International Journal of Medicinal Plant Research ISSN: 2169-303X Vol. 2 (4), pp. 187-189, April, 2013. Available online at © http://internationalscholarsjournals.org International Scholars Journals

Full Length Research Paper

Effect of growth regulators on the *in vitro* multiplication of *Viola odorata*

Zahoor. A. Kaloo*, Rukhsana Akhtar, Zafrul-Haq and B. A. Wafai

Department of Botany University of Kashmir, India.

Accepted 23 September, 2012

The present work as undertaken to study the effect of auxins and Cytokinins on the *in vitro* multiplication of nodal cuttings with axillary buds and runner tips with apical buds of *Viola odorata* a medicinal herb growing in Kashmir and western Himalayas. The growth medium used was MS (1962) basal medium supplemented with BAP, Kn and NAA at varying concentrations. The multiplication rate of shoots increased with increasing the concentration of BAP and Kn. However the optimum results were obtained on MS medium supplemented with a combination of 15 µm NAA and 10 µm BAP. Nodal segments showed better results than runner tips.

Key words: Nodal segment, benzylaminopurine, naptheleaciticacid, kinetin, multiplication, *viola odorata.*

INTRODUCTION

Viola odorata Linn (sweet violet) belongs to family *violaceae*, is an evergreen perennial herb growing about 10 cm tall. It flowers in late winter. The flowers are nodding, deep violet and sweet scented. It is distributed in Kashmir and western Himalayan regions at an altitude of 1500 to 1800 m asl, The herb is well known for its Pharmaceutical importance in Ayurvedic and Unani medicinal system. It is used for treatment of whooping cough. Its drug is also anti-inflammatory, diaphoretic, diuretic, emollient, expectorant, antipyretic and laxative. It contains salicylic acid which is used to make aspirin hence effective for the treatment of headaches, migraine and visominia. The roots of the plant yield an alkaloid violin which is used as an expectorant. There is a general felling that the populations of *V. odorata* are decreasing at an alarming rate. The plants of this genus are known to hybridize at intra- and inter-specific levels very freely in nature. Therefore, taxonomically it has become very difficult to distinguish between the different species, with the result that the drug is highly adulterated with other congeners viz. *Viola biflora*, *V. cinerea*, *V. pilosa*, *V. cauescens* and *V. sylvestris*. This is why the efficacy of the drug claimed so far is now being questioned.

It is with this backdrop that the genuine plants of *V. odorata* need to be conserved and plant tissue culture techniques provide the most efficient and reliable method for multiplication and conservation of such plants.

MATERIALS AND METHODS

The explants used in the present study were (auxiliary buds and apical buds) as these preformed buds are the potential source for the large scale clonal propagation of the plants. These were excised from authentic plants of *V. odorata* washed first under tape water with extron detergent and then with double distilled water. These explants were disinfected by 0.1% $HgCl_2$ solution for 4 to 5 min and then thoroughly washed with autoclaved double distilled water under laminar airflow. These were then cut into 2 to 3 cm long segments and inoculated on sterilized culture medium in suitable culture vials.

The culture medium used was MS basal supplemented with different concentrations and combinations of auxins and cytokinins. The pH of the medium was adjusted to 5.8 with 0.1 N NaOH / HCl. The media were sterilized by autoclaving at 15lbs pressure and 121°C for 15 min. After inoculation the cultures were maintained under controlled conditions (16/8 h photoperiod, 24 ± 2°C temperature, light intensity of 500lx and relative humidity of 80%).

*Corresponding author. E-mail: zakallu@yahoo.com.

Abbrevations: MS; Murashige and skoog medium, **BAP;** benzyle aminopurine, **Kn;** Kinetin, **NAA;** napthalene acetic acid.

Table 1. Effect of different growth regulators on the multiplication of axillary buds of *V. odorata.*

Growth medium	%age of buds sprouted	Shoot length (mm)±SD
MS basal	0	0.00
MS+BAP (µM)		
5	30	5.00 ± 0.3
10	40	10.00 ± 1.7
15	45	20.0 ± 1.3
20	40	15.0 ± 1.2
MS + Kn (µM)		
5	30	10.00 ± 1.4
10	35	13.25 ± 1.7
15	40	15.00 ± 2.2
20	35	10.00 ± 1.6
MS+NAA+BAP(µM)		
5 +10	60	15.00 ± 3.2
10 + 10	70	20.00 ± 2.8
15 +10	90	25.00 ± 2.8
20 + 10	80	15.00 ± 2.7

LSD (P=0.05%) 5.71965; data scored at the end of 4 ± 1 weeks: 10 replicates per treatment.

Figure 1. *V. odorata* plant.

Figure 2a. Multiplication of auxillary buds of *V. odorata* on 15µM NAA and 10µM BAP.

Figure 2b. Control.

