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Organogenic plant regeneration and ecorestoration of Salvadora Persica-An Ethnomedicinal Facultative Halophyte

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ABSTRACT

Salvadora persica plants, showing relatively high salt tolerance were used for micro propagation and generation of plantlets from callus cultures. Present investigation has been focused on efficient in vitro propagation protocol for this valuable plant species. immature cotyledon explants of in vitro grown one-month-old seedlings serves as explants source for callus induction. Maximum callus proliferation was obtained on Murashige and skoog's medium supplemented with 2, 4D and Kn. Three weeks old callus was used for shoot regeneration. The maximum shoot regeneration was achieved in four weeks when callus was cultured on MS medium fortified with BAP and NAA. Regenerated shoots were excised and multiplied (25.5+1.5 shoots/explants)on MS medium supplemented with NAA (0.1 mg/l) and BAP (1.0 mg/l) along with adenine sulphate (40 mg /l). Although slight root induction was observed on the same hormonal regime, however for maximum root induction, multiple shoots were divided into single shoots and were rooted (5.1+ 0.49 rootlets/ shoot)on half strength MS medium fortified with indole-3- butyric acid (3.0mg/l) and activated charcoal (0.2%). The establishment of in vitro grown plantlets into pots containing a mixture of sand: soil (1:1 v/v) was 90% .Plantlets got hardened by keeping them in hardening unit for few days. Hardened plats established very well in the nursery. Though originated from callus these plants did not show any morphological variations and were similar to their parent donor plant.

Keywords: Salvadora persica, callus cultures, meswak ,organogenesis, halophyte, salt tolerance.

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INTRODUCTION

Salvadora persica L. (Meswak) belongs to family salvadoraceae is a facultative halophyte, because it occurs in both non-saline to very highly saline habitat. It is a potential source for seed oil and identified as a predominant species in highly saline habitats of coastal and inland black soils. The high fatty acid as a valuable substitute to coconut oil for quality soap making. The oil is non- edible due to the presence of dibenzyl thiourea and a glucoside glucote opelin and its decomposition products. The ripe fruits (particularly the parthenocarpic berries) of this species are eaten raw, the seeds contain about 30-40 percent oil. The tender twigs, leaves and roots have many pharmaceutical applications as they contain salvadoricine, salvadourea, di-benzyl thiourea, rutin , quercitin trim ethylamine, thioglucoside, Potash, chloine etc. [1, 2]. Besides, this plant is also suitable for ageoforestry as it stops wind breaks and helps in land reclamation [3-5] . Aside from its diverse utility there are several limitations . At the same time very few reports are available on *in vitro* plant propagation of this plant species . In the propagation of this plant species as it is a cross- pollinated crop shows high variability and viability of seeds is also very low. Thus, in view of the multifaceted advantages of *Salvadora persica*, its high degree of salt tolerance (its luxurious growth on highly saline soils), pharmaceutical and economical values, it becomes imperative to evolve some alternative methods for plant propagations. Moreover for further research into the biochemical compositions and potential medicinal values of this, an efficient *in vitro* regeneration system for the production of plants is required because field-grown plants may be subjected to seasonal and somatic variations, infestations of bacteria, fungi and insects as well as environmental pollution that can affect the medicinal value of the harvested tissues.

Earlier we have reported regeneration of *Salvadora persica* plants via various explants shoot tips [6]; Cotyledonary hodes [7]; nodal segments [8].

These protocols are useful for clonal propagation of Salvadora plants. However, high frequency, consistent and high number of shoots regeneration of plants for Biomass enhancement, organogenesis is required for this plant species.

MATERIALS AND METHODS

The seeds of *salvadora persica* L. were collected from JNV University , Jodhpur.

Seed Germination

The seeds were rinsed with 70% alcohol for 5-10 minutes followed by teepol for 3-5 times with sterile distilled water. After that they were inoculated in glass culture tubes (2.5x 15cm, Borosil, India) containing 20 ml of ½ MS medium supplemented with 3% sucrose and 0.8% agar (Qualigen, India). The pH of the medium was adjusted to 5.8 before autoclaving at 15 psi and at 121oC for 15 to 20 minutes. All the cultures were incubated at 25+2oC with a 16 h photoperiod (30 umol.m-2S-2) provided by cool white fluorescent tubes.

