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IN VITRO FLOWERING IN ROSA INDICA L.

P.T. PRATHEESH[*1] AND M. ANIL KUMAR[2]

[*1]School of Biosciences, Mahatma Gandhi University, Kottayam, India.

[2]Department of Botany, Union Christian College, Alwaye, India.

*Corresponding Author Email: pratheeshpt@yahoo.com

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ABSTRACT

A protocol for In vitro flowering in Rosa indica L. from nodal explants by the addition of silver nitrate was established. Multiple shoot formation of up to 5 shoots were obtained on Murashige and Skoog (MS) medium supplemented with 0.5 mg/1 IAA and 1 mg/1 BA. Regenerated shoots cultured on MS medium containing various concentrations of BA, IAA, and sucrose did not flower. The multiple shoots obtained showed gradual yellowing of leaves which was rectified by the addition of different concentrations of silver nitrate. Addition of silver nitrate in the regeneration medium along with three week interval subculture period induced in vitro flowering in Rosa indica L. The 3-week intervals for two consecutive subcultures on this MS medium supplemented with 0.5 mg/l IAA, 1 mg/l BA and 50 mg/l silver nitrate under photoperiod 16/8 (light/dark cycle) were efficient for flower induction. Shoots readily rooted on 1/4 MS medium devoid of growth regulators. Rooted plantlets were hardened and established in pots at 100% survival.

KEYWORDS: *Micropropagation, silver nitrate, in vitro flowering, nodal culture.*

INTRODUCTION

Rose is the queen of flowers. The popularity of rose as a garden plant linked with its use in the production of cut flowers and as a source of rose oils for the cosmetic industry makes it one of the most important floricultural crop. Floricultural crops are usually vegetative propagated in order to maintain cultivar fidelity. At the same time efficient regeneration systems must be designed to minimize somaclonal variants. *In vitro* propagation protocols have been established for many rose cultivars using shoot tip and auxillary bud explants [1,2]. We have already developed an efficient protocol for micropropagation of *Rosa indica* L[3]. Several other successful attempts to induce *in vitro* flowering other species of roses have also been reported [4,5], but *in vitro* flowering has not been reported earlier in *Rosa indica* L.

The transition from vegetative growth to flowering is a crucial point in higher plant development. It is known to be under the control of the so called switch-on mechanism. Basically this mechanism is governed by ensembles of flowering time and meristem identity genes with a complex hierarchy [6]. The mechanism has not been fully elucidated. It may be influenced by various factors affecting plant development[7], including hormones [8]. An *in vitro* system is considered a conventional tool to study the switch-on flowering mechanism. Establishing a reliable *in vitro* protocol to induce flowering in roses is important for the study of molecular and genetic mechanism of flower induction and for assisting rose breeding programs.

MATERIALS AND METHODS

Plant materials

Rosa indica L. was used in the experiment. Nodal explants were collected from the actively growing branches of *Rosa indica* L. and surface sterilized in 0.1 % aqueous solution of mercuric chloride for 12 minutes followed by washing three times in sterile distilled water for 5 minutes each. Nodal explants of 0.5 cm were carefully excised from the shoots and inoculated on to the culture medium.

Medium preparation and culture conditions

Murashige and Skoog [9] (MS) medium supplemented with hormonal combinations of auxins and cytokines. The pH of all media was adjusted to 5.8 with 1 N NaOH or 1 N HCl before autoclaving at 121°C for 20 min. The culture tubes were incubated at 25 ± 2°C for a photoperiod of 16

hours per day with a light intensity of 2500 lux and 60% humidity unless otherwise stated.

Regenerating explants were sub-cultured at regular interval of three weeks. The regenerated explants were sub-cultured to fresh media with growth regulator free $^1/_4$ MS medium. After development of adequate length of shoot and roots, the plantlets were transferred to screw capped jars containing sterile vermiculite for 2 weeks for hardening off.

