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# *In vitro* Flowering in *Rauvolfia tetraphylla* L.

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**Abstract:** *Rauvolfia tetraphylla* L. is an economically important medicinal plant because of the presence of various alkaloids, which are in use of cardiovascular and psychiatric treatments. Flowering is a complex process up regulated by external and internal factors and its induction under aseptic *in vitro* culture is extensively rare. Nodal segments and shoot tips were cultured on MS medium supplemented with various concentrations of BAP and $GA_3$. Maximum number of multiple shoots (4.56±0.098) was obtained at 4.44 μM of BAP after two weeks of inoculation. When $GA_3$ was used in combination with BAP, *in vitro* flowering was observed. Flower bud production and flower induction was noticed at 4.44 μM of BAP with 4.3 μM of $GA_3$. However, maximum number of shoots was reduced to three in the same concentration.

**Key words:** Multiple shoot, *in vitro* flowering, *Rauvolfia tetraphylla* L.

## INTRODUCTION

The genus *Rauvolfia* belongs to the family Apocynaceae and five species of this genus are native to India (Bhattacharjee, 1998). *R. tetraphylla* L. is economically important which is cultivated on commercial scale in India (Farooqi and Sreeramu, 2001). The plant is important because of the presence of nearly 30 alkaloids in this plant root. Among them reserpine holds the first place. Other frequently reported alkaloids are ajmalicine, reserpitine, sarpagine, deserpidine, rescinnamine, serpentine, ajmalidine, alloyohimbine, chandrine, corynathine, iscajmaline, neo ajmaline, papaverine, raunatine, raunoline, rauwolscine, reserpiline, reserpinine, reserpoxidine, serpinine, thambine and yohimbine (Farooqi and Sreeramu, 2001). Reserpine is acting as tranquilizer and also lowers the blood pressure. The alkaloid serpentine is a weak hypotensive agent. Sarpagine has only fleeting effect on blood pressure. Yohimbine is hypotensive, a cardiovascular depressant and hypnotic (Ramawat *et al*., 1999). Ajmaline stimulates respiration and intestinal movement and also useful in the treatment of arrhythmic heart disorders (Haverkamp, 2001). Extract of the plant root is valuable for intestinal troubles and believed to stimulate uterine contraction in cases of difficult delivery. Fruits yield black dye and the extract of the herb is mixed with castor oil applied to skin ailments (The useful plants of India, 1986). Due to overexploitation and lack of organized cultivation, the wild population has declined fast and the species is listed as endangered (Swarup and Arora, 2000). Literature availability on *in vitro* flowering in this plant is very scanty and seed germination rate is also very poor in this plant (Patil and Jayanthi, 1997). Sarma *et al*. (1999) observed initiation of flowering in *R. tetraphylla* L. in the shoot multiplication medium containing BA and KN but the response was poor. Hence the present study is undertaken with an objective to standardize a reproducible protocol for *in vitro* flowering in *R. tetraphylla* L.

## MATERIALS AND METHODS

Shoot tip and nodal explants were collected from medicinal plants garden, Department of Plant Science, Bharathidasan University, Trichy. The explants washed in running tap water for 2 min and then transferred to laminar air flow chamber. There the explants were treated with 70% ethyl alcohol (V/V) for 30 sec and rinsed with sterile distilled water for two times. 0.1% mercuric chloride (W/V) was used to treat the explants for 3-5 min and thoroughly washed with sterile distilled water for five times to remove the traces of sterilizing agents. MS (Murashige and Skoog, 1962) nutrient salts with $B_5$ vitamins (Gamborg *et al*., 1968) were used for medium preparation. For multiple shoot induction, cytokinins like BAP and Kinetin were used. *In vitro* flowering was noticed when $GA_3$ used in combination with cytokinins. 3% sucrose was added with the medium and the pH was adjusted to 5.7 before the addition of 0.8% agar. The explants were aseptically inoculated on MS medium and

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cultured in 1500-lux light. The temperature was maintained as 25±2°C and 16/8 (day/night) hours photoperiod was maintained. For every 21 days, the explants were transferred to freshly prepared culture medium.

