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CHANGES IN PEROXIDASE ISOENZYMES IN TOMATO GALLS INDUCED BY *MELOIDOGYNE INCOGNITA*[1]

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Abstract

All of the twelve isoperoxidases detectable for tomato somatic organs are present in the root systems. From the point of origin on starch gel electrophoretograms, those moved toward the cathode are in the order named 1C, 2C, 3C, 4C, 5C, and 6C. Those in the anodic end are coded as 1A, 2A, 3A, 4A, 5A and 6A. Only 5A, 6A and occasionally 4A are detected from a normal stem. Induction of stem galls with root knot nematodes, however, induces syntheses of cathodic 2C, 3C, 5C, 6C, occasionally 1C and anodic 2A as well as 4A isoperoxidases in addition to the two anodics regularly present in the normal stem. Syntheses of cathodic extra isoenzymes in stem galls are detectable 12 hours after inoculation. Highest number of isoperoxidases can be found in stem galls between two days and one month of age. Cathodic members decreased in the older galls. Gall development also enhances syntheses of the three anodic isoenzymes in normal stems and the stimulation persists to the four- month old galls. Induction of root galls by the nematodes increases the concentration of a few isoenzymes but does not qualitatively alter the isoperoxidase population of a normal root system.

Changes in isoenzyme patterns have been repeatedly detected from malignant tumors of man and animals. Brain tumors are known to exhibit alterd lactate dehydrogenase (LDH) isoenzyme activities (Sherwin *et al.*, 1968). Characteristic LDH isoenzyme shifts have been convincingly demonstrated in human bronchogenic (Langvad, 1968) and uterine carcinoma developments (Widy-kierska & Roszkowski, 1969). Sera from prostate carcinoma patients were found to exhibit one additional acid phosphatase isoenzyme band (Rozenszajn *et al.*, 1968). Rat liver tumor cells show an aldolase isoenzyme pattern distinctly different from those of normal livers (Matsushita *et al.*, 1968)

Tumor-forming hybrid of tobacco (*Nicotiana glauca.* × *N. longidorfii*) carries enzymes and proteins of characteristic electrophoretic patterns which are not equivalent to either one of the parental species (Bhatia *et al.*, 1967). No other isoenzyme studies for plant tumors or galls is known to us, though changes

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in isoenzyme patterns attributable to plant diseases other than tumors and galls have been reported in many places (Shannon, 1968).

Isoenzymes have been used effectively as markers in genetic and developmental studies (Shannon, 1968 and Scandalios, 1969). It has even been proposed that isoenzymes are involved directly in genetic regulatory control mechanism and therefore have very much to do with tissue differentiations (Shannon, 1968). Since gall formation induced by root knot nematodes involves abnormal developments (Huang & Maggenti, 1969a and b) as well as differentiation (Huang & Lin, 1970), the isoenzyme changes associated with the disease development were studied.

Materials and Methods

Tomato (*Lycopersicon esculentum*) stem galls used throughout this work were artificially induced with *Meloidogyne incognita* according to the method previously described (Huang & Lin, 1970). The tomatoes were grown in a greenhouse where diurnal temperature ranged between 25–35°C. The infected (galled) stem portions were periodically harvested after inoculation and analysesed for their peroxidase isoenzyme patterns. Uninfected stem portions in the vicinity of a stem gall were also harvested to serve as controls. Since the control and galled stems were from the same individuals, changes in isoenzyme patterns thus detected in the galls are not due to genetical variation.

To prepare them for electrophoretic analyses, the tissues were freshly excised and rinsed thoroughly with distilled water. About 1–3 g of the tissues were then ground in pre chilled mortars which had previously been heated to 160°C for an hour in an oven. The homogenate was applied to electrophoretic field without further purification.

Separations of the enzymes were done with the slightly modified version of Smithies (1955) starch gel zonal electrophoresis described by Endo (1966). 12.5% starch (NBC Batch No. 9200) gel was prepared with 0.03 M borate buffer at pH 8.5 and solidified in the plastic molds (Toyo model HA–1, internal dimension 6×20×200 mm) at 4°. The tissue homogenate was picked up (saturated) with a 6×18 mm filter paper strip (Whatman # 1) which was then inserted at the midpoint of a solidified starch gel. A D.C. current of 10 V/cm (Ca. 1.7 mA/cm) was then applied to the gels in cold (5–8°) for four hours using 0.3M borate at pH 8.5 as electrode buffer.

