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Short Communications

Cloning of nitric oxide associated 1 (NOA1) transcript from oil palm (*Elaeis guineensis*) and its expression during *Ganoderma* infection

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SUMMARY

Nitric oxide associated 1 (NOA1) protein is implicated in plant disease resistance and nitric oxide (NO) biosynthesis. A full-length cDNA encoding of NOA1 protein from oil palm (*Elaeis guineensis*) was isolated and designated as *EgNOA1*. Sequence analysis suggested that *EgNOA1* was a circular permutated GTPase with high similarity to the bacterial YqeH protein of the YawG/YlqF family. The gene expression of *EgNOA1* and NO production in oil palm root tissues treated with *Ganoderma boninense*, the causal agent of basal stem rot (BSR) disease were profiled to investigate the involvement of *EgNOA1* during fungal infection and association with NO biosynthesis. Real-time PCR (qPCR) analysis revealed that the transcript abundance of *EgNOA1* in root tissues was increased by *G. boninense* treatment. NO burst in *Ganoderma*-treated root tissue was detected using Griess reagent, in advance of the up-regulation of the *EgNOA1* transcript. This indicates that NO production was independent of *EgNOA1*. However, the induced expression of *EgNOA1* in *Ganoderma*-treated root tissues implies that it might be involved in plant defense responses against pathogen infection.

Introduction

Nitric oxide (NO) is an ubiquitous biological messenger involved in a broad spectrum of plant physiological processes such as seed germination, pollen tube growth, stomatal movement, leaf maturation and senescence, floral transition, root organogenesis, stress tolerance and disease resistance (Ferrarini et al., 2008; FrÖhlich and Durner, 2011). The rapid burst of NO as elicited by avirulent pathogen in mouse-ear cress/*Pseudomonas syringae* pv. tomato and oat/*Puccinia coronata* pv. *avenea* is one of the hallmark events that mediates defense responses (Tada et al., 2004). The NO mediated plant defense responses include hypersensitive response (HR), activation of salicylic acid (SA) and jasmonic acid (JA) signaling pathway, accumulation of antimicrobial compounds, cell wall lignification, modulation of defense related gene and post-translational protein modification (Ferrarini et al., 2008; Misra et al., 2010).

NO biosynthesis in plants is divided into oxidative and reductive pathways involving various enzymes and non-enzymatic mechanisms (FrÖhlich and Durner, 2011; Thakur and Sohal, 2013). Nitric oxide synthases (NOSs) (EC 1.14.13.39) are NO generators in the mammalian system, involving the oxidation of L-arginine to L-citrulline and NO. NOS homologs are widely distributed in animals, fungi, green algae and bacteria, but are absent in plants. Although NOS activity has been detected in plants but a novel plant NOS has not yet been identified. The *Arabidopsis thaliana* NO synthase 1 (*AtNOS1*) was initially reported as a putative plant NOS, but was later renamed *A. thaliana* NO associated 1 (*AtNOA1*), as it was soon indicated as a circular permuted GTPase (cpGTPase) of the YawG/YlqF family (Zemojtel et al., 2006; Moreau et al., 2008). The defective arginine-dependent NO synthesis activity in recombinant

Abbreviations: BSR, basal stem rot; CPG, circularly permutated GTPase; CTD, C-terminal domain; DAI, day after inoculation; C_q, quantification cycle; EST, expressed sequence tag; FFB, fresh fruit bunch; GTP, guanosine triphosphate GTPases; HAS, hydrophobic amino acid substituted for catalytic glutamine residue; HR, hypersensitive response; JA, jasmonic acid; NAD, NADH dehydrogenase subunit 5-like; Ni-NOR, nitrite-NO reductase; NO, nitric oxide; NOA1, nitric oxide associated 1; NR, nitrate reductase; NTC, non-template control; ORF, open reading frame; PAMP, pathogen associated molecule patterns; PRRs, PAMP recognition receptors; PTI, PAMP-triggered immunity; RACE, rapid amplification of cDNA ends; ROS, reactive oxygen species; RWB, rubber wood block; SA, salicylic acid; TRAFAC, after translation factors; UTR, untranslated region; ZBD, zinc-binding domain.

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AtNOS1 protein and the contradictory NO accumulation responses in NOA1-silenced mutants have affirmed that *AtNOS1* is not an authentic NOS per se (Van Ree et al., 2011). The involvement of NOA1 in NO biosynthesis is currently identified as sucrose dependent and nitrate reductase (NR)-related (Gas et al., 2009; Van Ree et al., 2011). The indirect regulatory effect of NOA1 in NO production was demonstrated with impaired NO accumulation in NOA1-silenced *A. thaliana* and *Nicotiana benthamiana* (Guo et al., 2003; Asai and Yoshioka, 2009).

Apart from NO biosynthesis, NOA1 is implicated in plastid function, ribosome biogenesis, and enhanced tolerance to both abiotic and biotic stresses (Flores-Perez et al., 2008; Qiao et al., 2009; Liu et al., 2010). Increased susceptibility to pathogens in NOA1 knockout *A. thaliana and N. benthamiana* had demonstrated the role of NOA1 in disease resistance (Zeidler et al., 2004; Asai and Yoshioka, 2009). The NOA1 mediated defense mechanism was observed be to associated with the induced accumulation of oleic acid, jasmonates and carbon-based secondary metabolites, expression of salicylic acid (SA)-defense responsive gene and elevated NO production (Kato et al., 2008; Wünsche et al., 2011; Mandal et al., 2012).

