CIBTech Journal of Bio-Protocols ISSN: 2319 – 3840 (Online)
An Open Access, Online Journal Available at http://www.cibtech.org/cjbp.htm
2018 Vol. (7) January-April, pp.1-4/Kaur et al.
Research Article

IN VITRO PROPAGATION AND FLOWERING IN *STELLARIA MEDIA* L.

[1]Preetpal Kaur, [2]Shruti Srivastava, [3]Surbhi Mewara, [3]Khushbu Sharma, [1]Hanwant Singh and [1]Vineet Soni*
[1]Department of Botany, Mohanlal Sukhadia University, Udaipur, India
[2]Department of Biotechnology, JECRC University, Jaipur, India
[3]Department of Biotechnology, Amity University, Jaipur, India
**Author for Correspondence: vineetsonijnu@gmail.com*

ABSTRACT
An efficient and reproducible protocol for *in vitro* regeneration and flowering of *Stellaria media* was established through nodal explants. Multiple shoot proliferation was observed on MS medium supplemented with 1.0 mg/L BAP. High frequency root induction was observed on half-strength MS medium augmented with 0.5 mg/L IBA. *In vitro* flowering was initiated on medium having BAP (2.5 mg/L) and adenine sulfate (100 mg/L). The present investigation describes hormonal regulation of morphogenesis in vitro in nodal explants of *S. media*. The study has also laid a preliminary foundation for a further research to understand the complex mechanism of floral development.

Keywords: *In vitro propagation, Rhizogenesis, In vitro flowering, MS medium, Stellaria media*

INTRODUCTION
The transition from vegetative stage to reproductive stage of growth is one of the most critical events in the life of a plant. Flowering is the most elusive and fascinating of all plant developmental processes. It is a complex process which is influenced by a multitude of environmental and physiological factors and its occurrence *in vitro* is of crucial importance as it provides a clear insight for studying flower initiation and development as well as provides an opportunity for conducting micro breeding. *In vitro* flowering bears immense importance in plant breeding where pollen can be used from rare stock, thus provide a platform for recombination of genetic material via *in vitro* fertilization in otherwise non hybridizable lines. *In vitro* flowering also facilitates the understanding of physiology of flowering and largely depends upon the level and interaction of exo-and endogenous phytohormones, sugars, minerals and phenolics. The ability to control flowering *in vitro* would be important for molecular and genetic studies aimed at elucidating the mechanisms of flower induction (Jaime *et al*., 2013). The application of cytokinins, photoperiod and subculture time to promote *in vitro* flowering is well documented in many plant species (Vu *et al*., 2006; Wang *et al*., 2002). Several successful attempts to induce in vitro flowering of several plant species have been reported, such as *Rosa indica* (Pratheesh and Kumar, 2012), *Cleome viscosa* (Rathore and Shekhawat, 2013), *Ceropegia pusilla* (Kalimuthu and Prabakaran, 2013), *Swertia chirayita* (Sharma *et al*., 2014), *Blepharis maderaspatensis* (Drisyadas *et al*., 2014), *Dendrobium huoshanense* (Lee and Chen, 2014), *Lens culinaris* (Mobini *et al*., 2015), *Vicia faba* (Mobini *et al*., 2015) and *Tridax procumbens* (Kaur *et al*., 2017).
Stellaria media L. (Caryophyllaceae; Fig. 1A) is an edible plant used in folk medicine as an antiviral drug (Slavokhotova *et al*., 2011; 2014). Adapted to diverse environments, *S. media i*s supposed to be resistant to biotic and abiotic stress. The present investigation was undertaken to develop an efficient protocol for *in vitro* propagation of *S. media* through nodal explants. Efforts were also made to induce *in vitro* flowering in this medicinal plant species.

MATERIALS AND METHODS
Plant material and surface sterilization: Nodal explants were collected from healthy plants of *S. media* growing in the University College of Science, Udaipur (India). The excised nodal explants were washed thoroughly under running tap water for 30 min to eliminate dust particles and then with 5% teepol for 8 - 10 min and rinsed several times in sterile distilled water. Then, the explants were treated with an antifungal agent (Bavistin) for 1 hour and the rinsed three times with sterile distilled water. Thereafter, the

explants were surface sterilized in a laminar flow cabinet with aqueous solution of 0.1% $HgCl_2$ for 3 min and finally washed with sterile distilled water for 2–3 times.