At the end of the run, the gel blocks were horizontally sliced into two and the bottom halves were stained for 20 min. with a reagent containing 0.03% H_2O_2, 0.1% benzidine acetate in 0.1 M tris-acetic buffer at pH 4.0 for peroxidases (Endo, 1966). The staining solution was prepared fresh each time.

Results

Isoperoxidase populations in various tomato organs

So that aberrations of pathological origin might be recognized, normal tomato vegetative organs were investigated for their respective isoperoxidase populations. Analysis of ten random plants with the electrophoretic technique indicated that twelve isoperoxidases are recognizable for a normal tomato. Since all of the twelve isoenzymes are represented in the root extract, electrophoretograms prepared for this organ were taken as a model based on which zymographic bands were named. According to their relative positions from the point of origin, the bands migrating toward cathode are named 1C, 2C, 3C, 4C, 5C and 6C respectively. Similarly, those moved toward anode are respectively coded as 1A, 2A, 3A, 4A, 5A and 6A (Fig. 1).

Organ specific distribution of isoperoxidases in tomato is evident in Fig. 1. Whereas all of the twelve isoenzymes are present in the root systems, only a small number of them are detectable from the above-ground somatic organs.

To be specifically noted is the isoenzyme population of normal stems whose pathological aberrations are to be analysed. No cathodic bands has ever been detected for this organ. Of the anodic isoenzymes, 5A and 6A appeared constantly whereas 4A occasionally and weakly, in normal stem preparations. 1A, 2A and 3A have never been detected from this organ.

Peticles carry isoperoxidase population similar to that of the stems. Except 5A, 6A and rarely 5C and 3C, no other isoenzymes are detected from leaf blades. From the shoot apex, only 4A, 5A and occasionally 6A are recognizable.

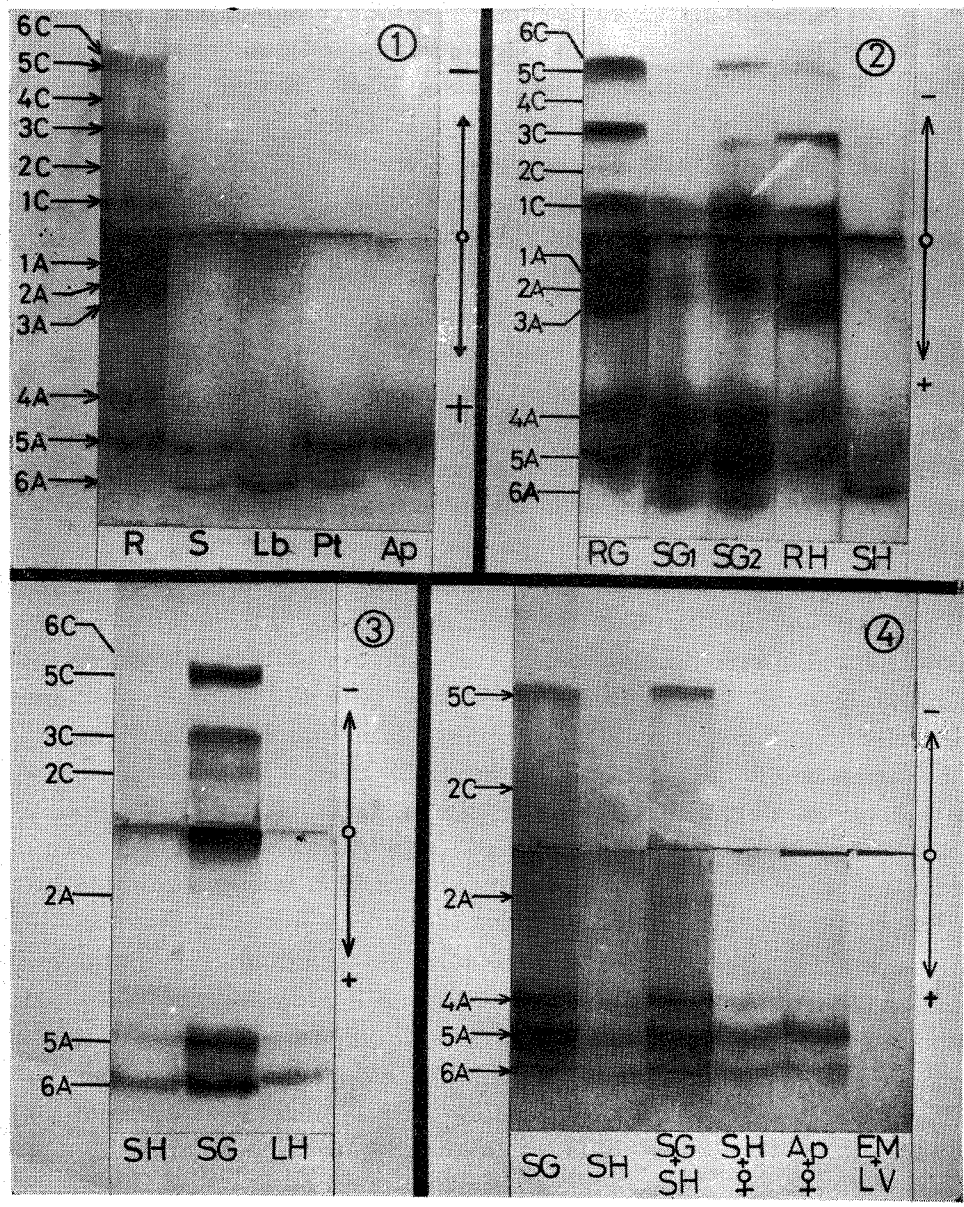
As shown in Fig. 1, band intensities (isoenzyme concentrations) are variable between isoenzymes of the same organs as well as between the same isoenzymes

