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The *de novo* Formation of Buds and Plantlets from Various Explants of *Ailanthus altissima* Mill. Cultured *in vitro*

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Abstract. The hypocotyls, cotyledons, leaf blades, whole leaves and petioles of seedlings of *Ailanthus altissima* are capable of producing callus and buds *in vitro*. Buds and callus were also obtained from whole leaves and internodes of 2-years old plantlets grown *in vitro*. From the calli buds differentiated and later, both from buds developing directly without a callus phase and also *via* a callus phase, well developed shoots were formed. The cultures were mainained on MS medium in 2 combinations: A) IAA – 0.2 mg l^{-1}, BAP – 1 mg l^{-1}, GA_3 – 0.5 mg l^{-1}, thiamine – 4 mg l^{-1} and sucrose 3 %; B) BAP – 0.5 mg l^{-1}, IAA – 1 mg l^{-1}, casein hydrolysate 400 mg l^{-1}, thiamine 4 mg l^{-1} and sucrose 3 %. Excised shoots, which had developed *de novo* in culture, produced roots when incubated on the basic mineral medium of MS with the addition of IAA. The regenerative potential of *A. altissima* is very high and this woody species seems to be an ideal object for various morphogenetic studies.

Additional index words: *in vitro* regeneration

The genus *Ailanthus* comprises about 15 species growing in east and south Asia and north and east Australia. *Ailanthus altissima* is a fast growing tree, even on a poor sandy soils. Adventitious buds are produced in abundance on its roots. It grows well in the industrial areas as it is resistant to smoke. *In vitro* morphogenesis of *A. altissima* has been studied by Caruso (1974) who cultured aseptic internodal segments in a defined nutrient media lacking growth regulators. He found that the presence of vascular tissues was a prerequisite for adventitious bud formation. Thus stem segments excised in the spring from young, soft shoots of this species and grown on a minimum nutrient medium, were capable of producing buds. The present paper describes high frequency organogenesis and plantlet formation from hypocotyls, cotyledons, petioles, leaf blades and internode segments from seedlings and 2-years old plantlets of the woody angiosperm *Ailanthus altissima*.

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MATERIAL AND METHODS

Embryos at the stage of torpedo were isolated from fruits of *Ailanthus altissima* cv. Erythrocarpum tree grown in the Botanical Garden in Poznań. The age of the tree was about 70 years. Before excision of the embryos the whole fruits were surface sterilized with 70 % ethanol for 1 min, then transferred to chlorine water for 15 minutes and subsequently washed 3 times with sterile distilled water. Embryos isolated under the stereo microscope were placed on the basic MS (Murashige and Skoog 1962) medium in 2 combinations: A) 3-indoleacetic acid (IAA) – 0.2 mg l^{-1}, 6-benzylaminopurine (BAP) – 1 mg l^{-1}, gibberellic acid (GA_3) – 0.5 mg l^{-1}, thiamine – 4 mg l^{-1} and sucrose 3 %; B) BAP – 0.5 mg l^{-1}, IAA – 1 mg l^{-1}, casein hydrolysate (CH) – 400 mg l^{-1} and sucrose 3 %. Both types of media contained agar in concentration 8 g l^{-1} and their pH was adjusted to 5.8. Embryos were cultured in 250 ml Erlenmayer flasks containing 25 ml culture medium. All cultures were incubated at 22–24 °C under continuous low-level diffuse light. Three weeks following the incubation of embryos fully formed seedlings developed. From those seedlings reaching a size of 2.0–2.5 cm the following explants were excised and transferred onto the same media: a) cotyledons; b) leaf blades, leaf blades with petioles and petioles only; c) hypocotyls when about 0.5 cm long. From plants regenerated from these explants and cultured over the next 2 years (1988–1989) the following explants were excised and transferred onto the same media: a) whole leaves; b) 0.5–1.0 cm internodes segments excised from different parts of the shoot. The number of cultured explants is shown in Table 1.

For histological investigations explants were fixed in Carnoy's solution, passed through an alcohol-xylene series and embedded in paraffin. Sections of 12–15 μm were cut and stained in safranin and fast green.

RESULTS

The incubated embryos started to germinate shortly after the transfer to the media. Swelling of the cotyledons and hypocotyls was noticed already on the third day and at the end of the second week small seedlings with roots and leaves were formed. Some of the swollen hypocotyls produced callus during the next 2 weeks and later small green buds started to differentiate on the callus (Fig. 1). The number of buds varied from several to 30 per explant. From these buds, when left intact on the explant, many shoots developed. In certain cases, calli were not produced but buds differentiated directly from the cortical tissue of the hypocotyl (Fig. 2). In these instances, 4–5 weeks subsequent to the inoculation of embryos, the hypocotyls of seedlings were covered with small buds. When excised and transferred to fresh medium these buds produced well developed plantlets. Medium "B" was much more suitable for the development of plantlets than medium "A".

