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Activity of indolyl-3-acetic acid oxidase and peroxidase in roots of carrot infested with *Meloidogyne hapla* Chitw.

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Abstract

IAA-oxidase and peroxidase activity was measured in storage and side roots of healthy and *M. hapla* infested carrots of two sultivars. Cultivar 'Perfekcja' is sensitive whereas cv. 'Slendero' is tolerant to the northern root-knot nematode. 3-, 4-, and 5-month-old plants were subjected to analyses.

In *M. hapla* infested plants of both cultivars IAA-oxidase inhibitors accumulated. Kinetics of IAA oxidation *in vivo* were the same in healthy and infested plants. IAA-oxidase activity *in vitro* was inhibited in crude extracts of the infested tissues, the inhibition being prevented by PVP. Peroxidase activity increased in secondary phloem and decreased in galled side roots of both cultivars when compared with healthy controls. In galled side roots of the youngest 3-month-old plants peroxidase activity was not decreased. IAA-oxidase inhibitors accumulated in the infested roots.

It is concluded that *M. hapla* has no direct effect on IAA-oxidase. Degree of tolerance to nematodes is correlated with the ratio of IAA-oxidase inhibitors to IAA-oxidase rather than with the absolute activity of IAA-oxidase.

INTRODUCTION

The root-knot nematode *Meloidogyne* spp. develops well in roots of many host plants (Brzeski 1971) which respond to the invading larvae by formation of both (1) multinucleate syncytia (giant cells) and (2) galls (Bird 1962).

There are many hypotheses explaining the machanism of formation of the gall (Dropkin 1969; Viglierchio 1971). According to one line of evidences, the galls are formed in the result of accumulation of active auxins and other growth regulators around the loci of the nematode feeding (Yu and Viglierchio 1964; Viglierchio and Yu 1965, 1968; Balasubramanian and Purushothaman 1972; Brueske and Bergeson 1972). The phytohormones, concentrated

locally, induce the cells of the pericycle and cortex to divide and enlarge (Viglierchio 1971).

The northern root-knot nematode, *Meloidogyne hapla* Chitw., develops well on fibrous side roots of carrot (Berbeć 1970). Knypl et al. (1975) have found that activity of IAA-oxidase decreased and IAA-oxidase inhibitors accumulated in the roots of infested carrots. They suggested that the galls might be formed as the result of decreased auxin catabolism in the tissues surrounding the places of nematode development. If this suggestion was true, varieties of carrot with different susceptibility to the nematode, should be characterized by either (1) different IAA-oxidase activity or (2) different ability to synthesize and/or accumulate the IAA-oxidase inhibitors in response to infestation.

The aim of this study was to analyse IAA-oxidase activity in two cultivars of carrot. The cultivar 'Perfekcja' is regarded to be very susceptible to *M. hapla,* whereas the cultivar 'Slendero' is regarded to be tolerant (Brzeski 1974a, b) since it shows little injury on infestation (Rohde 1972).

MATERIAL AND METHODS

1. Plants

The tests have been caried out in 1973 and 1974 using 2-, 3-, 4-, and 5-month-old carrots (*Daucus carota* L.) cv. 'Perfekcja' and cv. 'Slendero'. The seeds were sown into steam sterilized sandy soil in 8 cm plastic pots. One-month-old plants were infested with two egg-sacs of *Meloidogyne hapla* per pot. Control plants were not infested. The plants have been grown in a greenhouse of the Institute of Vegetable Crops, Skierniewice.

At the moment of analyses storage roots of three- to five-months-old plants cv. 'Perfekcja', infested with *M. hapla,* were branched. Many galls appeared on both the fibrous side root system and the storage root. The storage roots of infested cv. 'Slendero' were not branched and the galls were small (Brzeski 1974a, b; Knypl et al. 1975).

2. Plant material for analyses

The pots were transferred from Skierniewice to Łódź, and placed for a number of days in a growth room at 25°C. The plants were washed with tap water and divided into several parts. Fibrous side root system and the root-knot galls that appeared on the surface of the storage roots were dissected and collected separately. Leaves, the top hypocotyl part of the storage root (upper ca. 3 cm) and the main non-storage root were discared. Secondary phloem and pericycle, and the secondary xylem of the storage roots were separated by hand and collected.

