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# Superoxide dismutase as a tool for the molecular identification of plant parasitic nematodes

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**Summary.** Superoxide dismutase (SOD) is a constitutive family of enzymes produced by all aerobic organisms. Varying amounts of SOD activity have been found at all life stages of the most diffused plant parasitic nematodes. SOD is important to aerobic metabolism and parasitism of nematodes in that it catalyzes the first step of the neutralization of the highly toxic superoxide anion ($O_2^{\bullet-}$), which is largely produced in plant-nematode incompatible reactions. SOD has also been shown to be a significant tool to diagnose root-knot, cyst-, and longidorid nematodes. A high SOD polymorphism has been revealed by Native-Page on gradient polyacrylamide gels for *Meloidogyne* spp. and by isoelectrofocusing for *Globodera*, *Xiphinema* and *Longidorus* spp. The sensitivity of such procedures has been improved by using the PhastSystem (Amersham Biosciences, Piscata, NJ, USA), an automated equipment for electrophoresis. An accurate discrimination of species of all the nematode genera tested has been achieved and an attempt was made to group populations of the *Xiphinema americanum*-group and to detect *Globodera rostochiensis* and *G. pallida* pathotypes.

**Key words:** diagnostics, *Globodera*, *Longidorus*, *Meloidogyne*, *Xiphinema*.

## Introduction

Superoxide dismutase (SOD) belongs to a family of isozymes which convert the toxic superoxide anions ($O_2^{\bullet-}$) into hydrogen peroxide ($H_2O_2$), the first step of the cellular detoxification of the highly reactive oxygen species (ROS). ROS generation is a constitutive process in aerobic cells but it is enhanced in conditions of stress such as pathogen attack to plants (Baker and Orlandi, 1995). Plant-parasitic nematodes have to face the toxicity of such compounds during the parasitism of the host as well as in their own metabolism, since they are aerobic organisms. Generally, aerobic organisms have an active antioxidant enzyme system, which comprises SOD in association with catalase (CAT), ascorbate (APX) and glutathione peroxidase (GPX); the physiological role of these enzymes is to maintain the cellular level of ROS beneath the threshold which triggers cell death. The efficiency of such an enzyme system has already been related to the survival of helminth parasites in animal diseases (Clark *et al.,* 1986) and to the potential life span of the free-living nematode *Caenorhabditis elegans* (Vanfleteren, 1992). Accordingly, enzymes, such as SOD, CAT and APX, display high antioxidant activity in different genera of plant parasitic nematodes, such as *Meloidogyne*, *Globodera*, *Heterodera* and *Xiphinema* (Molinari and Miacola, 1997).

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**SOD for diagnosis of root-knot nematodes (RKN)**

Esbenshade and Triantaphyllou (1985) reported an extensive survey of populations from many parts of the world studied in terms of the enzyme electrophoresis isoforms of female extracts. They used a simplified version of the thin-slab method of polyacrylamide gel electrophoresis adapted for routine enzyme studies of *Meloidogyne* species. In the 291 populations screened, six phenotypes of SOD were identified of which four accounted for 97% of all samples. Seven bands of activity were detected, of which the first six, fast-migrating ones had a taxonomic value, whilst the seventh, slow-migrating band was insensitive to 1 mM KCN and was suspected to represent mitochondrial SOD. The four most common phenotypes specifically identified the most widespread tropical species (*M. incognita*, *M. javanica, M. arenaria*) and *M. hapla*. Another survey of 111 *Meloidogyne* populations identified five phenotypes and seven bands of SOD (Carneiro *et al.,* 2000). The authors of that paper claimed that it was not possible to differentiate *M. javanica* and *M. arenaria*, and also that *M. incognita* did not show a specific pattern. The pattern for *M. hapla* was not even mentioned, probably because this species was not screened for SOD. Actually, the detection of minor SOD bands, though useful to discriminate pattern differences, can be a problem using conventional electrophoresis technique and equipment, in part because of the negative staining procedure involved and in part because of the low enzymatic response (Esbenshade and Triantophyllou, 1985). In the laboratory of the Istituto per la Protezione delle Piante, C.N.R., in Bari, a technique has been developed that uses pre-cast 0.45-mm-thick mini-gels, with a separation zone of 3.8×3.3 cm, inserted in the automated PhastSystem equipment (Amersham Biosciences, Piscata, NJ, USA). This technique permits pre-programming of the separation method chosen, a constant temperature during the run, high resolution, high reproducibility, and easy processing of the data (Molinari, 2001). Moreover, 12–24 samples can be tested in about 40 min (30 min run + 10 min staining) at limited cost (16–23 euro). The best resolution of SOD bands for RKN samples was achieved using gels with a polyacrylamide gradient (8–25%) (Fig. 1). Samples consisted of extracts

