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An insight into the expression profile of defence-related genes in compatible and incompatible *Oryza sativa-Meloidogyne graminicola* interaction

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Abstract

The present study was conducted to investigate the comparative parasitic ability of root-knot nematode (RKN: *Meloidogyne graminicola*) in putatively resistant (Suraksha) and susceptible (Pusa 1121) cultivars of rice and to analyse the relative expression level of plant defence genes upon RKN infection in these cultivars. qRT-PCR analyses of RKN-infected roots and their corresponding shoots indicated that phytohormone-mediated host defences were activated during early infection in both susceptible and resistant plants, whereas it was apparently suppressed during later stage of infection in susceptible plants. Expression of defence genes in the shoot tissues of resistant plants suggested the successful induction of systemic acquired resistance. In parallel, lignin- and callose-mediated plant basal defence was activated in Suraksha. Information generated on the RKN-induced expression of defence genes in different rice varieties can be exploited to improve resistance in cultivated species of rice to RKN infection.

Key words: Pusa 1121, Suraksha, *Meloidogyne incognita*, salicylic acid, jasmonic acid, PR proteins

Introduction

Being one of the major staple food crop, rice (*Oryza sativa* L.) provides 20% of the world's dietary energy supply and is consumed predominantly in 17 countries of Asia, 9 countries in North and South America and 8 countries in Africa (FAO 2004). Over the last decades genetic diversity has been increased immensely in rice using improved breeding techniques in order to meet the increasing demand for food due to explosive population growth in India (Choudhary et al. 2013). Of late, Pusa 1121 (a high yielding basmati variety) has gained considerable adoption among the Indian farmers due to the potential of this variety to fetch higher income (Rice Knowledge Management Portal: www.rkmp.co.in). On the other hand, Suraksha (a derivative of the founder varieties TN1 and IR8) was developed in the Indian breeding programme for multiple pest and disease resistance (FAO 2003). Suraksha had shown resistance to rice gall midge infection which was linked to the phytohormone-mediated defence response of rice (Rawat et al. 2010, 2013).

Yield of rice is compromised by various biotic stressors including plant-parasitic nematodes. Estimated annual yield decline in rice due to nematode infection (predominantly by *M. graminicola* and *Hirschmaniella oryzae*) ranges from 10-25% (Bridge et al. 2005). Owing to changing climate and agricultural practices *M. graminicola* is emerging as a serious problem in aerobic rice cultivation in South-east Asia (Kyndt et al. 2014). RKN exhibitsprolonged and intricate relationships with rice, often eliciting complex modifications in host cell morphology and physiology.The second-stage juvenile (J2) of RKN invades rice root in the elongation zone and migrates towards the root tip, where it probes the selected vascular cells with its stylet and injects esophageal gland secretion into it, which results in the reorganization of these cells into a feeding cell called 'giant cell'. RKNs feed on giant cell for the remainder of its sedentary life cycle (Gheysen and Mitchum 2011). Root tissues around the giant cell become hypertrophied to form typical hook-shaped root galls that hinder the nutrient translocation from root to shoot. In well-drained soil, RKN completes its life cycle in 19

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days at 22-29°C temperature (Dutta et al. 2012).

Plant defence mechanism to pathogen invasion is a complex phenomenon which consists of multiple layers. Phytohormones such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play major roles for signalling networks associated with local and systemic plant defence responses (Bari and Jones, 2009, Pieterse et al. 2009). Accordingly, upon nematode infection, both constitutive and systemic defence of plant is activated which is regulated by the coordinated expression of phytohormones (SA/JA/ET), reactive oxygen species (ROS), MAPK-mediated cascades and WRKY transcription factors (Li et al. 2015). However, information on the role of plant-derived compounds that triggers the host (especially monocots) innate immunity to nematodes is still fragmented (Kyndt et al. 2013). In addition, resistance against RKNs, displayed by wild relatives of rice (*O. glaberrima*, *O. longistaminata*), is limitedly available in cultivated species of rice, i.e., *O. sativa* (Kyndtet al. 2014; Dimpka et al. 2015; Kumari et al. 2016).

