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Isoperoxidases in Jerusalem Artichoke in Relation to Tuberization and Dormancy

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Abstract. Peroxidase activity and isoenzyme pattern were investigated in buds and tubers of Jerusalem artichokes in relation to induction and breaking of dormancy. Peroxidase activity per unit soluble protein is the highest in the dormant stage. Conditions leading to growth, *i.e.* release of dormancy by the cold, stimulation of axial growth by gibberellic acid or stimulation of radial growth (tuberization) by kinetin, cause rapid loss of total peroxidase activity together with a decrease of intensity of the most cathodic isoperoxidases. Induction of dormancy by AMO-1618 increases peroxidase activity mainly through the same cathodic isoenzymes. The role of the cathodic isoperoxidases is discussed in relation to auxin catabolism and the genesis of oxygenation products inhibitory to plant growth.

Many descriptive data are available concerning changes in peroxidase activity and modifications in the isoperoxidase spectrum in relation to a large number of physiological processes (GALSTON and DAVIES 1969). Although the implication of peroxidase in biochemical reactions such as synthesis (RIDDLE and MAZELIS 1964) and degradation (GASPAR 1971) of auxin, hydroxylation of prolin (YIP 1964), oxidation of polyphenols (REID 1959) and formation of lignin (SIEGEL 1955), ethylene synthesis (MAPSON and WARDALE 1971), *etc.* has been described, the role and the participation of each of the different isoperoxidases in one or more of these reactions remains to be demonstrated. An understanding of the possible significance of such quantitative and qualitative changes in peroxidase during growth and development requires a compared detailed investigation of enzyme ontogeny (BIRECKA and GALSTON 1970) in different plant organs in varying conditions.

Jerusalem artichoke (*Helianthus tuberosus* L.) is an excellent material for studying morphogenesis since axial and radial growth can be controlled both in natural (field conditions) and artificial (*in vitro* on synthetic medium

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eventually supplied with growth regulators) conditions (COURDUROUX 1967). This material under the form of parenchymatous slices served quite often previously for many biochemical purposes and particularly for a study of peroxidase synthesis in response to wounding (BASTIN 1968). For the first time it is used here as a whole (buds plus parenchyme) for a peroxidase investigation in relation to tuberization and dormancy.

Material and Methods

Material samples were taken up from two origins. First, buds from field dormant (harvested in November and stored at 25 °C) and non-dormant (harvested in February and kept at 4 °C) tubers of *Helianthus tuberosus* L. (cv. D 19, Vilmorin Paris) were used. For the purpose of some experiments non-dormant tubers were transferred from cold to room temperature at different times before analysis. Second, buds or small tubers (buds plus parenchyme) from *in vitro* cultures were analysed. Naturally dormant tubers were obtained *in vitro* from buds of field dormant tubers cultivated on a knop medium (COURDUROUX 1967) at 25 °C in the dark. Artificially dormant tubers were obtained *in vitro* from non-dormant buds by a long drawn culture on an inhibiting medium (COURDUROUX 1968, COURDUROUX *et al.* 1972). For this purpose, either the sucrose concentration was increased from 0.1 M to 0.5 M (dormancy induced by osmotic inhibition) either AMO-1618 (1.3×10^{-5}M, plus sucrose 0.3 M) or ABA (3.6×10^{-5}M, plus sucrose 0.3 M), in presence or not of kinetin (2.3×10^{-5}M) was added to the standard medium. Kinetin in these conditions was found to increase the tuberization process by favorizing radial growth (COURDUROUX 1967).

Axial growth without breaking dormancy was stimulated (COURDUROUX *et al.* 1972) by transfering naturally or artificially dormant tubers on a medium supplemented with GA_3 (1.6×10^{-5}M). Release of dormancy was provoked by cold treatment at 4 °C for varying periods.