Silver nitrate treatment

The explants showed yellowing of leaves after the first subculture. Different concentrations of silver nitrate ($AgNO_3$) were supplemented (10, 20, 30, 40, 50, 60 and 70 mg/l) along with MS medium consisting of hormones (**Table-1**), for rectification of leaf yellowing in *Rosa indica* L.

Table-1 Effect of $AgNO_3$ on leaf yellowing and *in-vitro* flowering in *Rosa indica* L.

MS + Hormones (mg/l)	Silver nitrate ($AgNO_3$) Conc. in mg/l	Leaf yellowing*	*In vitro* flowering*
IAA – 0.5 BA – 1.0	10	+	-
	20	+	-
	30	-	-
	40	-	-
	50	-	+
	60	-	-
	70	-	-

*** Observation after second subculture of three week subculture cycle (6 explants per experiment)**

RESULTS AND CONCLUSION

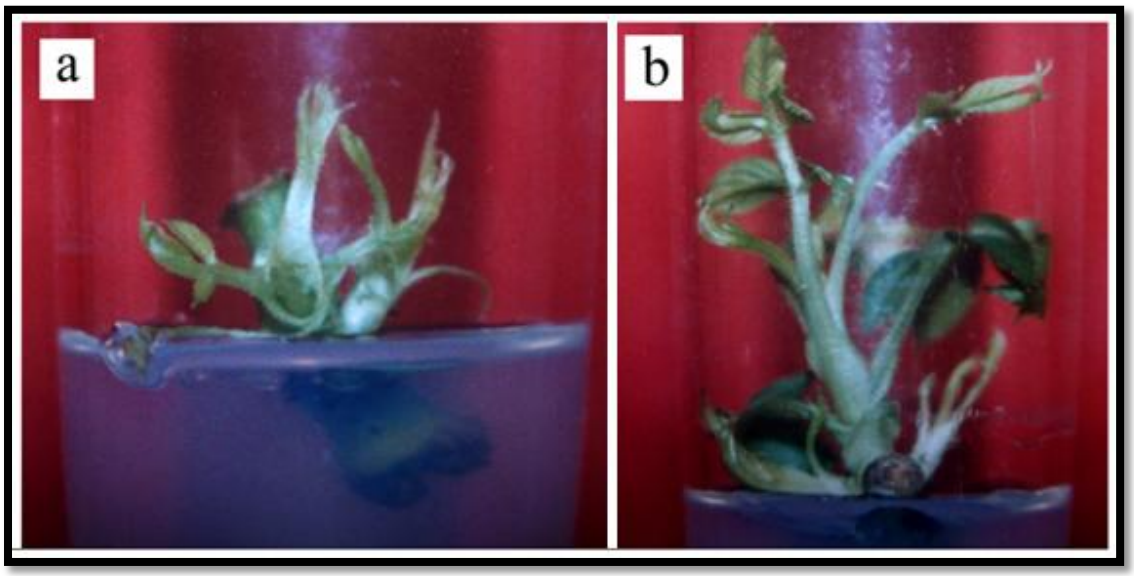
Nodal explants showed shoot initiation after seven days, maximum shoot multiplication was observed on explants cultured on MS medium supplemented with 0.5 mg/l 1AA and 1 mg/l BA (**Fig. 1a & 1b**). But the shoots showed initial signs of yellowing from the second sub-culture onwards (**Fig. 2a**), and this problem was rectified by the addition of 30 mg/l silver nitrate in MS medium supplemented with 0.5 mg/l IAA and 1 mg/l BA (**Fig. 2b**). The addition of silver nitrate has significantly decreased the yellowing of shoots and increased the rate of shoot multiplication.

