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Influence of amino acids on i*n vitro* flowering of *Rauvolfia tetraphylla* L.

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ABSTRACT

Rauvolfia tetraphylla L. is an endangered medicinal plant, widely used in both traditional and modern medicine to cure various ailments. The flowers of *R. tetraphylla* are used in the treatment of asthma along with leaves by the tribes of Tamil Nadu. The present study was undertaken with an objective to study the effect of amino acids on flowering response in *Rauvolfia tetraphylla* L. The nodal explants were collected and tested for *in vitro* flowering response by using medium prepared with MS nutrient salts and B_5 vitamins with different concentrations of GA_3 along with 4.44 µM BA. BA at 4.44 µM and GA_3 at 4.441 µM concentration produced more flower buds than the other tested concentrations. Proline at 0.66 mM concentration, along with the above said phytohormones combination induced 5.24 floral buds per explant from the node explants with 78% response within ten days of culture. This protocol may help for the continuous supply of flowers that are useful in herbal formulation preparations, moreover, flowers are produced within a short period time.

Keywords: Proline, glutamine, lysine, Benzyl adenine (BA), Gibberellic acid (GA_3)

INTRODUCTION

Flowering is a complicated process, which is regulated by external and internal factors and plants flower only after attaining maturity. In many plants, *in vitro* flowering is normally achieved by the addition of exogenous hormones to the culture medium (Bodhipadma and Leung, 2003). Interaction of various physical and chemical factors is needed for *in vitro* flowering, where this technique can be used to produce specific compounds of commercial importance from the floral organs (Zeng et al., 2013). The interaction of exogenous and endogenous phytohormones is also important in flowering (Joshi et al., 2009). Cytokinins may affect the flower development by controlling the expression of genes of floral meristem (Vaz and Gilberto, 2008), where apical meristems are converted into floral meristems (Sri Rama Murthy et al., 2012). Also, Cytokinins play a key role in regulation of cell division and organ formation, which influences flowering (Sharma et al., 2014). Herbal medicines are widely used in developing countries to treat various health problems (Ouedraogo et al., 2023), which has been used by 70% to 95% population as raw or as herbal preparations (Deguenon et al., 2023).

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Rauvolfia tetraphylla L. is an important, endangered (Gulab et al., 2022) medicinal plant belonging to the family Apocynaceae. This plant is threatened because of its extensive exploitation for a variety of medicinal uses (Hoque et al., 2020). All parts of this plant such as root, root bark, leaf, stem, fruit, seed and whole plant is used for treating various ailments due to the presence of various alkaloids (Mahalakshmi et al., 2019). Medicinal uses of this plant include reduction in hypertension, treatment in poisonous bites and induction of sedation (Bhattacharjee, 2004). The leaves and flowers of this plant are consumed for the treatment of asthma by tribes of south India (Iqbal et al., 2013). Only the report of our group (Anitha and Ranjitha Kumari, 2006) and Sarma et al. (1999) are available on *in vitro* flowering in *R. tetraphylla* L., but further efforts were not made to enhance the flowering response. As stated earlier, the flowers are useful in the treatment of asthma, enhanced flower production may be exploited commercially in future in the manufacture of drugs. Biotechnology is a powerful tool to overcome natural barriers and also helpful in the production of contamination free plant organs (Nanti et al., 2023). Hence, the present study was undertaken with the objective to study the effect of amino acids on floral induction in *Rauvolfia tetraphylla* L. under *in vitro* condition.

MATERIALS AND METHODS

Node explants of field grown plants were collected from the medicinal plants garden, Bharathidasan University, Tiruchirappalli, Tamil Nadu and thoroughly washed under running tap water. The explants were surface sterilized by using the protocol of Anitha and Ranjitha Kumari (2006). In short, 70% ethanol treatment for 30 seconds followed by treatment with 0.1% mercuric chloride for four minutes was carried out. Sterile distilled water was used to wash the explants during and after surface sterilization. The sterile explants were grown on medium consisting of MS (Murashige and Skoog, 1962) salts with B_5 (Gamborg et al., 1968) vitamins, BA (Benzyl adenine) (4.44 µM) and GA_3 (Gibberellic acid) (2.316-5.019 µM), 30 g/l sucrose and 8 g/l agar. The medium pH was adjusted to 5.7 and sterilized at 121°C for 15 minutes. The cultures were exposed to 16/8 hr light/ dark condition by using cool white fluorescent tubes (40 µM $m^{-2}s^{-1}$) and 60-70% relative humidity at 25 ± 1ºC. Using the best responding hormone combination, the effect of amino acids on flowering were tested; amino acids such as lysine (0.17-0.68 mM), glutamine (0.17-0.68 mM) and proline (0.22-1.1 mM) were added in the medium to check their efficacy on flowering. The results were observed periodically and the data were statistically analyzed as follows; the experiments were conducted in randomized block design; 15 replicates were used in each treatment and each experiment was repeated thrice. Data were subjected to one-way ANOVA and comparison of means were carried out with DMRT at 0.05% significance level using SPSS software version 10.0 (LEAD Technologies Inc., Chicago, USA-1999).