The plant material was surface sterilized with 0.1 per cent chloretone (1,1,1-frichloro-fert-butanol) for 15 min, washed with sterile water, blotted dry, and used for IAA-oxidase *in vitro* and *in vivo* assays, and for peroxidase *in vitro* assay.

3. Extraction of IAA-oxidase and peroxidase

One gram samples of sliced tissues were homogenized with prechilled mortar and pestle in 5 ml of cold sodium-phosphate buffer (0.2 M, pH 6.1) (Jacobson and Caplin 1967) alone or the buffer and hydrated insoluble polyvinyl-N-pirrolidone (Polyclar AT; PVP) as described (Knypl and Chylińska 1974). Peroxidase was extracted with the same buffer using no PVP. Preliminary tests revealed that PVP may lead to decreased peroxidase activity in the extracts (Knypl et al. 1975).

4. *In vivo* and *in vitro* IAA-oxidase assay

500 mg samples of the sliced tissues were surface sterilized with 0.1 per cent chloretone for 15 min, washed, blotted dry and placed in 10 ml aliquots of the reaction mixture consisting of 0.2 mM IAA, 0.1 mM $MnCl_2$, 0.1 mM 2,4-dichlorophenol and 2.5 ml sodium-phosphate buffer (0.2 M, pH 6.1). Penicillin G (50 $\mu g\ ml^{-1}$), streptomycin sulphate (50 $\mu g\ ml^{-1}$) and chloramphenicol (2.5 $\mu g\ ml^{-1}$) were added to the medium as bacteriostatics. Incubation was carried out in a laboratory water-bath shaker (100 oscillations min^{-1}) at 30°C in diffused day light. At zero time and at each 15 min intervals 0.5 ml aliquot of the reaction mixture was taken up and remaining IAA measured colorimetrically (Pilet and Chollet 1970).

Details of the IAA-oxidase *in vivo* assay (Galston and Dalberg 1954) have been described (Knypl and Chylińska 1974).

5. Determination of peroxidase activity

The peroxidase (quaiacol:H_2O_2 oxidoreductase, E.C.1.11.1.7) activity was assayed by the method of Gaspar and Lacoppe (1968). The increase in absorbance at 470 nm measured between 30 and 90 seconds after addition of 0.4 ml of 0.3 per cent hydrogen peroxide solution to the reaction mixture containing 2.9 to 2.5 ml of phosphate buffer 0.1 M, pH 5.4, 0.5 ml 1 per cent quaiacol, and 0.1 to 0.5 ml enzyme extract in a total volume of 3.5 ml.

6. Determination of protein

Protein from buffer extracts was precipited, purified (Knypl et al. 1975) and measured according to Lowry et al. (1951) using bovine serum albumin as standard.

RESULTS

1. Activity of IAA-oxidase *in vivo* and *in vitro*

Kinetics of IAA oxidation *in vivo* were basically the same irrespective healthy or *M. hapla* infested tissues were taken into consideration. As it can be seen from Fig. 1, slopes of the curves representing IAA-oxidation by comparable healthy and infested tissues are the same. The only one difference is that the lag-phases, preceding the initiation of IAA oxidation by the nematode infested tissues, are markedly longer when compared with the proper healthy control. The lag-phase in the case of *M. hapla* infested side roots cv. 'Slendero' was longer than six hours, and for that reason it was impossible to detected the enzyme activity *in vivo*. The only one exception of this general rule is the secondary xylem of the 3-month-old infested 'Perfekcja' plants, which showed shorter lag-phase than the healthy tissue (Fig. 1, curves XM and XH, respectively).

