

BRIEF COMMUNICATION

Changes in Isoperoxidases Induced by Hydroxyphenylacetic Acids

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Abstract. The effects of *o*-, *m*- and *p*-hydroxyphenylacetic acids on total peroxidase activity and isoperoxidase patterns of wheat coleoptile sections were studied. *o*- and *m*-HOPA reduced the total peroxidase activity while *p*-HOPA increased it. Electrophoresis in polyacrylamide gel revealed that *o*- and *m*-HOPA diminish the intensity of some anodic and cathodic bands, but that *p*-HOPA increases anodic and reduces cathodic isoperoxidases. These results may explain the apparently paradoxical positive effects of *p*-HOPA on both growth and total peroxidase activity.

In the study of the growth promoting action in plants of hydroxyphenylacetic acids (HOPA), it has been found that the three hydroxyderivatives (*o*-, *m*- and *p*-HOPA) all promoted elongation in a coleoptile section test, although with different kinetics (MATO 1980). However, only *o*- and *m*-HOPA inhibit the enzymic degradation on indole-3-acetic acid (IAA), while *p*-HOPA increases auxin-oxidase activity (MATO 1979). In an attempt to explain these complex effects, the investigations have been extended to examine the action of these three phenolics on total peroxidase activity, since peroxidase participates in auxin formation (GASPAR *et al.* 1973) and therefore has a growth regulatory function (OCKERSE and MUMFORD 1973). Further, as this enzyme exists in multiple molecular forms (SIEGEL and GALSTON 1967) and IAA produces changes in the isoperoxidase status (WHITMORE 1971), the modifications in isoenzyme patterns induced by HOPA were also analysed.

Seeds of *Triticum vulgare* L. cv. Rex were grown and coleoptiles selected as previously (MATO 1980). The apical 5 mm portion was removed and two 4 mm sections were cut off. Groups of 150 sections were incubated on 10 ml

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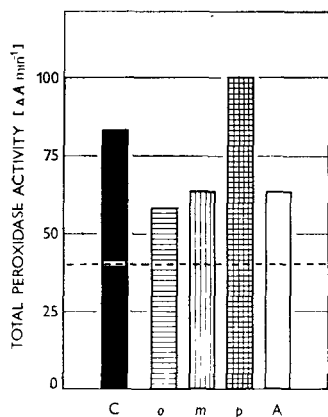


Fig. 1. Total peroxidase activity in wheat coleoptile sections incubated 20 h without (C) or with IAA 10^{-5} M (A); *o*-, *m*- and *p*-HOPA $5 \cdot 10^{-4}$ M. Dotted line: peroxidase activity before incubation.

of the test solution (phosphate-citrate buffer pH 5.0 with 2% sucrose) with or without the phenolics, for 20 h in darkness at 24 °C.

For enzyme extraction, coleoptile section lots were ground with 10 ml phosphate buffer pH 7.0 and the homogenates centrifuged at 10 000 *g* for 20 min. Total peroxidase activity was determined in this crude extract using guaiacol as substrate.

Peroxidase isoenzymes were separated by electrophoresis using 7.5% polyacrylamide gels. The bridge buffers were Tris-HCl pH 8.1 for the anodic separations and Tris-glycine pH 8.5 for the cathodic ones (COUPE *et al.* 1972). A current of 4 mA per tube was applied for 2 h in the former and 4 h in the latter. Before electrophoresis the crude extracts were concentrated 4x by ultrafiltration on Amicon membrane (FLEURIET and MACHEIX 1980). The gels were stained by supplying H_2O_2 and either guaiacol, pyrogallol or *o*-dianisidine as substrate in phosphate-citrate buffer pH 5.2.

The data for total peroxidase activity (Fig. 1) show that *o*- and *m*-HOPA reduce peroxidase activity with respect to the control, though less than IAA does. These effects are in accord with their action on growth because both phenolics as well as IAA, promote coleoptile elongation (MATO 1980). However, *p*-HOPA, also a growth stimulator (MATO 1980), increased the total peroxidase activity in coleoptiles. Apparently, there is no agreement between its effects on growth and those on peroxidases.

Electrophoresis revealed a greater number of anodic isoperoxidases than cathodic ones with all substrates used (Fig. 2). Anodic bands also presented a uniform distribution from the top to the bottom of the gels, while cathodic separation resulted in a more localized migration. This suggests greater structural similarity among cathodic than anodic isoenzymes. Similar results have been reported (GORDON and HENDERSON 1973) for oat coleoptiles.

