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PRODUCTION OF QUASSINOIDS BY TISSUE CULTURES OF *AILANTHUS ALTISSIMA*

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Key Word Index—*Ailanthus altissima*; Simaroubaceae; plant tissue culture; auxins; biosynthesis; quassinoids; ailanthone.

Abstract—Callus cultures derived from a young shoot and a flower bud of *Ailanthus altissima* have been examined for quassinoid production (ailanthone). The biosynthetic potential of the calli from the two sources was related to the explant origin as well as to the addition of exogenous auxins to the culture medium. This is the first report of quassinoid production in cell culture.

INTRODUCTION

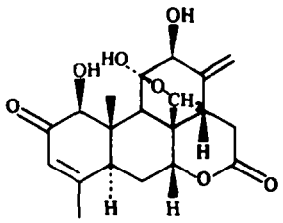
Ailanthone (1) is the major quassinoid of *Ailanthus attissima*. It was first isolated from the seeds of this tree [1].

Apart from the study of Anderson *et al.* [2], who failed to detect the presence of quassinoids in tissue or cell-suspension cultures of aseptic cotyledon seedlings of *A. altissima* (Mill.) Swingel, the production of these mevalonate-derived compounds by Simaroubaceae tissue cultures has not yet been investigated. Because of the pharmaceutical interest in the biologically active compounds of this family [3], the *in vitro* tissue culture of *A. altissima* was reinvestigated in order to establish if it is possible to promote the production of ailanthone. Two factors were studied: (1) explant origin (stem and anther from the same tree) and (2) the addition to the culture medium of the exogenous auxins, 2,4-dichlorophenoxyacetic acid (2,4-D), indol-3-acetic acid (IAA) and indolebutyric acid (IBA).

RESULTS AND DISCUSSION

The major quassinoid isolated from the callus tissue extracts was identified as ailanthone (1) by HPLC and TLC using an authentic sample of this quassinoid and by comparison with the published ^{1}H NMR [4] and MS [5] data.

Stem and anther explants of *A. altissima* were cultivated on solid Murashige and Skoog medium in the presence of 1 μM 2,4-D. Ailanthone was only detected in the strain derived from the anther callus (Table 1).



1 Ailanthone

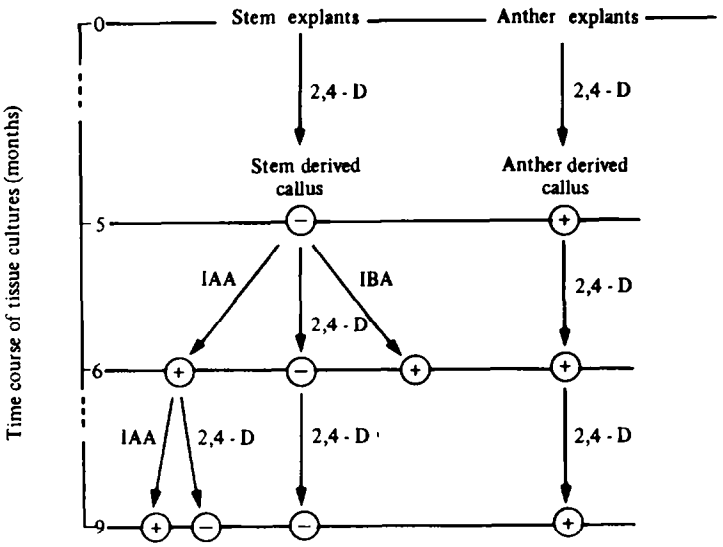
To test the influence of the addition of another exogenous auxin to the culture medium, the strain from stem cultures (which do not produce ailanthone) were sub-divided and transfered on to the same basal medium supplemented with either 1 μM 2,4-D or 20 μM IAA (Table 1). This last medium induced the production of ailanthone which was thus associated with the addition of IAA to the culture medium. Such an effect has been reported previously for the production of diterpenes [6] and of shikonin derivatives [7] which also derive from mevalonate.