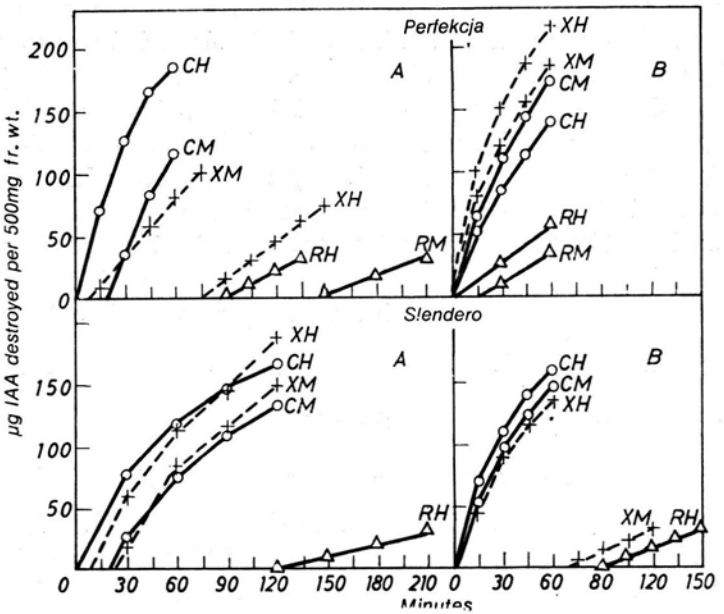

Fig. 1. Kinetics of IAA destruction *in vivo* by tissue samples of healthy (H) and *M. hapla* (M) infested carrots cv. 'Perfekcja' and cv. 'Slendero'
A — 3-month-old plants; B — 5-month-old plants. C — secondary phloem, X — secondary xylem, R — fibrous side roots
In the case of side roots of infested plants cv. 'Slendero' (RM), the lag period was longer than 360 min

The lag-phases preceding IAA oxidation *in vivo* by the oldest five -month-old plants were generally shorter than those showed by younger three-month-old plants (Fig. 1 B). In crude buffer extracts of the

storage root tissues of three-month-old healthy plants of both carrot cultivars the IAA-oxidase activity was significantly higher than in the extracts of corresponding tissues of nematode infested plants. This was especially true for the secondary phloem and secondary xylem tissues of cv. 'Perfekcja'. In contrast, there was no significant difference between IAA *in vitro* oxidation by buffer extracts of healthy and *M. hapla* infested side roots of a given cultivar (Table 1).

If healthy plants are taken into consideration, the cultivar 'Perfekcja' is characterized by higher IAA-oxidase activity in the secondary phloem tissue and lower enzyme activity in the side roots as compared with the cultivar 'Slendero' (Table 1).

Table 1

Activity of IAA-oxidase *in vitro* in roots of healthy (H) and *M. hapla* (M) infested carrot plants cv. 'Perfekcja' and cv. 'Slendoro'.

The plants were grown for 3-, 4-, and 5-months in a greenhouse since June to December 1973. IAA-oxidase was extracted with 0.2 M sodium-phosphate buffer pH 6.1 alone B; 3-month-old plants, or the buffer and hydrated PVP B+PVP; 4-, and 5-month-old plants

Tissues	µg IAA destroyed 30 min^{-1} 100 mg^{-1} fr. wt.		
	Age of plants, months		
	3	4	5
	B	B + PVP	B + PVP
cv. 'Perfekcja'			
Secondary phloem H	153	96	66
Secondary phloem M	49	120	100
Secondary xylem H	65	85	93
Secondary xylem M	28	62	80
Side roots H	35	105	100
Side roots M	35	115	110
cv. 'Slendero'			
Secondary phloem H	56	72	84
Secondary phloem M	42	71	86
Secondary xylem H	62	60	64
Secondary xylem M	25	60	60
Side roots H	85	70	78
Side roots M	70	115	96

The level of endogenous IAA-oxidase inhibitors increases with age of carrot plants (Jacobson and Caplin 1967). If the inhibitors were discarded by means of grinding the tissues with PVP, the IAA-oxidase

activity in the infested tissues was found to be either the same or higher than in the healthy control tissues (Table 1). The increment of enzyme activity due to grinding with PVP was especially high in side roots of infested plants cv. 'Slendero'.

2. Peroxidase activity and protein content

Peroxidase activity (Table 2) was measured in extracts of the same tissues as used for IAA-oxidase *in vitro* assays, but PVP was omitted during extraction. Peroxidase activity, expressed per 100 μg protein, in tissues of healthy plants of both cultivars increases with age of the plants, partly because the level of extractable protein decreases (Table 3).

Table 2

Peroxidase activity in roots of healthy (H) and *M. hapla* (M) infested carrot plants cv. 'Perfekcja' and cv. 'Slendero'

Peroxidase was extracted with 0.2 M phosphate buffer pH 6.1 without PVP and the activity determined as described in Material and Methods

Tissues	Age of plants, months		
	3	4	5
	Proxidase, [ΔA_{470} min^{-1} 100 μg^{-1} protein] $\times 10^{-3}$		
	cv. 'Perfekcja'		
Secondary phloem H	150	180	400
Secondary phloem M	260	320	750
Secondary xylem H	65	110	130
Secondary xylem M	60	90	130
Side roots H	700	1040	2110
Side roots M	670	1120	1420
	cv. 'Slendero'		
Secondary phloem H	100	150	460
Secondary phloem M	180	400	1530
Secondary xylem H	280	470	130
Secondary xylem M	60	80	190
Side roots H	310	1550	2950
Side roots M	330	920	1220

