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Implications of Plant Growth Regulators on Induction of *In vitro* flowering in *Aerva lanata* (L.) Juss. ex Schult.

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ABSTRACT

In vitro flowering was successfully induced from the shoots regenerated in cultures of *Aerva lanata*. *In vitro* flowering method can be monitored effectively in studying floral transition and flower development from vegetative to reproductive phase. The nodal shoot segments were used as explants in the present study. The explants were surface sterilized using $HgCl_2$ and inoculated on Murashige and Skoog's (MS) medium supplemented with 6-benzylaminopurine (BAP) for bud break. Maximum numbers of shoots were induced on MS medium supplemented with 1.0 mg l^{-1} BAP. The cultures were multiplied by continuous subculturing of shoots with mother explants on fresh MS medium with BAP and Kinetin (Kin). Rooting was achieved on one fourth MS medium supplemented with 2.0 mg l^{-1} indole-3-butyric acid (IBA). The multiplied shoots were subjected to various concentrations of different plant growth regulators and photoperiod regimes to induce flowering *in vitro*. The maximum numbers of *in vitro* flowers were recorded from the shoots on full strength MS medium with 0.5 mg l^{-1} BAP and 0.1 mg l^{-1} indole-3-acetic acid (IAA) on 12/12 h/d photoperiod.

***Keywords*:** *In vitro* flowering; *Aerva lanata*; Spectral flux photon density

1. INTRODUCTION

Aerva lanata (L.) Juss. ex Schult. Belongs to the family Amaranthaceae, is an important medicinal herb, commonly known as stone breaking plant or Chaya (Khare, 2007). This perennial herb grows up to the height of 80 cm and indigenous to the India, Sri Lanka, South Asia, Saudi Arabia and South Africa (Tushar *et al.,* 2008). It is a rare (Sudeesh, 2012) and vulnerable plant species (Rajanna *et al.,* 2011; Nandagopal *et al.,* 2015). The stem covered with minute white hairs and many hairy white flowers occur on axils, measuring the length of 8 to 20 mm. The flowers are normally self-pollinated and flowering during May to October (Krishnan *et al.,* 2009).

It is extensively used by the Ayurvedic practitioners for many pathological conditions. The plant is used to cure headache, scabies, cough, jaundice, cholera, nasal bleeding, fractures, spermatorrhoea, scorpion stings, snake bite, burns etc. (Joshi, 2007). It is well documented for its anti-inflammatory, diuretic (Vetrichelvan *et al.,* 2000), urolithiatic (Soundararajan *et al.,* 2006), antinociceptive (Venkatesh *et al.,* 2009), antiasthmatic (Deepa *et al.,* 2009), anthelmintic (Rajesh *et al.,* 2010), hepatoprotective (Ramachandra *et al.,* 2011), antimicrobial (Muthukumaran *et al.,* 2011), antidiarrheal (Rai *et al.,* 2011) and antioxidant (Battu and Kumar, 2012) activities.

A. lanata is granted with various important chemical compounds like alkaloids, flavonoids, phenols, tannin, solanin and chaconine, aervine, aervoside and aervolanine. Higher concentration of these bioactive compounds is reported in the roots as compared to the aerial parts of the plant (Priya and Chaturvedi, 2012).

The reproductive phase of a plant is possible when genetic factors, photoperiod and environmental responses are hospitable (Tissarat and Galletta, 1995). By altering the favorable conditions for flowering, plant can be induced to attain flowering phase in early stage. *In vitro* flowering technology enables understanding life cycle of plant easier (Gielis *et al.,* 2002). The significant remarks of *in vitro* flowering in various plant species were explored enormously, which compress the life cycle from 40 years to 3-6 month time duration (Ramanayake *et al.,* 2001; Lin *et al.,* 2007). The induction of flowers *in vitro* can be used effectively in studying the transition of vegetative parts to the flower buds in plants. *In vitro* flowering in culture can serve as important tool for studying flower induction, flower development and to control breeding programs in species with long juvenile periods. Direct amplification of reproductive organs using *in vitro* protocols was commercially exploited for secondary metabolite production (Lin *et al.,* 2003).