To confirm unequivocally the influence of IAA on the initiation of quassinoid production as well on the maintainance of the metabolic pathway associated with its biosynthesis, a part of the stem-derived callus cultivated in the presence of IAA was transfered onto the same medium, whilst another part was transfered onto a medium containing 2,4-D (Table 1, Step 3). Again ailanthone was only recovered when IAA was present in the culture medium and was not detected in the medium supplemented with 2,4-D addition. Thus the effect of IAA on quassinoid production is reversible.

No general inhibitory activity could be attributed to 2,4-D, since it did not interfere with the biosynthesis of ailanthone by the strain derived from anther culture. This fact proves that other factors than IAA must be implicated in quassinoid production. Indeed, the biosynthesis of ailanthone is also activated in stem-derived callus when IBA is substituted for IAA (Table 1, Step 2).

In addition, histological studies were performed to check for possible morphological differentiation which could occur when the exogenous auxins were changed. However, no observable differences were recorded. These results provide evidence for the view that the biochemical differentiation initiated by either IAA or IBA treatment is not correlated to morphological differentiation. Thus the production of ailanthone by undifferentiated cells is possible and is not necessarily associated with organ

Table 1. Influence of explant origin and exogenous auxin treatments (IAA, 2,4-D, IBA) on ailanthone biosynthesis by tissue cultures of *A. altissima*



⊕ Ailanthone present [~ 15 μg (by HPLC)/100 g fr. wt]; ⊖, ailanthone undetectable by HPLC.

differentiation as in the whole plant; indeed ailanthone was detected in the root bark extract of the tree from which the original explants were removed.

As the yields of ailanthone by callus tissue culture of *A. altissima* under these experimental conditions is very low, studies on the factors which could enhance its production are now in progress.

EXPERIMENTAL

Plant material and methods of culture. A young stem and a flower bud of *A. altissima* were collected in May 1985 from a tree growing in the Experimental Botanical Garden, J. Massart of the Université Libre de Bruxelles, Belgium.

The two different explants were surface sterilized in 70% EtOH (5 min) and 1% $HgCl_2$ (3 min) and then rinsed 3 × with sterile distilled water. Calluses were then initiated on solid basal Murashige and Skoog medium [8] supplemented with 0.1 μM kinetin, PVP (1%), charcoal (0.3%) and different auxins: 1 μM 2,4-D or 20 μM IAA or 20 μM IBA. The pH of the medium was adjusted to 5.6 with 0.1 M NaOH before autoclaving (110°, 20 min).

The cultures were maintained at 24 ± 1° in continuous light (2000 lx) and subcultured every 30 days onto fresh medium. The calli had to be sub-cultured 5 × in order to provide enough material for the experiments.

Extraction and qualitative determination of ailanthone (1). Fresh calli (± 40 g) were harvested after 30 days of culture and extracted by maceration in MeOH. After filtration, the methanolic soln was diluted (1:1) with H_2O and extracted (× 2) with $CHCl_3$. The $CHCl_3$ soln was dried (Na_2SO_4) and, after removal of the solvent, the residue was taken up with 2 ml $CHCl_3$ and transfered onto a silica gel column (8 g), prepared with $CHCl_3$. The column was eluted with $CHCl_3$–MeOH (19:1) and the fractions which contained 1 (TLC control) were further subjected to prep. TLC with $CHCl_3$–MeOH (1:1). The pertinent band was eluted with MeOH and the methanolic extract dissolved in 0.5 ml MeOH and analysed by HPLC; Hibar® column (15 cm × 4.6 mm i.d.) prepacked with Lichrosorb® RP-18 (mean particle size 5 μm), H_2O–MeOH–HOAC (75:24:0.5), flow rate 1 ml/min, detection at 254 nm, ailanthone R_t = 6 min. The same method was used for the analysis of the root bark of *A. altissima*. For small callus samples (< 15 g) CC on silica gel was not performed.

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