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Evaluation of Nitric Oxide Synthase Status During Disease Progression in Resistant and Susceptible Varieties of *Sesamum indicum* Against *Macrophomina phaseolina*

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ABSTRACT

Nitric oxide (NO) is an important molecule in animal and plant system which is produced from L-arginine catalyzed by the enzyme nitric oxide synthase (NOS). NO in plants has been implicated to perform a significant role in several biological systems including defense. The earliest event in the pathogenic recognition is rapid accumulation of reactive oxygen species (ROS) and NO in animals. Likewise, resistant plant pathogen interaction is followed by induction of ROS and reactive nitrogen species like NO. NO has been shown to induce programmed cell death (PCD), although the versatility of NO action in plants is still to be well defined. In the present study we selected a resistant and susceptible variety of *Sesamum indicum*, Rama and Tilittoma, respectively that were challenged with *Macrophomina phaseolina* (Tassi) Goid. NOS activity of the treated and healthy plants was estimated at every 3 days interval. In the susceptible treated plants a positive correlation was observed between symptom severity and decreased NOS activity whereas a reverse relation was shown by the resistant plants. In the susceptible plants NOS activity decreased up to 55% whereas in resistant plants it was increased up to 11% over the control. In our earlier study it was found that in different compatible host pathogen combination of fungal bacterial and viral component, pathogenesis-related NOS activity was cytosolic and according to kinetics the NOS activity was blocked competitively during diseased condition. We conclude that NOS activity is an important component of resistance or susceptibility of a plant against a pathogen, using sesame as a new model plant.

Keywords: disease protection, plant defense, sesame, susceptibility, systemic acquired resistance

INTRODUCTION

The past few years have seen a dramatic change in our understanding of molecular principles of disease resistance (Zeidler *et al*. 2004). Principles of plant defense mechanism like hypersensitive reaction (Delledonne *et al*. 1998), programmed cell death (Greenberg 1996), production of different signal molecules like salicylate, jasmonate, ethylene (Klessig *et al*. 2000; Marcos *et al*. 2003) and establishment of systemic acquired resistance (Durrant and Dong 2004) have been followed by a new group of researchers to explain these phenomena in the light of nitric oxide synthase (NOS) activity. Nitric oxide (NO), catalyzed by NOS from L-arginine is well studied in animal systems as a key molecule in various physiologic and pathologic conditions (Salerno 1996). Some basic analogies at the molecular level regarding signal transduction of NO in animal to that of plants are being unveiling gradually (Durner *et al*. 1998;). The involvement of NOS in plant systems is a recent field of work (Ninnemann and Maier 1996) and is expanding rapidly. The importance of this molecule has been found to be pertinent in regulation of growth and hormonal signaling (Leshem 1996), root growth (Gouvea *et al*. 1997), phytoalexin accumulation (Noritake 1996), stimulation of seed germination, de-etiolation and inhibition of hypocotyl elongation (Beligni and Lamattina 2000). NO has now been shown to mediate defense responses of plants against pathogens (Delledonne *et al*. 1998; Zeidler *et al*. 2004; Teixeira da Silva 2006).

We have already shown that cytosolic NOS activity of the host was inhibited during diseased condition in susceptible host-pathogen (fungal/bacterial/viral) combinations (Acharya and Acharya 2002a, 2002b; Acharya *et al*. 2005). In this study, the nature of NOS activity has been investigated during disease progression in the resistant variety (var. Rama) and susceptible variety (var. Tilottoma) of *Sesamum indicum* L. when challenged with stem rot-causing pathogen *Macrophpmina phaseolina* (Tassi.) Goid.

MATERIALS AND METHODS

Plant material

Seeds of resistant and susceptible varieties of sesame plants were collected from Pulses and Oil Seeds Research Station, Baharampur, West Bengal, India and planted in our laboratory garden. After 2 weeks, the emerged seedlings were transferred to plastic pots containing 3 kg of silt loam soil and were grown at a temperature of 30 ± 2°C for further experiments.

