Rooting Experiments on Stem Cuttings of *Evolvulus Alsinoides* L.

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Abstract: The present propagation study has been attempted to enumerate the rooting response of stem cuttings of economic valuable medicinal plant species *Evolvulus alsinoides*. The different concentrations of IAA, IBA and NAA at the range of 1000 to 5000 ppm using quick dip method during winter and summer seasons. The maximum rooting 80% was observed in IBA 2000 ppm. The root length showed variation when the cuttings were observed for various concentrations of auxin treatments. The root length obtained maximum in the same IBA 2000 ppm and reached up to 19 cm – 21cm during winter season.

Key Words: Auxin, Rooting, Medicinal plants.

INTRODUCTION

The vegetative propagation of plants is useful for maintaining the desired genetic constitutions, multiplications of plants and development of clones. Micro-propagation has provided to be an alteration for the multiplication of selected genotypes and phenotypes of several medicinal and aromatic plants (Bajaj *et al.*, 1988.). Plant micro propagation standardised in-*vitro* culture has been considered a promising tool to obtain homozygous plant material to serve as approximate sources of drugs (Rout *et al.*, 2000; Lima *et al.*, 2001).

Evolvulus alsinoides , the slender dwarf morning glory, is belongs to the family Convolvulaceae. The species inhabits a wide range of habitats and a number of varieties are recognised. This species is one of the plants included in Dasapushpam, the ten sacred flowers of Kerala. The herbaceous plant used in traditional medicine of East Asia for its purported psychotropic and no tropic properties. It is used as a brain tonic Ayurveda and Unani Medicines (Indhumol *et al.*, 2013). The plant having antimicrobial properties and act as a curative medicine for gastro and neuro problems. It is cure for Alzheimer's disease, against loss of memory. This species used against ulcer and Ashma. So the clonal attempt of propagation of this plant is highly useful for mankind.

MATERIALS AND METHODS

The plants of *Evolvulus alsinoides* were collected from Chittanavasal region of Pudukkottai district. The experiment was conducted during the year 2016. Semi hard wood cuttings were collected during summer (April - May) and winter (November - December) seasons. Nodal cuttings of 20cm long were taken with 3-4 nodes and 2-3 excised leaves on the top. The cuttings were treated with Bavistin to avoid fungal insertion in mist house. After that the cuttings were washed with distilled water and treated with IBA, IAA and NAA rooting hormones with different concentrations of 1000 – 5000 ppm.

The solutions of growth regulators in various concentrations were prepared and the basal parts on the bundle cuttings (50 cuttings in each bundle) were dipped in the respective 20 minutes. Stem cuttings were also kept as control without any hormone treatment. The treated cuttings were kept in the beds of the mist chamber containing same as the rooting medium.

The mist chamber contains congenial microclimate conditions for the growth of cuttings (70 – 80 percentage RH) and temperature 37 ± 2° C. The data on percentage of rooting for stem cuttings were recorded on 40 days after treatment with different concentrations for both the seasons.

RESULT AND DISCUSSION

The present study show encouraging results under the influence of growth regulators and water medium. Observations were made periodically to record any development changes in the cuttings. Nearly 2 weeks after first sprouting was observed in terminals which were treated with IBA 2000 ppm. The rooting was seen in about 21 days after treatment. The rooting was observed in treated cuttings only but not in control. The percentage of rooting was observed to be the different in NAA and IBA concentrations. The root length with IBA 2000 ppm was recorded maximum root length noticed (Table-1) 19 - 20 cm. Whereas with NAA 2000 ppm recorded 11 – 12 cm. Earlier reports suggest that the rooting response using stem cuttings of the medicinal plant endangered tree species *Sterculia urens* (Kesava Reddy 1994 and Purohit and Dave 1996) *Vicoa indica* (Sivasubramanian *et al*., 2011.), *Premna serratifolia* (Ravindar Singh *et al*., 2011).

Table 1: Rooting response of stem cuttings of *Evolvulus alsinoides* L.

S.No.	Various Treatments	Number of Cuttings treated	Number of rooted cuttings	Percentage of rooting	Mean number of root per plant	Mean length of roots per plant (in cm)
1	Control	30	5	12	2.2 ± 0.3	6.1 ± 1.0
2	IBA 2000ppm	30	24	80	9.1 ± 2.1	**19 ± 1.1**
3	NAA 2000ppm	30	18	60	5.1 ± 1.6	14.5 ± 0.6

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PLANT REGENERATION FROM SEED CULTURE OF *CUCUMIS DIPSACEUS* Ehrenb.

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Abstract: The present paper focuses on *in vitro* regeneration method for the tendril climber and economic important medicinal plant Cucumis dipsaceus Ehrenb. Was established. The seeds were treated with GA_3 produced *in vitro* grown seedlings within ten days intervals. Among the different treatments water as well as GA_3 induced the highest percentage 86% maturation and germination of embryos. The *in vitro* generated shoots turned brown at base and gradually turned black resulting in reduced plantlets rooting. Addition pinch of charcoal to the rooting medium resulted in increased rooting percentage. The rooted plants, transferred to a soil mix had a survival rate of 91% in the green house. All the plants that were transferred to wetlands survived.