Recent studies reveal that there are various factors, which facilitates *in vitro* flowering in cultures. It depends upon the level and interaction of *in vitro* environment such as growth regulators, temperature, light regime, sugars, minerals and phenolics (Shekhawat, 2012; Murthy *et al.,* 2012). *In vitro* flowering clearly explains about the time-effective studies on various aspects of flowering, and it has been achieved in number of commercial, ornamental, food crops and medicinal plants such as *Rosa hybrida* (Kanchanapoom *et al.,* 2009), *Vitex negundo* (Rathore and Shekhawat, 2011), *Arnebia hispidissima* (Shekhawat, 2012), *Elaeis guineensis* (Nizam and Chato, 2012) and *Dendrocalamus hamiltonii* (Kaur *et al.,* 2014) etc.

A. lanata has been reported to be seasonal, and seen after the onset of monsoon (July) and disappear by the beginning of January (Pullaiah *et al.,* 2000). Dormancy and seasonal appearance pushed this plant in rare category. *In vitro* flowering in *A. lanata* was demonstrated for the first time in this investigation.

It could aid to combat the problems faced by conventional propagation due to prolonged duration of development, low rate of fruit setting and poor seed germination.

In vitro flowering and fruiting can play significant role in this plant, because it has a narrow germplasm base and continuously exploited for its various medicinal properties, susceptibility for fugal and bacterial infections, low rate of fruit set and poor seed germination as well as habitat destruction for commercial activities (Rajanna *et al.,* 2011; Nandagopal *et al.,* 2015). Therefore, there is a need to study the *in vitro* flowering protocol for *A. lanata*, which will help in germplasm conservation and storage of this valuable medicinal plant within short period of time.

2. MATERIALS AND METHODS

2. 1. Plant material and sterilization of explants

The mature and healthy plants were collected from the East Coast region of Pondicherry (India), washed in running tap water to remove dirt particles. Nodal segments containing 2-3 alternate nodes with active lateral buds were selected as explants The nodal shoot segments were primarily disinfected with 80% ethyl alcohol for 3-4 minutes and then treated with 0.1% (w/v) solution of Bavistin (systemic fungicide; BASF India Ltd, India). The explants were finally surface sterilized by immersing in 0.1% (w/v) mercuric chloride solution for 4-5 min and rinsed in sterile distilled water till the explants are free from sterilant. The sterilized explants were inoculated onto culture medium for establishment of cultures.

2. 2. Nutrient medium and culture conditions

Culture medium was prepared using MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.8% agar, ascorbic acid 50 mg l^{-1}, citric acid, adenine sulphate and arginine 25 mg l^{-1} (HiMedia, India). The pH of the medium was adjusted to 5.8 ±0.02 using 0.1 N NaOH or HCl before autoclaving at 1.05 kg/cm^2, 121 ^{o}C for 15 min. The cultures were incubated in a controlled temperature culture room (25 ± 2°C) for a photoperiod of 16 hrs with a relative humidity 65-70%.

2. 3. Induction of *in vitro* flowers

The *in vitro* regenerated shoots were excised with explants and multiplied on different strength of MS salts with various concentrations (0.5 – 3.0 mg l^{-1}) of shoot and root inducing plant hormones (BAP, Kin, IBA and Naphthalene acetic acid) to study the impact of hormones on *in vitro* flowering. *In vitro* multiplied healthy and sturdy shoots were separated and studied rooting efficacy on root inducing media and *in vitro* flowering on different media concentrations and photoperiods. The cultures were transferred to fresh medium to induce adventitious shoots and roots. The rooted plantlets were placed in sterile soilrite® and moistened with 1/4th MS macrosalts solution and hardened off for 4-5 weeks in the greenhouse.