Fig. 1. Electrophoretograms of isoperoxidases from various somatic organs of a normal tomato, R=root system. S=stem. Lb=leaf blade. Pt=petioles. Ap=shoot apex. Cathodic bands 2C and 4C of root appeared on the gel weakly thus not clearly shown in the photograph.

Fig. 2. Electrophoretograms of isoperoxidases from a tomato showing changes in the isoenzyme populations due to ***Meloidogyne incognita*** induced gall formation RG=root galls of approximately two months old. RH=normal roots. SG_1=stem gall four months after inoculation. SG_2=stem gall one month after inoculation. SH=healthy stem. All of these tissues are of one single plant. Note also the differences in intensities between the bands of RG and RH.

Fig. 3. Electrophoretograms of isoperoxidases from a tomato showing ***M. incognita*** induced changes in isoenzyme populations. SH=healthy stem portion immediately beneath SG. SG=root knot nematode-induced stem gall two days after inoculation. LH=leaf and petiole. All of the tissues are from the same plant.

Fig. 4. Electrophoretograms of isoperoxidases showing that the changes in isoenzyme populations in stem galls are not due to egg mass or nematode bodies themselves. SG=stem gall, SH=healthy stem, AP=shoot apex, all from the same plant. Globule females (♀), egg mass (EM) and second stage larvae (LV) of ***M. incognita*** are from root galls of another tomato plant.


①
6C
5C
4C
3C
2C
1C
1A
2A
3A
4A
5A
6A
R S Lb Pt Ap
②
RG SG1 SG2 RH SH
③
SH SG LH
④
SG SH SG+SH SH♀ Ap♀ EM+LV

from different organs. Intensities of given isoenzyme bands, however, appeared to be specific for organs from which the enzymes were detected.

Isoperoxidase populations in nematode-induced galls

With respect to peroxidase isoenzyme population, no qualitative difference can be detected between normal roots and nematode-induced root galls. Judging from the band intensities on electrophoretograms, however, differences in isoenzyme concentrations undoubtedly exist (Fig. 2).