Culture media and growth conditions: The sterilized nodal explants were cultured on MS Medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and various combinations/ concentrations of plant growth regulators to induce morphogenetic response. The pH of the media was adjusted 5.8 before autoclaving 121 ^{0}C for 15 min. All the cultures were maintained at 25 ± 2 ^{0}C and 65 - 70% relative humidity with photoperiod of 16-h.

In vitro rhizogenesis and hardening: Obtained shoots were used for *root* induction on MS media supplemented with NAA, IAA, or 2,4-D respectively to determine an appropriate auxin for rooting. The shoots were cultured on MS supplemented with various auxins viz. IBA, IAA and NAA. Well rooted plantlets, derived from nodal explants, were gently washed in sterile water and transferred to plastic cups (10 cm × 8 cm) containing sterilized mixture of sterile soil, sand and coco peat (1:2:1). The plantlets covered in transparent polyethylene bags were kept for 4 weeks in growth chamber at 25 ± 2 ^{0}C with 16 h photoperiod. The plantlets were irrigated with tap water. The hardened plants were subsequently transferred to large pots containing normal garden soil and were maintained in an open greenhouse without environmental conditioning for 4 weeks.

In vitro flowering: In order to inducing flowering, *in vitro* developed shoots were transferred on medium containing various combinations and concentrations of plant growth regulators. Each treatment was replicated 12 times and all experiments were repeated at least thrice.

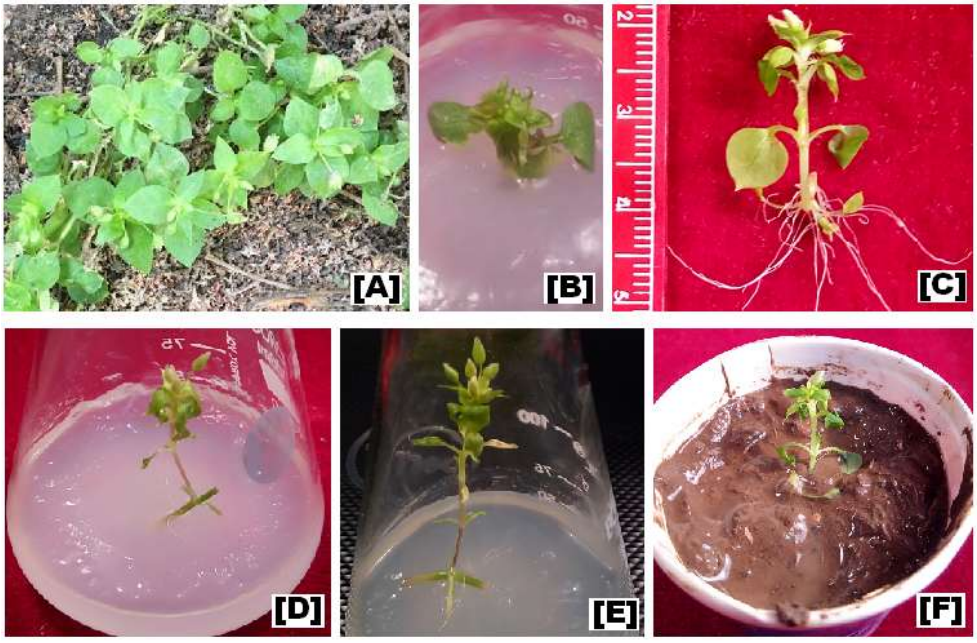

Figure 1: (A) *Stellaria media,* growing in natural habitat; (B) Nodal explant showing multiple shoot induction, (C) well developed shoots and roots, and (D-E) *in vitro* flowering. (F)- hardened plant.

RESULTS

In vitro establishment and multiplication

Multiple shoots were recorded on all explants after 3 weeks of culture, on MS medium containing various concentrations of BA and kinetin. High frequency multiple shoot bud formation was achieved when nodal explants were cultured on medium augmented with 1.0 mg/L BAP(Fig. 1B). Higher concentration of BAP showed an inhibitory effect on multiple shoot induction in *S. media*. Similar, adverse effect of higher concentrations of BAP on *in vitro* shoot proliferation has been reported in *Albizia chinesis* (Sinha *et al*., 2000), *Pterocarpus marsupium* (Anis *et al*., 2005), *Arachis hypogaea* (Banerjee *et al*., 2007), *Doritis*

pulcherrima (Mondal *et al*., 2013) and *Salvia splendens* (Sharma *et al*., 2014). Other plant growth regulators, singly or in combinations could not induce shoot proliferation in nodal explants of *S. media*.

