In vitro shoot response of *Rauvolfia serpentina* to the type and concentration of cytokinin

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Abstract.. *Rauvolfia serpentina* is widely recognized for its use as a raw material in hypertension and antihypertensive medications, including reserpine. Since this plant is used directly from the natural world, cultivation activities are necessary. Seeds for cultivation must be consistent, high-quality, and free of pests and diseases. Thus, a suitable propagation technique is required. In vitro propagation is one method that can produce homogeneous plants with a relatively high rate of multiplication. Cytokinin-family regulatory molecules are crucial for in vitro proliferation techniques. The aim of this research was to determine the optimal type and concentration of cytokinin for the in vitro induction of *R. serpentina* shoots. This study employed a completely randomized factorial design. The first factor was the type of cytokinin (Benzylaminopurine (BA), Zeatin, Kinetin, and 2iP), and the second factor was the cytokinin concentration (0, 0.5, 1.0, 1.5 mg/l). Each treatment was replicated 10 times. The results showed that the best cytokinin for *R. serpentina* shoot induction in vitro was BA at a concentration of 0.5 mg/l. This treatment produced a greater number of shoots and leaves, taller shoots compared to other treatments, and resulted in more well-developed plant visualization.

1 Introduction

Rauvolfia serpentina belongs to the family Apocynaceae and is a potential medicinal plant for development because it is used as raw material for pharmaceuticals. This is due to the fact that *R. serpentina* contains 21 types of alkaloids, including reserpine, rescinnamine, deserpidine, serpentine, yohimbine, and ajmaline, which can be used as treatments for high blood pressure, as tranquilizers, and for circulatory system disorders [1].

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R. serpentina is utilized in traditional medicine for treating conditions such as shortness of breath, stomach pain, dysentery, headaches, and snake bites. It can also be used to reduce fever, lower high blood pressure, and treat dysentery, cholera, loss of appetite, intestinal inflammation, and more [2][3][4].

The amount of *R. serpentina* simplicia used domestically was 6,898 kg in 2000, representing a 25.89% yearly growth [5]. Because *R. serpentina* is taken straight from the wild, it is regarded as rare. The species is included in Appendix II of CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora), which lists species that are not currently threatened with extinction but may do so if trade is not regulated. The species is listed as threatened by the International Union for Conservation of Nature (IUCN). The fact that roots of *R. serpentina* are employed as medicinal materials adds to its rarity by making ordinary propagation difficult and restricting its range [6].

Conventional propagation of *R. serpentina* is very limited, with seed germination and stem cutting percentages both below 15%. The stiff seed shell is the cause of the low growth percentage and extremely limited capacity for seed germination.. This makes seed propagation of *R. serpentina* suboptimal. To balance the demand for *R. serpentina* simplicia and prevent its extinction, conservation and cultivation efforts are necessary. Therefore, mass production of seedlings is needed. In vitro culture is one potential technique that can generate a lot of seedlings in a short amount of time [[7].

In vitro culture is a technique for growing plant parts such as protoplasts, cells, tissues, or organs in a suitable medium under aseptic conditions [8]. Growth regulators are one of the factors that affect the success of tissue culture. Plant tissues' biological processes are largely regulated by growth regulators [9]. The chemical molecules known as growth regulators are non-nutritive substances that, when present in small amounts, can promote the growth and development of plants and are crucial in improving the metabolism of explants [9].

One commonly used group of growth regulators in tissue culture is cytokinins. The cytokinins commonly used in tissue culture include 2-IP (2-Isopentenyl adenine), zeatin, BA (benzil adenin), and kinetin (6-furfurylaminopurine). Generally, cytokinins function in cell division, cell enlargement, delaying flower and fruit aging, and root and shoot differentiation [10]. This description serves as the basis for the study's investigation of the impact of various cytokinin formulations on the in vitro growth and provision of seedlings for *R. serpentina* (L.) Benth. ex Kurz explants. Finding the right cytokinin type and concentration for *R. serpentina* in vitro shoot induction is the aim of this study.

2 Methodology

The plant material used in this study was in vitro shoots of *R. serpentina* that were cultivated for 14 days on MS (Murashige and Skoog) medium without the use of growth regulators. A factorial totally randomized design with two components was employed in this investigation. Two factors were identified: the kind of cytokinin (BA, Zeatin, Kinetin, and 2iP) and its concentration (0, 0.5, 1, and 1.5 mg/l). There were ten duplicates of each therapy. The pH of each treatment medium was brought down to 5.8 and added 30 g/l sucrose and 2.5 g/l gelrite as supplements. Subculture on the same media was carried out after the plants were 2 weeks old.

