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Rapid and Mass Propagation of the Economically Important Desert Plant *Capparis decidua* for its Afforestation Program

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Abstract: *Capparis decidua* is an important constituent of the desert ecosystem and plays vital roles in the rural economy of western Rajasthan. It provides people with food (vegetable and pickle), medicine, fodder, wood for carving, fuel etc. Natural populations of *Capparis decidua* are being depleted, since its fruits are used as a food. Hence planting stock is not available in required quantities. The present study was undertaken with an aim to set up a protocol for *in vitro* propagation. The season of explants showed direct influence on bud break. Nodal segments of mature superior selected trees were collected. The nodal shoot explants harvested during the months of March, April, September and October were found to be best for establishment of cultures of *Capparis decidua*. Surface sterilized explants were inoculated onto MS medium supplemented with 17.76μM BAP + 0.54μM NAA + ascorbic acid (50 mg/l) + adenine sulphate (50 mg/l) + citric acid (30 mg/l) for axillary bud break. The proliferated shoots were excised from mother explants and further multiplied on MS medium supplemented with 8.88 μM BAP + 0.57 μM IAA + additives. These *in vitro* regenerated shoots were elongated on MS medium + additives. Elongated *in vitro* shoots were *in vitro* rooted on one fourth strength MS medium supplemented with 4.9μM IBA + additives and incubated at 31°C, where 94% of *in vitro* shoots rooted. Rooted plantlets were hardened, acclimatized and established in soil, where they exhibited normal growth. The protocol developed is able to produce superior planting stock for various afforestation and reforestation programmes.

Key Words: Afforestation, *Capparis decidua*, Reforestation

1. Introduction

Capparis decidua (Forsk.) Edgew, commonly known as Kair, is an important indigenous shrub. In India, Kair can be found in the dry regions of Rajasthan, Gujarat, Punjab, Haryana, Madhya Pradesh and many parts of Tamil Nadu, Karnataka and Andhra Pradesh. It is a small much branched tree or shrub of the Thar desert and arid regions in Southern Asia with a mass of slender, leafless branches, with small caduceus leaves which are found only on young shoots. The tree rarely exceeds a height of five meters. The mature fruits serve as a valuable and integral source of nutrition for villagers of arid and semiarid regions, and the immature fruits are collected from natural stands and serve an additional source of income and nutrition for the rural poor (Singh and Singh, 2011).

The tree provides hard, heavy and termite resistant timber (Gupta *et al*., 1989). The seed contains 20% of high quality edible oil. The stem and root bark extracts contain isocodonocarpine and other alkaloids which are effective in treating asthma, inflammation, cough etc. (Ahmed *et al*., 1989). The immature fruits are used to cure stomach problems. So diverse are the uses of every part of *Capparis decidua* that it is called as "Sandal Wood" of the desert in India (Harsh and Tiwari, 1998).

The plant is facing depletion of natural populations due to overexploitation, change in climate condition, and the cutting of plants in natural habitat for urbanization and agricultural reasons (Anon, 2001; Khan *et al*., 2003). To maintain and sustain forest vegetation, conventional approaches like grafting, layering and cutting have been used for propagation. Nonetheless, these conventional methods of plant propagation have limited applicability (Yadav and Singh, 2011; Yadav *et al*., 2012). There is wide genetic variability in the field population of *Capparis decidua* as it is an open pollinated plant. The seeds have a short period of viability. Micropropagation offers a rapid means of afforestation, multiplying woody biomass, and conservation of elite and rare germplasm (Bajaj, 1986; Karp, 1994). The technique of cell and tissue culture, under controlled and defined conditions, has contributed in raising new plants, manipulation of plant without conventional breeding mechanism and methods, shortening germination and development phase of plants. Genetically poor gene pool is only now available. Plant tissue culture offers an opportunity for rapid multiplication of desired tree species.

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2. Material and Methods

2.1. *In vitro* shoot induction

2.1.1. Plant material

Explants in the form of nodal segments (2.0-3.5 cm) were collected from healthy, disease free mature field grown planta of *Capparis decidua* for axillary bud induction and proliferation from places near Sarnau village (Rajasthan).