***In vitro rhizogenesis*:** High frequency root induction was induced on half-strength MS medium augmented with 0.5 mg/L IBA (Fig. 1C). Similarly, the effect of IBA on rhizogenesis*in vitro* has been reported in *Plectranthus bourneae* (Thaniarasu *et al.,* 2015), *Passiflora foetida* (Shekhawat *et al.,* 2015), *Morinda coreia* (Shekhawat *et al*., 2015), *Ceropegia evansii* (Chavan *et al.,* 2015).

***In vitro flowering*:** Flowering is considered to be a complex process regulated by both internal and external factors and its induction under *in vitro* culture is extensively rare. In the present study, *in vitro* flowering was observed on MS medium containing 2.5 mg/L BAP and adenine sulfate (100 mg/L). The flowers produced *in vitro* appeared morphologically normal, white in color. Four to six flower buds were produced for each *in vitro* cultured plantlets (Fig. 1D-E). Plantlets having well developed root-shoot system were successfully hardened for acclimatization *in vivo* (Fig. 1F).

DISCUSSION

In the present study, BAP at low concentration (1.0 mg/L) was found to be effective for multiple shoot proliferation from nodal explants of *S. media*. IBA at low concentration (0.5 mg/L) induced high frequency root induction. In the present study, BAP (2.5 mg/L) along with adenine sulfate (100 mg/L) initiated *in vitro* flowering in shoots regenerated from nodal explants of *S. media*. Other concentrations of BAP and adenine sulfate could not trigger floral development *in vitro*. BAP is widely used for *in vitro* flowering in a number of plant species (Lin *et al*., 2004, Taylor *et al*., 2005, Anitha and Kumari, 2006; Saritha and Naidu, 2007; Jana and Shekhawat, 2011). The developed *in vitro* protocol can be successfully used for large-scale multiplication of *S. media*. The study provides deeper insight into the complex mechanism of floral induction and has laid a preliminary foundation for a further research of *in vitro* flowering of *S. media*.

ACKNOWLEDGEMENT

V. Soni and P. Kaur are grateful to Prof. P.L. Swarnkar, Prof. Reto Strasser and Prof. B. Robert for constant blessing and academic encouragement.

REFERENCES

Anis M, Husain MK and Shahzad A (2005). *In vitro* plantlet regeneration of *Pterocarpus marsupium* Roxb., an endangered leguminous tree. *Current Science* 88 861–863.

Anitha S, Kumari BDR (2006). *In vitro* flowering in *Rauvolfiatetraphylla* L. *Pakistan Journal of Biological Sciences* **9**(3) 422–424.

Banerjee P, Maity S, Maiti SS and Banerjee N (2007). Influence of genotype on *in vitro* multiplication potential of *Arachis hypogaea* L. *Acta Botanica Croatica* **66** 15–23.

Chavan JJ, Gaikwad NB, Kshirsagar PR, Umdale SD, Bhat KV, Dixit GB and Yadav SR (2015). Highly efficient *in vitro* proliferation and genetic stability analysis of micropropagated*Ceropegiaevansii* by RAPD and ISSR markers: A critically endangered plant of Western Ghats. *Plant Biosystems* **149** 442–450.

Drisyadas P1, Smitha RB, Benjamin S and Madhusoodanan PV (2014). Induction of *in-vitro* fowering and callogenesis in *Blepharis maderaspatensis* L. (Acanthaceae), a medicinal plant. Annals of Plant Sciences **3**(08) 799-803.

Jaime A, Silva T, Kerbauy GB, Zeng S, Chen Z and Duan J (2013). *In vitro* flowering of orchids. *Critical Reviews in Biotechnology* 34(1) 56–76.

Jana S and Shekhawat GS (2011). Plant growth regulators, adenine sulfate and carbohydrates regulate organogenesis and *in vitro* flowering of *Anethum graveolens*. *Acta Physiologiae Plantarum* **33**(2) 305–311.

Kalimuthu K and Prabhakaran R (2013). *In vitro* flowering from nodal explants of *Ceropegiapusilla* Wight and Arn. *International Journal of Botany and Research* **3**(3) 35–42.