When detection was conducted with guaiacol, treatments with IAA, *o*- and *m*-HOPA diminished the size and intensity of two anodic bands (A_1 , A_2) and of the cathodic band (C_2); however, *p*-HOPA treatment, which reduced the cathodic isoenzyme C_2 , caused induction of the bands A_1 and A_2 (Fig. 2). In gels stained with pyrogallol two isoenzymes (A_2 , A_3) appeared with greater intensity in extracts from coleoptiles treated with *p*-HOPA than in the control, whereas their intensity diminished in treatments with

o-, *m*-HOPA and IAA. The intensity of the band C_2 decreased in all treatments (Fig. 2). When isoenzymes were located with *o*-dianisidine, the band A_2 was diminished in samples incubated with *o*-, *m*-HOPA and IAA but it was increased when *p*-HOPA was present. The band C_2 was reduced in all treatments (Fig. 2).

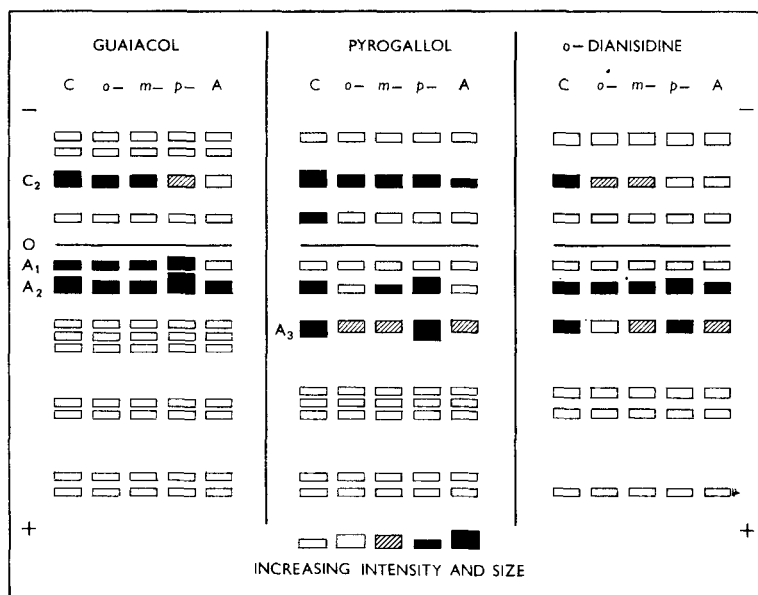


Fig. 2. Diagram of isoperoxidases from wheat coleoptile segments separated by polyacrylamide gel electrophoresis, after 20 h of incubation. C: Control; *o*-, *m*-, *p*-HOPA $5 \cdot 10^{-4}$ M; A: IAA 10^{-5} M.

It is known that cathodic peroxidases present IAA-oxidase activity (GORDON and HENDERSON 1973) and that this activity may also be associated with some anodic isoenzymes (WHITMORE 1971). These observations may explain the action of HOPA on cell elongation and on peroxidase activity. In the investigations reported here, *o*- and *m*-HOPA, as well as IAA, decreased the activity of some anodic and cathodic bands, and these effects may be associated with their action as growth stimulators (MATO 1980). More important are the results obtained with *p*-HOPA, which enhanced the activity of anodic bands and decreased the intensity of the cathodic ones. These findings indicate a possible explanation of its promoting action on both coleoptile elongation and total peroxidase activity, namely, that the growth-promoting ability may correspond to the lesser intensity of the cathodic bands while the increase in total peroxidase activity must be the sum of the increased anodic and decreased cathodic isoenzyme activity.

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BOOK REVIEW

WILLIAMS, G. (ed.): ELSEVIER'S DICTIONARY OF WEEDS OF WESTERN EUROPE. — Elsevier Scientific Publishing Company, Amsterdam—Oxford—New York 1982. 320 pp. US \$ 81.50, Dfl. 175.—.

A search for the scientific or common name of a plant in a foreign language may be a difficult and time-consuming task for a research worker. Therefore multilanguage dictionaries and manuals facilitating this work are gratefully accepted. A new book of this group was published on the proposal of the Joint Panel on the Evaluation of Herbicides of the European Weed Research Council and the European Plant Protection Organisation, with the co-operation of the Education Committee of the European Weed Research Society.

The book, introduced by explanatory notes and a list of abbreviations, contains in the "Basic table" (144 pages) the names of 1043 weed species arranged alphabetically according to their Latin scientific names. Latin names are complemented with abbreviations of the authorities for these names, area of the distribution of the plant (northern, central, southern, western zones) and their importance in each zone. Synonyms of the scientific names are (often) given in Danish, German, English, Spanish, Finnish, French, Icelandic, Italian, Dutch/Flemish, Norwegian, Portuguese and Swedish. The second part of the dictionary (170 pages) consists of alphabetic indexes of the common names in the above mentioned eleven languages. The search in these indexes is complicated by special letters and order of letters in alphabets of different languages; for instance, æ, ø, å, ä, ö are placed at the end of alphabets of Scandinavian languages. This book deserves our attention as a very useful source of plant names. Further volumes devoted to weed species of Eastern Europe and, naturally, to crop species of the whole world would be certainly welcomed by most of the research workers as the need of good scientific dictionaries and manuals will hardly ever be fully satisfied.

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