

EFFECT OF AMLA JUICE (*EMBLICA OFFICINALIS*) ON IN VITRO SHOOT INDUCTION OF *VIOLA ODORATA* L.

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Abstract

The present work was undertaken to study the effect of Amla juice (*Emblica officinalis*) with combination of BAP and Adenine sulphate on *in vitro* shoot induction of medicinally important plant *Viola odorata* L. by using apical bud and axillary buds as an explant. *Viola odorata* L. is known for pharmaceutical importance in Unani and Ayurvedic medicinal system. MS (Murashige & Skoog 1962) medium was supplemented with different combination of BAP, amla juice and Adenine sulphate. *E. officinalis* contains higher amount of vitamin C and contains nicotinic acid, iron, minerals, protein and amino acids like glutamic acid, proline, cysteine. Number of shoot and number of shoot length (cm) were recorded after 40 days of inoculation. BAP with amla juice was found effective in shoot induction. Maximum number of shoots (11) were recorded at MS medium supplemented with 2.5mg/l BAP+30mg adenine sulphate+2ml amla juice/l and maximum shoot length (5.38) also recorded at same concentration.

Keywords: *Viola odorata* L., Shoot induction, In vitro, Amla juice

1. Introduction

Viola odorata L. belongs to family Violaceae. It is commonly known as sweet violet in English and in Indo Pakistan called as Banafsha. *Viola odorata* is native to North Africa, Europe and Asia. In Pakistan, it is found in Kaghan, Swat, Hazara, Nathiagali, and Chitral at the height of 1500- 2000 meters (Siddiqi, *et. al.* 2012). *Viola odorata* L. is pubescent herb about 15cm in height. Leaves are broadly, green, tough, cordate in shape. Leaves are arranged in rosette at the base of plant. Flower are solitary, axillary, violet coloured sweet scented (Salve, *et. al.* 2014). *Viola odorata* L. contains different compounds such as Vitamin C, Flavonoids, coumarins, mucilage, methyl salicylate, glycosides, saponins, alkaloids, tannins. It is effective in throat and chest discomfort, sore throat, dyspnea, pneumonia, whooping cough and acute bronchitis (Tripathi, *et. al.* 2015). *Viola odorata* L. is known for its Pharmaceutical importance in Unani and Ayurvedic medicinal system. Its drug is also anti-

inflammatory, expectorant, emollient, antipyretic, expectorant and laxative. It contains salicylic acid which is used to make aspirin hence effective for the treatment of headaches, migraine (Kaloo *et. al.* 2013).

E. officinalis has been reported that fruits of *E. officinalis* contains higher amount of **vitamin C** and contain minerals, protein and amino acids like glutamic acid, proline, cystanin. *E. officinalis* is considered to be a powerful rasayana (rejuvenator) and to be useful in delaying the degenerative as well as a senescence process. (Bhandari & Kamdod, 2012). Vitamin C, commonly known as L-ascorbic acid (LAA) has been proven to be highly beneficial. It is established that Vitamin C, is an abundant antioxidant in plants. In *in vitro* cultures LAA primarily used as an antioxidant, to prevent browning of tissues (Horton, 2006).

2. Material and Method

2.1 Plant material

The plant *Viola odorata* L. was collected from Sanjeevinivatika of Department of Horticulture, UAS, GKVK, University of Agriculture Science Bangalore - 65, India.

2.2 Explants preparation

In the present study apical bud is used as explant. Apical bud is source for the large scale clonal propagation of the plants. The excised apical buds were washed in tap water with laboline for 15 minutes then explants were sterilized by 70% alcohol then treated with 0.1% HgCl₂ for 1 min followed by several time washing with DDW under aseptic condition.

2.3 Media Preparation

For inoculation of explants the MS media (Murashige & Skoog 1962) was supplemented with cytokinin (6-Benzylaminopurine), adenine sulphate and amla juice (Amla fruits were harvested from field. fruits were first washed with tap water after Double distilled water. later juice was extracted from the fruits using grinder further extract was filtered using muslin cloth and extract was stored in refrigerator.) . pH of the medium was adjusted at 5.8 and prior to autoclaving, added 0.6% agar agar for solidification (Himedia, Mumbai), then autoclaving at 15psi for 30mins of MS media were carried out.

2.4 Inoculation

After complete preparation of MS media apical buds were cut with the help of sterilized scissor were inoculated on MS medium with the help of forceps in the laminar air flow chamber.

