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IN VITRO PROPAGATION OF *GERBERA JAMESONII* ADLAM

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Gerbera jamesonii Adlam is an ornamental plant of Asteraceae. It is an herbaceous perennial, native of South Africa and is popularly known as "Barberton daisy" or "Transvaal daisy". It has a short flowering season but the market demand is heavy. Hence, in the present study *in vitro* culture trials of this herb were carried out using stem tip explants on MS medium supplemented with different concentrations of auxins and cytokinins. The stem tip explants cultured on MS medium without auxin and cytokinin did not show any callus formation within 12 days of inoculation. MS medium fortified with IAA (2 mg/l) +BAP (10 mg/l) developed six shoots whereas the other cultures with Kn developed only four shoots. Micro shoots on MS medium amended with IAA developed more roots as compare to IBA after four weeks of inoculation and incubation.

Keywords: Callus; *Gerbera;* Growth regulators.

Introduction

The present study on *Gerbera jamesonii* Adlam was taken to standardize a protocol for indirect regeneration of plantlets using stem tip explants, since in the conventional methods it can be cultivated only in two months of the year (June-July)[1,2]. It has been reported that shoot organogenesis via callus culture can be used as an effective method for multiplication of medicinal plants[3-5]. Domeg[6] reported micropropagation of *Melia azadirachta* L. through shoot tip culture. Gurumurti and Jagadees[7] have successfully and widely investigated the microprogation from similar shoot tip explants in *Azadirachta indica.*

Gerbera jamesonii Adlam is grown in gardens throught the world. It is one of the most popular ornamental plant in the world, both as a cut flower and as a pot plant, and therefore is of considerable economic importance. Understandably, several attempts were made to regenerate it *in vitro* because plant tissue culture offers several advantages over conventional methods. The storage of *in vitro* rooted plants could enable better exploitation of equipment and labour in laboratories, and therefore improve their economical results.

Material and Methods

In vitro studies on *Gerbera jamesonii* Adlam were carried out using stem tip explants. The plants were collected from the garden of science form at Noonankuppam in their natural surroundings. The stem tips from a healthy plant were excised under water and then they were surface sterilized by constant agitation for 7 min in 0.1% $HgCl_2$ solution followed sterilized distilled water. These surface sterilized stem by rinsing with tips were transferred to commercially available Murashige and Skoog medium[8] supplemented with various concentrations of Auxins, Indole acetic acid (IAA), Indole butryic acid, Cytokinin, 6 benzyl aminopurine (BAP) and Kinetin (Kn). The pH was adjusted to 5.8 and 0.8% (w/v) agar was added as solidifying agent. Explants were placed on MS medium under perfectly sterilized conditions and were incubated at a temperature of 25±2°C and at a relative humidity of 65-70 percent. The cultures were kept under white light at an intensity of 2000 Lux (at the level of culture tubes), provided from white fluorescent lamps (Philips, India) with 12 hours photoperiodic duration.

Results and Discussion

The stem tip explants of *G jamesonii* is processed and inoculated into the culture tubes containing MS medium fortified with or without auxins and cytokinins. The inoculated culture tubes were transferred to growth chambers provided with required light. The inoculated stem tip explants cultured on MS medium with auxin and cytokinin showed the growth of callus after 12 days of inoculation. The stem tip explants on MS medium without auxin and cytokinin did not show any callus formation within 12 days suggesting importance of growth regulator in the induction of callus. The callus further turned into shoot buds after 3 weeks of inoculation. The number of

Table 1. Effect of IAA and BAP on the production of callus and shoot development from shoot tips of *Gerbera jamesonii* Adlam on MS medium.

Sr. No.	IAA (mg/l)	BAP (mg/l)	Number of shoots (After 3 weeks)
1	0.0	0.0	0
2	0.5	1.0	1
3	1.0	2.0	3
4	1.5	5.0	4
5	2.0	10.0	6
6	2.5	15.0	2

Table 2. Effect of IAA and Kn on the production of callus and shoot development from shoot tips of *Gerbera jamesonii* Adlam on MS medium.

