In Vitro Culture of Spilanthes Acmella (L.) Murray

Anil Vishnu Dusane[a], Swapnal K. Patil[b], Bohara Khushboo M[c]., Dongre Shivdeep [d]., Shivani A Dusane[e]

[a]Associate Professor Sir Parashurambhau College, Pune, India

Abstract

Spilanthes acmella (L.) Murrayis awell-known anti-toothache plant. This plant is an extensive source of various bioactive chemical. S. Acmella has immense utilization as medicine, cosmetic and health supplement. This plant species has variety of therapeutic properties viz. Anti-asthmatic, anti-rheumatic, diuretic, anti-inflammation, anti-cancer, anti-diabetic, anti-allergic, wound healing, analgesic, anti-obesity and antioxidant. This plant has low seed germination and low success rate of vegetative propagation methods. The in vitro propagation is the only method to meet the world-wide demand. In the present investigation the callus culture of S. Acmella obtained from leaf disc while the shoots are obtained from nodal sector. The different growth regulators such as BAP, NAA, 2,4Dichlorophenoxyacetic acid (2-4D) influenced the growth of callus and shoots. The leaf disc and nodal sectors grown on Murashige and Skoog (MS) culture medium with 1.0, 1.5, 2.0 and 2.5 mg/l concentrations of BAP, Napthalene acetic acid (NAA), 2,4 Dichlorophenoxyacetic acid (2-4D) shown variety of responses at different combinations. The nodal sectors (explants) on MS medium supplemented with BAP (2mg/l) and NAA (0.5mg/l) has shown highest proliferation of shoots.

KEYWORDS: Anti-toothache plant, BAP**,** bioactive metabolites, in vitro culture, Spilanthes acmella

Introduction

Spilanthes acmella (L.) Murray (Asteraceae) is 40-60 centimeters tall short-lived herb. It is native to Brazil and it is widely distributed in tropical and subtropical regions. Its flowers and leaves have pungent taste and when touched it is accompanied by tingling sensation and numbness. It is used as a vegetable (Tiwari et al., 2011) in many countries. It is an important plant drug in traditional medicine and still a popular choice for primary health care (Vanamala et al., 2012). S. Acmella contains highly valuable bioactive chemicals such as alkaloids, tannins, essential oils and flavonoids. Generally, whole plant or leaves or flowers are used as a folk remedy for toothache, rheumatism and fever. This plant species has a varied therapeutic properties such as anti-asthmatic, anti-rheumatic, diuretic, anti-inflammatory, anti-cancer, anti-diabetic, anti-allergic, wound healing, anti-pyretic, analgesic, anti-microbial and anti-obesity (Jain et al., 2006; Abascal and Yarnell, 2010; Chakraborty et al., 2010; Kumar et al.,2010; Arora et al., 2011). It is also use as beauty care product and dietary supplementsdue to its antioxidant property (Tyagi and Delanty, 2003).

The study on S. acmella revealed ‘Spilanthol’ has immense commercial value (Baruahand Leclercq, 1993; Singh and Chaturvedi, 2012). The Spilanthol is the main

constituent isolated from many parts of S. acmella used as anti-inflammatory activity (Sharma, 2003; Wu et al., 2008).This plant species has a low rate of germination and low success rate of vegetative propagation (Joshi et al., 2015). The worldwide high demand and rapid depletion and over exploitation of plant created need for in vitro micropropagation.

Material and methods

Freshly prepared stocks of Murashige and Skoog (1962) were used to prepare the medium. The
MS was supplemented with BAP, NAA and 2-4-D. Sucrose was added in medium (30g/lit) and was gelled with 7.5g agar. The pH of the media was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCl before autoclaving at 1.06kg/cm^{-2} and 121 ^{0}C for 15 min.
Healthy plants of S. acmellawere collected from the Botanical garden of S.P. College, Pune campus. Explants were taken in the form of leaf disc and nodal sectors.Surface sterilization of the explants was carried out with Sodium hypochlorite and 0.1% $HgCl_2$. Inoculation of aseptic ex-plant i.e. leaf discs and nodal sectors was done on MS medium supplemented with BAP, NAA and 2-4-D. The cultures were maintained at 22 ^{0}C temperature and 12 hours of photoperiod. The callus initiation and shoots proliferation resulted after few days and subsequently sub-culturing was carried out.

