IN VITRO AXILLARY SHOOT FORMATION AND ROOTING IN BLACK WALNUT MATURE EMBRYOS

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Abstract.--Mature embryos of black walnut were grown on a nutrient medium containing benzylamino-purine (BAP) at 2 mg/1, indoleacetylphenylalanine (IAAPhe) at 2 mg/1, or these two growth regulators in combination, each at 2 mg/l. The development of axillary shoots was maximal when both growth regulators were used. BAP alone gave some axillary bud release while IAAPhe alone produced a long slender stem axis which was devoid of axillary shoots. Excised axillary shoots formed adventitious roots readily when cultured on one-half strength MS basal medium containing indolebutyric acid at 1 mg/l.

Additional keywords; Embryo culture, axillary bud development, Juglans nigra.

Although great strides have been made in tissue and organ culture of most herbaceous species of interest, many important tree species have undergone in vitro organogenesis only rarely, if at all. Black walnut (Juglans nigra L.) is an example of such a recalcitrant species. Seedlings of tree species, however, have historically been easier to manipulate in culture and black walnut is no exception. Rodriguez (1982a) observed multiple shoots in black walnut seedlings provided with a high concentration of benzylaminopurine (BAP), but the primary shoot system was abnormally conical. The combination of indolebutyric acid (IBA) and BAP was beneficial in producing more normal appearing shoots on subcultured specimens of black walnut, although no rooting was reported. Wood (1982) also observed shoot proliferation in axillary buds of the pecan seedling, and the synthetic growth regular; tors BAP and IBA were more effective than the combination of native hormones isopentenyladenine (2ip) and indoleacetic acid (IAA). A relatively common procedure is to stimulate root formation by transferring the newly formed shoots to a nutrient medium containing IBA, American chestnut (Keys and Cech, 1981) and European chestnut (Rodriguez, 1982b) are examples of two species which have been manipulated in this manner.

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Synthetic aryl esters of auxin, such as phenyl indolebutyrate, have also shown promise in stimulating rooting in pine seedling cuttings (Haissig, 1979). More recently, amino acid conjugates of IAA have been used in combination with a cytokinin to induce bud formation and root formation in certain herbaceous plants. The conjugated form of IAA is resistant to enzymatic degradation and thus it provides a slow release form of IAA (Hangarter and Good, 1981).

This paper describes the use of an amino acid conjugate, namely, indoleacetylphenylalanine (IAAPhe), alone and in combination with BAP, for the purpose of stimulating axillary shoot formation in mature embryos of black walnut. This approach was taken in order to set the stage for rooting and plantlet formation in this important tree species.

MATERIAL AND METHODS

Stratified black walnut (Juglans nigra L.) seeds were kindly furnished by Dr. J.W. Van Sambeek, Research Plant Physiologist, USDA Forest Service, Carbondale, Ill. Embryos were removed and dipped in 95% ethanol, stirred in 15% commercial Clorox for 20 minutes and rinsed 3 times in sterile, distilled water. The embryos were aseptically transferred to petri dishes (15 X 100 mm) or test tubes (25 X 150 mm) containing a basal nutrient medium (Murashige and Skoog, 1962) with BAP at 2 mg/1, or IAAPhe at 2 mg/1, or a combination of these growth regulators, each at 2 mg/l. Sucrose was added at 3% (W/V) and the media were solidified with 0.7% agar. Test tubes containing a bridge of filter paper, the base of which was immersed in liquid media with ingredients as above, were also used in a few experiments.

The specimens were kept at 24 °C in a 16-hour photoperiod under 1100 lux emanating from cool-white lags. The temperature of the dark period (8 hours) was kept at 20 °C. In an attempt to stimulate rooting, any visible axillary shoots and in a few cases terminal shoots of seedlings averaging 9 weeks old were excised and placed on one-half strength MS medium with IBA at 1 mg/l. These specimens were kept either in the light-dark regime as described above or in continuous darkness at 24 °C.

RESULTS AND DISCUSSION

Axillary Shoot Development

The combination of IAAPhe and BAP was superior to either BAP or IAAPhe alone in stimulating the development of axillary shoots of black walnut seedlings. The results from a representative experiment are seen in Table 1.

