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CHANGES IN OXYGEN UPTAKE OF MITOCHONDRIA FROM SUSCEPTIBLE AND RESISTANT TOMATO ROOTS INFESTED BY *MELOIDOGYNE INCOGNITA*

by
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Changes in the energy balance in resistant-reacting plants are associated with a marked increase in their biosynthetic activity. A manifestation of this enhanced metabolic activity is the increase of oxygen consumption by resistant plants attacked by pathogens (Smedegard-Petersen, 1980). However, it is also commonly observed that oxygen consumption in susceptible plants is affected by pathogen attack (Smedegard-Petersen, 1977, 1982).

When root-knot nematodes invade susceptible tomato cultivars they induce giant cells which contain more protein and nucleic acid than normal cells (Owens and Novotny, 1960). Giant cells are characterized by a high biosynthetic activity which is considered to be supported by an increased oxidative metabolism. Nematode infestation of resistant cultivars gives rise to a hypersensitive reaction (Kaplan and Keen, 1980; Bleve-Zacheo *et al.*, 1982) which leads to the formation of necrotic areas surrounding the pathogen. Such changes are accompanied by increased activities of many oxidative enzymes such as polyphenol oxidases, ascorbic acid oxidase and peroxidases (Zacheo *et al.*, 1982; Hutcheson and Buchanan, 1983) which are not directly linked to terminal oxidations involved in the energy-yielding processes.

Zacheo and Molinari (1987) found that oxygen consumption of tomato roots excised from very young seedlings of the susceptible cv. Roma VF infested by the root-knot *Meloidogyne incognita* (Kofoid *et* White) Chitw. was higher than in uninfested roots; oxygen consumption decreased in the resistant cv. Rossol exposed to infestation.

An investigation on the oxygen uptake of mitochondria isolated from the roots of resistant and susceptible tomato cultivars, uninfested or infested by *M. incognita*, was undertaken to ascertain changes in biochemical activities of the organelles that could be related to the defence reaction phenomenon.

Materials and Methods

Seeds of tomato (*Lycopersicon esculentum* Mill.) cvs Roma VF (susceptible) and Rossol (resistant) were germinated in sterilized quartz sand. Six days after germination half of the seedlings were each inoculated with 50 second stage active juveniles of *M. incognita* race 2, the other half was left uninfested as a control. Six days after inoculation, when the seedlings were 12 days old, the mitochondria were extracted from the roots.

Infested and uninfested roots (15-20 g) were carefully separated from the shoots and cut into very small pieces in an ice-cold grinding medium consisting of 0.3 M mannitol, 1 mM EDTA, 40 mM Tris-HCl (pH 7.4) and 0.1% BSA. The suspension (1:3 root weight/medium volume) was homogenized at low speed in a Potter homogenizer, using a teflon pestle, and then quickly filtered through four layers of gauze. The filtrate was centrifuged for 2 min at 48,000 g (Palmer and Kirk, 1974). The pellet was suspended in a washing medium consisting of 0.3 M mannitol, 10 mM phosphate buffer (pH 7.2) and 0.1% BSA. The suspension was subjected to an accelerating force up to 13,000 g to collapse nuclei, cell walls and heavy particles. The supernatant was further centrifuged for 2 min at 48,000 g. The pellet of crude mitochondria was resuspended in the same medium which provided about 5 mg of proteins.

This mitochondrial suspension was immediately used for oxygen uptake measurements.

Oxygen uptake was measured at 25°C using a Rank (Rank Bros., Cambridge, U.K.) O_2 electrode in 1 ml of an assay medium consisting of 0.3 M mannitol, 10 mM phosphate buffer (pH 7.0), 5 mM $MgCl_2$, 10 mM KCl, 0.1% BSA and approximately 0.5 mg/ml mitochondrial proteins.

Oxidation of malate was carried out after incubation of mitochondria with 0.1 mM thiamine pyrophosphate and 25 μM coenzyme A for about 2 min in the assay medium; 0.5 mM ATP was used similarly for succinate oxidation (Oestreicher *et al.*, 1973; Wiskich and Day, 1982).

Mitochondrial respiration was uncoupled by adding 0.4 μM FCCP (carbonyl cyanide p-trifluoromethoxyphenylhydrazone). The CLAM-sensi-

tive component was calculated by the degree of inhibition of 0.5 mM m-CLAM (m-chlorobenzhydroxamic acid) on the uncoupled respiration of mitochondria and indicated as the contribution of the alternative pathway (Lambers *et al.*, 1983).

