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In vitro Micropropagation of Medicinal plant *Portulaca grandiflora*

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ABSTRACT

According to the WHO about 80% population of the world depend on medicinal plants for remedies. Medicinal plants are exploited for therapeutic medicine and have been used by Ayurveda, Unani and Siddha. In Ayurveda, Charak and Sushruta described various plants along with their medicinal values. *Portulaca grandiflora*, is a medicinal ornamental plant in the family Portulacaceae, *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. In the present study micropropagation of *Portulaca grandiflora was* done using nodal explants, inoculated on MS medium containing kinetin (1.0-4.0mg/l). Axillary bud proliferation was observed within 05 days**.** After 3 weeks regenerated shoots shown fresh growth with 3-4 new nodes on each concentration of kinetin supplemented Murashige & Skoog medium. No callus initiation was observed on any of the Kinetin supplemented media**.** Out of the four concentration 2.0 mg/l Kinetin shown maximum percentage of Axillary bud proliferation i.e. 60%, whereas higher concentration of Kinetin shown stunted growth. Regenerated shoots were transferred into Murashige & Skoog media supplemented with 1.0- 4.0mg/Indole Butyric Acid (IBA) for root initiation. Root initiation was observed after 5 days of subculturing. Maximum percentage of root initiation and plantlet regeneration were recorded in 2.0mg/l IBA i.e. 90%, whereas minimum percentage observed on 4.0mg/l i.e 40%. Regenerated Plantlets were transferred to green house and finally to the field.

Key words: Micropropagation, *Portulaca grandiflora*, Regeneration

1. INTRODUCTION

Plants are good source of medicine. According to the WHO about 80% population of the world depend on traditional remedies. Medicinal plants are exploited for therapeutic medicine and have been used by Ayurveda, Unani and Siddha[1]. In Ayurveda, Charak and Sushruta described various plants along with their medicinal values. India exports more than 32,000 tones of medicinal plants raw material of worth US$ 46 millions annually. In India, most of the pharmaceuticals industries draw almost 90% of its supply from wild[2]. Due to this fact a good number of medicinal plants are depleting in the natural habitat. In addition to this, deforestation, rapid agricultural and urban development is responsible for the disappearance of medicinal plants. 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[3,4]. Latha and Seeni discussed about the conservation of medicinal plants and their mass propagation has been achieved through *in vitro* techniques with significance[5].

In vitro propagation of medicinal plant species hold tremendous potential for the production of high quality plant based medicines[6]. The availability of the shoot cultures throughout the year and the circumvention of surface sterilizing procedures with great assurance of non-contaminated cultures make them ideal source for various purposes. Micropropagated plants can be tested or sold with the assurance that they are genetically uniform.

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. 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 species.

2. MATERIALS AND METHODS

In the present study *Portulaca grandiflora* Plants were collected from CIMAP, Lucknow and maintained at botanical garden of Omcar India, Gwalior.

Murashige and Skoog's (HiMedia) was used as basic nutrient medium for the Preparation of MS medium[7]. 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.

For Shoot initiation MS Media supplemented with various concentration of kinetin (1-4mg/l) were used for shoot initiation, as shoot developed regenerated shoots were removed from culture tubes and washed with distilled water carefully then inserted into another fresh MS media containing 1-4mg/l IBA (indole butyric acid) for root initiation. 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 & number of plantlets per explant. 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. RESULTS AND DISCUSSION

In the present study Nodal explants were inoculated on MS medium containing kinetin (1.0-4.0mg/l) (Table 1). Axillary bud proliferation was observed within 05 days (Fig 1). After 3 weeks regenerated shoots shown fresh growth with 2-4 new nodes. No callus initiation was observed on any of the Kn supplemented media (Fig 1). Out of the four concentration 2.0 mg/l Kn shown maximum percentage of Axillary bud proliferation i.e. 60%, whereas higher concentration shown stunted growth. Regenerated shoots were transferred into MS media supplemented with 1.0-4.0mg/IBA for root initiation. Root initiation was observed after 5 days of subculturing (Fig 2). Maximum percentage of root initiation and plantlet regeneration were recorded in 2.0mg/l IBA i.e. 90%, whereas minimum percentage observed on 4.0mg/i.e 40% (Table 2).

Fig.1 Shoot initiation in kinetin supplemented MS Medium

Fig 2. Root initiation and plantlet regeneration in IBA Supplemented MS Medium

Fig 3. Regenerated plants were transferred to small cup

Table 1: Effect of Kinetin on multiple shoot bud initiation and using nodal explant of *Portulaca grandiflora.*

S.No.	Concentrations of Kn (mg/l)	% response ± S.E	
		Shoot initiation	No. of shoots per explant
1	1.0	38.12±0.14	5.0±0.19
2	2.0	60.14±0.23	15±0.24
3	3.0	56.12±0.44	9±0,22
4	4.0	40.0±0.22	4±0.18

Table.2: Effect of IBA on Plantlet regeneration in *Portulaca grandiflora*

S.No.	Concentrations of IBA (mg/l)	% response ± S.E	
		Plantlet Regeneration	No. of Roots per Shoot
1	1.0	60.22±0.14	15.0±0.13
2	2.0	90.27±0.28	18±0.14
3	3.0	76.16±0.31	9±0,33
4	4.0	40.20±0.22	4±0.14

In the present investigation cytokinins (KIN) was used in 1.0-4.0mg/l for in *vitro* multiple shoot initiation and plantlet regeneration in *Portulaca grandiflora.* In general, bud break and development of shoots from stem node explants is a function of cytokinin activity[8]. In the present study axillary shoot buds were formed under the influence of Kinetin. Similar results have been reported in *Bacopa monnieri* and Portulaca grandiflora plants[9,10]. In the present investigation root initiation was observed in lower concentration of IBA and similar result were observed[11].

When regenerated plantlets were of 2-4 cm long, they 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.3) 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 100% established in the field conditions.

4. CONCLUSION

Plants are very good source of medicine. Medicinal plants are used by all system of medicine like ayurveda, Unani and Siddha. *Portulaca grandiflora* is a medicinal ornamental plant and it is used for the treatment of various diseases. Due to its medicinal values its demand in pharmaceutical industries is increasing. Tissue culture play a vital role in the fast multiplication of plant. Therefore in the present study we developed a short and easy method for fast multiplication of this medicinal plant.

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