Earlier, we had identified some resistant and susceptible genotypes of *O. sativa* using a unique *in vitro* screening assay employing pluronic gel medium (Kumari et al. 2016). Additionally, we had compared the expression profile of defence genes in susceptible (Pusa 1121) and resistant (Vandana) genotypes upon *M. graminicola* infection to determine the factors governing resistance/susceptibility in monocot plants to RKN infection (Kumari et al. 2016). In continuation with that study, in the present investigation, we have assessed the comparative infectivity of *M. graminicola* in putatively susceptible (Pusa 1121) and resistant (Suraksha) genotypes of *O. sativa*. Subsequently, the relative expression of plant defence genes (that regulate both induced and systemic defence) duringearly and late infection of *M. graminicola* in these genotypes was analysed. Our study provided some interesting contrasts among the transcriptional alteration of defence genes (ET and JA pathway, PR and WRKY genes were found to be the major player in regulating resistance response) in susceptible and resistant cultivars of rice upon RKN infection.

Materials and methods

Culturing of nematodes

A pure culture of an Indian isolate of *M. graminicola* Golden & Birchfield was maintained on rice (cv. Pusa 1121) in a glasshouse. Egg masses were collected from the galled roots of infected plant using sterilized forceps and were hatched in a Petri dish containing sterile water. Freshly hatched J2s were used for all the experiments.

Nematode infection assays in Pluronic gel medium

Seeds of Pusa 1121 and Suraksha (kindly provided by the Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi) were surface-sterilized with 70% ethanol. Seedlings of 3-4 days old were used for the infection bioassay. Pluronic F-127 (PF-127) (Sigma-Aldrich) gel was prepared as described previously (Reynolds et al. 2011; Dutta et al. 2011). Twenty five ml of 23% PF-127 were poured into 110×25 mm Petri dish containing seven, uniformly distributed seedlings of the identical variety at 15°C. Approximately 30 J2s of *M. graminicola* were inoculated at the root tip of each seedling using a pipette tip followed by setting of gel at the room temperature. Petri dishes were incubated at 28°C with 16:8 h light:dark photoperiod in an incubation chamber. Six plates for each variety were included in each experiment and each experiment was repeated at least twice.

Inoculated plantlets were harvested from the PF-127 medium daily starting from the 1 dpi (days post inoculation) up to 15 dpi. Roots were stained with acid fuchsin (Byrd et al. 1983) and dissected under the microscope to identify the different developmental stages of RKN. Photographs were taken in a Zeiss AxiocamMRm microscope. To determine the reproductive potential of *M. graminicola* in susceptible and resistant varieties, respective nematode multiplication factor [(number of eggmasses × number of eggs per egg mass) ÷ nematode inoculum level] in different varieties was calculated.

Expression analysis of defence genes in susceptible and resistant plants upon RKN infection

Root tip of the rice cultivars, Pusa 1121 and Suraksha were inoculated with the 30 J2s of *M. graminicola* in PF-127 medium as documented above. At 2 and 6 dpi, plantlets were harvested from the medium, excised root tips and shoots were immediately frozen in liquid nitrogen and stored at –80°C until use. Total RNA was isolated from the root tip and shoot of infected and non-infected rice seedlings using NucleoSpin total RNA Kit (Macherey-Nagel, Germany). Quality and quantity of the extracted RNA was assessed using Nanodrop ND-1000 spectrophotometer (Thermo Scientific). Approximately 500ng of the purified RNA

was reverse transcribed to cDNA using cDNA synthesis Kit (SuperscriptVILO, Invitrogen). cDNA was used for amplification of candidate defence genes of rice. Primer details are given in Table 1.

relative gene expression in susceptible and resistant cultivars, mean Ct values were obtained and fold change values were calculated using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The non-infected rice was used as the control.