Mitochondrial proteins were determined by the method of Lowry *et al.*, (1951). Values of Tables I - II are obtained by the means of three experiments.

Results and Discussion

Fig. 1 shows the oxygen uptake traces of mitochondria isolated from resistant (cv. Rossol - 1a) or susceptible (cv. Roma VF - 1b) tomato roots uninfested (continuous line) and infested (dotted line) by *M. incognita*.

Table I - *Percentage of increase or decrease in coupled and uncoupled respiration of mitochondria isolated from resistant or susceptible tomato roots infested by* Meloidogyne incognita *compared with mitochondria isolated from uninfested tissues.*

Substrate oxidation	cv. Rossol (resistant)		cv. Roma VF (susceptible)	
	Coupled	Uncoupled	Coupled	Uncoupled
Duroquinol	—13	—18	+54	+76
NADH	—17	—17	+50	+50
Malate	—21	—20	+13	+11
Succinate	—18	—10	+18	+23

Table II - *Percentage of effective contribution of the alternative pathway (CLAM-sensitive component) on uncoupled respiration of mitochondria isolated from resistant and susceptible tomato roots uninfested or infested by* Meloidogyne incognita.

Substrate oxidation	cv. Rossol (resistant)		cv. Roma VF (susceptible)	
	Uninfested	Infested	Uninfested	Infested
Duroquinol	0	0	0	0
NADH	4	14	0	14
Malate	33	6	28	25
Succinate	20	17	4	16