The African oil palm (*Elaeis guineensis*) is commercially cultivated for vegetable oil, biofuel and oleochemicals. The outbreak of the destructive basal stem rot (BSR) disease in the oil palm nursery and plantations, caused by the white rot fungi, *Ganoderma* species is a major challenge to oil palm cultivation (Paterson et al., 2009). BSR disease has resulted in severe economic loss due to reduction in fresh fruit bunch (FFB) and collapse of standing palms (Chung, 2011). *Ganoderma* is spread by airborne basidiospores, insects assisted spore transfer and mycelial root contact (Sanderson, 2005; Paterson, 2007). The current disease management strategies include soil mounding, mechanical removal, clean clearing, legume cover crops, fungicide application, biological control, fertilizer and biofertilizer input management to prolong the lifespan of infected palms, but these are not effective in disease eradication (Chung, 2011). The low effectiveness of these approaches is caused by the resistant pseudosclerotia structure of the *Ganoderma* fungi and the lack of early disease symptoms (Rees et al., 2009).

The different susceptibility to *Ganoderma* infection exhibited by planting materials from different geographical origins such as Cameroon, Nigeria and Zaire has unraveled the prospect of oil palm genetic improvement programs (Durand-Gasselin et al., 2005). Breeding of resistant or tolerant planting materials is effective in disease eradication or delay of disease development. This reduces *Ganoderma* inoculum for subsequent replantings. Chitinase, glucanase, isoflavone reductase, metallothioneins, metallothionein-like protein, early methionine-labeled polypeptides and stearoyl-acyl carrier protein desaturase (SAD) have been reported to express differentially after *Ganoderma* infection (Yeoh et al., 2012; Tan et al., 2013; Tee et al., 2013). Nevertheless, the identification of defense associated genes against *Ganoderma* is ongoing. These genes are important in providing insight into the molecular events during plant–pathogen interaction and for the development of expressed markers for large scale screening of resistant or tolerant planting materials.

In the present study, a transcript encoding NOA1 protein from oil palm, designated as *EgNOA1*, was isolated and characterized. Oil palm seedlings were artificially inoculated with *Ganoderma boninense* to investigate the expression profile of *EgNOA1* in root tissue and its potential as expressed markers for early disease detection and/or fungal resistance. In addition, the association between *EgNOA1* gene expression and NO biosynthesis was also investigated.

Materials and methods

Isolation of full length cDNA sequence

Total RNA was extracted from three month old oil palm (*Elaeis guineensis* Jacq., Dura × Pisifera, GH500 series) root tissue by adopting the protocol outlined in Chan et al. (2007). RNA samples were treated with DNase I (Fermentas, Lithuania) according to manufacturer's instructions to eliminate traces of genomic DNA contamination. The integrity of RNA samples was examined using agarose gel electrophoresis and purity was assessed using the NanoDrop ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, USA) at absorbance ratio of A260/280 and A260/230. Gene specific primers were designed according to two expressed sequence tags (ESTs) (i.e. EL694732 and 17871) retrieved from the oil palm EST library (Ho et al., 2007) that matched (E-value < 10^{-5}) the NOA1 protein in the GenBank non-redundant protein database. Gene specific primers were designed using Primer3 Input software (version 0.4.0) (www.bioinfopop.ufv.br/sistema/primer3). The primers designed for 5′- and 3′-rapid amplification of cDNA ends (RACE) PCR were: 17871-R1 (5′-TTC CTG GAA CAA CCC TTG GTC-3′) and EL694732-F1 (5′-TTC CTG GAA CAA CCC TTG GTC-3′), respectively. The primers used for nested 5′- and 3′-RACE PCR were: 17871-R2 (5′-TCC TGG AAC AAC CCT TGG TCC CAT TG-3′) and EL694732-F2 (5′-TCC TGG AAC AAC CCT TGG TCC CAT TG-3′), respectively. Template for RACE-PCR was synthesized from 10 μg of total RNA using ExactSTART Eukaryotic mRNA 5′- and 3′-RACE kit (Epicentre, USA) according to manufacturer's instructions. RACE-PCR products were cloned into the pDrive vector (Qiagen, German) and sequenced using T7 and SP6 primers. The resulting full-length cDNA sequence was designated as *EgNOA1*.

Bioinformatics analysis

Sequence similarity and catalytic domain of *EgNOA1* was analyzed using Basic Local Alignment Search Tool (BLASTx) and conserved domain database (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) at the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov). Molecular weight and isoelectric point of the *EgNOA1* protein was calculated using ExPASy ProtParam program (www.expasy.ch/tools/protparam.htm). Subcellular location of *EgNOA1* was predicted using WoLFPSORT (www.wolfpsort.org). Sequence alignment of different plant NOA homologs was performed using the ClustalW method (www.ebi.ac.uk/clustalw). The phylogenetic tree was constructed using neighbour joining method implemented in Molecular Evolutionary Genetics Analysis (MEGA) version 5.1 program (Tamura et al., 2011).

Plant materials and treatments

A total of 70 4-month-old oil palm seedlings (*E. guineensis* Jacq., Dura × Pisifera, GH500 series) was purchased from Sime Darby Seeds & Agricultural Services Sdn. Bhd. (Banting, Malaysia). The seedlings were equally divided for two treatments, either treated with one-month-old *Ganoderma boninense* PER71 colonized rubber wood blocks (RWB) or sterilized RWBs (served as control). Inoculation was carried out through direct sitting technique by adopting the protocol outlined by Zaiton (2006). Five seedlings were harvested from both treatments at 3, 7, 14, 21, 28, 56 and 96 days after inoculation (DAI). The roots were washed and examined for visible symptoms of *G. boninense* infection, including mycelial colonization on root surfaces and necrotic lesions. Samples of roots were flash frozen in liquid nitrogen and stored at −80 °C until required.