2. 4. Impact of salt strength and growth regulators in *in vitro* flowering

The shoots with 5-7 nodes were selected from multiplication stage and inoculated into full strength, half and one-fourth strength MS medium to formulate proper floral induction

medium for *A. lanata*. The basal MS medium was supplemented with cytokinin (BAP and Kin) and auxin (IAA, IBA and NAA) at various concentrations and combinations to test the best combination of plant growth regulators in flower induction *in vitro*.

2. 5. Influence of photoperiod on flower induction *in vitro*

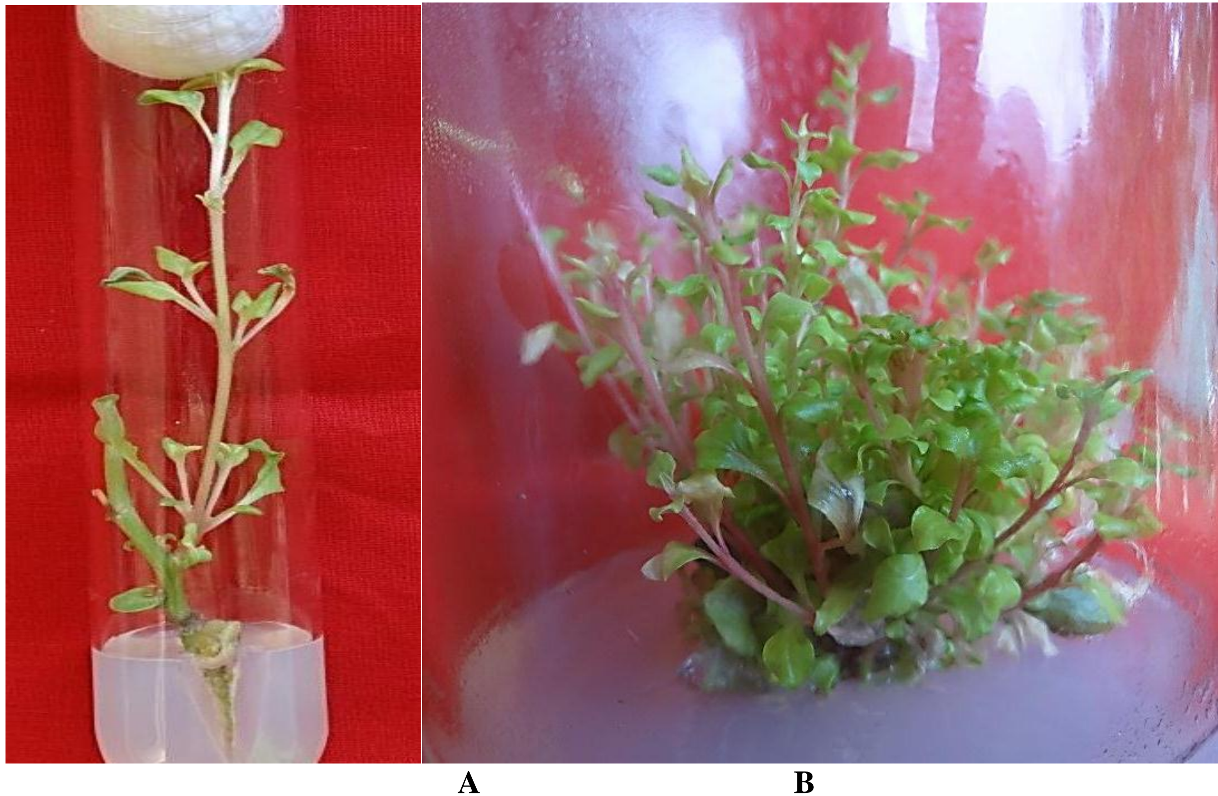
The cultures were incubated at different photoperiods (light/dark regims) such as 12/12, 16/8 and 8/16 to monitor flowering efficacy of *in vitro* regenerated shoots under illumination of 30 µmol $m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD) provided by cool-white fluorescent lights (Philips, India).