Table 1. Primers used for qRT-PCR analysis

Gene	Locus ID	Forward primer (5′-3′)	Reverse primer (5′-3′)
Os18srRNA	GenBank:AF069218	CGCGCAAATTACCCAATCCTGACA	TCCCGAAGGCCAACGTAAATAGGA
Os-actin	RAP-DB: Os03g0718100	CTCTCAGCACATTCCAGCAG	AGGAGGACGGCGATAACAG
OsMAPK5a	RAP-DB: Os03g17700	GTCTGCTCCGTGATGAAC	TGATGCCTATGATGTTCTCG
OsMAPK6	RAP-DB: Os06g0154500	GATACATTCGCCAACTTCC	CAGTGATGCCAGGTAAGG
OsMPAK20	RAP-DB: Os01t0629900	TCAACTCCAATTCCTGCCAAG	AACAACTCTTCCTGGTCTTGC
OsPAL1	RAP-DB:Os02g41630	TGTGCGTGCTTCTGCTGCTG	AGGGTGTTGATGCGCACGAG
OsICS1	RAP-DB:Os09g19734	TGTCCCCACAAAGGCATCCTGG	TGGCCCTCAACCTTTAAACATGCC
OsEDS1	RAP-DB: Os09t0392100	CAGGAGAGGCAGTGTTAATCG	GCAAGCGGAGTAAGTGGTATG
OsPAD4	RAP-DB: Os11t0195500	TCAGAGGCAAGGCAGTAGTG	ACCGCTCACGCAGGATAG
OsNPR1	RAP-DB: Os01t0194300	AGAAGTCATTGCCTCCAG	ACATCGTCAGAGTCAAGG
OsAos2	RAP-DB: Os03t0225900	GCGAGAGACGGAGAACCC	CGACGAGCAACAGCCTTC
OsJMT1	RAP-DB:Os06g0314600	CACGGTCAGTCCAAAGATGA	CTCAACCGTTTTGGCAAACT
OsJAMYB	RAP-DB:Os11g45740	GAGGACCAGAGTGCAAAAGC	CATGGCATCCTTGAACCTCT
OsACS1	RAP-DB:Os03g51740	GATGGTCTCGGATGATCACA	GTCGGGGGAAAACTGAAAAT
OsACO7	RAP-DB:Os01g39860	GGACTACTACCAGGGCACCA	GATTAGCGCACGCGATTTTA
OsEIN2	RAP-DB:Os07g06130	TAGGGGGACTTTGACCATTG	TGGAAGGGACCAGAAGTGTT
OsERF1	RAP-DB:Os04g46220	AAGGGTCATAATTCGCGTCA	TCCACACCACAAGACATCGT
OsPR1a	GenBank: EF061246	AACTTCGTCGGCCAATCTC	CATGCATAAACACGTAGCATAGC
OsPR1b	GenBank:EF061247	TACGACTACGCCTCCAACA	CCGGCTTATAGTTGCATGTGA
OsPR10	GenBank:AF274850	ACGCCTAAGATGAAGAGGAATAC	CTCAAACGCCACGAGAATTTG
OsWRKY13	RAP-DB: Os01t0750100	GCCAGCGGAGAACGAATC	CTCCTCCTGCTTCACAACC
OsWRKY45	RAP-DB: Os05g0322900	AATTCGGTGGTCGTCAAGAA	AAGTAGGCCTTTGGGTGCTT
OsRbohB	RAP-DB:Os01g0360200	CTGGACAGGACCAAGAGCAG	ATCTTGAACGGAGCAGCACA
OsRAC1	RAP-DB:Os01t0229400	GCTTCTTCCATAATAACAACG	AGTTTCTTTCTGGTTACATCC

To analyse the transcript abundance of candidate defence genes, quantitative real-time PCR (qRT-PCR) was carried out in a realplex^2 thermal cycler (Eppendorf) using SYBR Green Supermix Kit (Eurogentec). The cycling conditions were as follows: a hot start of 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Additionally, a melt curve programme (95°C for 15 s, 60°C for 15 s) was followed to ensure the specificity of amplification. Two constitutively expressed genes of *O. sativa*, *18S rRNA* and *actin*, were used for normalization of qRT-PCR data. At least two biological and three technical replicates were used for each of the samples. In order to determine the

The normalized fold change values were $\log_2$-transformed and those values were used for the construction of bar graphs (separately for root and shoot data). For simplified illustration expression data of root and shoot tissues were combined in a heat map.Heat map was generated using the Heatplus package available at the Bioconductor using the function *regHeatmap* in the R statistical environment.

Statistical analysis

Data of the bioassay experiments were subjected to one way Analysis of variance. Results are reported as significant or non-significant based on Duncan's

multiple comparison test with significance level at $P< 0.05$ using SAS software (version 9.3). Regarding qRT-PCR data, significant differential expression between infected plants and control tissue was determined by student's *t*-test at $P < 0.05$.

Results and discussion

Strategies deployed by phytonematodes to successfully invade and colonize the host plants include suppression of host immune responses. Compared to the large number of reports regarding molecular interplay between dicots and RKNs (Kyndt et al. 2013), our understanding on the RKN-induced defence response in monocots are rather limited. Along with that, novel sources of natural resistance to RKN are wanting inthe cultivated species of rice (*O. sativa*) (Kyndt et al. 2014). In the present study, Pusa 1121 and Suraksha were used as the candidate genotypes to understand their compatible and incompatible interaction with RKN, respectively.