Fungal material

The fungal pathogen (*Macrophpmina phaseolina* (Tassi.) Goid.) was obtained from the culture collection of Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta.

Chemicals

Chemicals were of analytical grade and purchased from Merck, Mumbai, India.

Inoculation

One-month-old plants of resistant and susceptible varieties were soil inoculated with pathogen using a talc-based formulation. Inoc-

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Short Communication

ulum was grown in potato dextrose broth at 30 ± 2°C for 9 days. The mycelial mat was harvested, blended in a coffee blender at a low speed for 20 sec and mixed with 50 ml of sterile distilled water and filtered through cheese cloth. 50 ml of this suspension was mixed with 100 g of sterile magnesium trisilicate ($Mg_2Si_3O_8$) and 1 g of carboxymethyl cellulose. 5 g of this talc based formulation was mixed with 3 kg of soil. Inoculum density was determined by serial dilution technique (Russin *et al*. 1995) and was found to be 10 CFU/g of soil.

Determination of NO formation

Activity of NOS was detected by measuring the amount of nitric oxide produced by this enzyme based on the method described by Jia *et al*. (1996) by using scanning Hitachi 330 spectrophotometer. Here, NO was quantified spectrophotometrically based on the principle of conversion of oxyhemoglobin to methemoglobin which shows a change in absorbance at 575 nm. 60 ± 5 mg of intact leaf tissue was incubated in a reaction mixture containing L-arginine (10 μM), hemoglobin (30 μM) in a total volume of 2 ml of 10 mM phosphate buffer (pH 7.4) at 37°C. After incubation NO content in the reaction mixture was assayed. Tissues taken from the non-treated plants served as the control set. Results obtained from the treated plants were compared with their respective control sets.

Statistical analyses

Results are presented as the mean ± SD (Standard deviation) of at least 5 experiments each with 3 replicates. Data were analyzed by the Student's *t*-test following one-way ANOVA and $P<0.01$ was considered significant.

RESULTS AND DISCUSSION

The NOS activity which was estimated by quantification of NO produced by this enzyme was performed at a regular interval of 3 days throughout the experimental period. Resistant and susceptible plants were grouped into treated and control sets. Intact tissues from all the sets were used as the source of NOS enzyme. Activity of this enzyme was measured as described in the materials and methods section and compared with control set (**Fig. 1**).

It is clear from **Fig. 1** that there was no significant difference between the basal NOS activity of resistant and susceptible varieties of *S. indicum* during their healthy condition. NOS activity remained within the range of 8-9 pmol/g tissue/h and maintained a relatively steady state throughout the experimental period. But when they were challenged with pathogen, NOS activity increased in resistant plants and decreased in susceptible plants in relation to basal levels (**Fig. 1**). NOS activity increased up to 11% more than the control in treated resistant plants and decreased up to 55% less than the control in treated susceptible plants.

