*Plant & Cell Physiol.* 16: 1175–1178 (1975)

Short communication

# Root georeaction affected by exo-β-D-(1,3)-glucanase

Paul-Emile Pilet

Institute of Plant Physiology and Biology of the University, 1005 Lausanne, Pl. de la Riponne, Switzerland

(Received September 8, 1975)

Two hour pretreatment with exo-β-D-(1,3)-glucanase cause (at a concentration of 0.4 unit/ml) significant stimulation of the georeaction of maize apical root segments, horizontally placed for 7 hr. Implications of this enzyme effect on cell wall extension are discussed.

The action of the cell wall extension of X-βG, which occurs in many plants (*2*, *3*, *6*, *8*), is still discussed. X-βG may induce elongation of segments from *Pisum* stem and *Avena* coleoptile (*9*, *24*, *25*). This enzyme, as well as auxin (*10*, *20*), increases reversible extensibility of the cell walls (*9*, *11*). But the X-βG effect is not additive to the IAA action when given together (*10*) and it has been postulated that IAA induces both activation and formation of X-βG (*12*). In contrast, there has been a number of reports in which the role of glucanases in growth responses has been questioned (*4*, *5*, *22*). Recently, nojirimycin was reported as being a potent inhibitor of IAA-induced growth of excised *Avena* coleoptile and *Pisum* epicotyl segments. Since nojirimycin is an inhibitor of X-βG, this enzyme may act in IAA-induced changes of non-cellulosic wall glucan (*14*). These observations support the hypothesis that IAA-induced wall extension may be mediated by X-βG (*13*).

On the other hand, the root georeaction has to be related to the changes of root elongation (*1*, *15*). It seems clear now, at least for *Zea* roots, that the downward bending of horizontally placed root segments is due to an asymmetrical extension of the walls of the cortical cells from the extending zone of the root (*19*).

The aim of the work reported in this paper was to determine, by physiological experiments, whether X-βG could act on maize root georeaction and, consequently, on the extension of the cell wall of the cortical cells from the elongating zone of the roots. Effects of X-βG on root growth will be discussed in relation to light and darkness in another paper.

The conditions for germination of maize (*Zea mays* L. cv. Kelvedon 33) fruits have been described previously (*17*). When the primary roots reached a length of 15±3 mm, seedlings were placed vertically with their roots immersed (2 hr, dark, 25±1°C) in a buffered solution (pH 5.6) with or without X-βG. Then, after

Abbreviations: X-βG, exo-β-D-(1,3)-glucanase; IAA, indole-3-acetic acid.

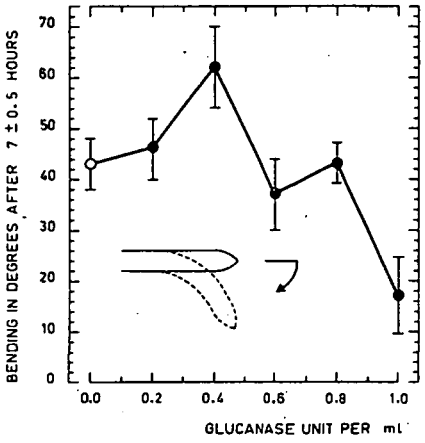


Fig. 1.

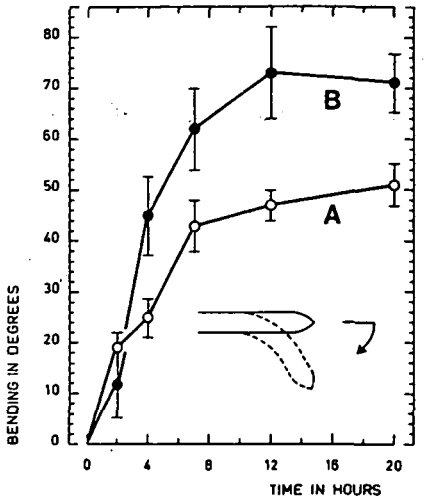


Fig. 2.

