Acta Bot. Neerl. 37(4), December 1988, p. 533–535

Effect of several growth regulators and of aetiolation on chestnut lignification

T. DÍAZ, I. IGLESIAS, E. GONZÁLEZ, A. SEGURA and M. CORTIZO

Department of Plant Biology, Faculty of Pharmacy, University of Santiago, Spain

SUMMARY

The effects of the growth regulators α-naphthalenacetic acid (NAA), 3-indolebutyric acid (IBA) and maleic hydrazide (MH) on the lignification of 2-month-old chestnut sprouts (F_4 of *Castanea sativa* Mill. × *Castanea crenata* Siebb. et Zucc.), both with and without aetiolation, were studied. The results obtained show that aetiolation on its own has no effect on lignification.

Treatments with IBA + NAA or with MH significantly promoted lignification. When, however, auxines were administered in combination with aetiolation, the promotion effect was significantly stronger.

Key-words: aetiolation, *Castanea* hybrids, lignification, phytohormones.

INTRODUCTION

Lignin, a stable structural component of wood, is a product of the polymerization of phenolic units that pervade the polysaccharides of cell walls (Higuchi 1981). The lignification process is affected by several factors. Many studies have shown a direct relationship between this process and the levels of peroxidase, 3-indoleacetic acid and other growth regulators (Gautheret 1963; Harking and Obst 1973; Parups 1964); light can also affect this process (Grand *et al.* 1979; Gross 1977). However, few data are available on the role of lignins on the growth process of plants (Zeroni & Hall 1980).

The present paper describes the effects of α-naphthalenacetic acid (NAA), 3-indolebutyric acid (IBA), and maleic hydrazide (MH), either alone or combined with aetiolation, on the lignification of 2-month-old chestnut sprouts.

MATERIALS AND METHODS

Two-month-old chestnut sprouts, from chestnut stumps or from the clone T-13, hybrid of the F_4 of *Castanea sativa* Mill. × *Castanea crenata* Siebb. et Zucc. were used. In order to observe the effects of growth regulators on lignification, the following experiments were performed, using 45 sprouts for each treatment.

(a) Application, by leaf spraying, of MH in doses of 1000 mg l^{-1}, mixed with 0·1% of the non-ionic moistener Agral (ICI Plant Protection Division).

*Abbreviations: IBA, 3-indolebutyric acid; MH, maleic hydrazide; NAA, α-naphthalenacetic acid.

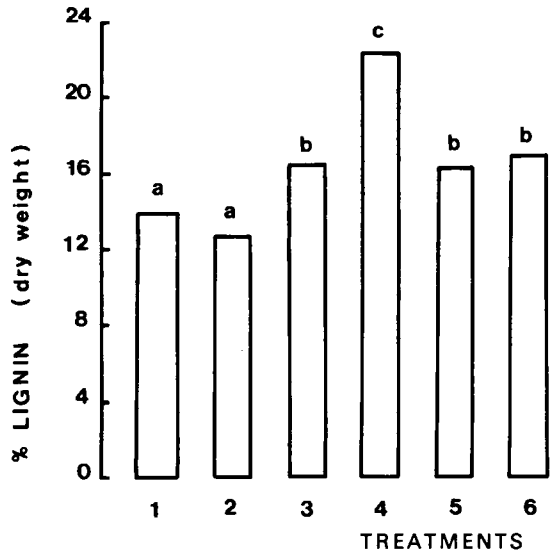

Fig. 1. Effect of the different treatments on the percentage of dry weight lignin (arcsin transformed). (1) control (light); (2) control (aetiolation); (3) IBA + NAA; (4) IBA + NAA + aetiolation; (5) MH; (6) MH + aetiolation. Means followed by the same letter are not statistically different at the 1% level (Duncan's multiple range test).

(b) Basal application of 3 mg g^{-1} IBA + 3 mg g^{-1} NAA in vaseline (over 1–7 cm). Treatments (a) and (b) were carried out in both the light and the dark (aluminium paper).

(c) Non-treated sprouts served as a control.

After 1 month the lignin content was determined by spectrophotometry (Alibert & Boudet 1979). The lignin standard employed was obtained by the dioxan method (Hergert 1960).

Percentages were transformed into arcsin percentages before statistical analysis (Sokal & Rohlf 1979). Differences were tested by analysis of variance and by Duncan's multiple range test (Rodriguez-Maya 1977).