Table 1
The number of selected explants producing callus and buds

Type of explants	Number of explants	Number of explants producing callus[+]	Number of explants directly producing buds without a proceeding callus phase
Hypocotyls of 2–4 weeks old seedlings	82	48	7
Cotyledons of 10–14 days old seedlings	70	46	4
Leaf blades only and leaf blades with petioles from 3-week old seedlings	82	44	7
Leaf blades only and leaf blades with petioles from 2-years old plantlets	84	56	4
Internodes of 2-years old plantlets	38	20	–

[+] Calli developing from all the explants were capable of producing buds and plantlets

Development of buds and plantlets from the excised organs

a) Cotyledons. Whole cotyledons were cut off from 2 weeks-old seedlings and cultured on both media "A" and "B". Irrespective of the medium, 2 weeks after inoculation, callus formed first on the cut surface of the cotyledons and later over the whole surface. Callus grew intensively and, on the 4th week of culture, many buds started to differentiate. From those buds during the next 2 weeks of culture shoots have developed (Fig. 3). Some cotyledons produced buds directly (Fig. 4) but in these cases no further differentiation into plantlets was observed.

b) Hypocotyls. Hypocotyls produced callus and by the 5th week of culture buds started to differentiate on it and 2 weeks later many shoots developed. No direct development of buds occurred from the hypocotyls.

c) Leaf blades, leaf blades with petioles and petioles only. Irrespective of whether isolated from 2–3 week old seedlings or from 2-years old plantlets cultured *in vitro* (Fig. 5) and irrespective of their size, the explants produced buds and callus (Fig. 6). Calli were found at the base of the leaves as well as over their surface. As with the hypocotyls and cotyledons, callus grew abundantly on these leaf explants and, between the 5th and 6th week of culture, buds started to differentiate and shoots later developed (Fig. 8). In some cases when leaves were cultured with the petioles attached, bundles of buds developed

from the cut ends of the petioles (Fig. 7). Such buds developed into shoots which, after transferring to the basic MS medium, grew into plants.

d) Internodes. Segments excised from the internodes of plants grown *in vitro* initially formed callus. This mainly developed over cut surface but also it rarely grew over the epidermis. From the callus buds and shoots developed during the next 6–8 weeks of culture.

In order to obtain rooted plants capable of developing further in soil, it was necessary to culture the shoots, irrespective of the type of explant from which they had differentiated, on the basic mineral medium with the addition of IAA at the concentration of 0.2–0.5 mg l^{-1}. Plants which possessed a rich root system were directly transferred (after thoroughly washing off with water the remnants of the agar medium) into soil in pots (Fig. 9). After 2 to 3 months growth in pots, the plants were transferred into the soil in the garden.

DISCUSSION

The technique of tissue culture offers extraordinarily good possibilities for vegetative propagation of *Ailanthus altissima*. This species seems to be an ideal object for studies of *in vitro* morphogenetic potential of woody plant. Our investigations have shown that all the explants are capable of regenerating buds either directly or, more commonly, *via* a callus stage. These buds later differentiate into shoots which can be excised and grown into rooted plants. The capacity of producing buds is possessed with by explants from the 2–3 week old seedlings and by those taken from 2 year old plants grown in the sterile conditions. Caruso (1974) reported that the presence of vascular tissue in stem explants of *A. altissima* was a prerequisite for bud formation. Those observations were made on explants from young shoots and cultured on a minimum nutrient medium which lacked growth regulators. Our experimental material was excised from young seedlings and from 2-years old plantlets cultured *in vitro*. Irrespective of the type of explants utilised regeneration was always noticed. However, both of the two media utilised contained growth regulators which were indispensable for the high rate of regeneration. The level of BAP had a definite effect on the initiation of buds in all the cultured explants. Neither normal or even abnormal buds were observed on the explants when they were cultured on BAP-free medium. These results indicate that BAP is involved in the induction of meristematic tissues and differentiation of shoots, but this requirement for exogenus BAP was not high. Shoot production occurred already on the medium containing 0.2 mg l^{-1} BAP, however, (as our preliminary observations reveal, not published as yet) with the higher concentrations of 2 and 4 mg l^{-1} leaves and fragment of internodes from 2-years old plants are capable of producing an abundant mass of buds. The importance of BAP in the process of micropropagation *in vitro* of woody species is well known from previous workers

(Chalupa 1975, Oka and Ohyama 1981, Sharma and Chandra 1988, Rutledge and Douglas 1988, Vilaplana and Mullins 1989, Douglas *et al.* 1989).

On the basis of our observations it can be presumed that *Ailanthus altissima* represents a woody species with a high, possibly unique capacity to regenerate. This species could be used on a much wider scale as an experimental woody material in various studies. The high regenerative potency of its organs and tissues, the speed in which buds and shoots develop as well as the ease with which rooting of the excised shoots occurs – all these features show that this species might be a very convenient object for various morphogenetical studies.