Peroxidase activity in the secondary phloem of *M. hapla* infested plants of both cultivars is strikingly higher than in corresponding heathly control, the effect being regular and independent on age of the plants. The enzyme activity in galled side roots, in contrast to the secondary phloem, is either the same as in the healthy roots (three-month-old plants) or lower by 40 to 60 per cent (five-month-old plants). This decrease is mainly due to the fact that the galled roots of five-month-old plants

contain about twice as much soluble protein as corresponding healthy roots (Table 3). There was no such a decrease of peroxidase activity when the result were calculated per a unit of fresh weight (data not shown).

Table 3

Soluble protein contents in healthy (H) and *M. hapla* (M) infested roots of 3-, 4-, and 5-month-old carrot plants

Protein was extracted with 0,2 M phosphate buffer pH 6,1 and measured by the method of Lowry et al. (1951)

Tissues	Protein, μg 100 mg^{-1} fr. wt.		
	Age of plants, months		
	3	4	5
cv. 'Perfekcja'			
Secondary phloem H	181	100	75
Secondary phloem M	211	137	73
Secondary xylem H	132	81	62
Secondary xylem M	136	89	60
Side roots H	90	52	44
Side roots M	130	82	81
cv. 'Slendero'			
Secondary phloem H	190	87	44
Secondary phloem M	185	78	45
Secondary xylem H	89	56	31
Secondary xylem M	102	51	32
Side roots H	181	67	31
Side roots M	182	70	82

DISCUSSION

It has been found that (1) activity of IAA-oxidase both *in vivo* and *in vitro* is higher in storage tissues and side roots of healthy carrot cv. 'Perfekcja' as compared with cv. 'Slendero' except of the side roots of the youngest three-month-old plants in which the relation *in vitro*, in contrast to the *in vivo* (Fig. 1), is reverse, *i.e.* the side roots of cv. 'Slendero' are characterized by higher IAA-oxidase activity than side roots of cv. 'Perfekcja' (Table 1). The inconsistence of results of the *in vivo* and *in vitro* tests was possibly due to a fact that IAA-oxidase activity was assayed in crude buffer extracts, containing many polyphenolic inhibitors and activators of the enzyme (Hare 1964; Jacobson and Caplin 1967). If majority of the polyphenols were discared by grinding the tissues with PVP, side roots of young carrot cv. 'Slendero' showed

lower IAA-oxidase activity when compared to cv. 'Perfekcja' (K n y p l et al. 1975).

(2) Kinetics of IAA destruction *in vivo* by corresponding tissues of healthy and *M. hapla* infested plants are basically the same. *In vitro*, however, the infested tissues show generally lower IAA-oxidase activity than healthy controls, if buffer extracts are taken into consideration (Table 1). When PVP is included into extracting buffer, the infested tissues have the same or higher IAA-oxidase activity as the healthy controls. It can be thus inferred that lower IAA-oxidase activity in crude extracts of nematode infested tissues is an artefact due to presence of polyphenols, espacially of chlorogenic acid (K n y p l et al. 1975) which inhibit the enzyme activity (H a r e 1964).

(3) The level of IAA-oxidase inhibitors markedly increases in the infested tissues as manifested by the longer lag phases preceding IAA-oxidation both *in vivo* (Fig. 1) and *in vitro*, especially in side roots and galls (K n y p l et al. 1975).

(4) Peroxidase activity per unit of protein increases in secondary phloem and decreases in side roots of *M. hapla* infested plants of both cultivars as compared to healthy controls. However, in the side roots of the youngest three-month-old plants peroxidase activity is nearly the same in the infested plants as in the healthy plants. Since in the galled roots the level of soluble protein increased, the peroxidase activity per unit of fresh weight increased (Table 2 and 3) under influence of nematodes. If younger, two-month-old plants were taken into analyses, peroxidase activity in the galled side roots was markedly higher than in control roots (K n y p l et al. 1975).