Induction of stem galls with root knot nematodes increased the number of peroxidase isoenzymes of a normal stem tissue (Figs 2 & 3). Since each comparison was made between the stem gall and healthy stem tissue of the same plant, the difierence obviously is not genetical.

All the isoperoxidases throughout this report are named according to the healthy root model (Fig. 1). Wherever in doubt, a portion of that tissue in question was homogenized with equal amount of normal roots and the mixture run side by side with the extracts of normal roots as well as the suspected tissue in the same electrophoretic field. Comparison of these electrophoretograms correctly identifies the unknown isoenzymes relative to their counterparts of normal roots.

A question naturally arises as to whether the extra isoenzymes in a stem gall are the contributions from the nematode corpes. To clarify this, young globule females were dissected out of root galls, rinsed three times with cold distilled H_2O and homogenized with healthy stem and shoot apex tissues respectively at the ratio of 50 ♀/g tissues. The ratio was chosen because it was determined to be the maximum nematode content in the stem galls we have been working with. The isoenymes in these homogenates as well as those of egg mass and larvae were then compared with those of a stem gall from the same plant. Results indicated that the extra isoenzymes in the stem galls are not accounted for by the nematode corpses in the former (Fig. 4).

As controls to our method of inoculation, the following experiments were carried out from time to time. (1) Tomato stems were wrapped with black sand in exactly the same manner as if they were to be inoculated. Instead of nematode inoculum, however, tap water was injected. (2) Stems were punctured with a sharp needle followed immediately by the same treatment mentioned in (1). Both of the treatments failed to provide tomato stems with the extra isoenzymes observed for stem galls.

To investigate isoenzyme patterns of stem galls at various stages of development, a batch of tomato stems was inoculated with *M. incognita* and periodically harvested to assay for isoperoxidases. So that interference from genetical variations can be recognized, a portion of uninoculated stem from

the same plant was assayed simultaneously with a stem gall each time to serve as a control. The uninoculated stem controls were always those stem tissues immediately above and below a gall. As shown in Table 1, changes in isoenzyme patterns are detectable as early as twelve hrs. after inoculation. At any stage of development, stem galls contain extra isoenzymes in addition to those detectable for the uninoculated counterparts. It is to be noted, however, that all isoenzymes in stem galls are detectable from uninoculated roots but the reverse is not true.

The following patterns can be deduced from Table 1. (1) Except a few irregularities, species of extra isoenzymes synthesized in stem gall are quite constant, at least during the first month of gall development. (2) Gall development does not inhibit syntheses of the three anodic isoperoxidases detectable in normal stems. It instead seemed to enhance them and the stimulation persisted to the four-month old galls. (3) Highest number of isoenzymes are found in stem galls between two days to one month old. The period correlates with the most active period of gall development.

Discussion

All of the twelve isoperoxidases of the tomato are detectable from root systems. However, only three to five of them appeared in the above-ground tissues. Since organs of the same plants were compared to one another in all of the assays, the discrepancy in isoenzyme constituents between organs can not be genetical. The results are in good conformity with many reports about the organ-specific distribution of isoperoxidases in a plant (Scandalios, 1964, Evans & Alldridge, 1965 and Upadya & Yee, 1968).

Root knot nematode infections enhance synthesis of at least five isoperoxidases which otherwise are not found, or present in quantities too minute to be detected, in a tomato stem. To the best of our knowledge, this is the first report on isoenzyme changes due to gall-formation in plants.

Differential synthesis of isoenzymes in various organs and tissues of a plant, confirmed in this report for tomato, has been generally believed to play a vital role in differentiation (Shannon, 1968, Scandalios, 1969). Based on the regulator and operator gene concept proposed by Jacob and Monod (1961), Shannon (1968) suggested that the differential synthesis of isoenzymes are the primary results of the regulatory mechanisms of gene activity. Induction of certain isoenzymes in tomato stem galls by root knot nematodes, therefore, must be the results of interferences with the host plant's normal regulatory systems of gene activity. It is premature to speculate at this time, however, on the mechanism of the interference, inasmuch as the so-called regulation of gene activity has not yet been clearly elucidated.

Table 1.

