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Effect Of Auxin On Plant Regeneration In Portulaca Grandiflora

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Abstract
According to the WHO about 80% population of the world rely on medicinal plants for remedies. Medicinal plants are exploited for therapeutic medicine and have been used by Ayurveda, Unani and Siddha. Portulaca grandiflora, is a medicinal ornamental plant in the family Portulacaceae, Portulaca has many medicinal properties against cirrhosis of the liver, pharyngeal pain and swelling, scurvy, snake and insect bites, burns, scalds, and eczema. In the present investigation complete plant regeneration was achieved only in single medium i.e. auxin (Indole butyric acid). Nodal explants of Portulaca grandiflora were inoculated on MS medium containing IBA (1.0-4.0mg/l). Axillary bud proliferation was observed within 05 days and root initiation was also observed on the same medium within 10 days of inoculation. No callus initiation was observed on any of the IBA supplemented media. After 3 weeks regenerated shoots attain height of 2-3 inch transferred to green house and finally to the field. Out of the four concentration used 3.0 mg/l IBA Axillary bud proliferation (86.12 %,) and plantlet regeneration (89.23). Regenerated plantlets were successfully transferred to green house and finally to field with 90% survival rete.
Keywords: *Micropropagation, Portulaca grandiflora, Regeneration*

1- Introduction

Plants are good source of therapeutic medicine. According to the WHO about 80% population of the world depend on traditional remedies (Task force, 2000). Medicinal plants are exploited for therapeutic medicine and have been used by Ayurveda, Unani and Siddha (Jadhav, 2008). India exports more than 32,000 tones of medicinal plants raw material of worth US$ 46 million annually. Most of the pharmaceuticals industries draw almost 90% of its supply from natural habitat. Due to this fact a good number of medicinal plants are depleting in the natural habitat, as a result of this plants are becoming threatened. Therefore there is a urgent need for exist conservation of medicinal plant. Various conservation strategies have been employed, Out of them tissue culture has been used globally for the exits conservation of plants. Conservation of medicinal plants and their mass propagation has been achieved through *in vitro* techniques with significance (Arora & Bhojwani., 1989; Sharma *et al*., 1993; Latha & Seeni., 1994)

Portulaca grandiflora, is a medicinal ornamental plant in the family Portulacaceae, native to Argentina, southern Brazil, and Uruguay and often cultivated in gardens. It has many common names, including rose moss, eleven o'clock, Mexican rose etc. It is a small, but fast-growing annual plant growing upto 30 cm tall. The leaves are thick and fleshy, up to 2.5 cm long, arranged alternately or in small

clusters. The flowers are 2.5–3 cm diameter with five petals, variably red, orange, pink, white, and yellow.

Portulaca has many medicinal properties. It is used for the treatment of cirrhosis of the liver, pharyngeal pain and swelling. Leaves used for scurvy. Fresh juice of leaves and stems applied to snake and insect bites, burns, scalds, and eczema (Kirtikar, K.K., Basu, B.D., 1987) In Chinese medicine, used to treat various tumors. One of the ingredients of the Chinese herbal medicine, Tumoclear formulated for tumor and cancer care. In Thai medicine, aerial parts used for treatment of sore throat, skin rash and detoxification. Leaves and flowers worn around the neck to relieve muscle spasms and neck stiffness.

In the present study an efficient Protocol has been developed for micropropagation and plant regeneration of *Portulaca grandiflora* using nodal explants. The protocol could go along way in the further biotechnological improvement and commercial exploitation of this important plant specie.

2- Material & Methodology

In the present study *Portulaca grandiflora* Plants were collected from CIMAP, Lucknow and maintained at botanical garden of Omcar India, Gwalior. Murashige & Skoog's (1964)(HiMedia) was used as basic nutrient medium.. The disease free, young and healthy nodal explants were selected for carrying out study. Explant washed under running tap water for 30 minutes in order to wash off the external dust/contaminants. In the next step explants were soaked in an aqueous solution containing 0.1 % $Hgcl_2$ for 5 minutes in Laminar flow hood. This was followed by gentle wash in sterile double distilled water for 1-1 minutes for two cycles. Sterilized explants were transferred aseptically to sterilized glass plate under the laminar flow hood. Then nodal explants cut into 1-2 cm. in size and insert into media in the test tube.