Flower buds were initiated from the shoots after two weeks subculture in 50 mg/l silver nitrate containing medium (**Fig. 3a**). The *in vitro* flowering response was studied using different concentrations of silver nitrate in MS medium augmented with 0.5 mg/1 IAA and 1 mg/1 BA, this hormone concentrations. A single flower bud has been observed from a single shoot. Optimums of 5 flower buds were obtained from 5 shoots within a single culture on MS medium supplemented with 0.5 mg/1 IAA, 1 mg/1 BA and 50 mg/l silver nitrate. Little flower buds were noticed on MS medium fortified with 50 mg/l silver nitrate along with 1 mg/l IAA and 5 mg/1 BA. The flower buds began blooming after third subculture of three week interval in the culture media (**Fig. 3b**). The *in vitro* flowering was observed only in explants supplemented with 50 mg/l silver nitrate (**Table-1**). *In vitro* flowering in the present study were observed after the third subculture of three week interval, a similar result was reported by

Kanchanapoom in earlier research work [10], they have also report that photo period did not have any effect on *in vitro* flowering in rose. The age of mother plant can be a possible factor in governing *in vitro* flower induction [11].

Micro propagation of shoots from nodal explants

Fig. 1 (a) Nodal explants showing shoot initiation on MS media after 1 week (b) Multiple shoot formation in MS medium 0.5 mg/1 IAA, 1 mg/1 BA.



Effect of silver nitrate on leaf yellowing

Fig. 2 - Yellowing of leaves rectification using $AgNO_3$
(a) Before silver nitrate treatment.
(b) Green leaves after treatment with 30 mg/l silver nitrate.

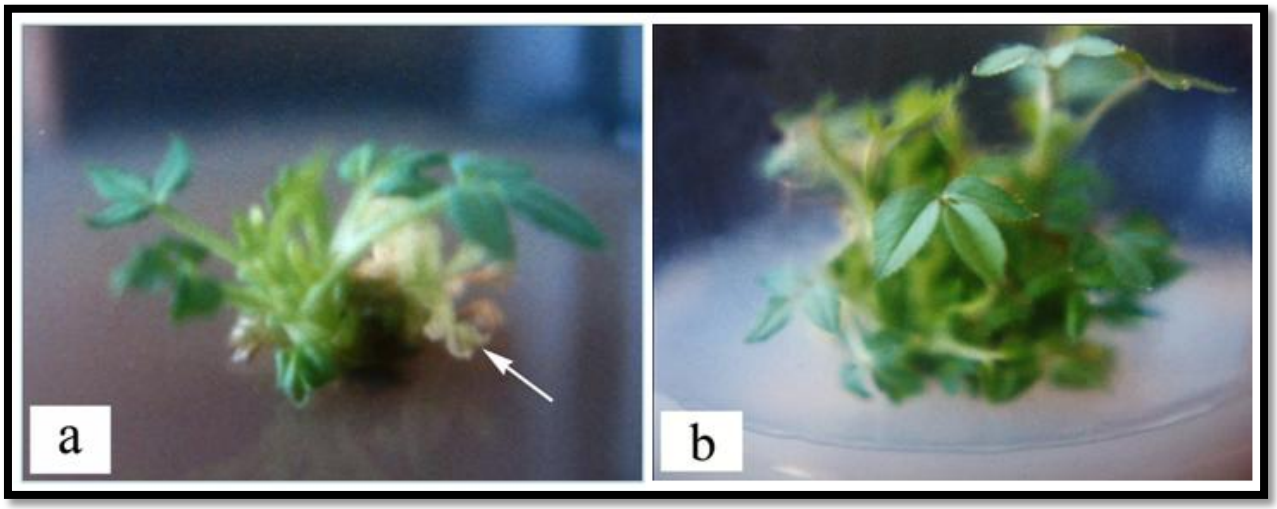
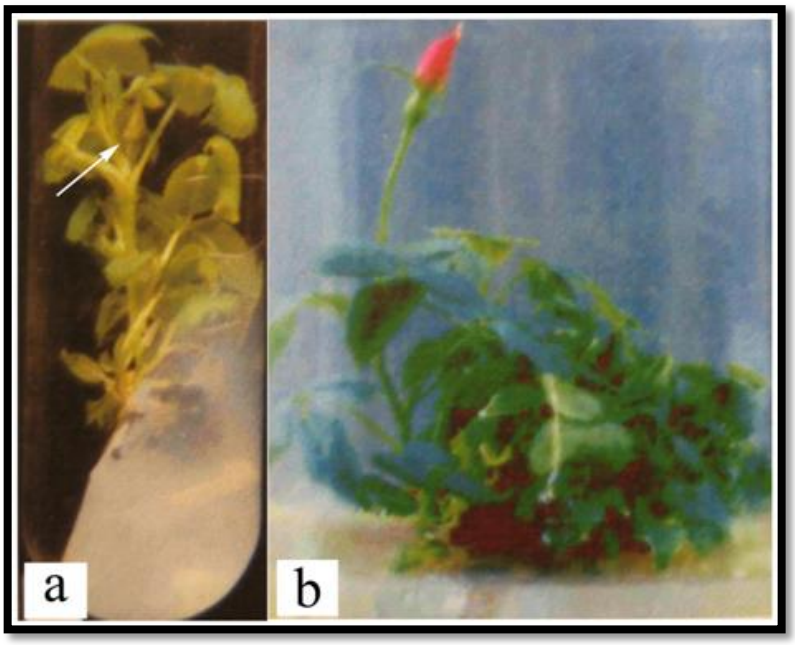