The number of roots, number of leaves, shoot height (cm), and number of shoots were the variables that were observed. After the second subculture or during the fourth week, observations were made. An F-test was used at a 5% significance level to statistically examine the data gathered from observations of *R. serpentina* explants.

3 Results and Discussion

Generally, the plant growth response to cytokinin treatment was observed through changes in plant height and the emergence of new shoots. Each treatment produced different responses across the various observed parameters.

3.1 Number of Shoots

The number of shoots was observed in the fourth week after culture. This measure is crucial because the number of shoots produced indicates the success of the multiplication activity. According to the ANOVA results, the interaction between the type and concentration of cytokinin had a significant effect on the number of shoots. Table 1 shows that increasing the concentration of cytokinin resulted in an increased number of shoots. The best treatment was BA 0.5 mg/l, which produced 4.3 shoots, more than any other treatment. Table 1 also indicates that BA produced more shoots than Kinetin, Zeatin, and 2iP. A synthetic cytokinin made from adenine, BA is very effective at encouraging the growth of new shoots. Chlorophyll production, tissue and organ differentiation, cell division, and other physiological responses are all influenced by BA [11]. Stronger than other cytokinins like kinetin or 2-iP, the BA is a cytokinin that plays a major role in shoot development and multiplication [12].

Table 1. The effect of cytokinin type and concentration on the number of shoots in *R. serpentina* cultur

Concentration of growth regulators (mg/l)	Types of growth regulators			
	BA	Zeatin	Kinetin	2IP
0	1.3a	1.1a	1.1a	1.1a
0.5	4.3d	3.5c	1.4a	1.2a
1	3.5c	2.5b	1.9ab	2.1b
1.5	2.5b	2.5b	2.9bc	2.4b

There were 3.5 and 2.5 fewer shoots when the concentration of BA was increased to 1 mg/l and up to 1.5 mg/l, respectively. This also happened when zeatin was administered; at 0.5 mg/l, zeatin produced 3.5 shoots, but as the concentration of zeatin increased, fewer shoots were produced. Because high quantities of cytokinins can interfere with food absorption and decrease explant growth, it appears that employing growth regulators at higher concentrations inhibits shoot growth (Kusmianto, 2008).

3.2 Plant Height

Because of the nutrients in the medium, the shoots are growing and developing, as seen by the increase in shoot height. The measurement of shoot height serves as a gauge for the growth that the administered treatments have produced. During the in vitro culture phase, the growth rate pattern can be explained by the increase in shoot height.

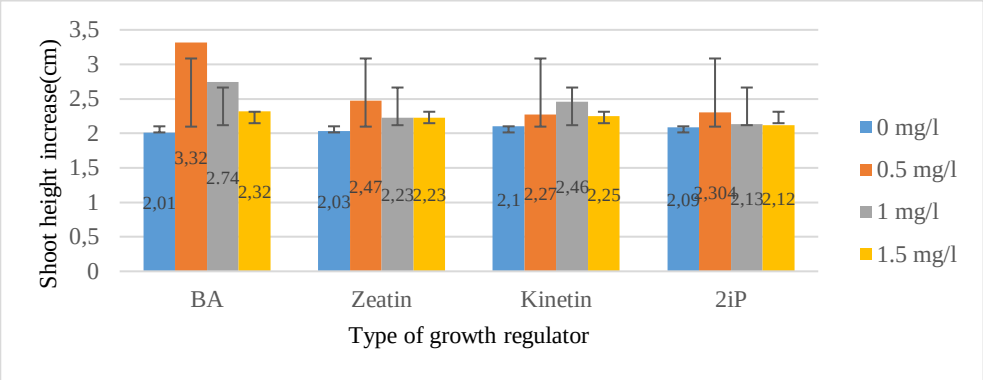

Fig. 1. The effect of cytokinin type and concentration on the shoot height of *R. serpentina* Culture

When compared to other treatments, the BA therapy often had the most positive outcomes. Because cytokinins and auxins work synergistically as growth regulators, using them in the same treatment media can promote shoot proliferation [14] [15].

The BA 0.5 mg/l treatment provided the best response in terms of plant height. The shoot height produced with BA 0.5 mg/l treatment was 3.32 cm (Figure 1). Treatments with Zeatin, Kinetin, and 2iP showed similar plant heights across various cytokinin concentrations. However, compared to the treatment without cytokinin, the addition of cytokinin resulted in taller shoots. By encouraging cell division and preventing elongation, cytokinins can promote shoot growth, resulting in comparable shoot lengths for the three cytokinin types [16][17] .

