

## Production of $\beta$ -Carboline Alkaloids in Transformed Root Cultures of *Peganum harmala* L.

I. N. Kuzovkina, A. Gohar, and I. E. Alterman  
K. A. Timirjazev Institute of Plant Physiology, U.S.S.R.  
Academy of Science, Botanicheskaya 35,  
Moscow 127 276, U.S.S.R.

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*Peganum harmala*, Hairy Root Cultures,  $\beta$ -Carboline Alkaloids

Hairy root cultures of *Peganum harmala* were established by genetic transformation with *Agrobacterium rhizogenes* strain A4. In contrast to suspension cultures the root cultures contained high levels of  $\beta$ -carboline alkaloids (1–1.5% of dry mass).

The expression of secondary pathways in plant cells is often closely related to and dependent upon morphological differentiation. Consequently, morphologically undifferentiated cell cultures of higher plants produce sometimes low levels or even lack desired products. For example,  $\beta$ -carboline alkaloid formation in cell suspension cultures of *Peganum harmala* was lost in long term cultures [1] and was only inducible in rather young, somehow differentiated cell cultures [2]. Heterotrophic rather than photoautotrophic cell cultures were able to synthesize small amounts of harmane alkaloids [3]. Indeed, Gröger [4] had suggested that roots of *P. harmala* should be the tissue where the harmane alkaloids are formed. Root cultures of most dicots are nowadays rather easy to establish by transforming plant tissue with *Agrobacterium rhizogenes*. In addition, it has been shown that these root cultures may contain a similar spectrum of secondary metabolites as roots of the intact plant [5, 6]. We describe here that hairy root cultures of *P. harmala* are indeed able to synthesize appreciable amounts of  $\beta$ -carboline alkaloids.

## Materials and Methods

Two-month-old seedlings of *P. harmala* grown under sterile conditions were infected with *Agrobacterium rhizogenes* strains (A4, 15834 or 8196,

respectively). After formation of roots at the sites of wounding, the stem segments with the roots were cultured on phytohormone free Murashige-Skoog (MS) medium [7] supplemented with 500 mg claforan<sup>R</sup>/l (Hoechst/Glaxo; cefotaxime-Na). After removal of the bacteria from the root culture by the antibiotic, the roots were detached and subcultivated on antibiotic free MS-medium. Every 3 weeks roots with the best lateral growth were transferred to fresh medium.

For the identification of the alkaloids 500 mg freeze dried cells were repeatedly extracted with MeOH. The combined extracts were concentrated and chromatographed on silica gel plates with CHCl<sub>3</sub>:MeOH:25% NH<sub>4</sub>OH 4:1:0.1. The fluorescent zones having the same  $R_f$ -values as the authentic alkaloids (harmane, harmaline, harmol, harmalol from Sigma) were scraped off and eluted with MeOH. The absorption and the fluorescence spectra of the eluted compounds was identical to those of the authentic material. Quantification of the main alkaloids in the eluates were determined by calibrations curves at 243 nm (harmane) and 390 nm (harmalol).

## Results and Discussion

Of the three different *A. rhizogenes* strains only A4 produced roots at the wounding sites. Among 30 wounded plants 15 showed after 2 weeks first callus like structures from which during the next 2 weeks roots appeared. Thus 4 weeks after wound infection the seemingly transformed roots were placed on phytohormone free nutrient agar containing the antibiotic claforan<sup>R</sup>. After two passages of 3 weeks on the antibiotic containing medium, most roots could be maintained in the absence of claforan<sup>R</sup> when no regrowth of bacteria was observed. In the presence of claforan<sup>R</sup> the roots were rather short and thick and growth was low. In the absence of the antibiotic longer and branched roots emerged. Growth of the roots was better on a filter paper above viscose wool embedded in liquid MS-medium than on a corresponding agar medium. For improving the growth of the transformed root cultures further, nitrogen levels of the medium were reduced. Indeed MS-medium with 1/2 of the nitrogen level resulted in thinner roots and better growth. Thus the maintenance medium was changed into MS-medium with the KNO<sub>3</sub> and

Requests for reprints to Dr. I. N. Kuzovkina.

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$\text{NH}_4\text{NO}_3$  concentrations reduced by 50%. However, growth rates of the root cultures varied depending on the piece of root used as inoculum. Pieces with many lateral roots showed up to 20-fold increases of fresh weight within 3 weeks.

The pattern of adventive root formation, the permanent and good growth in the absence of phytohormones and the deviation from geotropical orientation were reasonable indications that transformed root cultures were obtained. Appearances of roots isolated from plants or of roots induced from callus cultures were quite different when cultured on phytohormone free medium, and they died after the second or third passage on phytohormone-free medium. In addition, Dr. Ondjei detected recently mannopine in the transformed root cultures. Thus the transformation of the roots by the *A. rhizogenes* A4 strain seems to be clear without having performed hybridization experiments.

All roots showed the fluorescence typical for  $\beta$ -carboline alkaloids (harmine/harmalol) when viewed at 365 nm under UV-light. TLC analysis showed that harmine (blue fluorescent) is the main alkaloidal constituent, followed by harmalol. Harmol and to a lesser extent harmaline were also always present. Some quantitative data of various passages of the best growing root culture are shown in the Table. There are indications that the appearance of the roots and the alkaloid levels are somehow related. The short and thick roots contained less  $\beta$ -carboline alkaloids, while the thinner and longer roots, formed in the presence of reduced nitrogen levels, yielded distinctly higher alkaloid yields. Under these conditions the total

Table.  $\beta$ -carboline alkaloid levels of a transformed root culture of *Peganum harmala* after three weeks cultivation on MS-medium (6th, 7th passage) or on MS-medium with 1/2 N (12th passage) in comparison to the content of the best callus culture of our collection (5 years in culture).

	Harmine mg/g dry mass	Harmalol
Transformed root culture		
6th passage	2.65	1.03
7th passage	2.74	1.24
12th passage	12.85	2.46
Callus culture	0.44	0.51

$\beta$ -carboline levels varied between 1.0–1.5% of dry mass. These rather high levels suggest that root cultures could become a useful tool for studying the biosynthesis of  $\beta$ -carboline alkaloids. Due to the low and decreasing levels of  $\beta$ -carbolines in callus and suspension cultures, cell cultures have not successfully been applied for this purpose [1]. TLC analysis of root culture extracts showed also the presence of several other, strongly fluorescent compounds which were absent in callus and suspension cultures.

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