2.1.2. Surface sterilization

Explants were washed with Tween-80 and followed by bavistin and streptomycin. Further, surface sterilization was carried out by 0.1% $HgCl_2$ for 3-10 min and finally they were washed thrice with autoclaved distilled water.

2.1.3. Effect of plant growth regulators on shoot induction

Nodal segments were inoculated on MS (Murashige and Skoog, 1962) medium supplemented with 3% (w/v) sucrose (Himedia, India) and solidified with 0.8% Agar (Himedia, India). Medium was enriched with ascorbic acid (50 mg/l), citric acid (30 mg/l), and adenine sulphate (50 mg/l). The medium was then incorporated with different plant growth regulators. Cytokinins BAP and Kn were tested either alone or in combination with auxin NAA. The pH of the medium was adjusted to 5.8 and autoclaved at 15 lb pressure for 20 minutes at 121°C.

2.2. *In vitro* shoot multiplication

Multiplication of the shoots starts after bud break initiation from *in vitro* cultured plants. After 3-4 weeks *in vitro* regenerated shoots of the cultures attain the height of 2-3 cm which were cut into shoot segments containing at least two nodes 1.5 to 2 cm in length and carefully subcultured on MS medium supplemented with various concentration of cytokinin BAP and Kn alone and in combination with auxin IAA and NAA. Additives such as ascorbic acid, citric acid, and adenine sulphate for shoot multiplication were also used in the medium. The multiple shoots proliferated from cultures were repeatedly transferred to fresh cultured medium. Repeated transfer was done after 25-30 days. Shoot produced were either used for further multiplication of shoots or for elongation.

2.3. *In vitro* rooting

Well developed *in vitro* shoots of 5-6 cm length were used for various *in vitro* rooting experiments. These *in vitro* shoots were transferred to MS medium supplemented with auxins in order to obtain *in vitro* rooting. Different auxins (IBA, NAA and IAA) were tested at different concentration in the medium for *in vitro* rooting. Effect of strength of MS medium (1/4×, 1/3×, 1/2×, 3/4×, 1×) was investigated for *in vitro* rooting of *in vitro* raised shoots.

2.4. Hardening and acclimatization of *in vitro* propagated plantlets

The tissue culture raised plants are heterotrophic in their mode of nutrition and cannot withstand harsh environmental conditions without proper hardening and acclimatization. The rooted plantlets were taken out from the flasks, washed thoroughly with water to remove all traces of medium attached to the roots and then transferred to glass bottles containing 1/3 volume of soilrite. These plantlets were nurtured with half strength MS medium (without organics) twice a week for three weeks and were kept in a tissue culture room. After three weeks, these bottle were shifted to a mist chamber having relative humidity 80-90% with a temperature of 30 ± 2°C. The caps of bottles were removed and plantlets were allowed to remain in the bottle for 3-4 days before they were transferred to polybags containing a mixture of sand, soil and farmyard manure in ratio of 1:1:1. In the mist chamber, the plants were kept for four weeks and were irrigated with half strength MS medium. Finally, hardened plants were transferred to bigger pots and transferred to a net house.

All cultures were incubated at 28 ± 2°C temperature and relative humidity of 70 ± 2%, maintained in culture rooms by air conditioners. All cultures were kept under illumination of 16 hrs photoperiod with light intensity of 2500 lux, obtained by white cool fluorescent tubes of 40 watts (Philips, India).

Data collected were analyzed using CRD design of experiments because it is one of the most widely used and acceptable designs in the case of tissue culture experiments (controlled set of conditions and for homogenous materials) which gives the best analysis. Experiments were repeated thrice and data represent the mean of three experiments. Each treatment consisted of a minimum of twelve replicates. Degree of variation was represented by mean, standard deviation and standard error.