Fig. 1 *The effect of different concentrations of X-βG (2 hr pretreatment of intact roots) on the georeaction (curvature in degrees ± standard error) of maize apical root segments horizontally placed for 7±0.5 hr.* The bending (53.7±4.6) of the controlled segments, not pretreated for 2 hr with the solution, was found to be not significantly different from those of the control assay (0.0 unit).

Fig. 2 *Georeaction (curvature in degrees ± standard error) of maize apical root segments in relation to time (0 to 20 hr).* Two-hour pretreatment of intact roots with a solution without (A) or with (B) 0.4 unit of X-βG.

careful washing with tap water, only rectilinear segments were mounted horizontally in plastic frames with their basal cut ends covered with moist filter paper (pH 6.1, *18*). The frames were kept in closed boxes (90±5% relative humidity; white light of 0.9±0.006 J $m^{-2}$ $s^{-1}$; 22±1°C). Bending of the root segments was recorded (after 7±0.5 hr) by shadow photographs. Each result represents the mean response of at least 45 roots. Standard errors of the mean have been calculated and significant differences assessed by the *t*-test.

The enzyme preparation containing exo-β-D-(1,3)-glucanase (EC 3.2.1.6) was kindly supplied by Dr. M. Horisberger (Soc. Ass. Techn. Nestlé, La Tour de Peilz, Switzerland). X-βG was isolated from the culture of *Basidiomycete* species QM 806 and purified (*7*). Its activity was referred to as 1 unit/ml when 1 mole of glucose was produced from 0.05% laminarin incubated (15 min) in the enzyme solution (50 mM acetate buffer, pH 4.8; 37°C; final volume: 1 ml). The specific activity of X-βG was 90.0 units per mg protein.

The georeaction (after 7±0.5 hr) of the horizontal root segments prepared from intact roots pretreated 2 hr with X-βG was then analyzed. The changes of the downward curvature, in relation to several concentrations of X-βG, are reported in Fig. 1. As can be seen, the reaction is significantly enhanced with 0.4 unit of X-βG but significantly inhibited with 1.0 unit of X-βG. A similar concentration effect (except for the values of X-βG doses which are difficult to compare) has been obtained for the elongation of oat coleoptile segments (*10*) and pear pollen tubes (*21*).

When the georeaction is studied in relation to time (Fig. 2A), note that the curving is very rapid for the first few hours, as previously observed (*16*, *23*). Pretreatment with X-$\beta$G (at the maximum activating concentration of 0.4 unit) only acts, as shown in Fig. 2B, on the first phase of the georeaction, significantly increasing (except for the first 2 hr, which is difficult to explain) the downward bending.

From the present data, we can assume that exogenous X-$\beta$G, at certain concentrations, stimulates the georeaction of maize roots and, consequently, may act on the extension of the wall of the upper cortical cells located in the elongating part of the root (*19*). But it remains to be proved whether endogenous $\beta$-(1,3)-glucanase really controls root cell elongation.

## References


(*1*) Audus, L. J. and M. E. Brownbridge: Studies on the geotropism of roots I. Growth rate distribution during response and the effects of applied auxin. *J. Exp. Bot.* 8: 105–124 (1957).

(*2*) Buchala, A. J. and H. Meier: $\beta$-Glucanases and non-cellulosic glucans in the developing oat plant. *Planta* 111: 245–252 (1973).

(*3*) Clarke, A. E. and B. A. Stone: $\beta$-1,3-Glucan hydrolases from the grape vine (*Vitis vinifera*) and other plants. *Phytochem.* 1: 175–188 (1975).

(*4*) Cleland, R.: Auxin and wall extensibility: Reversibility of auxin-induced wall-loosening process. *Science* 160: 192–194 (1968).

(*5*) Datko, A. H. and G. A. Maclachlan: Indoleacetic acid and the synthesis of glucanases and pectic enzymes. *Plant Physiol.* 43: 735–742 (1968).

