Enrichment of *Solanum khasianum* Callus Generating Rootlets with Steroidal Glycoalkaloids

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Undifferentiated callus cultures of *Solanum khasianum* contain only traces of steroidal glycoalkaloids, whereas cultures which have just started to generate rootlets contain these compounds at a level of up to 5.2% of the dry weight. Only 0.34% steroidal glycoalkaloids occur in the seeds; they consist predominantly of solasonine and solanidine as well as traces of an unidentified compound. Conversely, steroidal glycoalkaloids from tissue cultures starting organogenesis contain the unidentified compound as a major fraction, in addition to small amounts of solasonine and solanidine.

Introduction

Steroidal glycoalkaloids are gaining importance in view of their suitability as substitutes for diosgenin in the synthesis of steroid hormones [1]. Such alkaloids occur in intact plants belonging to several species of the genus *Solanum*, viz. *S. khasianum*, *S. aviculare*, *S. laciniatum* and *S. xanthocarpum* [2–6].

Recently, a few studies have been published on steroidal glycoalkaloids in tissue cultures of *Solanum spp.* Thus, Heble *et al.* [7, 8] have identified solasonine, β-sitosterol and diosgenin in tissue cultures of *S. xanthocarpum.* The same authors have studied the influence of different plant hormones on the cultures and reported changes in steroidal contents indicating biochemical regulation by auxins [9]. Kadkade and Madrid [10] have identified solasodine, solasonine and solamargine in callus cultures of *S. acculeatissimum.*

It is well known that tissue cultures are much less active than intact plants with regard to the synthesis of secondary metabolites. Hence, it is certainly of interest to study whether or not cultures which have started organogenesis show higher biosynthetic activities than undifferentiated cultures. In the present communication, we describe the establishment of callus cultures from seedlings of *S. khasianum*, as well as the generation of organs in these cultures. The contents and patterns of steroidal glycoalkaloids in undifferentiated cultures and in those starting organogenesis, as well as in seeds of this plant are compared.

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Materials and Methods

Callus cultures of *S. khasianum* were initiated from sterile seedlings on B_5-medium [11] as well as MS-medium [12]. The cultures were incubated at 30 °C in the dark, and were transferred onto fresh media every 2–3 weeks. For inducing organogenesis, calli of the third generation were transferred onto B_5-medium [12] which was modified by supplementing it with casein hydrolyzate; 3 g/l, yeast extract; 2 g/l, and higher levels of sucrose, 40 g/l. Organogenesis started after 5 to 8 weeks incubation at 30 °C in the dark.

Undifferentiated calli, as well as calli which had generated rootlets, 3–5 mm long, were harvested and their steroidal glycoalkaloids were extracted [13] and weighed. Steroidal glycoalkaloids of the seeds were also determined. The compounds were resolved by TLC on Silica Gel G (E. Merck, Darmstadt, Germany) using chloroform-methanol, 19 : 1, by vol., as solvent [10]. The fractions were detected by spraying the chromatograms with 50% aqueous H_2SO_4 and heating at 120 °C for 5–10 minutes whereby characteristic red to purple colors developed. Detection was also done by spraying the plates with Clarke's reagent [14]. The individual steroidal glycoalkaloids or aglycones were identified by comparing their chromatographic behavior to that of authentic samples. The compounds were isolated by preparative TLC on 0.5 mm layers, their melting points were determined and their infra-red spectra were recorded and compared to the spectra of authentic samples.

Results and Discussion

The initiation of callus cultures from seedlings of *S. khasianum* is faster and the growth is more vigorous on B_5-medium than on MS-medium. When calli of the third generation are transferred onto B_5-medium, supplemented with casein hydrolyzate, yeast extract and higher levels of sugar, the undifferentiated tissues start to generate organs after 5–8 weeks.

Table I. Steroidal glycoalkaloids in seeds and callus cultures of *Solanum khasianum*.

	Seeds	Undifferentiated cultures	Cultures generating rootlets
Total alkaloids *	0.34%	trace	5.2%
Major compounds	solasonine, solanidine		unidentified
Minor compounds	unidentified		solasonine, solanidine

* Values are expressed in % dry weight

At first, rootlets develop and become covered densely with root hairs; and at later stages shoots are generated. Thus, complete plants are produced from callus cultures.

It is interesting that cultures of *S. khasianum* can generate organs in the absence of supplemented cytokinetins which are known to be essential for organogenesis, particularly shoot generation in cultures of other plants [15]. Probably, cultures of *S. khasianum* synthesize adequate levels of cytokinetins and hence, do not need any external source of the compounds for generating organs.

Undifferentiated callus cultures of *S. khasianum* contain only traces of steroidal glycoalkaloids (Table I). This result agrees with the well established fact that cell cultures frequently lack secondary metabolites which are synthesized in the intact plants. On the other hand, callus cultures which have started to generate rootlets contain up to 5.2% steroidal glycoalkaloids. This value is higher than that found for the seeds (Table I) and is comparable to the value reported for mature berries of the same plant [6]. In this context, it is to be noted that, with the onset of organogenesis enrichments were recorded in levels of erucic acid [16], pyrethrins [17] and α-solanine and α-chaconine [18] in cell cultures of *Brassica napus*, *Tanacetum cinerarifolium* and *Solanum tuberosum*, respectively. It is thus apparent that the morphological differentiation in cell cultures is associated with biochemical "differentiation".

Thin-layer chromatography of steroidal glycoalkaloids and aglycones from seeds and differentiating cultures of *S. khasianum* reveals the presence of three fractions. We have identified solasonine (m. p. 279–284 °C) and solanidine (m. p. 216–218 °C) after they had been separated by preparative TLC and crystallized as needles from aqueous methanol and chloroform, respectively. The identity of these compounds was confirmed by comparing their infra-red spectra to those of authentic samples. The third fraction could not be identified. Its solubility characteristics and migration rates during chromatography were similar to those known for steroidal glycoalkaloids. Moreover, it gave the reactions characteristic of steroidal glycoalkaloids on spraying the chromatograms with sulfuric acid and with Clarke's reagent [14]. Solasonine and solanidine are predominant in seeds of *S. khasianum* (Table I), whereas the unidentified glycoalkaloid occurs only in traces. Conversely, in callus cultures starting to generate rootlets, the unidentified compound predominates, whereas solasonine and solanidine are present only at low levels.

The results of the present communication and of related earlier studies [16–18] show clearly that, in plant cell cultures, the onset of organogenesis should be considered as one of the tools for stimulating the biosynthesis of secondary metabolites.

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