Shoot Bud Regeneration Studies

Various explants such as cotyledons, epicotyls, hypocotyls and leaves were excised from one month old *invitro* seedlings and were tested for organogenic callus induction. However immotile cotyledons were found to be the best for optional callus induction .Each cotyledon was cut into 3-4 Pieces of size 1x1 cm. These were inoculated either in the culture tubes or in sterile plastic dishes in contact with MS medium [9] supplemented with 3% sucrose and 0.8% agar. Basal medium supplemented with 2,4D (0.5-5.0 mg/l)+Kn (0.05-2.05 mg/l) were used for callus culture from immatuse cotyledon explants. After 3 weeks, the calli proliferated upon the cotyledon explants. The primary calli were subjected to two successive subcultures at three week intervals. Primary and sub cultured calli were transferred to MS medium fortified with various concentrations of BAP+ NAA to induce plant regenration. All growth regulators were added prior to autoclaving the medium. The conditions of sterilization of media and incubation of cultures were as described above.

Elongation and rooting of shoots

Multiple shoots (3 cm length) that formed on MS medium supplemented with various concentrations of BAP and NAA were separated and transferred to same media regime along with activated charcoal (0.1%) for elongation. Although slight rooting was also observed on the same media. However, for optimal root induction, isolated elongated shoots were transferred to ½ MS medium along with IBA (0.5-5.0 mg/l) and activated charcoal (0.2%).

Acclimatization and Hardening Procedures

In vitro regenerated plantlets with well-developed shoot (56 cm) and root (4- 6cm) were hardendened in pots containing autoclaved mixture of sand: soil (1:1) and acclimatized for 2 weeds under natural diffuse sunlight and 70% humidity. After that they were established in garden soil where they grew well.

Statistical Analysis

Each tube or bottle with three or five explants was taken as one replicate. Each experiment consisted of ten replicates for each treatment and all experiments were repeated at least twice. These were then compared by one way ANOVA and individual treatment means were compared by student's t-test [10].

RESULTS AND DISCUSSION

Different explants viz, epicotyl, hypocotyl cotyledon, leaf were cultured *in vitro* to find out the most suitable explant for callus induction. Although all these explants showed slight callusing response. Both epicotyl and leaf explants produced callus put the frequencies was low (<25%) and the texture of the calli were non-organogenic (Brown and fragile), which on further sub culturing becomes brown and declined to grow. However calli produced via hypocotyl

explant was whitish brown and watery. Thus among the different explants tested, acotyledons were found to be the best for callus induction. The suitability of cotyledon explant on organogenic plant regeneration was also noticed by .Tripathy and Reddy 2002.Callus induction via immature cotyledon explants was observed on MS media fortified with various concentrations and combinations of cytokinins and auxins within 2-3 weeks of cultures. Wide variations were observed in percentage of callus formation and average fresh wt. of callus. The highest percentage of callus formation (85%) was observed in MS media having 2.5 mg/l 2-4D and 0.5mg/l Kn .Similarly at this particular hormonal regime, highest callus growth and terms of fresh wt. (991+9.9 mg) was observed. Whereas highest dry wt. of calli (142+0.34 mg) was noticed in MS media fortified with Kn (1.0 mg/l). Produced callus was lush green in colour and later gave rise to several shoot buds. Positive response for shoot bud regeneration was observed when calli were cultured on MS media having both BAP and NAA (Table-1). However media containing BAP or NAA alone failed to induce differentiation and shoot growth. MS media with BAP (1.0 mg/l) and NAA (0.1 mg/l) gave best results for adventitious shoot bud induction frequency (5%), number of shoots (25.5+1.51)per explant and shoot length (7.20 + 0.69). Addition of adenine sulphate (40 mg/l) enhanced the growth of the shoots with dark green leaves. These shoot buds first appeared as nodular growth within 3-4 weeds of culture and after another week nodular structures change into shoot buds. Interaction of callusing media and shoot regeneration media on shoot induction frequency shoed that a maximum of 17- 18% shoot induction frequency was obtained from calluses induced on MS media containing 2.5 mg/l 2-4D and 0.5 mg/l Kn and regenerated on MS media containing BAP (1.0 mg/l)and NAA (0.1 mg/l). (Fig.)

Table 1: Effect of various concentration and combination of auxins cytokinins on callus initiation from cotyledon explant of *Salvadora persica* L.

Hormonal Treatments (mg/l)	Days of Callus initiation	% of Callus formation	Colour of callus	Texture of Callus	Fresh wt of Callus (mg)+SD	Dry wt of Callus (mg)+SD
MS +NAA 1.5	20	21	PY	C	325+7.9	25+0.22
MS +NAA 2.0	20	28	PY	C	330+6.2	40+0.34
MS +NAA 3.0	20	32	PY	C	330+6.2	35+0.24
MS +2,4-D 1.0	12	55	LB	F	495+10.0	123+0.30
MS +2,4-D 2.0	12	56	LB	F	532+10.7	125+0.28
MS +2,4-D 2.5	12	67	LB	F	592+10.2	127+0.34
MS +2,4-D 3.0	12	58	LB	F	458+3.9	103+0.42
MS+KN 0.25	12	50	LG	F	680+6.8	110+0.40
MS+KN 0.5	12	52	LG	F	695+6.2	7+0.32
MS+KN 1.0	12	65	LG	F	702+5.3	142+0.34
MS+KN 2.5	12	50	LG	F	582+10.7	110+0.28
MS + BAP 0.25	O8	35	DG	C	394+7.8	59+0.34
MS + BAP 0.5	O8	30	DG	C	294+6.2	65+0.26
MS + BAP 2.5	O8	30	DG	C	288+6.1	59+0.22
MS +2,4-D (2.5))+KN 0.25	12	71	LG	F	98.0+9.8	95+0.31
MS +2,4-D (2.5))+KN 0.5	12	85	LG	F	991+9.9	98+0.37