Fig. 3 (a) Flower buds observed after second subculture in culture medium.
(b) In vitro flowering of the Rosa indica L. in MS media supplemented with 0.5 mg/1 IAA, 1mg/1 BA and 50 mg/l silver nitrate.



Flowering is a complex process resulted by external and internal factors and its induction under aseptic *in vitro* culture is exclusively rare. In the present investigation flowering has been induced by the presence of lower concentrations of auxin (0.5mg/l IAA) and higher concentrations of cytokine (1 mg/1 BA) along with the presence of additional nitrate in the form of silver nitrate. Cytokine promotes the transition in some higher plants to their reproductive stage *in vitro* [12,13]. BA is widely used for *in vitro* flowering in many roses [14], and a number of other plant species [15,16]. These data suggested that cytokine activates the switch on mechanism for flower induction under the experimental conditions.

The ability of explants to form flowers *in vitro* depends on many external, internal, chemical and physical factors and virtually all these factors interact in various complex and unpredicted ways [17,18,19]. Combination of genetic and environmental factors play an important role in flowering response *in vitro*[20]. *In vitro* flowering has been reported in *Ocimitm basilicum* on half strength MS medium supplemented with 5 mg/1 BA and 1 mg/l IAA. Similar results were reported in *Vitex* where they used 1.5 mg/1 BA in combination with 0.1 mg/1 NAA [21].

According to Handro low concentrations of auxins induced flowering in *Streptocarpus nobilis*[22]. On the contrary in the present investigation auxins alone did not induce flowering. Substitution of nitrates in MS medium resulted in *vitro* flowering in *Circhonium intybus* [23]. Silver nitrate induced flowering has been reported in *Rotida aquatic* [24]. Similar results was obtained in the present study with *Rosa indica* L. The addition of $AgN0_3$ in the medium along with low concentration of auxin induced *in vitro* flowering.

An efficient protocol for *in vitro* flowering in *Rosa indica* L. was developed in this study. More studies need to be conducted for the evaluation of molecular mechanism behind in vitro flowering by silver nitrate. The protocol developed in this study will certainly help accelerating the rose breeding programs for the production of novel hybrid clones, more reliable culture regimes need to be elucidated in future.