2. 6. Data collection and statistical analysis

All the experiments were conducted with minimum of 10 replicates per treatment and were repeated thrice. The values were compared by one-way ANOVA, and the significance ($p < 0.05$) of differences was calculated by Duncan's Multiple Range Test using SPSS (version 16.0). The data were recorded every 4 weeks of interval.

3. RESULTS AND DISCUSSION

3. 1. Establishment of cultures



A B

Fig. 1. A. Initiation of shoots from the nodal explants. B. Multiplication of shoots on MS medium.

Shoots were regenerated from all the explants cultured on full strength MS medium supplemented with 1.5 mg l^{-1} BAP. The response of explants with BAP was reported better compared to Kin in terms of shoot induction and shoot number (Fig. 1A). The initiated shoots were multiplied on full strength MS medium by repeated subculture and incorporation of 1.0 mg l^{-1} BAP and 0.2 mg l^{-1} NAA in the medium (Fig. 1B). The developed healthy shoots were rooted on one fourth strength MS medium augmented with 2.0 mg l^{-1} IBA. The rooted plantlets were acclimatized successfully in soilrite® under the greenhouse conditions with 92% survival rate of plants.

3. 2. *In vitro* flowering

There are many physico-chemical factors which affect the *in vitro* flowering mechanism in plants. Carbohydrates, growth regulators, light and pH of the culture medium are considered as important factors for *in vitro* flowering (Heylen and Vendrig, 1988). Conversion of vegetative phase into a reproductive phase was successfully achieved by photoperiod, growth regulators and flower induction medium. Incorporation of cytokinins associated with better response in floral induction. To study the various aspects of flowering in *A. lanata*, the isolated shoots from multiplication stage were cultured in different inorganic and organic salts strength of MS media containing various combinations of plant growth regulators. There are many reports about the application of exogenous plant growth regulators in the culture medium to induce flower *in vitro* (Sharma *et al.,* 2008; Jana and Shekhawat, 2011; Shekhawat, 2012).

The nodes of *in vitro* shoots showed profuse flowering in the form of heads of spike-lets and each spikelet had tiny clusters hermaphrodite florets. *In vitro* flowering bears immense importance in selective hybridization especially in using pollen from rare stocks and may be the first step towards the possibility of recombining genetic material via *in vitro* fertilization in otherwise non hybridizable lines. The effect of different plant growth regulators in in vitro flowering was reviewed in number of plant species (Naor *et al.,* 2004; Murthy *et al.,* 2012).

3. 3. Influence of growth regulators

In vitro flowering has been induced by BAP and Kin both at low concentrations in this study, but BAP was found more effective than Kin. Maximum percentage (92%) of inflorescence induction on shoots occurred when the healthy mature shoots from multiplication phase were shifted to half strength MS medium supplemented with 0.5 mg l^{-1} BAP and 0.1 mg l^{-1} IAA. Similar results were observed in *Bacopa chamaedryoides* (Haque and Ghosh, 2013).

Kolar and Senkova (2008) reported that the reduced mineral nutrient have accelerated *in vitro* flowering in *Arabidopsis thaliana.* Half strength MS medium or reduced nitrogen level enhanced *in vitro* flowering in many agriculturally important as well as medicinal plants like *Orichophragmus violaceus* (Luo and Lan, 2000), tomato (Dielen *et al.,* 2001) etc. In contrast, sucrose and nitrogen concentrations in the medium were not affected the rate of *in vitro* flowering in *Bambusa edulis* (Lin *et al.,* 2003).

There was no development of inflorescence in full strength MS medium, but promoted number of shoots in cultures. Inhibition of *in vitro* flowering at higher concentration of nitrogen and carbohydrate in MS media was also reported in *Lycopersicon esculentum* by Dielen *et al.,* (2001).