Results and Discussion

Explant Response

The surface sterilization treatment with Sodium hypochlorite to explants was found ineffective and shown high percentage of contamination. Treatment with 0.1% $HgCl_2$ for 1 min proved quite effective in providing 80% of the aseptic cultures while higher exposure time of 0.1% $HgCl_2$ proved lethal for the explants. The results obtained with different concentration of MS medium with BAP, NAA and 2-4-D with leaf discs and nodal sector has been tabulated in Table no.1 and Table no.2.

Table no.1: Effects NAA and 2,4-D on callus induction of S. acmella (leaf discs)

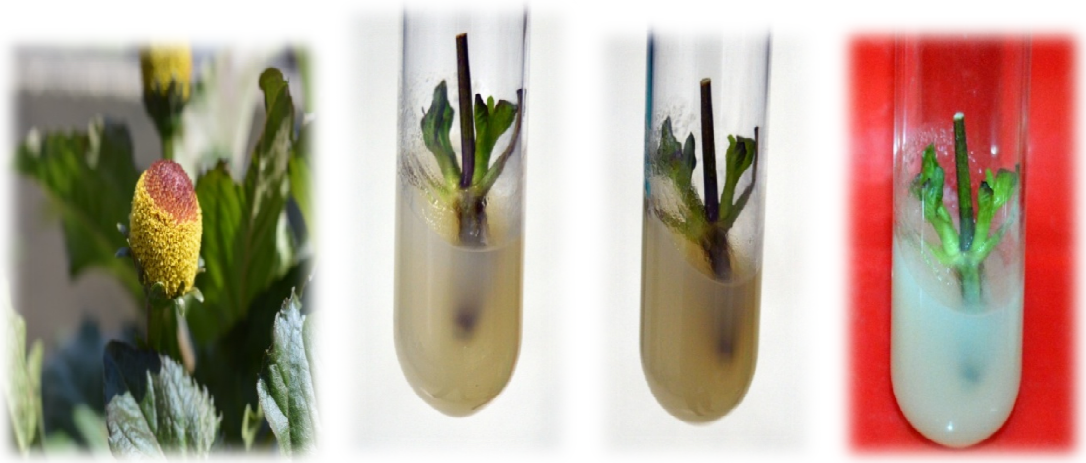
No of tubes inoculated	Medium	Explants	No of tubes showing callus induction	Average days taken for callus induction	% of callus induction
25	MS+NAA+2,4-D	Leaf disc	1	10	4.0%
25	MS+0.5NAA+0.52,4-D	Leaf disc	2	10	8.0%
25	MS+1 NAA+1 2,4-D	Leaf disc	4	10	16.0%
25	MS+1.5 NAA+1.5 2,4-D	Leaf disc	5	10	20.0%

25	MS+2NAA+22,4-D	Leaf disc	7	10	28.0%
25	MS+2.5NAA+2.52,4-D	Leaf disc	8	10	32.0%
25	MS+3NAA+32,4-D	Leaf disc	3	10	12.0%

Table no.2: Effects BAP and NAA on shoot proliferation S. acmella (Nodal sector)

No of tubes inoculated	Medium	Explants	No of tubes showing shoot induction	Average days taken for shoot induction	% of shoot proliferation
25	MS+0.5BAP+0.5NAA	Nodal sector	3	14	12.0%
25	MS+1BAP+0.5NAA	Nodal sector	10	14	40.0%
25	MS+1.5BAP+0.5NAA	Nodal sector	20	14	80.0%
25	MS+2BAP+0.5NAA	Nodal sector	25	14	100.0%
25	MS+2.5BAP+0.5NAA	Nodal sector	11	14	44.0%
	MS+3BAP+0.5NAA	Nodal sector	3	14	12.0%

32% of all the inoculated test tubes shown callus induction. The average 10 days were taken for callus induction with leaf disc callus induction. However, the nodal sector shown 100% of shoot induction with MS+2BAP+0.5NAA. The average 14 days were taken for shoot proliferation. The multiple shoots formation was also observed in few inoculated tubes.