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REFERENCES

1. Rout GR, Debata BK and Das P, *In vitro* mass scale propagation of *Rosa hybrida* cv. Landora, Current Sci, 5 8: 876- 878, (1989).
2. Skirvin RM , Chu MC and Young HJ, Rose, in *Handbook of plant cell culture,* Vol V, edited by Ammirato P V, Evans D A, Sharp WR , Bajaj YPS (Mc Graw-Hill Publishing Co. New York).pp 716-743, (1990).
3. Kumar MA and Pratheesh PT, *In vitro* shoot multiplication in *Rosa indica* L. Plant Cell Biotech. And Mol. Biol. 5:73-76, (2004).
4. Vu NH, Anh PH, Nhut DT, 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, (2006).
5. Wang GY, Yuan MF, Hong Y, *In vitro* flower induction in roses. *In Vitro* Cellular and Developmental Biology. Plant, 38:513-518, (2002).
6. Nilson O, Weigel D, Modulating the tilling of flowering, Curr Op in Biotechnol, 8 : 195-199, (1997).
7. Chory J, Chattarjee M, Cook RK, Elich T, FankJiauser C, From seed germination to flowering, light controls plant development via pigment phytochrome, in Proc Nail Acad Sci, (USA) 12066-12071.(1996).
8. Bernier G, Havelango A, Houssa C, Petitjean A and Lejeune P, Physiological signals that induce flowering, Plant Cell, 5:147-1150, (1993).
9. Murashige T and Skoog F, A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant, 15:473-497, (1962).
10. Kanchanapoom K, Posayapisit N and Kanchanapoom K, *In vitro* flowering from cultured nodal explants of rose (Rosa hybrid L.) Not. Bot. Hort. Cluj, 37(2): 261-263, (2009).
11. Demeulemeester MCA and DeProfit MP, *In vivo* and *in vitro* flowering response of chicory (Cichorium intybus L.): influence of plant age and vernalization. Plant Cell reports. 18:781-785, (1999).
12. Paek KY, Shin GB and Kim JJ, Exploitation of temperate *Cymbidiiims* and establishment of micropropagation system, J Kor Soc Hort Sci, 30: 234-237, (1989) .
13. Wang GY, Xu ZH, Chia TP and Chua NH, *In vitro* flowering of orchid *(Dendrobium candidum)* in Biotechnology in Agriculture, edited by B You(Kluwer, Amsterdam) pp 373-378, (1993).
14. Dobres M, Williams L, Gail R, Micropropagation of rose plants. United States patent 5, 843.782, (1998).
15. Lin CS , Lin CC and Chang WC, Effect of thidiazuron on vegetative tissue-derived somatic embryogenesis and flowering of bamboo *Bambusa edulis*. Plant Cell, Tissue and Organ Culture. 76:75-82, (2004).
16. Taylor NJ, Light ME and Van Staden J, *In vitro* flowering of *Kniphofia leucocephala*: influences of cytokinins. Plant Cell, Tissue and Organ Culture. 83:327-333, (2005).
17. Tran Than Van M, Direct flower neoformaiion from superficial tissue or small explants of *Nicotiana tabaccum* L. Planta, 115: 87-92, (1973).
18. Scorza R and Janick J, *In vitro* flowering of *Passiflora suherosa* L, J Am Soc Hort Sci,105:127-130, (1980).
19. Croes AF, Creemer MT, Vanden Ende G, Kemp A and Barendse G M W, Tissue age as an endogenous factor controlling *in vitro* bud formation in explants from the inflorescence *of Nicotiana tabaccum* E, J Exp Biol, 36 :1771-1 779, (1985).
20. Tisserat Band Galletta PD, Production of Cucumber fruits from the culture of "Market more-76" Plantlets. Plant Cell Reports,, 13: 37-40, (1993).
21. Thiruvengadam M and Jayabalan N, *In vitro* flower ng of *Vitex negundo* L. a medicinal plant, Plant Cell Biotechnol Mol Biol, 2: 67-70, (2001).
22. Handro W, Effect of some growth regulators on *in vitro* flowering *of Streptococcus nobilis ,* Plant Cell Rep, 2:133-136. (1983).
23. Bais HP , Sudha G, Ravishankar GA, Influence of putricine, silver nitrate and polyamine on the morphogenetic response in imtransfoniusd and transformed tissue *of Circhonium intybus* and their regenerants. Plant Cell Rep, 2: 547-555, (2001).
24. Chithra M, Martin KP, Sunandakumari C , Madhusudhanan PV, Silver nitrate induced rooting and flowering *in vitro* on rare rheophytic woody medicinal plant, *Rotula aquatica* Lour , J Biotechnol, 3:418-421, (2004).

***Corresponding Author:**
***P.T. PRATHEESH*[*1]**
School of Biosciences,
Mahatma Gandhi University,
Kottayam, India.