Kaur P, Sharma A and Soni V (2017). Micropropagation and induction of *in vitro* flowering in *Tridax procumbens* L. *Biotechnological Research* **3**(4) 85**–**88.
Lee PL and Chen JT (2014). Plant regeneration via callus culture and subsequent *in vitro* flowering of *Dendrobium huoshanense*. *Acta Physiologiae Plantarum* **36** 2619–2625.
Levy YY and Dean C (1998). The transition to flowering. *Plant Cell* **10** 1973–1990.
Lin CS, Lin CC and Chang WC (2004). Effect of thidiazuron on vegetative tissue-derived somatic embryogenesis and flowering of bamboo *Bambu saedulis*. *Plant Cell, Tissue and Organ Culture* 76 75–82.
Mobini SH, Lulsdorf M, Warkentin TD and Vandenberg A (2015). Plant growth regulators improve *in vitro* flowering and rapid generation advancement in lentil and faba bean. *In Vitro Cellular and Developmental Biology - Plant* **51**(1) 71–79.
Mondal T, Aditya S and Banerjee N (2013). *In vitro* axillary shoot regeneration and direct protocorm-like body induction from axenic shoot tips of *Doritispul cherrima* Lindl. *Plant Tissue Culture and Biotechnology,* **23** 251–261.
Murashige T and Skoog FA (1962). Revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15** 473–497.
Pratheesh PT and Kumar MA (2012). *In vitro* flowering in *Rosa indica* L. *International Journal of Pharmacy and Biological Sciences* **2**(1) 196–200.
Rathore NS and Shekhawat NS (2013). *In vitro* flowering and seed production in regenerated shoots of *Cleome viscosa*. *Industrial Crops and Products* **50** 232–236.
Saritha KV and Naidu CV (2007). *In vitro* flowering of *Withania somnifera* Dunal.—an important antitumor medicinal plant. *Plant Science* **172** (4) 847–851.
Sharma S, Shahzad A, Kumar J and Anis M (2014). *In vitro* propagation and synseed production of scarlet salvia (*Salvia splendens*). *Rendiconti Lincei*. **25** 359–368.
Sharma V, Kamal B, Srivastava N, Dobriyal AK and Jadon VS (2014). *In vitro* flower induction from shoots regenerated from cultured axillary buds of endangered medicinal herb *Swertia chirayita* H. Karst. *Biotechnology Research International* **5** (2014) 264690.
Shekhawat M S, Manokari M and Ravindran CP (2015). An improved micropropagation protocol by *ex vitro* rooting of *Passiflo raedulis* Sims. f. flavicarpa Deg. through nodal Segment culture. *Scientifica (Cairo)* **2015** 1–8.
Shekhawat MS, Kannan N and Manokari M (2015). *In vitro* propagation of traditional medicinal and dye yielding plant *Morindacoreia* Buch.–Ham. *South African Journal of Botany* 100 43–50.
Sinha RK, Majumdar K and Sinha S (2000). *In vitro* differentiation and plant regeneration of *Albizia chinensis* (OBS.) MERR. *In Vitro Cellular & Developmental Biology – Plant* **36** 370–373.
Slavokhotova AA, Odintsova TI, Rogozhin EA, Musolyamov AK, Andreev YA, Grishin EV and Egorov TA (2011). Isolation, molecular cloning and antimicrobial activity of novel defensing from common chickweed (*Stellaria media* L.) seeds. *Biochimie* **93** 450–456.
Slavokhotova AA, Rogozhin EA and Musolyamov AK (2014). Novel antifungal α-hairpinin peptide from *Stellaria media* seeds: structure, biosynthesis, gene structure and evolution. *Plant Mol Biol* **84** 189.
Taylor NJ, Light ME and Staden JV (2005). *In vitro* flowering *of Kniphofia leucocephala*: influences of cytokinins. *Plant Cell, Tissue and Organ Culture* **83** 327–333.
Thaniarasu R, Kumar TS and Rao MV (2015). *In vitro* propagation of *Plectranthusbourneae* Gamble- an endemic redlisted plant. *Plant Tissue Culture and Biotechnology* **25** 273–284.
Vu NH, Anh PH and Nhut DT (2006). The role of sucrose and different cytokinins in the *in vitro* floral morphogenesis of rose (hybrid tea) cv. 'First Prize'. *Plant Cell Tissue and Organ Culture* **87** 315–320.
Wang GY, Yuan MF and Hong Y (2002). *In vitro* flower induction in roses. *In Vitro Cellular and Developmental Biology- Plant* **38** 513–518.