Sr. No.	IAA (mg/l)	BAP (mg/l)	Number of shoots (After 3 weeks)
1	0.0	0.0	0
2	0.5	1.0	1
3	1.0	2.0	2
4	1.5	5.0	3
5	2.0	10.0	4
6	2.5	15.0	2

Table 3. Induction of rooting in micro shoots of *Gerbera jamesonii* Adlam in vitro using IAA and IBA on MS medium after 1 week.

Sr. No.	IAA (mg/l)	Number of Roots	IBA (mg/l)	Number of Roots
1	0.0	-	0.0	-
2	0.5	2	0.5	1
3	1.0	5	1.0	2
4	1.5	3	1.5	1
5	2.0	2	2.0	2

IBA- Indole butyric acid

shoot buds differs with the amount of IAA and cytokinin in the MS medium. MS medium supplemented with IAA+BAP showed more buds compared to the other cultures where MS medium fortified with IAA+Kn (Table-1&2). MS medium fortified with IAA (2 mg/l)+BAP (10 mg/l) developed six shoots whereas the other culture with Kn developed only four shoots. Increasing the level of BAP up to 10 mg/l to the MS medium containing IAA showed increased production of shoots but beyond that level the number of shoots decreased with increased concentration of BAP.

The shoot buds from the primary cultures were transferred to rooting medium in the laminar airflow chamber at most care. The rooting medium (MS) is fortified only with auxins (IAA/IBA) and no cytokinin. Since auxins are necessary for the induction of root, four concentration of IAA/IBA is tried individually. Micro shoots from MS medium amended with IAA developed more roots compared to the other cultures with IBA after four weeks of inoculation and incubation. The number of roots were five in cultures containing MS medium fortified with IAA (1 mg/l) and were only two in other cultures amended with IBA (1 mg/l) (Table -3). The plantlets developed after 4-5 weeks of inoculation were transferred to polystyrene cups containing a mixture of vermiculite and sand after washing them carefully to remove adhering agar. The plantlets were subjected to hardening in the growth chamber. After 10 days of hardening plantlets were transferred to earthenware pots containing garden soil. After 2 weeks of such hardening treatment the plants become fully acclimatized to the prevailing day and night condition and they were healthy.

The present study on *G. jamesonii* was taken to standardize a protocol for indirect regeneration of plantlets using stem tip explants.

In Aristalochiaceae, *in vitro* differentiation of shoots as well as plantlets have been reported from a variety of explants such as leaves[9], stem[10], nodal segments[9] and shoot tip[9]. *Solanum trilobatum* was tested for its morphogenetic potential through 3 different explant viz., axillary buds, shoot tips and internodal segments cultured on MS medium supplemented with auxins and cytokinins[12]. Plant propagation by tissue culture is usually aimed at the possibly highest multiplication rate[13] without vitrification and mutation. In order to obtain high multiplication rates, relatively large amounts of cytokinins are used in multiplication. For some plants the multiplication rate decrease with increasing the concentration of cytokinin in the medium[14]. In the micropropagation of numerous plants, BA is much more effective than Kinetin.

Hormonal interactions between auxins and cytokinins are primary mechanism of apical dominance[16], and exogenous applications of cytokinin can stimulate lateral bud growth[17]. Application of synthetic cytokinin, BA, can induce the outgrowth of lateral buds in hosta[18], and effects formed from BA- induced buds can be removed from the mother plant within 30 days of BA application and rooted under intermittent mist[19]. These findings suggest that BA might facilitate conventional propagation methods by increasing the number of offsets available.

The present study revealed the same where the cultures were grown on MS medium amended with auxins and cytokinins showed more multiplication of shoots from callus. The highest multiplications of shoot buds were seen in culture of MS medium fortified with IAA (2 mg/l) and BAP (10 mg/l). Viswanath and Jayanthi[20] observed an average of 4 shoots per culture of *Rauvolfia micrantha* and *R. tetraphylla* within 15 days of incubation on MS medium supplemented with BA (10 mg/l).

Generally, micro shoots require an auxin to develop roots. In *G. jamesonii,* rooting was best achieved on MS medium supplemented with IAA (2.0 mg/l) though both IAA and IBA were tried. Viswanath and Jayanthi[20] reported the improved frequency of rooting in both the cultures of *R. micrantha* and *R. tetraphylla* on MS medium supplemented with IBA (2.0 mg/l).

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