Flowering was observed when the cultures were 3 months age of *in vitro* shoots (Fig. 2). The highest number of flowers per shoot was 9.4 in this medium concentration. But very less percentage of shoots (20%) with maximum 1.7 flowers recorded at same concentration of Kin (Table 1). Increase in concentration of plant growth regulators resulted in declining shoot numbers as well as flower buds. In this investigation, each node of *in vitro* shoot resulted in flowering. The flowers remained on plant for a week, and then they fall down on the medium.

Fig. 2. Induction of flower buds *in vitro*.

Table 1. Effect of plant growth regulators in induction of flower bud *in vitro*.

Conc. of BAP and Kin (mg l^{-1})	Percentage of explants producing flower buds	Number of flower buds
Control (0.0)	0	0.0 ± 0.0^{a}
BAP		
0.5	92	9.4 ± 0.6^{d}

1.0	48	5.0 ± 1.3^c
2.0	0	0.0 ± 0.0^a
3.0	0	0.0 ± 0.0^a
Kin		
0.5	20	1.7 ± 0.4^b
1.0	0	0.0 ± 0.0^a
2.0	0	0.0 ± 0.0^a
3.0	0	0.0 ± 0.0^a

Note: Medium: Full strength MS medium + 0.1 mg l^{-1} IAA, The different alphabet within column shows significant difference by DMRT at 0.5% level.

The shoots were developed roots in this medium combination but less in number and length compared to one fourth strength MS medium with 2.0 mg l^{-1} IBA. It was observed that BAP is required for induction of flowers *in vitro*. There was no flower induction, when plantlets cultured in medium devoid of BAP. Recent studies proved that controlled *in vitro* conditions, plant growth regulators at different ratios enabled the formation of inflorescences (Lu *et al.,* 2000; Lin *et al.,* 2004; Sharma *et al.,* 2008).

3. 4. Influence of photoperiod on *in vitro* flowering

Effect of photoperiods on shoots was recorded after 4 weeks of culture incubation. Length of photoperiod and age of the *in vitro* plants had a significant effect on flower development from shoots. Among the photoperiods studied for flower induction *in vitro*, 12/12 hrs light/dark cycles with 30 µmol $m^{-2}s^{-1}$ PPFD was found the best in this study and maximum numbers of flower buds were recorded (Fig. 3, Table 2) in this photoperiod. In contrast to this report, Kanchanapoom *et al*. (2009) stated that the length of photoperiod not affected *in vitro* flowering in *Rosa hybrida*. The findings of present investigation agreed with reports of Vaz *et al.* (2004), Shekhawat *et al.* (2012) and Haque and Ghosh, (2013). It was observed that the *in vitro* induced flowers exhibited same morphology as mother plants in the wild. The induced flowers were white and had normal structures.

Table 2. Effect of photoperiod on flower bud induction.

Photoperiod (light/dark regims)	**Percentage of flower buds induction**
12/12	87
16/8	51
8/16	26

Fig. 3. Flowers on *in vitro* multiplied shoots.

In vitro flowering has been successfully induced in various endangered and threatened plants like, *Spathoglottis plicata* (Murthy *et al.,* 2006), *Dendrobium nobile* (Wang *et al.,* 2009), *Ceropegia spiralis* (Murthy *et al.,* 2010), *C. pusilla* (Murthy and Kondamudi, 2010), *C. attenuata* (Chavan *et al.,* 2011), *Rauvolfia serpentina* (Mondal *et al.,* 2011), *Vitex negundo* (Rathore and Shekhawat, 2011), *Ceropegia noorjahaniae* (Chavan *et al.,* 2014) and *C. fimbriifera* (Desai *et al.,* 2014). This is the first report on *in vitro* flower induction in *A. lanata*.

4. CONCLUSION

The mechanism of *in vitro* flowering using juvenile explants has been achieved in *A. lanata*, a rare and vulnerable species. This plant has a narrow germplasm base and continuously exploited for its varied advantages and medicinal properties. Furthermore it was found that the *in vitro* flowers were highly comparable to those produced by the mature plants maintained in the greenhouse. This study could help in conservation of germplasm of this valuable medicinal plant and understand the mechanism of flowering in plants.

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