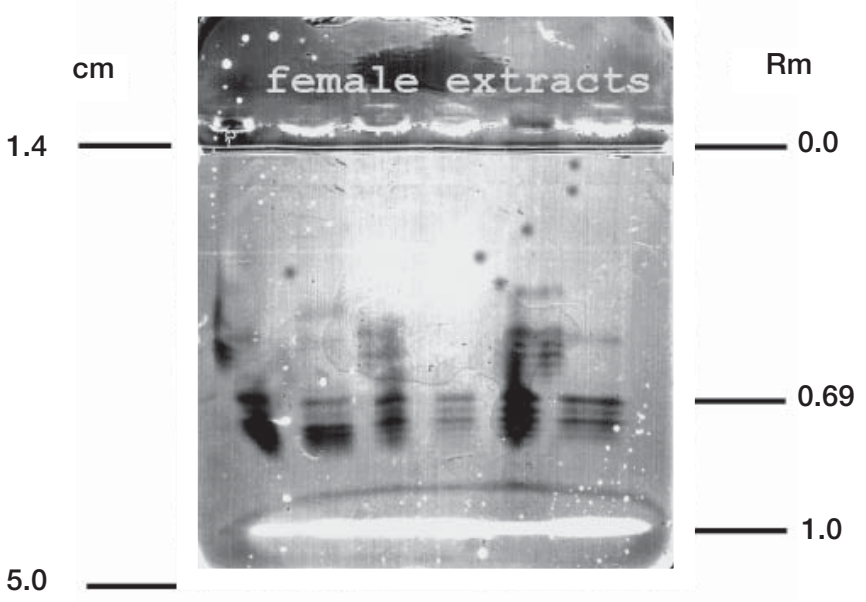


Fig. 1. Native-Page gel of *Meloidogyne* female extracts stained for SOD activity. The polyacrylamide gradient (8–25%) pre-cast mini-gel shows a 3.6-cm-long separation zone; 3.8 $\mu$l samples were loaded. Bands were tentatively divided into fast-migrating (Rm≥0.69) and slow-migrating (Rm<0.69). After SOD staining the mini-gel was dried and directly scanned into a digital image. This image was turned into a negative in order to show black SOD bands over a gray background.

of about 15 females and gels were directly scanned and converted into negative image files in order to have black SOD bands over a gray background. Bands were divided into fast-migrating (Rm≥0.69) and slow-migrating (Rm<0.69) since the pattern of fast-migrating bands seemed to be suitable for species determination, while the slow-migrating bands were possibly indicators of intra-specific polymorphism.

Discrimination of the four main RKN species (*M. incognita*, *M. javanica, M. arenaria* and *M. hapla*) by Native-Page and SOD staining of the female extracts is apparent from the specific patterns of the fast-migrating bands (Fig. 2A). SOD activity that was detected in the females did not come from enzymes ingested by feeding since the same bands were also seen with the egg masses and the juveniles of the nematode populations (Fig. 2B, C). Slow-migrating bands can detect *M. incognita* populations selected on *Mi*-bearing tomato cultivars (Molinari, 2004).

**SOD for diagnosis of cyst-nematodes**

High SOD activity was associated with marked polymorphism of the SOD bands as detected by Native-Page and by isoelectrofocusing of cyst extracts of *Heterodera* spp. (Molinari *et al.,* 1996). *Globodera* cysts revealed high SOD polymorphism when proteins were separated according to their charge by isoelectrofocusing (personal communication).