A B C D

Fig. 1. Shoot regeneration of S.acmella A- Plant, B- Shoot proliferation after 6 days, C- after 8 days, D-after 10 days.

Singh and Chaturvedi (2010) reported clonal propagation of S. acmella developed from the nodes on full strength MS basal medium. They also recorded maximum percentage of cultures showing shoot proliferation with 6-benzylaminopurine (BAP). Interestingly, higher concentration (>2 mg/l BAP) resulted in slow growth of shoots. In the present investigation the MS medium supplemented with BAP (2.mg/l) and NAA (0.5mg/l) favored maximum rate i.e. 100% of shoot multiplication with no adventitious roots. Interestingly some of the multiple shoots were also recorded. The spilanthol (an antiseptic alkylamide) extracted from S. acemella has immense commercial value in cosmetic industry. The regeneration protocol developed in the present investigation would provide a basis for germplasm conservation and mass cultivation of the plant for later biotechnological use.

Conclusion

The result presented here indicates that in vitro regeneration of complete plantlets is possible using nodal segments as an explants Ayurvedic preparations have great demand across the globe. Peoples are rushing towards safer medicines now a day. Increasing demand of medicinal plants could not supply sufficient plant material for the extraction of ayurvedic medicines. In vitro studies aresuitable option for multiplication of rootstock of plant material and to fulfill the need of the peoples.

References:

- Abascal K, Yarnell E. Treatment for recurrent aphthous stomatitis. Alternat Complement Ther. 2010; 16:100–106.
- Arora S, Vijay S, Kumar D. Phytochemical and antimicrobial studies on the leaves of Spilanthesacmella. J Chem Pharm Res. 2011;3:145–150.
- Baruah RN., Leclercq PA (1993) Characterization of the essential oil from flower heads of Spilanthesacmella. J. Essent. 1993:Oil Res., 5: 693-695

- Chakraborty A, Devi BRK, Thokchom I, Sanjebam R, Khumbong S. Preliminary studies on local anesthetic and antipyretic activities of SpilanthesacmellaMurr. in experimental animal models. Indian J Pharmacol. 2010;42:277–279
- Demarne F, Passaro G. Use of an Acmellaoleracea extract for the botulinum toxin-like effect thereof in an anti-wrinkle cosmetic composition. US patent. 2009
- Jain JB, Kumane SC, Bhattacharya S. Medicinal flora of Madhya Pradesh and Chattisgarh-A review. Indian J TraditKnowl. 2006;5:237–242.
- JoshiVeenu, KishanLalTiwari and Shailesh Kumar Jadhav. In vitro propagation of Spilanthesacmella (L.) Murray using semisolid and liquid medium Indian Journal of Biotechnology 2015: Vol 1 (4):112 -116
- Kumar BNS, Swamy BMV, Swamy A, Murali A. A review on natural diuretics. Res J Pharm BiolChem Sci. 2010;1:615–634.
- Sharma R. Medicinal plants of India - an encyclopedia. Delhi: Daya Publishing House; 2003.
- Singh Mithilesh and RakhiChaturvedi Optimization of Spilanthusacmella L. cultivation by Invitro nodal segment culture ActaHorticulturae2010 No.865:109-114
- SinghMithilesh and RakhiChaturvediScreening and quantification of an antiseptic alkylamide, spilanthol from in vitro cell and tissue cultures of SpilanthesacmellaMurr. Industrial crops and Products 2012; 36(1): 321-328
- Tiwari KL, Jadhav SK, Joshi V. An updated review on medicinal herb genus Spilanthes. Chin J Integr Med. 2011;9:1170–1178.
- Tyagi A, Delanty N. Herbal remedies, dietary supplements, and seizures. Epilepsia. 2003;44:228–235.
- Vanamala U, Elumalai A, Eswaraiah MC, Shaik A. An updated review on diuretic plants. Int J Pharm Biol Arch.2012;3:29–31.
- Wu LC, Fan NC, Lin MH, Chu IR, Huang SJ, Hu CY, et al. Antiinflammatory effect of spilanthol from Spilanthesacmella on murine macrophage by down-regulating LPS-induced inflammatory mediators. J Agric Food Chem. 2008;56:2341–2349.