mg l^{-1} of BAP. Shoot segment with a single node was found to be the best explant for induction of multiple shoots over the apical shoots and inflorescence segments. On an average, 4-6 shoots were induced (Fig. 1) from each nodal shoot segment, whereas 2-3 shoots differentiated from apical shoot segment. Inflorescence segments did not respond. The effect of growth regulators on multiple shoot

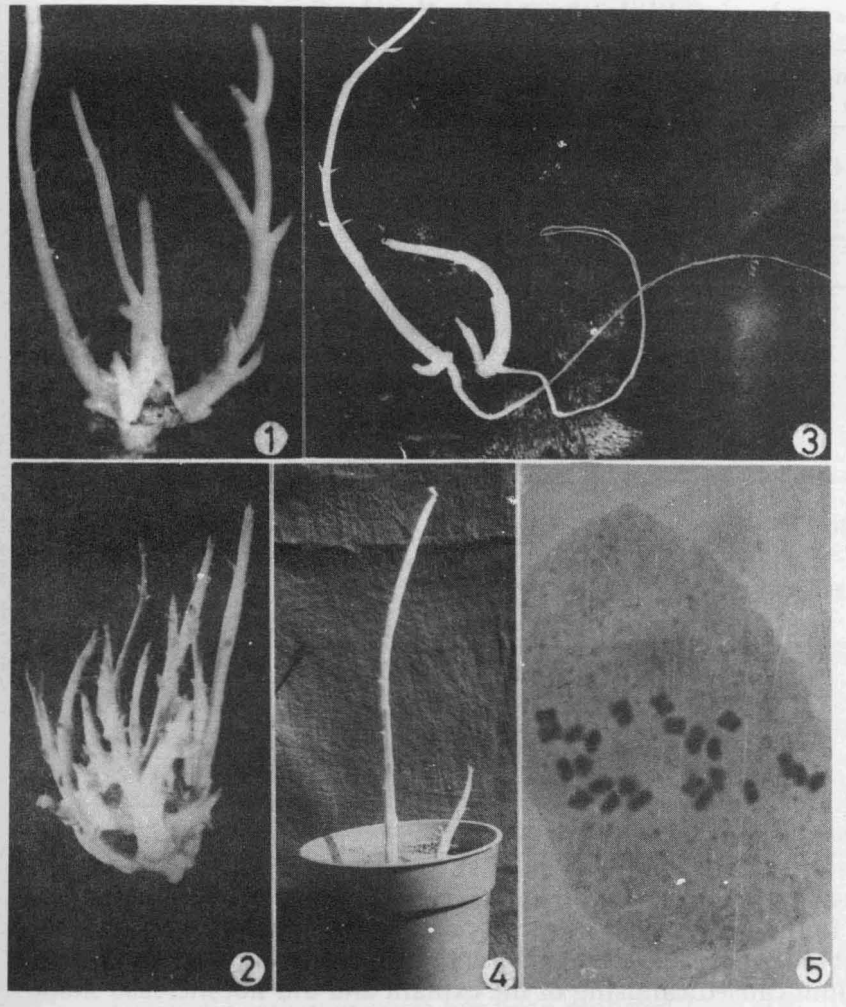

Fig. 1. Multiple shoot induction in *Caralluma edulis* from nodal segment on MS medium supplemented with IAA (0.25 mgl^{-1}) and BAP (2.5 mgl^{-1}).

Fig. 2. Multiplication of shoots on MS + IAA (0.25 mgl^{-1}) BAP (2.5 mgl^{-1}) + GA_3 (2.5 mgl^{-1}).

Fig. 3. Rooted shoots of *C. edulis* on half- strength MS medium containing IBA (1.0 mgl^{-1}).

Fig. 4. Five week old potted plant of *C. edulis*.

Fig. 5. Chromosomes 2V = 24) of a root tip cell of *in vitro* raised plantlet of *C. edulis*.

Table 1 : Combined effect of auxin + cytokinins on multiple shoot induction in *C. edulis*

Auxin + Cytokinin (mg l^{-1})	Number of shoots	Length of shoots (cm)	Callusing
IAA + BAP			
0.1 + 0.5	1.32	1.01	–
0.1 + 1.0	2.08	1.9	–
0.1 + 2.5	3.3	2.2	+
0.1 + 5.0	4.0	1.9	+
0.25 + 0.5	2.05	2.2	–
0.25 + 1.0	3.64	2.6	–
0.25 + 2.5	5.2	3.4	–
0.5 + 0.5	1.5	0.9	+
0.5 + 1.0	2.2	1.5	+
0.5 + 2.5	3.7	1.9	+
0.5 + 5.0	4.5	2.0	+
IAA + Kinetin			
0.1 + 0.5	0.91	0.7	–
0.1 + 1.0	1.72	1.0	+
0.1 + 2.5	1.79	1.2	+
0.1 + 5.0	2.24	1.3	+
0.25 + 0.5	2.9	1.5	+
0.25 + 1.0	3.0	1.8	+
0.25 + 2.5	3.2	2.3	+

inductions is summarised in Table 1. BAP and IAA combination was found better than kinetin and IAA combination for multiple shoot induction. Replacement of IAA by NAA in the shoot induction medium caused callusing of the explant and did not increase shoot number or length. Differentiated shoots were cut into small segments (2.0–2.5 cm) and cultured on fresh medium for further multiplication. The number of shoots per explant increased to 8 and the shoot length to 6-8 cm when 0.25 mg l^{-1} IAA, 2.5 mg l^{-1} BAP and 2.5 mg l^{-1} of GA_3 were incorporated in the culture medium (Fig. 2). Thus, incorporation of GA_3 in shoot multiplication medium caused considerable elongation of shoots (Table 2). Among the media tested for root induction, half strength MS medium was found to be the best followed by one fourth strength MS basal and

White's basal medium (Table 3). About 85-90 per cent of the shoots rooted on half strength MS medium containing 1.0 mg l^{-1} of IBA within three weeks (Fig. 3). It is estimated on the basis of present study that 50-60 plantlets can be obtained from single explant within 60 days.

Table 2 : Effect of GA_3 on shoot elongation of *C. edulis* cultured on MS medium containing 0.25 mg l^{-1} of IAA + 2.5 mg l^{-1} of BAP

GA_3 (mg l^{-1})	Number of shoots	Length of shoots (cm)
0.1	5.2	4.97
0.5	5.85	5.43
1.0	7.7	5.93
2.5	8.06	6.91
5.0	6.49	6.05

Table 3 : Root induction and elongation on *C. edulis* shoots cultured on different media containing IBA 1.0 mg l^{-1}

Medium	Number of roots	Length of root (cm)
MS full	1-2	3-4
MS/2	2-4	5-6
MS/4	1-3	4-6
White's	1-2	2-3

In vitro produced plantlets were analyzed for chromosome number using root tips. The *in vitro* regenerated plants showed chromosome number (2N = 24) similar to mother plants (Fig. 5). No chromosome abberation was recorded.

In several plants of Asclepiadaceae, plant regeneration through somatic embryogenesis has been reported (Rao *et al.*, 1991; Prabhudesai and Narayanswamy, 1974; Wilson and Mahlberg, 1977). Bapat *et al.* (1986) reported regeneration of plants from protoplasts derived calli. Thus, there is strong possibility of *C. edulis* being regenerated from callus, cells and protoplasts. It will help increasing genetic variability of this rare and highly drought and heat resistant edible plant of Indian desert. It is suggested that protocol developed for micropropagation and multiplication of *C. edulis* can be used for *in vitro* conservation of germplasm of this valuable plant.

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