(*6*) Heyn, A. N. J.: Glucanase activity in coleoptiles of *Avena*. *Arch. Biochem. Biophys.* 132: 442–449 (1969).

(*7*) Huotari, F. I., T. E. Nelson, F. S. Smith and S. Kirkwood: Purification of an exo-$\beta$-D-(1→3)-glucanase from Basidiomycete species QM 806. *J. Biol. Chem.* 243: 952–956 (1968).

(*8*) Kivilaan, A., R. S. Bandurski and A. Schulze: A partial characterization of an autolytically solubilized cell wall glucan. *Plant Physiol.* 48: 389–393 (1971).

(*9*) Masuda, Y.: Role of cell-wall-degrading enzymes in cell-wall loosening in oat coleoptiles. *Planta* 83: 171–184 (1968).

(*10*) Masuda, Y., S. Oi and Y. Satomura: Further studies on the role of cell-wall-degrading enzymes in cell-wall loosening in oat coleoptiles. *Plant & Cell Physiol.* 11: 631–638 (1970).

(*11*) Masuda, Y. and S. Wada: Effect of $\beta$-1,3-glucanase on the elongation growth of oat coleoptile. *Bot. Mag.* Tokyo 80: 100–102 (1967).

(*12*) Masuda, Y. and R. Yamamoto: Effect of auxin on $\beta$-1,3-glucanase activity in *Avena* coleoptile. *Development, Growth and Differentiation* 11: 287–296 (1970).

(*13*) Nevins, D. J.: The effect of nojirimycin on plant growth and its implications concerning a role for exo-$\beta$-glucanases in auxin-induced cell expansion. *Plant & Cell Physiol.* 16: 347–356 (1975).

(*14*) Nevins, D. J.: The in vitro stimulation of IAA-induced modification of *Avena* cell wall polysaccharides by an exo-glucanase. ibid. 16: 495–503 (1975).

(*15*) Pilet, P. E.: Root cap and root growth. *Planta* 106: 169–171 (1972).

(*16*) Pilet, P. E.: Control by the root cap on growth and georeaction of roots. In *Plant Growth Substances 1973*, Proc. 8th Intern. Conf., p. 1104–1110, Hirokawa Publ. Co. Tokyo, 1974.

(*17*) Pilet, P. E.: Effects of light on the georeaction and growth inhibitor content of roots. *Physiol. Plant.* 33: 94–97 (1975).

(*18*) Pilet, P. E.: Abscisic acid as a root growth inhibitor: physiological analyses. *Planta* 122: 299–302 (1975).

(*19*) Pilet, P. E. and A. Nougarède: Root cell georeaction and growth inhibition. *Plant Sci. Letters* 3: 331–334 (1974).

(*20*) Pilet, P. E. and J. Cl. Roland: Growth and extensibility of collenchyma cells. ibid. 2: 203–207 (1974).

(*21*) Roggen, H. P. J. R. and R. G. Stanley: Cell-wall-hydrolysing enzymes in wall formation as measured by pollen-tube extension. *Planta* 84: 295–303 (1969).

(*22*) Ruesink, A. W.: Polysaccharidases and the control of cell-wall elongation. ibid. 89: 95–107 (1969).

(*23*) Shaw, S. and M. B. Wilkins: The source and lateral transport of growth inhibitors in geotropically stimulated roots of *Zea mays* and *Pisum sativum*. ibid. 109: 11–26 (1973).

(*24*) Tanimoto, E. and Y. Masuda: Effect of auxin on cell wall degrading enzymes. *Physiol. Plant.* 21: 820–826 (1968).

(*25*) Wada, S., E. Tanimoto and Y. Masuda: Cell elongation and metabolic turnover of the cell wall as affected by auxin and cell wall degrading enzymes. *Plant & Cell Physiol.* 9: 369–376 (1968).