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# The influence of cytokinins on proliferation and polyphenol accumulation in shoot cultures of *Scutellaria altissima* L.

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ABSTRACT

*S. altissima* shoots were cultivated on MS (Murashige and Skoog) solid medium supplemented with IAA (indole-3-acetic acid, 0.57 μM) and various concentrations of cytokinin: zeatin, kinetin, BAP (6-benzylaminopurine) (1, 2, 4, 8 μM) or TDZ (thidiazuron) (0.2, 0.5, 1 μM). The effects on shoot proliferation and their accumulation of pharmacologically valuable phenolic compounds (baicalin, wogonoside, luteolin, luteolin-7-*O*-glucoside, verbascoside) were evaluated. Ultra-high performance liquid chromatography (UHPLC) was used for quantitative analysis of the compounds accumulated in the plant biomass. The metabolites were identified by comparing their retention time, UV spectra and mass spectra with those of the standard compounds and published data. The highest metabolite contents were recorded in shoots treated with TDZ at a concentration of 1 μM; under these conditions, the total level of all evaluated flavones expressed as the sum of baicalin, wogonoside, luteolin and luteolin-7-*O*-glucoside (17.35 mg/g dry wt) was more than twice that of those grown on MS cytokinin-free medium (7.55 mg/g dry wt). Verbascoside accumulation was also stimulated by 1 μM TDZ; its level was about six times higher than that found on the control medium (6.2 mg/g dry wt *vs.* 1.03 mg/g dry wt). The highest number of shoots (5.5–6.4 per explant within five weeks) was achieved with 2–8 μM BAP.



## 1. Introduction

The genus *Scutellaria* (Lamiaceae) comprises over 350 species, distributed across East Asia, Europe and the United States. *Scutellaria* plants have been used for hundreds of years as culinary herbs, garden flowers and medicinal plants for the treatment of allergy, inflammatory diseases, hyperlipidemia, arteriosclerosis, hypertension and hepatitis (Shang et al., 2010). One such *Scutellaria* species is *S. altissima,* commonly known as tall skullcap (Beshko et al., 1975). The plant grows in the mountain regions of South Europe and East Asia. It is a perennial herb with a 80–100 cm stem that flowers in June-August (Tutin et al., 1972). Its corolla is bluish or violet, but often with whitish lower lip. *S. altissima* has been used as medicinal plant in Turkey (Davis, 1982).

The properties of *Scutellaria* plants are mainly due to the presence of flavones such as baicalin, baicalein, wogonoside and wogonin, which exhibit pharmacologically and clinically important profiles (Shang et al., 2010). The ability of baicalin to protect hepatocytes against damage has been confirmed in numerous experimental models of liver inflammation induced by acetaminophen, carbon tetrachloride, lipopolysaccharide and D-galactosamine (Kim et al., 2010). The compound has also shown antiallergic properties and a scavenging effect in protecting the membrane of erythrocytes from free radical injuries (Gao et al., 1999). Extracts containing 21% baicalin inhibited the growth of lymphocytic leukemia cells, lymphoma and myeloma, and caused apoptosis in all tested cell lines (Kumagai et al., 2007). The flavone also markedly decreased levels of TNF-α (tumor necrosis factor-α) prevent viral infections. Baicalin has also shown antiviral activity in relation to HTLV-1 (human T-cell leukemia virus), and the mechanism of this action may rely on the *in vitro* inhibition of the retroviral reverse transcriptase activity (Li-Weber, 2009). Kitamura et al. (1998) have documented that baicalin inhibits HIV-1 infection and replication by inhibiting HIV-1 reverse transcriptase. Another flavone glycoside isolated from *Scutellaria* genus, wogonoside, has strong activity against lipid peroxidation and an inhibitory effect on histamine and IgE production (Lim, 2003). The compound can inhibit lipopolysaccharide-induced angiogenesis (Chen et al., 2009). In addition, the phenylethanoid glycosides verbascoside and martynoside have been isolated from some species of *Scutellaria.* Verbascoside has shown strong anti-inflammatory, antibacterial, antiviral, antioxidant and cytotoxic activity (Alipieva et al., 2014). Besides flavonoids and

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phenylethanoids, *S. altissima* contains diterpenoids with antifeedants and antifungal properties (Bruno et al., 1996).

Plant tissue culture represents a valuable alternative method for the micropropagation and production of therapeutically important products. Many examples are given in the literature for various bioactive metabolites, whose contents can be greater in tissue cultures than in wild plants (Stancheva et al., 2011; Döring and Petersen, 2014; Szopa and Ekiert, 2015).

Our earlier studies have described the successful establishment of shoot regenerating calli, shoot culture and whole plants of *S. altissima* (Grzegorczyk-Karolak et al., 2013, 2015a). The shoots cultivated on MS (Murashige and Skoog, 1962) medium supplemented with 0.57 μM IAA and 2 μM BAP produced important secondary metabolites (baicalin, wogonoside, luteolin, luteolin-7-*O*-glucoside, verbascoside), but their amounts were significantly lower in the shoots than in the organogenic callus. To enhance the biosynthesis of the metabolites, in the present study the influence of different cytokinins (BAP, zeatin, kinetin, TDZ) on *S. altissima* shoot proliferation and their production of polyphenolic compounds was evaluated.

The multiplication of shoot cultures of various plant species, and their capacity to produce secondary metabolites, varies depending on the type and concentration of cytokinin applied. However, no studies on the effects of cytoninin on tall skullcap cultures have yet been conducted. The aim of the present study was to develop an *in vitro* system of *S. altissima* which could contain high levels of bioactive metabolites. The polyphenol content of the shoot culture was determined by UHPLC examination, and the method was validated as part of the study.