## RESULTS AND DISCUSSION

The shoot bud production observed after 5-6 days of inoculation from shoot tip and nodal segments. Maximum 4.56±0.098 shoots were produced from each explant in the medium supplemented with 4.44 μM concentration of BAP. Shoot bud production was ceased when the concentration of BAP exceeds 8.88 μM. At 2.22 μM of BAP concentration growth of single shoot observed (Table 1). *In vitro* flowering was observed from the terminus of the regenerated shoot tip in the medium consisted 4.44 μM of BAP with 4.33 μM of $GA_3$ (Table 2).

In present work maximum response for multiple shoot production was observed with BAP alone and this is in consistent with the work of Patil and Jayanthi (1997). However Faisal and Anis (2002) observed optimum response with 10 μM of BAP + 0.5 μM of NAA.

In present work *in vitro* flowering was obtained with BAP and $GA_3$ combination but Sarma *et al.* (1999) reported that BAP and Kinetin combination has given optimum response for *in vitro* flowering in *Rauvolfia tetraphylla* L. In *Murraya paniculata* (L.) Jack, BAP alone induced floral bud formation (Jumin and Ahmed, 1999). In all these experiments BAP have been used alone or in combination with other hormones for the induction of *in vitro* flowering and it may be playing a major role in floral bud formation and maturation. This is further supported by Sudhakaran and Sivasankari (2002) in *Ocimum basilium* L., Wang *et al.* (2001) in *Momardia charantina* L. and Thiruvengadam and Jayabalan (2001) in *Vitex negundo* L. The usage of $GA_3$ for *in vitro* flowering is supported by the work of Stephen and Jayabalan (1998) who observed maximum flowering response in *Coriandrum sativum* L. on SH medium supplemented with NAA and $GA_3$.

Table 1: Multiple shoot induction in *Rauvolfia tetraphylla* L.

| Hormone concentration (μM) | Percentage of response | No. of shoots produced |
|---|---|---|
| BAP | | |
| 0.444 | 56 | $1.56\pm0.120^{de}$ |
| 2.22 | 60 | $2.24\pm0.075^{bc}$ |
| 4.44 | 95 | $4.56\pm0.098^{a}$ |
| 6.66 | 87 | $2.00\pm0.140^{c}$ |
| 8.88 | 43 | $0.52\pm0.049^{ef}$ |
| KN | | |
| 0.464 | 62 | $1.54\pm0.248^{e}$ |
| 2.32 | 60 | $1.74\pm0.268^{d}$ |
| 4.64 | 73 | $1.80\pm0.089^{cd}$ |
| 6.96 | 87 | $2.38\pm0.276^{b}$ |
| 9.28 | 90 | $2.24\pm0.216^{bc}$ |

Total number of explants used for each concentration = 120, Each experiment repeated thrice, Values are presented as Mean±Standard Error Means within a row followed by the same letters are not significant at p = 0.05 according to DMRT

Table 2: *In vitro* flowering in *Rauvolfia tetraphylla* L.

| Hormone concentration (μM) | | Percentage of response | No. of Shoots produced | No. of flowers produced |
|---|---|---|---|---|
| BAP | $GA_3$ | | | |
| 4.44 | 0.289 | 45 | $2.22\pm0.218^{b}$ | - |
| 4.44 | 1.44 | 48 | $2.48\pm0.278^{a}$ | - |
| 4.44 | 2.89 | 63 | $2.24\pm0.730^{ab}$ | $1.62\pm0.24^{ab}$ |
| 4.44 | 4.33 | 82 | $1.52\pm0.020^{c}$ | $2.22\pm0.45^{a}$ |
| 4.44 | 5.78 | 54 | $0.89\pm0.710^{d}$ | $0.71\pm0.20^{c}$ |

Total number of explants used for each concentration = 120, Each experiment repeated thrice, Values are presented as Mean±Standard Error Means within a row followed by the same letters are not significant at p = 0.05 according to DMRT

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