RESULTS

In the present work, *in vitro* flowering was obtained with BA and GA_3 combination. Among the various concentrations tested, GA_3 at 2.316 µM to 5.019 µM along with 4.44 µM of BA induced flowering in the explants. BA at 4.44 µM with GA_3 at 4.441 µM concentrations induced maximum floral bud production (3.21) and the flowering response was 65% in node explants (Table 1). Proline at 0.66 mM concentration, along with the above said phytohormones combination induced 5.24 floral buds with 78% response. The amino acids, lysine and glutamine also induced the flowering response in 73% and 68% explants respectively (Table 2). The time taken for floral bud induction in phytohormones (BA+GA_3) and the phytohormones with lysine or glutamine added medium was observed after twenty days of culture (Figure 1 -A, B and C), whereas in proline added medium, flower buds were directly induced in many explants within ten days of culture (Figure 1-D).

Table 1: Effect of BA and GA_3 on *in vitro* flowering in node explants of *Rauvolfia tetraphylla* L. cultured on MS+B_5 medium.

Phytohormone concentration BA + GA_3 (µM)	Flowering response (%)	Mean no. of floral buds/ explant
4.44+2.316	30^{i}	1.00^{i}
4.44+ 2.607	37^{h}	1.2^{gh}
4.44+2.996	43^{g}	1.32^{g}
4.44+3.285	48^{ef}	1.51^{f}
4.44+3.574	50^{e}	1.87^{d}
4.44+3.863	55^{c}	2.12^{bc}
4.44+4.152	59^{b}	2.69^{b}
4.44+4.441	65^{a}	3.21^{a}
4.44+4.73	54^{cd}	1.76^{de}
4.44+5.019	40^{gh}	1.00^{i}

Data represents the observation made after 20 days of inoculation in respective treatment
Treatment means followed by different letters are significantly different from each other at P≤0.05 according to Duncan's multiple range test (DMRT).

Table 2: Effect of amino acids along with BA and GA_3 on *in vitro* flowering in *Rauvolfia tetraphylla* L. cultured on MS+B_5 medium.

Amino acids	BA (4.44 µM) + GA_3 (4.441 µM) + Amino acid (mM)	Flowering response (%)	Mean no. of floral buds/ explant
Lysine	0.17	70^{bc}	2.68^{f}
	0.34	73^{b}	3.3^{d}
	0.51	60^{d}	3.0^{e}
	0.68	45^{f}	2.22^{hi}
Glutamine	0.17	68^{c}	2.3^{h}
	0.34	55^{e}	2.72^{ef}
	0.51	45^{f}	2.5^{g}
	0.68	20^{g}	2.42^{gh}

	0.22	68[c]	3.28[de]
	0.44	70[bc]	4.65[b]
Proline	0.66	78[a]	5.24[a]
	0.88	75[ab]	4.0[c]
	1.10	70[bc]	2.62[fg]

Data represents the observation made after 20 days of inoculation in respective treatment
Treatment means followed by different letters are significantly different from each other at P≤0.05 according to Duncan's multiple range test (DMRT).