It is well established that the recognition of a pathogen by the host is followed by production of reactive oxygen species (ROS). But severe oxidative stress provokes damage to almost every cellular component causing unprogrammed cell death (Heath 1987). On the other hand, resistant plant-pathogen combination very often leads to the induction of hypersensitive reaction (HR). There are evidences that NO plays a key role during HR (Daledonne *et al*. 1998; Durner *et al*. 1998). HR via NO is a typical example of programmed cell death (PCD) (Arsimowicz and Floryszak-Wieczorek 2007). Our result correlates these facts as it has been found that challenge of pathogen in the resistant plant aggravated NOS activity and thus increased production of NO up to a certain limit. This might be leading to PCD and thus provide resistance. The reverse happens in the case of susceptible plants where NOS activity is significantly blocked and the host might select unprogrammed cell death. This result finds hypothetical similarities with other studies regarding NO's function in biotic stress. Reports reveal that in *Arabidopsis* suspension cells, exogenous NO-induced cell death occur at concentrations similar to those generated by cells challenged by avirulent bacteria (Clarke *et al*. 2000). NO protects potato (*Solanum tuberosum*) plants against the noxious effect of pathogen (*Phytophthora infestans*) infection (Laxalt *et al*. 1997) and has been designated as an antioxidant molecule which could protect plants against several biotic and abiotic stresses (Beligni and Lamatina 2002). Earlier, we reported that in a different host-pathogen combination of *Brassica campestris* L. var. Sarson Prain, *Citrus aurantifolia* Swingle and *Ammomum subulatum* Roxburg vs. *Alternaria brassicae* (Bark.) Sacc., *Xanthomonas citri* Hasse. and chirke (mosaic streak) virus respectively showed similar kind of NOS kinetics having a competitive nature of inhibition (Acharya *et al*. 2005). In these cases, insufficient production of this antipathogenic molecule, i.e. NO, made the plants vulnerable to susceptibility. Another study strengthened this work where a symptomological field experiment of different common cultivated crop plants like *Lagenaria siceraria* (Molina) Standl., *Carica papaya* L., *Solanum melongena* L., *Trichosanthes angulina* L., *Abelmoschus esculentus* Moen., *Luffa acutangula* (L.) Roxb., *Piper betle* L., *Oryza sativa* var. *patnai* or *masuri*, *Colocasia esculenta* (L.) Schott. and *Raphanus sativus* L. showed a correlation between NOS activity and disease severity (Acharya 2007). In addition, we found that administration of sodium nitroprusside (SNP) by foliar spray 20 hrs before pathogen inoculation protected *Brassica campestris* and *Citrus aurantifolia* against fungal and bacte-

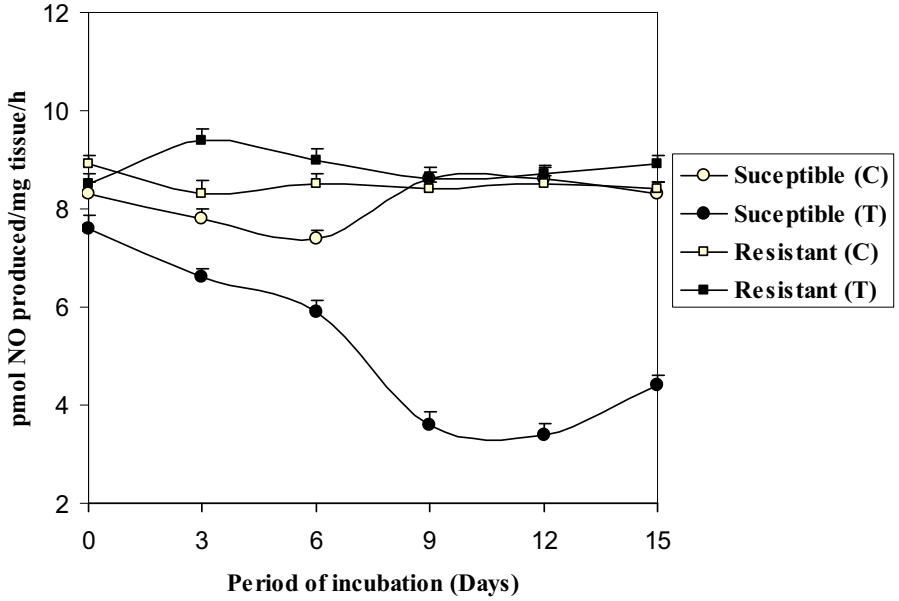

Fig. 1 Comparative production of NO in susceptible and resistant sesame plant treated with *Macrophomina phaseolina* (Tassi.) Goid. C=Control; T=Treated. Results are mean ± SD of three separate experiments, each in triplicate.

rial disease up to 72% and 65%, respectively (Acharya *et al*. 2005). So, cumulative findings of our study indicate that the status of NOS activity of a plant is directly related to its resistance or susceptibility. Further investigation is ongoing to assess how the level of NOS activity could be used as an indicator for detection of resistance and susceptibility of plants.

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