RESULTS

Micro-propagation protocols have been developed for several important medicinal plants. *Viola. odorata* (Figure .1) which is of tremendous medicinal importance can be propagated by tissue culture under the influence of various growth regulators. The results included in this paper depict the influence of auxins, cytokinins and their combinations on the multiplication of shoot tips and axillary buds. The nodal segments with axillary buds were taken as explants from juvenile runners and inoculated on MS medium supplemented with various concentrations of BAP and Kinetin. These hormones were found effective in inducing the sprouting of the buds at a concentration of 5 µM. The percentage sprouting increased with increasing the concentration of the hormones and the optimum results were obtained at 15 µM BAP and 15 µM Kinetin. However, when the nodal cuttings were treated with various combinations of NAA and BAP the results improved with maximum sprouting on 15 µM NAA and 10 µM BAP in 90% cultures .The optimum length of the *in vitro* raised shoots (25.00 ± 2.8 mm)was also achieved at this combination (Table 1, Figure 2a and b). The rate of shoot multiplication was also studied in case of runner

Figure 3a. Multiplication of runner tips of *V. odorata* on NAA+BAP 15µM each

Figure 3b. Control.

tips which showed less multiplication rate than axillary buds. With increasing concentration of BAP and Kn the multiplication rate enhanced with optimum results at 20 µM BAP and 20 µM Kinetin respectively for the two growth regulators. The combined effect of NAA and BAP was also observed in case of runner tips. The multiplication rate of shoots increased with increase in concentration of NAA and BAP with optimum results at 10 µM NAA and 15 µM BAP. In this combination about 80% buds sprouted which grew to an average shoot length of 15 mm on the same medium (Table 2 and Figure 3a and b).

DISCUSSION

The present *in vitro* studies on *V. odorata* were carried out to identify the factors which are responsible for *in vitro* micropropagation of this medicinally important plant which is over exploited for medicinal purposes. The axillary buds and the runner tips (apical buds) when subjected to *in vitro* studies did not produce any morphogenetic response on MS basal medium without any growth regulator thus signifying the importance of growth inducers which vary from species to species and explant to explant. During the present investigation it was established that the cytokinins (BAP and Kn) were effective in inducing the sprouting of the preformed buds (apical as well as leaf axillary) and their subsequent elongation into a shoot. Similar results have been achieved in *Cinnamomum camphor* (Babu et al., 2003), *Dioscorea zingiberensis* (Chen et al., 2003), *Ceropegia candelabrum* (Beena et al,, 2003), *Rotula aquatic* (Martin, 2003), Cedrela fissilis (Nunes et at., 2002). *Dioscorea deltoidea* (Kaloo and Shah,1998) thus highlighting the importance of cytokinins in the induction of shoot regeneration from buds which otherwise remain dormant under natural conditions of growth. Similarly the combined effect of NAA and BAP on multiplication of shoots has also been earlier reported in *Inula recemosa*

Table 2. Effect of different growth regulators on the multiplication of apical shoot of *V. odorata.*

Growth medium	age of Buds sprouted (%)	Shoot length (mm)±SD
MS+BAP (µM)		
10	20	7.00 ± 1.5
15	15	15.00 ± 1.8
20	30	21.00 ± 1.9
25	25	15.00 ± 1.3
MS+Kn(µM)		
10	15	10.00 ± 2.1
15	20	15.00 ± 2.1
20	30	20.00 ± 2.5
20	20	17.00 ± 1.7
MS+NAA+BAP(µM)		
5 + 15	50	10.00 ± 1.3
10 + 15	80	15.00 ± 1.8
15 + 15	75	17.00 ± 2.2
20 + 15	70	13.00 ± 1.7

LSD (P=0.05%) 4.25142; data scored at the end of 4 ± 1 week: 10 replicates per treatment.

(Kaloo and Shah.1997), *Piper longum* (Sonia and Das, 2002). Thus suggesting a viable system of shoot/ plant production which can be utilized for the micropropagation of the plant in question.

Conclusion

The effect of cytokinins (BAP and Kn) on the micro-propagation of *V. odorata* was studied and it was concluded that these hormones were more effective, when used in combination with auxins, in increasing the rate of shoot multiplication.

REFRENCES

Babu KN, Sajina A, Minoo D, John CZ, Mini PM, Tashar KV, Rema J, Ravindra PN (2003). Micro propagation of Camphor tree. Plant Cell, Tissue Organ Cult., 74(2): 179-183.

Beena MR, Martin KP, Kirti BP, Molly H (2003). Rapid *in vitro* propagation of medically important *Ceropegia candelabrum.* Plant Cell, Tissue Organ Cult., 72(3): 285-289.

Chen Y, Fau J, Yi F, Luo Z, Fu Y (2003). Rapid clonal propagation of *Dioscorea zingiberensis,* Plant Cell, Tissue Organ Cult., 73(1): 75-80.

Kaloo ZA, Shah AM (1997). Plant regeneration from shoot apical tips of *Inula racemosa* threatened medicinal plant species. Oriental Sci., 2(1): 17-22.

Kaloo ZA, Shah AM (1998). *In vitro* propagation of *Dioscorea deltoidea* –a rare plant species of medicinal value. Oriental Sci., 3(2): 7-12.

Martin KP (2003). Rapid *in vitro* multiplication anr *ex vitro* rooting of *Rotula aquatic* Lour., a rere rhoeophytic woody medicinal plant. Plant Cell Rep., 21(5): 415-420.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco culture. Physiol Plant, 15: 473-479.

Nunes E, da Costa V, de Castilho C, Netto MF, Maria VA (2002). *In vitro* culture of *Cesdrela fissilis vellozo*(Melaeeae). Plant Cell, Tissue Organ Cult., 70(3): 301-309.

Sonia EV, Das MR (2002). *In vitro* micropropagation of Piper longum-an important medicinal plant. Plant Cell Tissue Organ Cult., 70(3): 325-327.