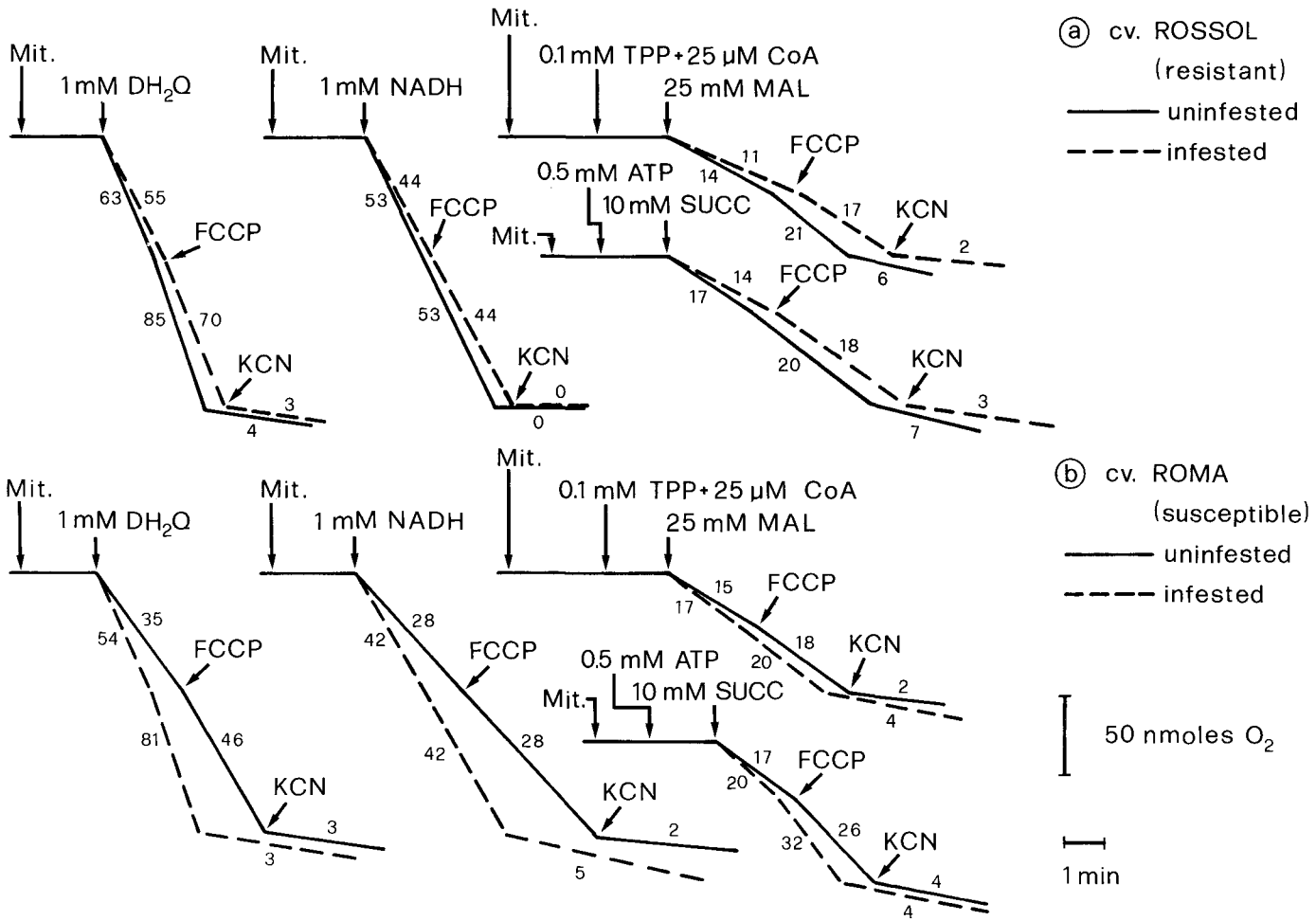

Fig. 1 - Oxygen uptake of mitochondria isolated from resistant (cv. Rossol - a) and susceptible (cv. Roma VF - b) tomato roots uninfested (continuous line) or infested (dotted line) by *M. incognita*. Assay methods as described in «Materials and Methods». FCCP and KCN were respectively 0.4 µM and 1 mM. Values are expressed in nmoles O_2 / min × mg prot. Additions are indicated by arrows.

Duroquinol (durohydroquinone), NADH, malate and succinate were chosen as respiratory substrates because they were generally found to be readily oxidized by plant mitochondria (Schonbaum *et al.*, 1971; Huq and Palmer, 1978; Palmer *et al.*, 1982; Palmer and Ward, 1985). A consistent difference was found in respiration rates of mitochondria isolated from infested tissues with respect to mitochondria isolated from uninfested ones. Oxygen consumption of resistant infested mitochondria (R.I.M.) was generally lower than that of resistant uninfested mitochondria (R.U.M.) (Fig. 1a) while oxygen consumption of susceptible infested mitochondria (S.I.M.) was generally higher than that of susceptible uninfested mitochondria (S.U.M.) (Fig. 1b). The CN-resistant respiration was very low with duroquinol and NADH but was more evident with malate and succinate as substrates. Again CN-resistant respiration seemed to be lower in R.I.M. than in R.U.M. and higher in S.I.M. than in S.U.M.

Table I clearly shows that, with all substrates tested, coupled and uncoupled respiration of mitochondria isolated from resistant infested tomato roots dropped by about 20% with respect to the control; on the contrary, coupled and uncoupled respiration of mitochondria isolated from susceptible infested tomato roots was increased by about 50% with respect to the control, with duroquinol and NADH as substrates. A smaller increase was found with malate (about 10%) and succinate (about 20%).

The per cent contribution of the alternative pathway to overall uncoupled respiration (CLAM-sensitive component) (Lambers *et al.*, 1983) of mitochondria from resistant and susceptible tomato roots, uninfested or infested by *M. incognita*, is shown in Table II. With malate and succinate as substrates the alternative pathway made only a minor contribution to R.I.M. respiration with respect to R.U.M.; however, there was an increase from 4% to 16% of the alternative pathway respiration in succinate oxidation by S.I.M. with respect to S.U.M.

The results obtained show that oxidation rates of the substrates tested are consistently changed in mitochondria from infested tissues compared with those of uninfested; thus it is reasonable to suppose that some properties of the oxidative circuit in root mitochondria undergo permanent variations when affected by nematode invasion. It is difficult from these preliminary data to understand which properties or possible enzymatic activities are involved in reaction to nematode infestation. However, it can be hypothesised that the oxidative capacity of the respiratory chain is affected by these changes since duroquinol oxidation of infested mitochondria did differ from uninfested mitochondria. It is clear that an altered oxidative capacity of the respiratory chain can in-

fluence the oxidation of any other substrate when the capacity of the respiratory chain is the rate limiting step of oxidation. In fact uncoupled respiration, in which the rate limiting step is the capacity of respiratory chain to transport electrons to oxygen, as reported by Nicholls (1982), is severally affected in infested mitochondria by all the substrates tested thus indicating a different capacity to transport electrons of mitochondria from infested tissues compared with mitochondria from uninfested tissues. A higher capacity in susceptible infested and a lower capacity in resistant infested mitochondria could be a reason why susceptible infested mitochondria are «more active» than their uninfested counterparts and resistant infested mitochondria are «less functional» than their uninfested counterparts.

This different behaviour can be related to the different reaction of resistant and susceptible tomato roots to nematode infestation. Finally, further investigations need to be done to gain further insight on the molecular nature of the inhibition or stimulation «sites» in the mitochondrial structure and on the recognition processes that enable resistant and susceptible mitochondria to change in an opposite manner when plants are infested by nematodes.

