RESEARCH ARTICLE

Effect of growth regulator combination on *in-vitro* regeneration of *Catharanthus roseus*

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ABSTRACT

Catharanthus roseus has high medicinal value and it is used as folk medicine for various ailments such as diabetes and high blood pressure. More over this plant also possesses anticancer and pain relieving properties and therefore it is used in pharmaceutical industries. Considering the medicinal value, it was proposed to manipulate this plant in tissue culture for micropropagation. Field grown plants were used as source of explants. Juvenile explants such as shoot tip and nodal sections were excised and used for regeneration. The explants were surface sterilized using various disinfectant like alcohol, Bavistin and sodium hypochloride at appropriate concentration. The explants were cut into desirable size and inoculated into regeneration medium consisting of Murashige and Skoog basal combination and various combination of phytohormone. The cultures were incubated at 16:8 hrs photoperiod and temperature of 27 + 20c. It has been observed that both the explants responded and initiate to produce multiple shoots in growth regulator combinations of BAP 2 mg/L and kinetin 1 mg/L. Callus induction was obtained from nodal section which were inoculated in medium containing MS + kinetin (1 mg/L) and NAA (2 mg/L) respectively. In all, 57 % of shoot tips were responded on medium containing BAP and kinetin combinations and in case of nodal explants 66 % explants were responded in MS medium supplemented with NAA and kinetin.

Keywords: Regeneration, Multiple shoot induction, In-vitro MS, *Catharanthus roseus*.

INTRODUCTION

Medicinal plants are of great interest in field of biotechnology since most of drug industries depend on it for production of pharmaceutical products. Human beings have exploited the plants for curing aliments since antiquity. Traditional system of medicine like. Ayurveda, Unani, Homeopathy & Siddha solely rely on phyto-pharmaceutical, that are obtained from selected medicinal plant (herb) based on traditional knowledge gained over a period of time & expertise by traditional healers. As the use of plant as medicines has a long history in the treatment of various diseases. *Catharanthus roseus* has been

used in folk medicine to treat diabetes & high blood pressure. As antidiabetic remedy it was believed to promote insulin production & increase utilization of sugar from food. However, in modern medicine alkaloids & chemo-therapeutic agent from *C. roseus* are known for anticancer pain relieving & properties. Main chemical components are ajmaline catharanthaine, leurosidine, vincristine, vinblastine, Vinorelbine, vindesine, vincamine. Vinca alkaloids are antimitotic & antimicrotubule agent. They are now produced synthetically are used as drugs in cancer therapy and as immunosuppressive drugs. These compounds are vinblastine vincrstine and vinorelbine Ross (2003). The tissue culture provides an affordable alternative for propogation of elite and rare material. An efficient and reproducible protocol is important for development of regeneration protocol. Keeping in view all fact, an experiment was designed to study the effect of different hormone combination on explants Moreno et al. (1995).

MATERIALS AND METHOD

Juvenile shoots from disease free , young and healthy catharanthus plant were obtained from mature plants of *Catharanthus roseus* growing the roadside and Garden's Shoot tip and nodal section were used as basic plant material for in-vitro regeneration studies. Explants were washed properly under running tap water to remove the dust contaminants. Surface sterilization was carried out by washing explants with liquid detergent for 10-20 minutes then they were washed under tap water to remove detergent traces. The explants were treated with 1% bavistin (antifungal agent) for 15-20 mins. Bavistin was then decanted and explants were washed with autoclaved doubled distilled water to remove traces of Bavistin. Explants were then treated with 0.1% sodium hypochlorite for 5-7 min with gentle shaking. After this Explants were removed from conical flask and placed in sterile Petri plate with tissue paper. The tissue paper will soak the water retain on the explants. The working MS basal medium was prepared by adding stock solution perform dilution according to requirement. Glucose & vitamin powder dissolved in sterile distilled water & added to the medium solution. For the shoot induction and callus induction media combinations BAP + Kinetin & NAA + Kinetin were used.

RESULT AND DISCUSSION

In-vitro shoot Induction from shoot tip explants

In this study 450 shoot tip explants were inoculated on medium which contain MS Basal Salt's along with 30 gm/L Glucose, 100 mg/l Inositol, 10 mg/L thiamine, 7gm/L Agar with added different combination and concentration of BAP and kinetin. In the medium containing MS +BAP (2mg/L)+ Kin (1mg/L) 246 shoot tip explants responded to multiple shoot induction. During initial period, 2-3 shoots per explants were observed however after 25-30 days of culture mass of multiple shoot bud developed in the form of cluster. The highest regeneration percentage was observed in the 8th lot in which out of 45 shoot tip explants 36 explants responded and the lowest regeneration percentage was observed in 3rd lot in which out of 45 shoot tip explants were 9 shoot tip explants responded to regeneration. Therefore the average regeneration percentage was recorded 54.57%.