Table 1.--Axillary shoot development

Medium	No. of seedlings	No. of seedlings forming axillary shoots	Total No. axillary shoots
Basal	5	0	0
Basal+BAP	5	4	11
Basal +IAAPhe	5	0	0
Basal + BAP + IAAPhe	5	5	20

Seedlings grown on the basal nutrient medium had healthy roots and the expanded leaves had larger surface areas. Basal medium with BAP alone resulted in some stunted root growth and smaller leaves. There was also a great deal of contrast in development between seedlings on basal medium and seedlings on medium supplemented with BAP and IAAPhe, as seen in Fig. 1. Seedlings provided with both of the growth regulators formed an average of 4 axillary shoots per seedling. Their roots, however, were swollen and slow growing, and the base of the stem axis was slightly swollen, as well.

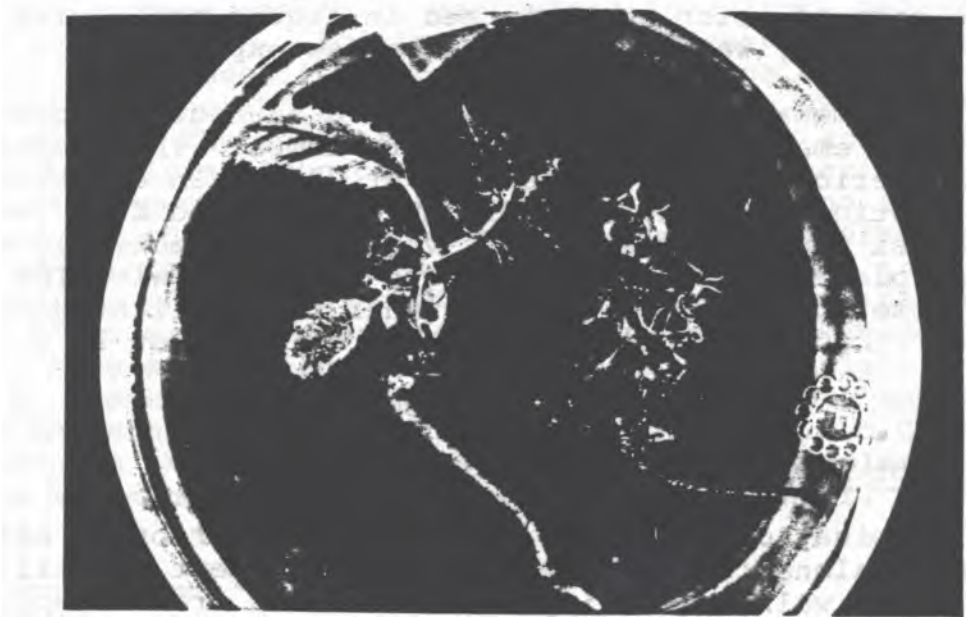

Fig. 1.--At left, seedling grown for 10 weeks on basal nutrient medium; at right, seedling grown for 9 weeks on basal medium plus BAP (2 mg/1) and IAAPhe (2 mg/1), X 1.

Seedlings on basal medium with IAAPhe alone had very long primary roots and elongated stem axes lacking axillary shoots (Fig. 2).

Fig.2--Elongated seedling on basal medium with IAAPhe at 2 mg/1, Xl.

Rooting

Over 50% of excised axillary shoots transferred to the IBA - supplemented medium, whether in the light-dark regime or in continuous darkness, formed adventitious roots. Root primordia were observed in many cases within 2 weeks of transfer. The technique of using a filter paper bridge worked well in keeping the base of the specimen moist and in stimulating rooting. However, the minor differences in success between shoots forming roots on filter paper and those on a solid medium did not seem to warrant the extra efforts required in keeping the filter paper arched above the liquid medium following autoclaving and during the insertion of the specimen into the test tube. Many rooted specimens were transferred to a nutrient medium, such as Hoagland's, which lacked growth regulators, and these plantlets displayed good growth of roots (Fig. 3).

Fig. 3.--Axillary shoot with adventitious roots on filter paper bridge moistened with Hoagland's liquid nutrient medium, X 1.4.

Terminal shoots obtained from seedlings on the basal medium also demonstrated the ability to form adventitious roots when excised and transferred to the rooting medium containing IBA.

CONCLUSIONS

The combination of BAP and IAAPhe proved to be most successful in stimulating the release of axillary shoots. Adventitious root primordia were observed on many excised axillary shoots within 2 weeks following their transfer to the nutrient medium with IBA. Roots were also produced on terminal shoot cuttings taken from seedlings on basal medium. The addition of growth regulators, however, is concluded to be beneficial in that the enhancement of axillary shoot development increases the number of potential propagules in this important tree species.

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