Generally 3—6 buds (from field tubers) or tubers (from the *in vitro* cultures) corresponding to 0.3 to 1.0 g fresh weight were used per sample. The plant material was homogenized (1 g for 1 ml) in a 0.1 M phosphate buffer, pH 6.2 and the suspension centrifuged at 10 000 *g* for 20 min The supernatant was analysed for soluble protein by the method of LOWRY *et al.* (1951), using bovine serum albumin as a standard. Gaïacolperoxidase activity (incubation mixtures: 8 ml phosphate buffer pH 6.2 + 1 ml H_2O_2 0.2 vol. + 1 ml gaïacol 1% + 0.1 ml enzyme) was determined spectrophotometrically by measuring the increase of absorbance at 420 nm and quantitated using horseradish peroxidase (Fluka) as a reference.

Peroxidase isoenzymes patterns were determined using vertical starch gel electrophoresis (detailed technique in DARIMONT and GASPAR 1972). The gel was developed with gaïacol and *o*-dianisidine.

Results

A prior comparative investigation of total peroxidase activity and isoperoxidase spectrum was made on buds from field dormant (stored at 25 °C) and non-dormant (stored either at 4 °C or at 25 °C) tubers (Fig. 1). It showed significant differences between the three samples. Buds from dormant tubers were characterized by the highest peroxidase activity and a general (except for C_1 and A^5) higher intensity of the isoenzymes; they furthermore contain two isoperoxidases (C_2 and A_4) which were not found in any other situation. Buds from non-dormant tubers kept at 4 °C had a lower total peroxidase activity corresponding to a decrease in intensity of a lot of isoenzymes (C_4, C_5, A_1, A_2, A_3, A_6) while three others (C_1, C_3, A_5) became more appearant. Storage of non-dormant tubers at 25 °C in conditions which allowed stolonisation (rapid growing process) had, as a main consequence, the disappearance of the two most cathodic isoperoxidases which was traduced by a considerably decreasing total enzyme activity.

In vitro natural dormant tubers or AMO induced dormant tubers exhibited a zymogram quite similar to this of buds from field dormant tubers, if we

except the absence of the isoperoxidases C_2 and A_4 linked with the "field" dormancy (Fig. 2). The same held true for *in vitro* tubers whose dormancy was induced either by ABA or osmotic inhibition. When such *in vitro* dormant tubers were treated by GA_3 for an increasing period of time — condition which led to axial stem growth — peroxidase changes took place (Fig. 2)

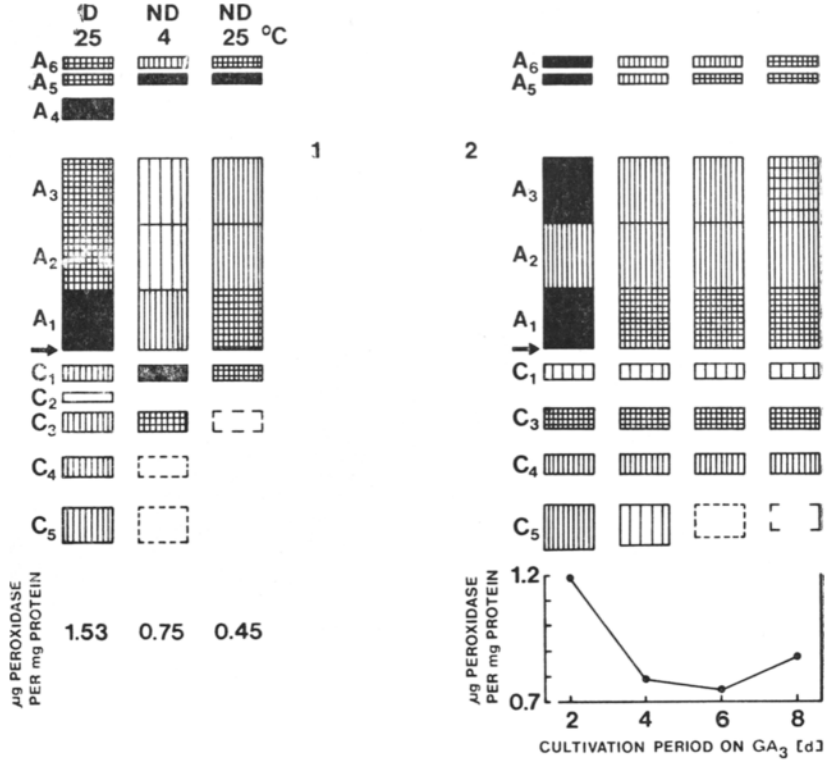

Fig. 1. Isoperoxidase zymograms (above) and total peroxidase activity (below) of buds from field dormant (D) and nondormant (ND) tubers stored at 25 or 4 °C (5 months for the D, 2 months for the ND).