Key Words: *In vitro*, medicinal plant, hardening

INTRODUCTION

Cucumis dipsaceus Ehrenb. Ex Spach is a species of flowering plant belonging to the family Cucurbitaceous. It has its origin in Ethiopia and is known by several common names like teasel gourd, Arabian cucumber, hedgehog, pepino-diablito, concombre porc-epic etc. Usually *Cucumis dipsaceus* are consumed as a leafy vegetable (Verdcourt and Trump, 1969), its fruit juice is topically applied to prevent hair loss (Rainer and Ashley, 2010). However, being a fruit growing in wild no attempt has been taken to evaluate the parameters which could support its use as vegetable. In India, herbal origin is used for various diseases and Indian folk medicine is used as prescriptions for therapeutic purposes such as wounds, inflammation, skin infection, leprosy, diarrhea, scabies, venereal disease, ulcers, snake bites (Hemamalini *et al.*, 2013). The country ranks sixth for harboring the largest number of threatened plant species. The IUCN Red list indicates that 91% plant species are threatened due to habitat loss and degradation (Hilton, 2001). Approximately 70% of India's medicinal plants are found in the tropical regions (Negi *et al.*, 2007). Conservation of bio- wealth and genetic resources is essential for ensuring future food of the Nation. In order to utilize plant species in a judicious way, a thorough knowledge of ecological needs of endangered and endemic plants is a prerequisite (Hawkes, 1976). This paper reports the protocol for improvement of micro propagation of this plant using seeds as the explants.

MATERIALS AND METHODS

The fruits were collected during the month of November 2014. The collected plant material was identified and their authenticity was confirmed by comparing the voucher specimen at the herbarium of Botanical survey of India (BSI), Southern circle Coimbatore, Tamil Nadu (No.BSI/SRC/5/23/2011-12/Tech-1466). Freshly collected fruits were cleaned to remove adhering dust and then dried under shade. The dried sample was powdered and used for further studies. The seeds were washed thoroughly in running tap water followed by glass distilled water. Surface sterilization was stone inside laminar air flow chamber with mercuric chloride (0.1%) for 5 minutes.

The followed by washing with sterile distilled water 3 - 4 times. The various treatments including GA3 treatment used for the seed germination studies. The cultures were incubated at 26 ± 2^0 c and photoperiod of 15 h at light intensity of 3000 lux provided by cool white fluorescent tubes.

Fig 1. Seeds development on the medicinal plant *Cucumis dipsaceus*

RESULT AND DISCUSSION

The present studies on *C.dipsaceus* were carried out to identify the factors for in vitro studies of this medicinally important plant which is over exploited for medicinal purposes. The studied explants show encouraging results under the influence of GA3 and growth regulators. The explants sterilized with 0.1% HgCl2 and different treatments shows variation in plantlet production. An initial dark period for 48 hours was found to be limiting factor for the establishment of cultures during present study. These cultures when exposed to fluorescent light of various photo period during the first 48 hours of incubation turn albino lost the green color. Some observations have been made Rukhsana, 2004. This suggests the inhibitory role that light has on the in vitro culture of many other angiosperm species (Razdan, 2001).

The plantlet production from seed explants on GA_3 medium within ten days of inoculation. However, in case of different growth regulators the response for production of plantlets. Mature showed saximum germination and they grew well on GA_3 medium while immature seeds showed abnormal growth with subsequent seedling death. However, the present survival of control plantlets was good 90% and when they were transferred to field they grew well. Similar result was observed in *Gnetum ula* (Siva Subramanian *et al*., 2005) *Aeschynomene indica* (Indira *et al*., 2012) and *Ginkgo biloba* (Aseesh *et al*., 2011). The resurgence of public interest in plant based medicine tied with the rapid expansion of pharmaceutical industries dictated an increased demand of medicinal plants, leading to exploitation that threatened the survival of many medicinal plants. Plant tissue culture provides propagation of plants which are rare or economically important, so that industrial demands could be met without disturbing the natural population of plants. The present investigation showed that a sufficient number of healthy plantlets of *C.dipsaceus* with 90% survival may be produced by applying GA_3 treatment within short period of time which could be an easy cost effective, rapid and promising method of macro propagation.

Table 1: Effect of pre-treatments on the germination behavior of seeds

Treatments	Duration	Germination Percentage	Days for Germination
IBA	2 minutes	55	20
	5 minutes	67	19
	10 minutes	59	22
NAA	2 minutes	69	17
	5 minutes	62	22
	10 minutes	64	19
IAA	2 minutes	59	25
	5 minutes	62	28
	10 minutes	57	32
Boiling Water	2 minutes	46	21
	5 minutes	57	27
	10 minutes	43	30
GA_3	2 minutes	77	16
	5 minutes	**86**	**10**
	10 minutes	82	21
Water medium	2 minutes	81	18
	5 minutes	**86**	**14**
	10 minutes	79	24
Acid treatment	2 minutes	23	21
	5 minutes	16	32
	10 minutes	11	27

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