**SOD for diagnosis of longidorid nematodes**

A method to identify species of the virus-vector ectoparasite genera *Xiphinema*, *Xiphidorus, Longidorus* and *Paralongidorus* was reported by Molinari *et al.* (1997). Isoelectrofocusing of a few individual extracts revealed that the SOD isoforms were highly polymorphic and that some newly described species and new records from South America had specific SOD patterns (Lamberti *et al.,* 1999a, 1999b, 2001; Crozzoli *et al.,* 2000). SOD patterns identified *Xiphinema* spp. in surveys carried out in Florida and Slovakia (Lamberti *et al.,* 1999c, 2002a, 2002b). Six *Longidorus* species from Switzerland were also distinguished in this way (Lamberti *et al.,* 2001).

Support for the classification of the so-called *X. americanum*-group, which contains many virus vector species of quarantine importance for Euro-

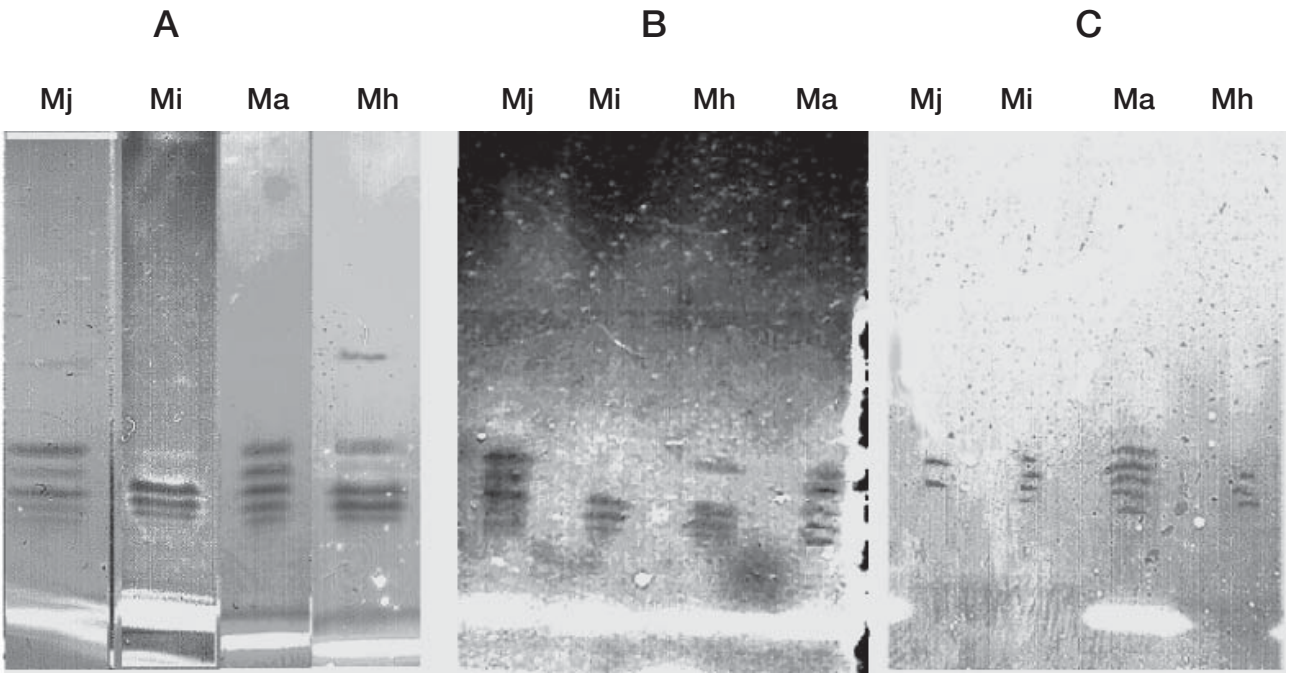


Fig. 2. Native-Page gel of *Meloidogyne javanica* (Mj), *M. incognita* (Mi), *M. arenaria* (Ma), and *M. hapla* (Mh) samples stained for SOD activity. A, Extracts of females; B, extracts of egg masses; C, extracts of invading juveniles.

pean countries, was provided by SOD patterns in a screening of about 120 populations ascribed to this complex group worldwide (Molinari, 1999; Molinari *et al.,* 2004). The grouping of different populations by means of SOD isozyme phenotypes completed an EU research project that had previously discriminated between putative species within the *X. americanum*-group by conventional means using morphometrics and clusters obtained on morphometric relationships (Lamberti *et al.,* 2000; Lamberti *et al.,* 2002c).

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