Table1: *In-vitro* Multiple shoot induction from shoot tip of *Catharanthus roseus* in BAP (2mg/L)+ Kinetin (1mg/L) Media Combination

Lot Number	No. of Explant inoculated	No. of Explant responded	Regeneration percentage (%)
Lot no.1	45	35	77.7%
Lot no.2	45	32	71.4%
Lot no.3	45	9	20%
Lot no.4	45	9	20%
Lot no.5	45	23	50%
Lot no.6	45	30	66.6%
Lot no.7	45	11	25%
Lot no.8	45	36	80%
Lot no.9	45	27	60%
Lot no.10	45	34	75%
TOTAL	**450**	**246**	**54.57%**

Table 2: Callus induction from nodal section of *Catharanthus roseus* in BAP (1mg/L) + NAA (2mg/L) media combination.

LOT NUMBER	No.of Explant inoculated	No. of Explant responded	Regeneration percentage (%)
Lot no.1	10	5	50%
Lot no.2	10	10	100%
Lot no.3	10	3	33.3%
Lot no.4	10	5	50%
Lot no.5	10	10	100%
Lot no.6	10	10	100%
TOTAL	**60**	**43**	**71.66%**

Fig. 1: In-vitro regeneration of *Catharanthus roseus*

In-vitro regeneration of *Catharanthus roseus*

At initial stage, less number of multiple shoots were observed which was green in colour. After 25-30 days mass of multiple shoots developed and callusing at the bottom of the shoot tips explant was observed.

Induction of callus from nodal section of *Catharanthus roseus*

The nodal section was collected from field. The sterilized nodal sections were inoculated on MS media containing BAP and NAA. In all total 60 explant were inoculated in BAP (1mg/L)+NAA (2mg/L) media out of which 43 explants were responded to callusing. The callus proliferation initiated within 15-20 days. At initial stage, pale yellow coloured callus was observed which turned brown coloured within 15-20 days. (Table2).

DISCUSSION

Plant material or Explant of *Catharanthus roseus* were surface sterilized by using liquid detergent, Bavistin, Sodium hypochlorite and sterile distilled water surface sterilized explants was then inoculated on suitable MS Medium supplemented with different vitamins and harmones. In the present study, different explants were screened for their regeneration potentiality. Shoot tip explant and nodal segment were used for induction of multiple shoots and callus induction.

In the present study Ms medium i.e. Murashige and skoog (1962) basal salts supplemented with 30 gm/l glucose, 100mg/L inositol, 10 mg/L thiamine and different concentration and combination of phytohormones like BAP (Benzyl-aminopurine), kinetin, NAA (Naphthalene acetic acid) were used. H.S. Taha et al. (2008) describes a simple, efficient protocol for *in -vitro* study for calli production, Direct shootlets regeneration & alkaloids determination on *Catharanthus roseus*, Calli were produced from shoot tip, leaf, stem & root explant on M.S. Media supplemented with 2-4D & Kin; NAA & BAP to study their effect on enhancement Calli production or direct shootlet regeneration. shoot tip explant have highest value of shootlet regeneration on modified MS media with 1 mg / l each of NAA & BAP than other explant. The highest value of total alkaloid was resulted from shoot tip derived direct shootlet regeneration and root derived from calli culture. In the present investigation multiple shoots were induced by using shoot tips. Multiple shoot induction was favorable that is maximum number of multiple shoots were observed when MS medium supplement with BAP (2mg/L)+Kin (1mg/L). Tanveer *et al* (2011*)* reported the use of different concentration of BAP, NAA and IBA for multiple shoot induction for *Catharanthus roseus*, of the different concentration BAP (0.5mg/L)+NAA (1.0mg/L) concentration proved to be optimal for production of maximum number of shoots. In the present study with multiple shoot induction, callusing at the bottom of shoots was also observed after 25-30 days. In present investigation callus was induced by using nodal segment. The callus induction was favourable when Ms medium supplemented with BAP (1mg/L)+NAA (2mg/L). In initial period pale yellow coloured callus turned brown within 15-20 days.

The protocol described in the present study can be directly used of large scale multiplication of *Catharanthus roseus*. All progeny will be true & to the type and virus free because shoot tip culture in tissue culture technique give rise to virus free & disease free progeny.

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