Fig. 2. Isoperoxidase zymograms (above) and total peroxidase activity (below) of whole *in vitro* dormant (dormancy induced by AMO-1618) tubers treated for varying periods by GA_3 (1.6×10^{-5}M) in presence of saccharose 0.1 M.

which remembered the differences between field dormant and non-dormant buds: general decrease in enzyme activity along the zymogram with particular loss at the cathodic side.

Kinetin which alone or in combination with AMO-1618 promotes mainly axial growth (tuberization) also caused a decrease in peroxidase activity (Fig. 3). Here obviously all the cathodic peroxidases were principally concerned while only minor modifications were observed at the anodic side.

Finally we examined the situation in presence of AMO-1618 and ABA *in vitro* (Fig. 4), conditions which led non-dormant buds to form dormant tubers. Both the two regulators induced a progressive gain in peroxidase

activity. This, through the zymograms, could be attributed (Fig. 4) to a strengthening of the most cathodic peroxidases to the detriment of the anodic side.

Discussion

Field or *in vitro* dormant tubers are characterized by a high peroxidase activity and by a considerably higher intensity of many peroxidases along

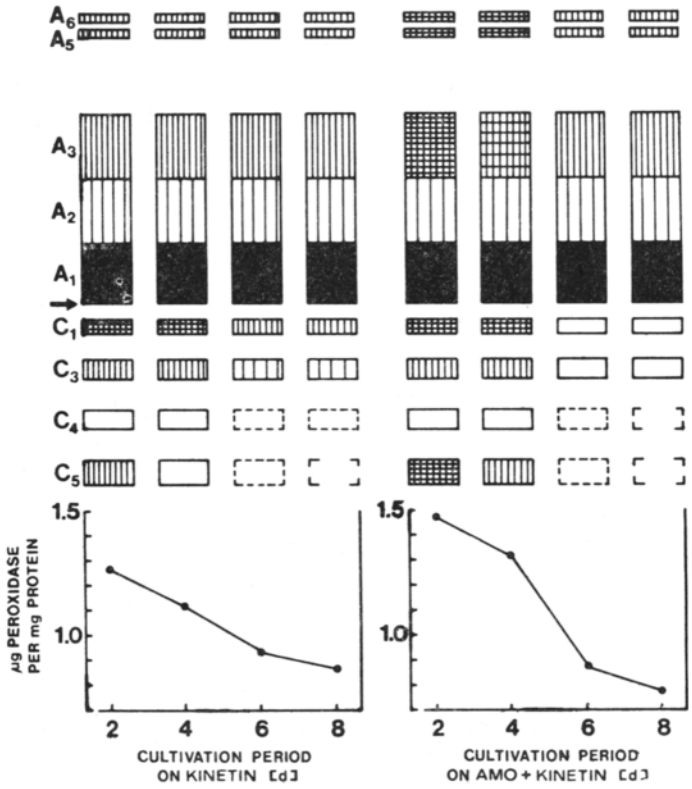

Fig. 3. Isoperoxidase zymograms (above) and total peroxidase activity (below) of buds (from field dormant tubers) *in vitro* cultivated for varying periods on kinetin (2.3×10^{-5}M) or AMO-1618 (1.3×10^{-5}M) plus kinetin (2.3×10^{-5}M), in presence of saccharose 0.3 M.