SUMMARY

Mitochondria were isolated from susceptible and resistant tomato roots uninfested and infested by *Meloidogyne incognita*. Oxygen uptake of infested mitochondria was lower in resistant and higher in susceptible cultivar compared with that of uninfested mitochondria. Duroquinol, NADH, malate and succinate were used as oxidable substrates. Changes in alternative pathway respiration were also detected.

LITERATURE CITED

BLEVE-ZACHEO T., ZACHEO G., MELILLO M.T. and LAMBERTI F., 1982. Ultrastructural aspects of the hypersensitive reaction in tomato root cells resistant to *Meloidogyne incognita*. *Nematol. medit., 10*: 81-90.

HUQ S. and PALMER J.M., 1978. Oxidation of durohydroquinone via the cyanide-insensitive respiratory pathway in higher plant mitochondria. *FEBS Lett., 92*: 317-320.

HUTCHESON S.W. and BUCHANAN B.B., 1983. Bioenergetic and metabolic disturbances in diseased plants. *In:* Biochemical Plant Pathology (J.A. Callow, Ed.), John Wiley & Sons Ltd, Hampshire, pp. 327-345.

KAPLAN D.T. and KEEN N.T., 1980. Mechanisms conferring plant incompatibility to nematodes. *Revue Nématol., 3*: 123-134.

LAMBERS H., DAY D.A. and AZCON-BIETO J., 1983. Cyanide-resistant respiration in roots and leaves. Measurements with intact tissues and isolated mitochondria. *Physiol. Plant., 58*: 148-154.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L. and RANDALL R.J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem., 193*: 265-275.

NICHOLLS D.G., 1982. Bionergetics. An introduction to the Chemiosmotic Theory. Academic Press, New York, pp. 190.

OESTREICHER G., HOGUE P., and SINGER T.P., 1973. Regulation of succinate dehydrogenase in higher plants II. Activation by substrates, reduced coenzyme Q, nucleotids and anions. *Plant Physiol., 52*: 622-626.

OWENS R.G. and NOVOTNY H.M., 1960. Physiological and biochemical studies on nematode galls. *Phytopathology, 50*: 650.

PALMER J.M. and KIRK B.I., 1974. The influence of osmolarity on the reduction of exogenous cytochrome c and permeability of the inner membrane of Jerusalem Artichoke mitochondria. *Biochem. J., 140*: 79-86.

PALMER J.M., SCHWITZGUEBEL J. and MØLLER I.M., 1982. Regulation of malate oxidation in plant mitochondria. Response to rotenone and exogenous NAD^+. *Biochem. J., 208*: 703-711.

PALMER J.M. and WARD J.A., 1985. The oxidation of NADH by plant mitochondria. *In:* Higher Plant Cell Respiration (R. Douce and D.A. Day, eds), Springer-Verlag, Berlin, Heidelberg, pp. 173-201.

SCHONBAUM G.R., BONNER W.D., JR., STOREY B.T. and BAHR J.T., 1971. Specific inhibition of the cyanide-insensitive respiratory pathway in plant mitochondria by hydroxamic acids. *Plant Physiol., 47*: 124-128.

SMEDEGARD-PETERSEN V., 1977. Respiratory changes of barley leaves infected with *Pyrenophora teres* or affected by isolated toxins of this fungus. *Physiol. Plant Pathol., 10*: 213-220.

SMEDEGARD-PETERSEN V., 1980. Increased demand for respiratory energy of barley leaves reacting hypersensitively against *Erysiphe graminis, Pyrenophora teres* and *Pyrenophora graminea. Phytopathol. Z., 99*: 54-62.

SMEDEGARD-PETERSEN V., 1982. The effect of defence reactions on the energy balance and yield of resistant plants. *In:* Active Defense Mechanism in Plants (R.K.S. Wood, ed.), New York and London, Plenum Press, pp. 299-315.

WISKICH J.T. and DAY D.A., 1982. Malate oxidation, rotenone-resistance, and alternative path activity in plant mitochondria. *Plant Physiol., 70*: 959-964.

ZACHEO G., BLEVE-ZACHEO T. and LAMBERTI F., 1982. Role of peroxidase and superoxide dismutase activity in resistant and susceptible tomato cultivars infested by *Meloidogyne incognita. Nematol. medit., 20*: 75-80.

ZACHEO G. and MOLINARI S., 1987. Relation between root respiration and seedling age in tomato cultivars infested by *Meloidogyne incognita. Ann. appl. Biol., 111*: in press.

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