3. Results and Discussion

In this report we describe a tissue culture procedure developed for rapid propagation of mature plants of *C. decidua*. The nodal shoot explants harvested during the month of March-April and September and October were found to be best for establishment of cultures of *Capparis decidua*. During sterilization, the shoots were sensitive to $HgCl_2$ treatment. The cut ends of all the explants exhibited browning in culture and subsequently the entire explant necrosed and died. Thus the brown portion was removed within 4-5 days of subculturing and explants were cultured on the same fresh medium to avoid further browning. Deora and Shekhawat (1995) and Chahar *et al*. (2010) have also reported that browning could be avoided by removing the necrotic portion

Table 1. Effect of BAP + 0.54 µM NAA concentration in MS medium supplemented with additives on *in vitro* axillary bud induction. Data recorded after 4 weeks.

BAP+NAA (0.54) (µM/l)	Response %	Mean shoot number	Mean Shoot Length (cm)
Control	15.53 ± 2.22^{e}	1.00 ± 0.00^{d}	1.00 ± 0.03^{e}
4.44	22.22 ± 2.22^{d}	1.44 ± 0.18^{cd}	1.26± 0.12^{d}
8.88	62.00 ± 2.00^{b}	1.79± 0.63^{bc}	1.99 ± 0.54^{bc}
13.32	66.66 ± 0.00^{ab}	2.13 ± 0.08^{b}	2.09 ± 0.04^{b}
17.76	**75.55 ± 2.22^{a}**	**3.03 ± 0.14^{a}**	**2.27 ± 0.03^{a}**
22.2	52.22 ± 4.44^{b}	3.10± 0.16^{a}	2.10 ± 0.03^{b}
26.66	51.11 ± 2.22^{c}	2.78 ± 0.20^{a}	1.89 ± 0.05^{c}
	F= 85.27**	**F= 20.66****	**F=57.41****

** = P<0.01, *= P<0.05. Values with common superscript are not significantly different at P<0.05, Duncan multiple range test.

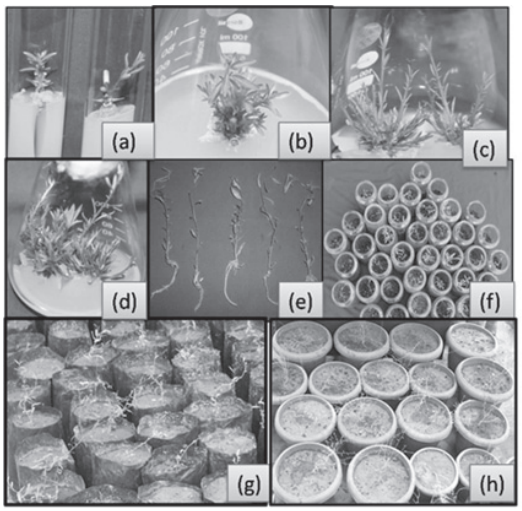

Fig. 1. A-H: a) Axillary bud induction using nodal explants collected from mature mother plants, b) Axillary bud proliferation after first subculturing (after 3-4 weeks), c) *In vitro* shoot multiplication after second subculturing (after 3-4 weeks), d) Elongated shoots before rooting, e) *In vitro* rooted plantlets, f) Hardened and acclimatized plantlets, g) Micropropagated plants transferred to poly-bags (after 3 months), h) Plants transferred to pots (after 6 months).

within 3-4 days and re-culturing the explants on the same fresh medium. In the present investigation, MS basal medium supplemented with 17.76 µM BAP + 0.54 µM NAA and ascorbic acid (50 mg/l), citric acid (30 mg/l), adenine sulphate (30 mg/l) was the most suitable for *in vitro* shoot induction (**Table 1**). Seventy five percent of the nodal explants responded with 3-4 shoots/explant within 3-4 weeks (**Fig. 1a**). Deora and Shekhawat, (1995) and Tyagi and Kothari, (1997) also observed best shoot proliferation from nodal explants in *Capparis decidua* on MS medium supplemented with BAP and NAA. The increased concentration of BAP showed increased number of shoots and shoot length in the presence of

Table 2. Effect of BAP concentration in MS medium supplemented with 0.57 µM IAA + additives on *in vitro* shoot multiplication of *C. decidua*. Data recorded after 4weeks.