the zymograms. If we consider the role of peroxidase in auxin catabolism (GALSTON and DAVIES 1969; GASPAR 1971), dormancy in the Jerusalem artichoke would be associated with a low auxin content and a high inhibitor-β level. We showed indeed that final auxin degradation products participate with ABA in the powerful inhibitory action of the so-called inhibitor-β (GASPAR *et al.*, 1972). Release of dormancy by cold (for field tubers) or stimulation of growth by GA_3 treatment (*in vitro*) corresponds to a decreasing peroxidase activity. This fits with the findings of other workers on other materials where release of dominance in buds (ALI and FLETCHER 1971) or

application of GA_3 (GALSTON and MACCUNE 1961; MACCUNE 1961; GASPAR and BOUILLENNE-WALRAND 1966) are known to lower the level of IAA-oxidase and to increase the level of diffusible auxin (NITSCH and NITSCH 1959; KURAISHI and MUIR 1963; GASPAR and BOUILLENNE-WALRAND 1966). The results presented in this paper are further evidence in favour of an auxin-sparing mechanism of GA_3 by the fact that growth is preceded by the disappearance or a strong decline in activity of the most cathodic isoperoxidases, those which were shown to have the greatest ability to destroy auxin by oxygenation (MAZZA *et al.* 1970; GASPAR 1971). Implication of gibberellin in the regulation of dormancy through a peroxidase mediated auxin metabolism could also be seen in the results obtain with the growth retardant AMO-1618 which is known to inhibit gibberellin biosynthesis (LANG 1970) and to induce dormancy in the present material (COURDUROUX 1967): this compound increases peroxidase activity by reinforcing the intensity of isoperoxidases at the cathodic side, as it does classicaly in other materials (HALEVY 1962; GASPAR 1970).

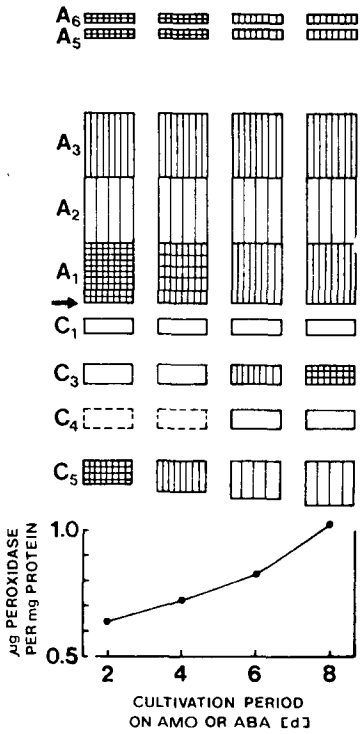

Fig. 4. Isoperoxidase zymograms (above) and total peroxidase activity (below) of buds (from field non dormant tubers) *in vitro* cultivated for varying periods on AMO-1618 (1.3×10^{-5}M) or ABA (2.6×10^{-5}M) in presence of saccharose 0.3 M.

We have to mention results (BOLDUC *et al.* 1970; OCKERSE *et al.* 1970; LEE 1971) which are in opposition of these from this series, i.e. an increase in the level of peroxidase-IAA oxidase after GA_3 treatment with an alternative interpretation (LEE 1971) of the GA_3 promoted growth. In this case, oxidation of IAA might lead to formation of intermediates and products more active than IAA in stimulating growth.

Such discrepancies can also be seen between the present effects of kinetin on the Jerusalem artichoke and these obtained on other materials (LEE 1971; GASPAR *et al.* 1972).

This too simple comparison between two kinds of results obtained with one growth regulator does not take into account the nature of the organ nor the physiological process involved nor the conditions of culturing and treatment. But it could indicate however that hormones may function in different capacities under different conditions.

Acknowledgements

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Při indukci a přerušení dormance byla sledována aktivita peroxidázy a spektrum isoenzymů v pupenech a hlízách topinamburu. Nejvyšší aktivita peroxidázy byla zjištěna ve stadiu dormance. Podmínky přispívající k růstu, např. přerušení dormance chladem, stimulace růstu osy kyselinou giberelovou, stimulace růstu hlíz kinetinem, působily rychlý pokles aktivity peroxidázy spolu se snížením intensity většiny katodických isoperoxidáz. Při indukci dormance AMO-1618 se aktivita peroxidázy a převážně stejných izoenzymů zvyšovala.