2.5 Culture Conditions

The cultures were incubated under at $25 \pm 2^{\circ}\text{C}$ & light (16 hours light and 8 hours dark). Details regarding quantity of callus color, type and number of days to callus formation were observed and results were recorded.

2.6 Experimental Design

Completely Randomized Design was used (Five replication)

2.7 Observation Recorded

Number of shoot and Shoot length were recorded after 45 days.

3. Results

In vitro protocol for shoot induction have been developed for different medicinal plants. one of them *Viola odorata* L. which has tremendous medicinal properties can be propagated through tissue culture by using different auxins and cytokinins. The results included in this paper depicts the influence of different combinations of BAP, adenine sulphate and amla juice on multiplication of apical bud and axillary bud. The nodal segments with apical buds and axillary bud were taken as explants from juvenile runners and inoculated on MS medium supplemented with different concentration of BAP, adenine sulphate and amla juice. Number of shoot and number of shoot length(cm) were recorded after 40 days of inoculation. BAP with amla juice was found effective in shoot induction. Maximum number of shoots (11) were recorded at MS medium supplemented with 2.5mg/l BAP+30mg adenine sulphate+2ml amla juice/l and maximum shoot length (5.38) also recorded at same concentration(Fig. a). 2.5ml/l amla juice was found effective for no. of shoot and shoot length when applied with 1.5mg/l BAP+30mg adenine sulphate as compare to (0.5, 1, 1.5, 2ml/l amla juice)(Table.1)(Fig. b)

Table 1. Effect of different concentration of BAP, Adenine sulphate and amla juice.

Treatments	No. of Shoot	Shoot length(cm)
T ₁ : 1.5 mg/l BAP +30mg adenine sulphate+0.5ml amla juice	3.4	3
T ₂ : 1.5 mg/l BAP+30mg adenine sulphate+1ml amla juice	3.8	3.74
T ₃ : 1.5 mg/l BAP+30mg adenine sulphate+1.5ml amla juice	3.8	3.1
T ₄ : 1.5 mg/l BAP+30mg adenine sulphate+2ml amla juice	4.4	3.62
T ₅ : 1.5 mg/l BAP+30mg adenine sulphate+2.5ml amla juice	5	4.46
T ₆ : 2.5 mg/l BAP+30mg adenine sulphate+0.5ml amla juice	4.4	3.42
T ₇ : 2.5 mg/l BAP+30mg adenine sulphate+1ml amla juice	5.2	3.68
T ₈ : 2.5 mg/l BAP+30mg adenine sulphate+1.5ml amla juice	6	3.7
T ₉ : 2.5 mg/l BAP+30mg adenine sulphate+2ml amla juice	11	5.38
T ₁₀ : 2.5mg/l BAP+30mg adenine sulphate+2.5ml amla juice	7.2	4.88
SE(m)	0.7170	0.2435



Fig. (a) Shoot induction on 2.5mg/l BAP+30mg Adenine sulphate+2ml/l Amla juice



Fig. (b) Shoot induction on 1.5mg/l BAP+30mg Adenine sulphate+2.5ml/l Amla juice

Discussion

The present in vitro studies of medicinal important plant *Viola odorata* L. were carried to identify role of amla juice on in vitro induction of shoot. Amla juice was extracted from amla fruit. Amla fruit contain vitamin C 6mg/g, Nicotinic acid 0.2μg/g Iron 12μg/g

phosphorous 0.02%, calcium 0.05%, mineral matter 0.7%, carbohydrates 14.1%. (Dasaroju & Gottumukkala, 2014) Several reports suggest that role of vitamin C in cell division and differentiation. (Arrigoni 1994, Edgar 1970). High concentration of ascorbate in meristems and its involvement in cell division has suggested for plant cells. (Arrigoni *et al.* 1997). (Kaloo *et al.*, 2013) reported when MS medium supplemented with 15 μ M BAP maximum shoot length 20.0 ± 1.3 (mm) was recorded in *Viola odorata* L. In present study maximum number of shoots (11) were recorded at MS medium supplemented with 2.5 mg/l BAP + 30 mg adenine sulphate + 2 ml amla juice/l and maximum shoot length (5.38) also recorded at same concentration.

Conclusion

The effect of amla juice with combination of BAP and adenine sulphate on shoot induction of *Viola odorata* L. was studied and it was concluded that amla juice was more effective, when used 2 ml/l amla juice in combination with BAP and adenine sulphate, in increasing in the shoot induction and shoot elongation.

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