Results of this study, listed in paragraphs (1) to (4), corroborate with the data reported earlier. V i g l i e r c h i o and Y u (1965) have found that IAA-oxidase activity increased in plants infested with *M. hapla*, *M. javanica* and *M. incognita*. The increment of enzymatic activity correlated with the rise of auxin content. According to H u a n g et al. (1971), peroxidase activity increased in galls, galled roots and the shoots of *M. incognita* infested tomato plants. Peroxidase activity increased in roots of *Bidens tripartita* parasitized by a free-living nematode *Longidorus africanus* (E p s t e i n 1972).

W i l s k i and G i e b e l (1971) have found that in response to *Heterodera rostochiensis* infestation, oxidation of IAA increases both in susceptible and resistant cultivars of potato. It is of interest that in roots of tomato resistant to *Heterodera rostochiensis* the IAA-oxidizing system is more active than in the roots of susceptible cultivars (W i l s k i and G i e b e l 1971).

Results of this study seem to indicate that *M. hapla* infestation has no direct effect on IAA-oxidase. Under the influence of the nematode,

however, IAA-oxidase inhibitors accumulate that probably retard auxin oxidation *in vivo*. Auxins in turn, accumulated locally, may induce tissue proliferation and lead to gall formation around the loci of nematode feeding.

Carrot cv. 'Slendero' is regarded to be less sensitive to *M. hapla* than cv. 'Perfekcja' (Brzeski 1974a, b). Since carrot cv. 'Slendero' has lower IAA-oxidase activity than cv. 'Perfekcja', it can be suggested that tolerance to nematodes is dependent on the ratio of IAA-oxidase to IAA-oxidase inhibitors rather than on absolute enzyme activity, especially in the early phases of the infection process (Knypl et al. 1975). Wilski and Giebel came to similar conclusion analysing the "IAA-oxidase: IAA-oxidase inhibitor" system of potato infested with *Heterodera*.

In next paper of this series data will be presented that carrot cv. 'Perfekcja' accumulates more IAA-oxidase inhibitors in response to *M. hapla*, than carrot cv. 'Slendero'.

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Aktywność IAA-oksydazy i peroksydazy w korzeniach marchwi porażonej mątwikiem północnym

Streszczenie

Obiektem analiz była marchew 3-, 4-, i 5-miesięczna odm. 'Perfekcja' i odm. 'Slendero', zdrowa i porażona przez mątwika północnego (*Meloidogyne hapla* Chitw.). Marchew odm. 'Perfekcja' jest bardzo wrażliwa w stosunku do nicienia, a odm. 'Slendero' jest odporniejsza.

Stwierdzono, że (1) w korzeniach bocznych marchwi zdrowej odm. 'Perfekcja'

zawartość białka, w przeliczeniu na 100 mg świeżej masy, jest niższa niż w korzeniach bocznych odm. 'Slendero'. (2) W zdrowej marchwi odm. 'Perfekcja' zawartość białka obniża się wolniej wraz z wiekiem niż w marchwi odm. 'Slendero'. (3) W roślinach porażonych przez nicienia zawartość białka jest wyższa niż w roślinach kontrolnych. (4) W korzeniu zapasowym marchwi odm. 'Perfekcja' aktywność IAA-oksydazy jest ogólnie wyższa niż w marchwi odm. 'Slendero'. (5) Aktywność IAA-oksydazy w nie oczyszczonych ekstraktach buforowych tkanek porażonych jest niższa niż w ekstraktach tkanek zdrowych, lecz po zastosowaniu PVP aktywność tego enzymu jest podobna lub wyższa w przypadku roślin porażonych niż zdrowych. (6) Pod wpływem porażenia przez nicienia w tkankach, zwłaszcza w korzeniach bocznych, gromadzą się inhibitory IAA-oksydazy, indukujące lag-fazę w procesie utleniania IAA. (7) Aktywność peroksydazy jest wyższa w zdrowej marchwi odm. 'Perfekcja' niż w odm. 'Slendero'. (8) Pod wpływem porażenia przez mątwika północnego aktywność peroksydazy silnie wzrasta w korzeniu zapasowym marchwi obu odmian; w porażonych korzeniach bocznych początkowo nie zmienia się, a następnie obniża.

Wnioskuje się, że *M. hapla* nie wpływa bezpośrednio na IAA-oksydazę. Stopień odporności marchwi na mątwika północnego zależy nie tyle od absolutnej aktywności IAA-oksydazy ile od stosunku aktywności IAA-oksydazy do zawartości jej inhibitorów.