BAP + IAA(0.57) (µM/l)	Mean shoot number	Mean shoot length (cm)	Multiplication Fold
Control	7.31 ± 0.14^{e}	1.62 ± 0.04^{d}	1.83 ± 0.04^{e}
4.44	9.78 ± 0.11^{d}	1.89 ± 0.03^{c}	2.44 ± 0.06^{d}
6.66	17.75 ± 0.87^{b}	2.10 ± 0.04^{b}	4.44 ± 0.05^{b}
8.88	**25.86 ± 0.22^{a}**	**3.02 ± 0.04^{a}**	**6.47 ± 0.04^{a}**
11.1	26.14 ± 0.24^{a}	1.93 ± 0.12^{c}	6.55 ± 0.09^{a}
13.32	15.19 ± 0.16^{c}	1.65 ± 0.04^{d}	3.81 ± 0.04^{c}
	F = 2025.88**	**F = 188.42****	**F = 2048.35****

** = P<0.01, *= P<0.05.Values with common superscript are not significantly different at P<0.05, Duncan multiple range test.

Table 3. Effect of IBA on *in vitro* rooting of *Capparis decidua*. One fourth strength MS medium supplemented with additives was used.

IBA (µM/l)	Response %	Mean root number	Mean root length (cm)
Control	4.44 ± 2.22^{e}	1.50 ± 0.50^{c}	0.20 ± 0.00^{d}
0.49	50.55 ±2.78^{d}	3.45 ± 0.43^{b}	2.07 ± 0.64^{a}
2.46	69.44 ± 2.78^{b}	2.29 ± 0.24^{b}	2.12 ± 0.04^{a}
4.92	**94.44 ± 2.78^{a}**	**5.82 ± 0.31^{a}**	**2.35 ± 0.07^{a}**
7.43	55.55 ± 2.78^{bc}	3.14 ± 0.16^{b}	1.70 ± 0.07^{b}
9.84	44.44 ± 2.78^{c}	1.75 ± 0.18^{c}	1.30 ± 0.07^{c}
	F= 134.19**	**F= 23.88****	**F=41.34****

** = P<0.01, *= P<0.05.Values with common superscript are not significantly different at P<0.05, Duncan multiple range test.

additives. However, in the absence of the additives a decrease in shoot regeneration response was observed from nodal explants. In the present study, axillary bud induction was more in number on BAP supplemented medium as compared to medium replaced with Kn. The efficiency of BAP for shoot initiation is also documented for a number of plant species (Kumar *et al*., 2010; Singh and Tiwari, 2012). The shoots proliferated from axillary buds were established on MS medium and were used for *in vitro* shoot multiplication (Fig. 1**b**). Best shoot multiplication was obtained on MS medium supplemented with 8.88 µM BAP + 0.57 µM IAA and ascorbic acid (50 mg/l), citric acid (30mg/l), and adenine sulphate (50 mg/l). A shoot multiplication rate of 6-7 fold was obtained in a period of four weeks (**Table 2** and Fig. 1**c**). Similar results have been reported earlier by Deora and Shekhawat (1995), Komalavalli and Rao (2000), and Phulwaria *et al*. (2012).

Elongated *in vitro* shoots of 5-6 cm length (Fig. 1**d**) were used for various *in vitro* rooting experiments. *In vitro* rooting was obtained when *in vitro* grown shoots were transferred on

MS medium supplemented with auxins (IBA, NAA and IAA). One fourth strength MS medium supplemented with 4.92 µM IBA and ascorbic acid (50 mg/l), citric acid (30 mg/l) and incubated at 31°C gave the maximum rooting response of 94.44% with an average of 5-6 roots per shoot (**Table 3** and Fig. 1**e**). Compared to NAA and IAA, IBA was found to be a better auxin for *in vitro* root induction. The present *in vitro* rooting percentage appears to be superior compared with previous reports published for the same plant species (Deora and Shekhawat, 1995; Tyagi and Kothari, 1997; Chahar *et al*., 2010). Plants were hardened and acclimatized and put into soil with 80% survival rate. (Figs. 1**f-h**)

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