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Figs. 1–9 at the end of the issue


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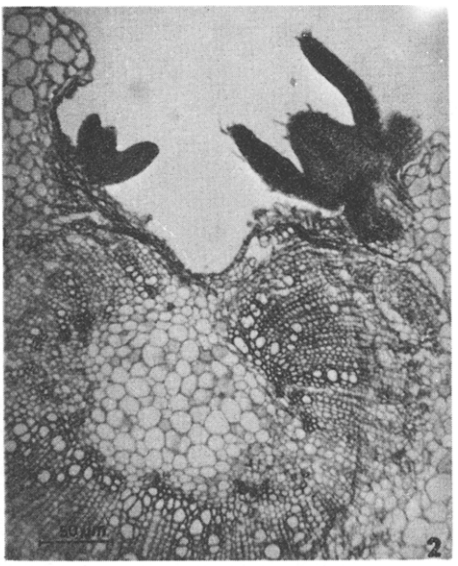
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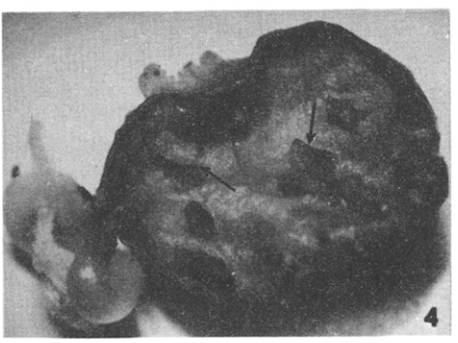
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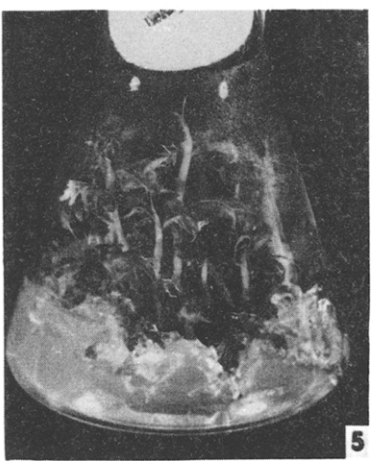
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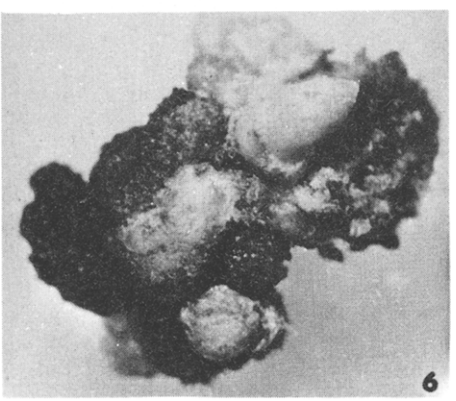
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AILANTHUS ALTISSIMA CULTURED IN VITRO

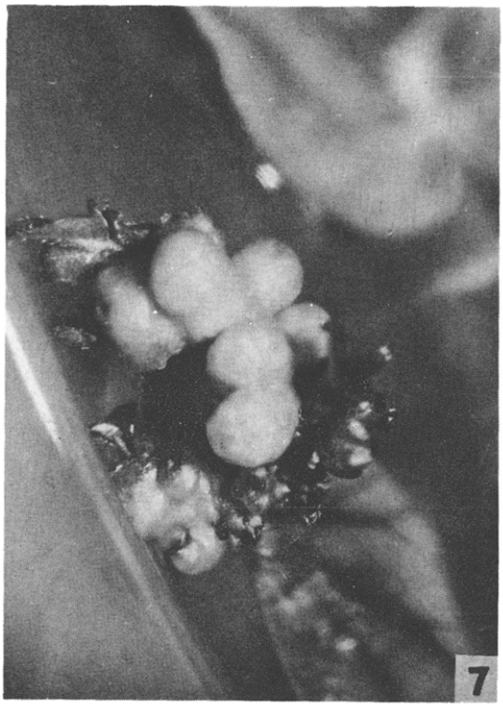

Fig. 1. Buds differentiating in the callus produced from the hypocotyl, 4 weeks of the culture.

Fig. 2. A direct differentiation of buds in the cortical tissue of the hypocotyl, 5 weeks of the culture.

Fig. 3. Development of shoots in the callus produced from the cotyledon, 6 weeks of the culture.

Fig. 4. Development of buds (arrow) directly from the cotyledon, 4 weeks of the culture.

Fig. 5. 2-years old plantlets cultured *in vitro*.

Fig. 6. Callus developing from a leaf of a 2-years old plantlet.

Fig. 7. A bundle of buds developing in the cut end of a petiole, 5 weeks of the culture.

Fig. 8. A development of shoots in a callus produced from the leaf blade, 6 weeks of the culture.

Fig. 9. Full developed plant obtained from the leaf of a 2-year old plantlet, 5 month after inoculation of the explant.