3.3 Number of Leaves

The development of leaves is crucial because the leaf axils are where new shoots will sprout. After four weeks of culture, leaves may grow on all treatments. Table 2 demonstrates that the number of leaves was significantly impacted by the interaction between the type and concentration of cytokinin. Out of all the treatments, the BA 0.5 mg/l treatment produced the highest yield of 11.0 leaves and was found to have a noteworthy impact. Other treatments with cytokinin produced less leaves at the same concentration. This suggests that BA offers a good leaf-formation-inducing reaction. The BA treatment produced more leaves than the kinetin treatment did in Photos tener, demonstrating another instance of this [18].

Table 2. The effect of cytokinin type and concentration on the number of leaves in *R. serpentina* culture

concentration of growth regulators (mg/l)	Types of growth regulators			
	BA	Zeatin	Kinetin	2IP
0	4.0a	4.17a	4.11a	4.15a
0.5	11.10f	6.80c	6.11c	5.11b
1	9.10e	5.80b	5.50b	4.70a
1.5	8.11d	5.20b	5.80b	6.40c

Compared to other treatments, the BA 0.3 mg/l treatment produced more leaves. There was less leaf formation when the BA concentration was raised to 0.5 mg/l. Table 2 demonstrates that 8.11 leaves were produced as a result of the BA 1.5 mg/l treatment. The response to other cytokinin therapies was comparable. For instance, there were nine leaves

when the concentration of zeatin was 1.5 mg/l; but, when the concentration was raised to 1.5 mg/l, there were only 5.2 leaves. Plant growth is inhibited by high growth regulator concentrations.

3.4 Number of Roots

For in vitro plant micropropagation to be successful during the acclimation stage, high-quality root formation is essential. Figure 2 illustrates that fewer roots are seen at greater cytokinin concentrations. The treatment without cytokinin had the greatest number of roots.

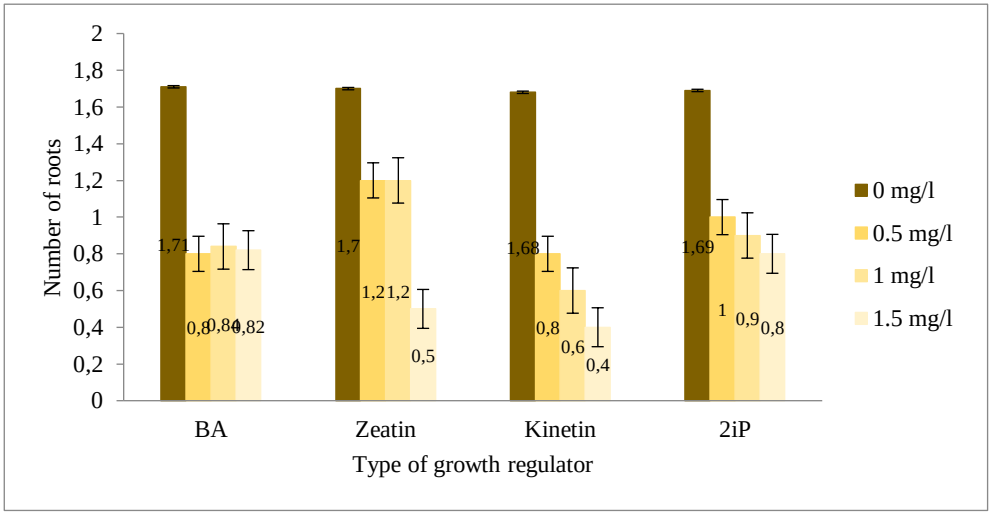

Fig. 2. The effect of cytokinin type and concentration on the number of roots of *R. serpentina* Culture

The number of roots significantly decreases as the concentration of BA increases, as seen in Figure 2. Treatments with zeatin, kinetikin, and 2iP have the same result. The number of roots decreases as Zeatin, Kinetin, and 2iP concentrations are increased. The correct ratio of auxin to nutrients determines the production of roots [19][20] [21]. Apart from the impact of exogenous auxin, genetic variations resulting from the utilized explants and their native cytokinin concentration also have an influence.

4 Conclusion

Equations From the research conducted, it is clear that BA, at a concentration of 0.5 mg/l, is the ideal cytokinin for shoot induction. Compared to previous treatments, this one produced more leaves and shoots that were both taller and more numerous. Increasing the cytokinin concentration can prevent leaves and shoots from developing. The application of cytokinin can also inhibit root growth, with the highest number of roots forming in treatments without cytokinin.

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