RESULTS AND DISCUSSION

The results obtained show (Fig. 1) that the 3-month-old sprouts (control) have a 13·7% dry weight of lignin. Aetiolation does not seem to affect lignification, since there are no significant differences between aetiolated and non-aetiolated sprouts. Phillips (1954) has stressed that illumination of the leaves is necessary in order to achieve lignification of the stem. Ishikawa & Takaichi (1957) have noticed, in bean plants, that the lignin content doubled when plants developed under white light. Vieitez (1973) has performed histological studies on the sclerenchyma of aetiolated chestnut sprouts, and has suggested that the absence of light caused a 6-month delay in the transformation of parenchyma into sclereid cells, as well as in the thickening of the secondary walls of the fibres. The different results obtained with the chestnut could be due to the different duration of the aetiolation. It therefore might be improper to assign a special role to light in lignification, and as Wardrop (1971) has pointed out, it is possible that light affects this process only indirectly.

With IBA + NAA, or with MH (Fig. 1) the lignin content was significantly higher than in the control. These results show that growth regulators favour lignification, which in the case of auxin agrees with the conclusions of other investigators. Using *Acer, Populus,* and *Fraxinus* sprouts, Wareing (1958) has found that IAA causes new xylem formation, together with vessel lignification. Sachs (1975) has noted that IAA induces the formation of lignificated xylem and has described this fact as being a direct consequence of the auxin treatment. Microscopic analysis of oat coleoptiles, however, has indicated that the application of MH leads to diminished lignification (Parups 1964).

The present study shows that application of aetiolation, together with the auxines (IBA + NAA), stimulates lignification, whereas aetiolation with MH alone has the same effect as the treatment of MH without aetiolation. The results indicate a possible synergism between light and auxin in the lignification process.

REFERENCES

Alibert, G. &, Boudet A. (1979): La lignification chez le Peuplier I. Mise au point d'une méthode de dosage et d'analyse monomerique des lignines. *Physiol. Vég.* **17:** 67–74.

Gautheret, R.J. (1963): Histophysiologie Végètale. Sur le déterminisme de la proliferation des cellules végètales cultivés *in vitro*: remarques sur l'interaction auxine–cinetine. *Copt. Rend.* **256:** 2071–2075.

Grand, C., Ranjeva, R., Boudet, A. & Alibert, G. (1979): Photoregulation of the incorporation of guaicyl units into lignin. *Planta,* **146:** 281–286.

Gross, G.G. (1977). Biosynthesis of lignin and related monomers. In: Loewus F.A. & Runeckles, V.C. (eds): *The Structure, Biosynthesis and Degradation of Wood.* Rec. Adv. Phytochem. 11: 141–184. Plenum Press, New York.

Harking, J.M. & Obst, T.R. (1973): Lignification in trees: indication of exclusive peroxidase participation. *Science,* **180:** 296–298.

Hergert, H.L. (1960): Infrared spectra of lignin and related compounds. II. Conifer lignin and model compounds. *J. Org. Chem.* **25:** 405–413.

Higuchi, T. (1981): Biosynthesis of lignin. In: Pirson, A. & Zimmermann, M.H. (eds) *Encyclopedia of Plant Physiology.* Springer-Verlag, Berlin.

Ishikawa, H. & Takaichi, K. (1957): Lignin and lignification. The formation of lignin in the young plants. *J. Japan. For. Soc.* **39:** 70–73.

Parups, E.V. (1964): The effect of maleic hydrazide on synthesis of lignin. *Can. J. Plant. Sci.* **44:** 252–258.

Phillips, E.W.J. (1954): Influence of leaf activity on the composition of the wood cell wall. *Nature,* **174:** 85–86.

Rodríguez-Maya, L. (1977): Comparación de medias e intervalos de confianza. In: *Instituto Nacional de Estadística (ed) Métodos estadísticos de investigación (Introducción al Análisis de la Varianza).* 21–32. Presidencia del Gobierno, Madrid.

Sachs, T. (1975): The control of the differentiation of vascular networks. *Ann. Bot.* **39:** 197–204.

Sokal, R.R. & Rohlf, F.J. (1979): Hipótesis del análisis de la varianza. In: Sokal, R.R. & Rohlf, F.J. (eds): *Biometría. Principios y métodos estadísticos en la investigación biológica.* 425–438. H. Blume, Madrid.

Vieitez, A.M. (1973): *Factores que afectan a la formación del tejido de esclerénquima en* Castanea sativa *Mill. Relaciones con su rizogénesis.* Doctoral Thesis, University of Santiago, Spain.

Wardrop, A.B. (1971): Occurrence and formation in plants. In: Sarkanen, K.V. & Ludwig, C.H. (eds): *Lignins: Occurrence, Formation, Structure and Reactions.* 19–41. Wiley-Interscience, New York.

Wareing, P.F. (1958): Interaction between indoleacetic and giberellic acids in cambial activity. *Nature,* **181:** 1744–1745.

Zeroni, M. & Hall, M.A. (1980): Molecular effects of hormone treatment on tissue. In: Pirson, A. & Zimmermann, M.H. (eds): *Encyclopedia of Plant Physiology.* Springer-Verlag, Berlin.