Isoperoxidase populations in Meloidogyne incognita induced tomato stem galls of various ages

	Isoperoxidases[2]	Healthy CK Roots	Healthy CK Stem	Ages of stem galls (time after inoculation[1]) 12H	24H	2D	5D	8D	11D	14D	19D	30D	36D	40D	61D	4M
Cathode	6C	++	−	−	−	+	++	++	++	++	++	++	−	−	−	+
	5C	++	−	−	−	++	+	++	−	++	++	+	++	−	++	−
	4C	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	3C	++	−	++	++	++	++	++	++	++	++	++	−	−	−	−
	2C	+	−	+	+	++	+	+	+	++	++	+	++	−	++	−
	1C	++	−	−	−	+	−	−	−	−	−	++	−	−	−	++
Anode	1A	++	−	−	−	−	−	−	−	−	−	−	−	−	−	+
	2A	++	−	−	−	+	+	+	+	+	+	−	−	−	++	++
	3A	++	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	4A	++	−(+)	−	−	−	+	++	++	++	++	++	++	++	++	++
	5A	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	6A	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++

1) H=hours; D=days, M=months.

2) −=peroxidase staining reaction negative. +=peroxidase staining weakly positive, of the intensity equivalent to that of 6A, 2C or 4C of root in Fig. 1. ++=peroxidase staining reaction positive, of the intensity equivalent to that of 5A, 4A, 1C, 3C, 5C or 6C of root in Fig. 1. ++=peroxidase staining strongly positive, of the intensity equivalent to that of 1A, 2A or 3A of root in Fig. 1. −(+)=peroxidase staining negative but occassionally weakly positive.

It is not known whether the synthesis of gall-specific isoperoxidases plays any role in root knot nematode-induced gall formation or whether it is merely the by product of the abnormal over-growth. Peroxidases carry out oxidations of a wide variety of compounds by H_2O_2 (Paul, 1963). Roles played by these enzymes in gall formation, therefore, will be a fertile area of research. We suggest that much can be gained through studies on the physiological meanings of individual isoenzymes.

Plant peroxidases have also been known to catalyze the oxidation of indole acetate by H_2O_2 (Endo, 1968). We have evidence to indicate that the gall-specific isoperoxidases also are capable of oxidizing indole acetate (unpublished data). Nematode-induced root galls are reported to contain higher level of indolar compounds compared to healthy roots (Bird, 1962, Balasubramanin and Rangaswami, 1962 and Yu and Viglierchio, 1964), though the roles played by the growth regulators in gall-formation have not been determined. How the gall tissues, with pronouncedly more peroxidases, still maintain the higher level of indolar compounds as reported, is indeed an intriguing problem and certainly deserves serious investigation.

Résumé

Les nêmatodes noueux des racines responsables des changements d'isoenzymes des galles de tomate

On retrouve dans le système radiculaire de la tomate les douze isoperoxydases pouvant être détectées dans les organes somatiques. En partant du point d'application sur les électrophorétogram-mes sur gel d'amidon, on a dénommé les ies isoperoxydases qui se déplacent vers la cathode 1 c, 2 c, 3 c, 4 c, 5 c and 6 c tandis que celles se déplacant vers l'anode ont été codées de 1 A à 6 A On retrouve dans la tige les 5 A et 6 A et occasionnellement 4 A. L'induction de galles sur la tige par des nématodes noueux des racines fait apparaître les 2 c. 3 c, 5 c, 6 c et occasionnellement le 1 c. En outre on détecte également les 2 A et 4 A en plus des deux isoperoxydases présents dans les tiges normales. La synthèse des isoenzymes cathodiques dans les galles est détectable 12h après l'inoculation. Le nombre le plus élevé d'isoperoxydases est détecté dans les galles âgées de deux jours à un mois. Le nombre des isoperoxydases cathodiques décroit dans les galles plus âgées. Le développement de galles stimule également, pendant une pèriode de 4 mois, la synthès des trois isoenzymes anodiques normalement prèsentes dans la tige. L'indution par des nèmatodes de galles sur les racines augmente la concentration de quelques isoenzymes mais n'affecte pas qualitativement le nombre d'isoperoxydases d'un système radiculaire normal.

Refereces

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