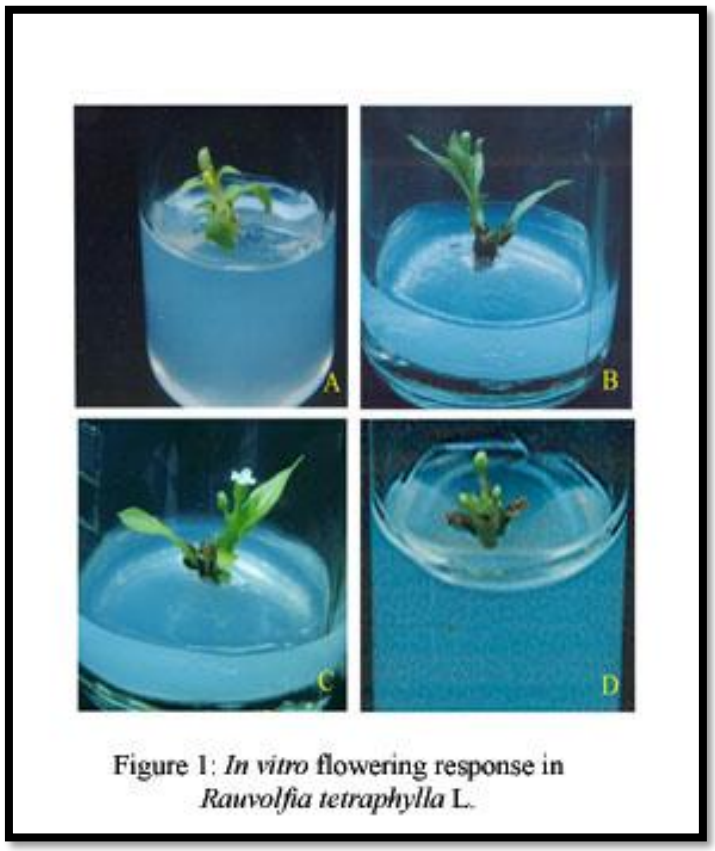

Figure 1: *In vitro* flowering response in *Rauvolfia tetraphylla* L.

A- Flower bud induction in node explant, B- Flower bud production in Glutamine supplied medium, C- Flowering in Glutamine supplied medium (after 25 days of culture), D- Flower bud production in Proline supplied medium (after 10 days of culture).

DISCUSSION

BA at 4.44 µM concentration with GA_3 at 4.441 µM induced maximum flowering response in node explants in the present experiment (Table 1). Consistent to present work, John Britto *et al*. (2003) noticed *in vitro* flowering response in *Ceropegia bulboa* with BA and GA_3 combinations. Though Sarma et al. (1999) observed flowering response in *R. tetraphylla* grown in the medium containing BA and KN, they reported that the response was poor. Lin et al. (2003) noticed that when

Ginseng (*Panax ginseng*) buds were incubated in B_5 medium supplemented with 1 mg/l BA and 1 mg/l GA, new inflorescences developed from the base of the explants. BA was reported to be superior for *in vitro* flower induction in *Swertia chirayita* (Sharma et al., 2014). BA along with casein hydrolysate was used for flowering *in vitro* in maize varieties (Joshi et al., 2009). In the present experiment BA and GA_3 combination was essential for flower bud production (Table 1). BA, when used alone, induced multiple shoot production without any flower bud production in *Rauvolfia tetraphylla* L. (Anitha and Ranjitha Kumari, 2006: Anitha et al., 2023). Yu et al. (2004) suggested that GA regulates the late-stage development of floral organs after the establishment of their identities within floral meristems. BA rich medium is reported to induce active cell division and morphogenic response (Kone et al., 2022). Addition of proline at 0.66 mM concentration, along with BA and GA_3 induced 5.24 floral buds in 78% explants (Table 2). Similarly, Virupakshi et al. (2002) observed enhanced flowering response in sugarcane with addition of 40 mg/l proline. Amino acids are the precursors of various metabolic pathways and thus their addition may be enhanced the physiological response of the explants.

Conclusion

In conclusion, the present study reported induction of flowering response in the node explants of *R. tetraphylla*. The flowering response was higher in the explants cultured on BA (4.44 µM) + GA_3 (4.441 µM) supplied MS+B_5 medium. Proline at 0.66mM concentration induced more flowering response when added with the above said medium within a short period of time (10 days.). As flowers of *R. tetraphylla* are used in the treatment of asthma, it's *in vitro* production at a very short duration is valuable for its continuous supply. Further studies may be conducted to elucidate the precise role of amino acids played in inducing flowering response.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

SA conducted the experiment and compiled all results, prepared this manuscript; GS gave suggestions in conducting the experiment and carried out corrections to improve this manuscript; BDRK Designed the experiment and guided in all processes.

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