In the present study single hormone i.e. auxin (Indole butyric acid) was used in 4 concentrations (1-4mg/l) for complete plant regeneration. All cultures tubes were kept at temperature conditions 25± 2 °C, with a photoperiod of 16 hours daylight and 8 hrs night break under the cool white fluorescent light of average 2000 lux (cool white fluorescent tube light 40 W GE). *In vitro* regenerated explants were taken out from test tube and washed thoroughly with distilled water and transferred to small pots containing vermicompost. Plants were covered with plastic bags for few days and gradually the plastic bag was removed as the plantlet is acclimatized to *in vivo* conditions were established. Finally the established *in vitro* regenerated plants were transfer to the green house for hardening.

In each experiment, per explants 20 replicates were inoculated and experiment was repeated thrice. The data was collected four weeks after inoculation for the type of response i.e. percentage frequency& plant regeneration. The data from all the replicates was pooled and mean percentage response was calculated. The standard error for the data of each experiment and each parameter were calculated

3- Result & Discussion

A simple one-step method of plantlet regeneration from nodal explants on MS semisolid medium supplemented with different concentrations of IBA has been experimented and reported by the author in this investigation. The regeneration occurred through direct organogenesis without callus formation

Nodal explants were inoculated on MS medium containing IBA (1.0-4.0mg/l) **Table.1.** Axillary bud proliferation was observed within 05 days of inoculation **(Fig.1).**Whereas root initiation was observed on the same medium within 10 days **(Fig. 2).** After 3 weeks regenerated shoots shown fresh growth wit h 2-4 new nodes and 10-15 roots per explants **(Fig.3).** No callus initiation was observed on any of the IBA supplemented media**.** Out of the four concentration 3.0 mg/l IBA shown maximum percentage of Axillary bud proliferation (86.12 %,) and plantlet

regeneration (89.23), whereas higher concentration shown stunted growth. In the present investigation single medium i.e. IBA was used for shoot initiation and plantlet regeneration in *Portulaca grandiflora.* Plant regeneration in single step of Trichosanthes *dioica* was also observed by Komal R. (2011). Whereas Jain & Bashir (2010) regenerated Portulaca *grandiflora* plant by two step method.

Regenerated plantlets were carefully taken out from test tube using a forceps. Gently the agar medium was removed from roots by pouring distilled water under aseptic conditions. If necessary, the regenerated plants were left in water for 30-60 minutes for separation of agar from roots. Initially, plants were kept on filter paper wick at least for one week for hardening and for further growth. Later they were transferred to the plastic cups or root trainers **(Fig.4)** containing mixture of autoclaved soil and manure in the ratio of 1:1. Plants were covered with the polythene bags and kept in the culture room at 25±1ºC under cool white fluorescent light of 2000 Lux. Plants were watered daily with simple sterile tap water. Polythene covers were gradually removed as the plants shown growth. Finally the *in vitro* regenerated plants were transferring to the green house for further hardening. Thousands of plants were successfully transferred to field which showed 90% established in the field conditions.

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Table.1:Effect of IBA (Indole Butyric Acid) on Plantlet regeneration using nodal explant of *Portulaca grandiflora.*

S.No.	Concentrations of IBA(mg/l)	% response ± S.E	
		Shoot initiation	Plantlet regeneration
1	1.0	36.12±0.12	30.2±0.14
2	2.0	66.14±0.13	58.11±0.16
3	3.0	86.12±0.19	79.23±0,22
4	4.0	40.0±0.14	38.14±0.18

Fig.1 Shoot initiation

Fig.2 Root initiation

Fig.3 Complete Plantlet

Fig.4 Regenerated plants were Transferred to small cup

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