## 2. Results and discussion

### 2.1. Effect of cytokinins on shoot propagation and biomass production

The effect of the cytokinins BAP, kinetin, TDZ and zeatin on axillary shoot proliferation, dry biomass and shoot morphology was evaluated after 5 weeks of culture. For all types and concentrations of cytokinins (except kinetin at the lower concentration of 1–4 μM), the mean number of *S. altissima* axillary shoots was higher than that observed on the control MS medium supplemented only with 0.57 μM IAA (1.3 per explant) (Fig. 1). It was also observed that MS medium devoid of exogenous growth regulators (both auxin and cytokinin) was ineffective for shoot induction and growth. The most effective cytokinin for shoot propagation was BAP. The highest mean number of shoots (6.4 per explant) was induced by BAP at 2 μM. In the presence of higher concentrations of the cytokinin (4–8 μM), the multiplication rate of *S. altissima* was also high: more than five shoots per explant. However, higher BAP concentrations resulted in hyperhydricity syndrome. Axillary shoots grown in the presence of 1 and 2 μM BAP were good quality without hyperhydric morphology, while 1.2% of multiplied shoots grown on media with 4 μM BAP, and 4.6% with 8 μM BAP, were hyperhydrated. In the presence of other cytokinins (zeatin, kinetin, TDZ), the multiplication rate of *S. altissima* shoots was lower and ranged from 1.3 for kinetin at a concentration of 1 μM–5.6 for 8 μM zeatin (Fig. 1).

Also for *Scutellaria integrifolia* (Joshee et al., 2007) and *S. alpina* (Grzegorczyk-Karolak et al., 2015b), the highest multiplication ratio was observed for shoots on MS medium supplemented with 2 μM BAP, while higher concentration of BAP (4.4 μM) has been reported to be more efficient for *S. discolor* shoot propagation (Sinha et al., 1999). Also TDZ has been used effectively in few *in vitro* cultivated *Scutellaria* species; however, this has been more for inducing shoot regeneration from callus than propagation from existing meristems (Li et al., 2000; Ozdemir et al., 2015; Grzegorczyk-Karolak et al., 2013). In our study, TDZ was not found to be valuable for *S. altissima* proliferation, giving only 3.9 shoots per plant when applied at 0.5 μM in the medium and 3.3 when 1 μM was used; in addition, more than 20% of the shoots obtained on these media were hyperhydric. Huetteman and Preece (1993) recommend the use of a lower concentration of TDZ, a non-purine cytokinin (0.001 μM–10 μM), than conventional cytokinins for shoot proliferation, as the use of higher levels of thidiazuron causes abnormalities or toxic effects.

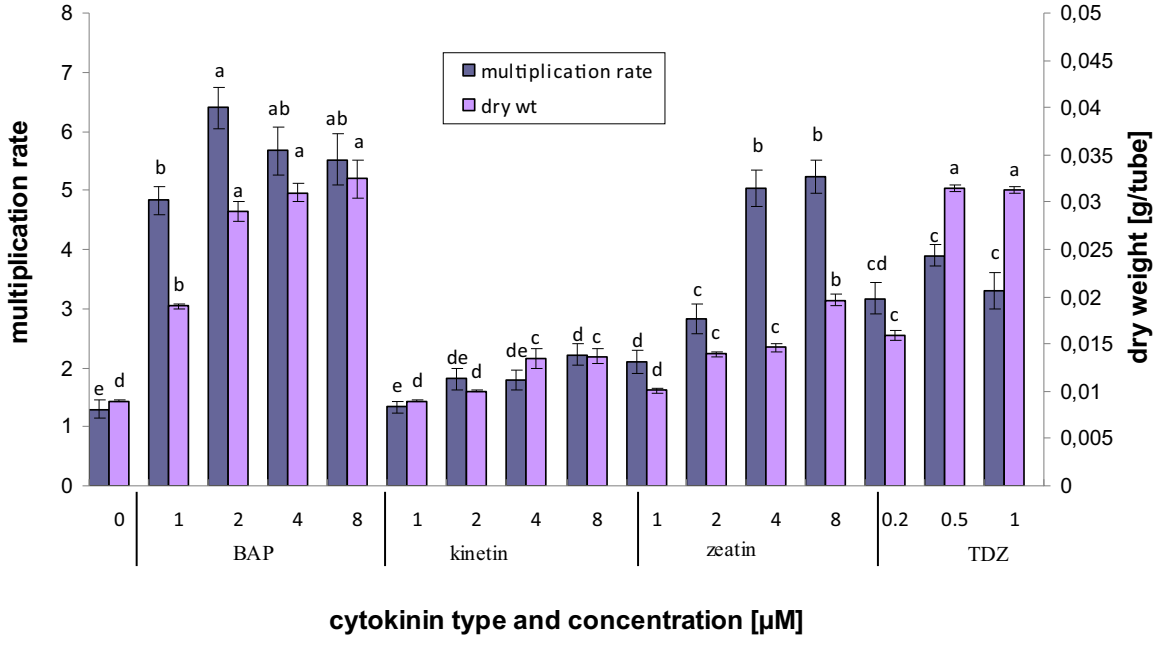


**Fig. 1.** The effect of various cytokinins and their concentrations on shoot proliferation of *S. altissima* and their biomass (dry wt) after five weeks of culture on MS medium with IAA (0.57 μM).
The results are expressed as means of three replicates ± SE. The means with the same letter for the same parameter do not differ significantly according to the Kruskal-Wallis test ($p \leq 0.05$).

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