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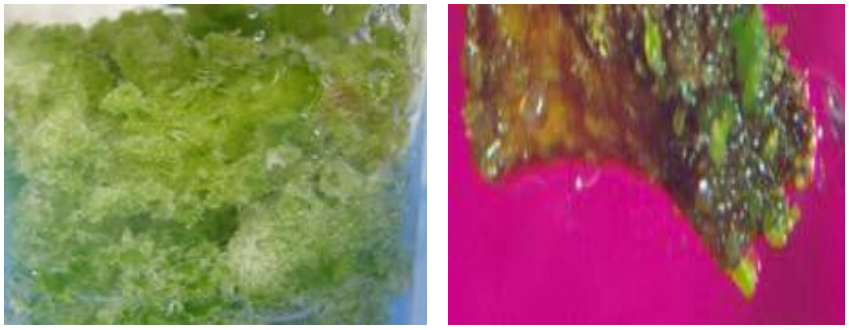
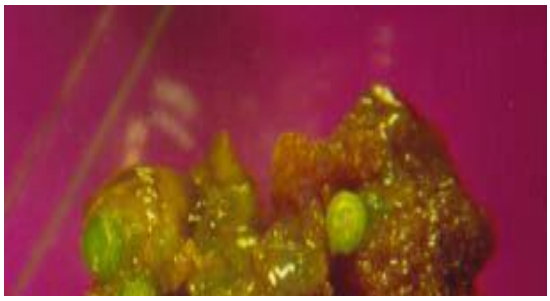
MS +2,4-D (2.5))+KN 1.0	12	76	LG	F	887+9.7	85+0.32
MS +2,4-D (2.5))+KN 2.0	12	56	LG	C	694+7.2	79+0.22
MS +2,4-D (2.5))+KN 2.5	12	49	LG	C	564+5.6	60+0.12

LG= Light Green **DG= Dark Green** **LB= Light Brown** **F= Friable**
PY= Pale Yellow **C= Compact**


(a-b)


(c)


(d-e)

Fig. Organogenic plant induction in Salvadora plant
(a) Callus induction (b) Organogenic callus (c) Shoot bud induction (d) Shoot regeneration (e) Root induction

Callusing media devoid of cytokinin gave significantly lower shoot regeneration response irrespective of the auxin concentration in callusing media. Thus it suggests the development of certain meristemoids in presence of cytokinin . When dedifferentiation process was taking place during callus induction in *Salvadora persica* L., which results in shoot induction in the prevalence of proper conditions.

Although on BAP and NAA supplemented media, several shoot buds were produced, however they were thin and small in length. Incorporation of activated charcoal (0.1%) Promoted both thickness as well as length of the shoots . Similarly, slight root induction was observed on the same differentiating hormonal regime. However for adventitious root induction shoots were excised and cultured on various strength of MS salts as well as varied hormonal regimes. Optimal root formation was observed on ½ MS media along with IBA (3.0 mg/l). This result is in accordance with that of Muthukumar et al., 2004; Natatrajan et al., 1999; Manickam et al; 2000. Here also addition of activated charcoal (0.2%) enhanced both number of roots as well as root length. Similar to our observation earlier we also reports best rooting response with activated charcoal [7].

Therefore callus free rooted plantlets were removed from culture tubes, washed thoroughly under tap water so as to remove the excessive media and later transferred to pots filled with mixture of sterile soil and manure in equal proportion. Plantlets in pots were irrigated with reduced strength ½ of MS salts, covered with plastic bags and kept in hardening unit at 30oC under high humidity (60-70%) for 10-15 days. After that, plantlets were transferred to bigger pots containing soil, sand manure irrigated with MS Salts (1/4 strength) and kept in green house. after one year the plants grow well and established successfully under natural conditions (up to 80%) .The regenerated plants did not show any detectable variations in the morphological characters as compared to donor plants.

Thus this work on shoot organogenesis from immature cotyledon explant of *Salvadora persica* L. (Meswak) can be used for high frequency and clonal propagation of this Ethan medicinal plant which is a highly salt tolerant and economically important arid zone plant species .

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