Firstly, in order to investigate the relative disease progression of RKN in susceptible and resistant cultivars, infection bioassay was conducted *in vitro* in pluronic gel medium, PF-127 (Dutta et al. 2011) that mimics natural three-dimensional soil environment. At 4 dpi, average number of galls per root system was comparatively higher in Pusa 1121 than Suraksha (Fig. 1). Accordingly, J2s had penetrated, developed to J3, J4 and adult female, reproduced by laying eggs in greater number and lesser incubation period in Pusa 1121 compared to Suraksha. Derived multiplication factor (MF) which reflects the parasitic ability of a nematode was significantly higher in Pusa 1121 compared to Suraksha (Fig. 2). These results re-established the fact that Suraksha is indeed a RKN resistant genotype in comparison to Pusa 1121.

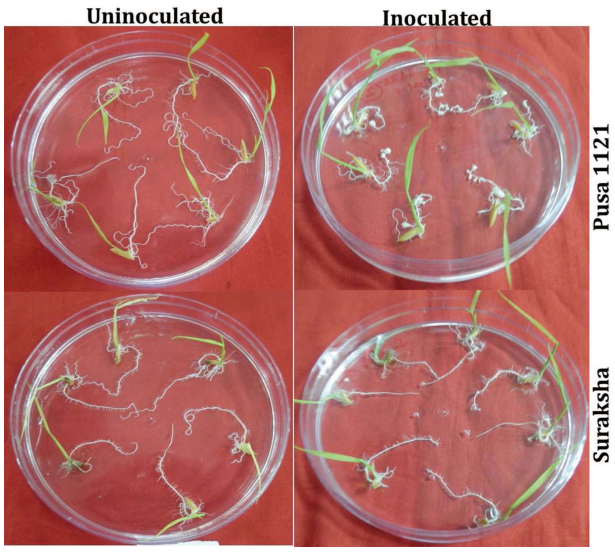


Fig. 1. RKN infected plantlets of Pusa 1121 and Suraksha in the Petri dish containing PF-127 medium at 4 dpi. Typical hook-like galls were observed in greater numbers in Pusa 1121 compared to Suraksha. Root tip of each seedling was inoculated with 30 J2 of *M. graminicola*

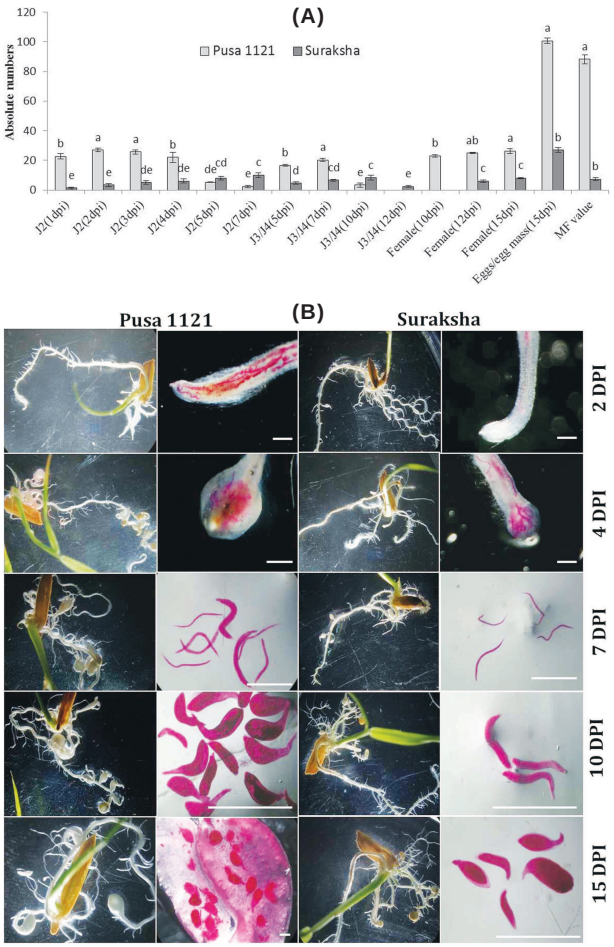


Fig. 2. (A) Comparative invasion, development and reproduction of *M. graminicola* in Pusa 1121 and Suraksha. Means ± standard errors are presented. Different letters within any parameter (J2, J3/J4, Females, Eggs/egg mass and MF) are significantly different at $P = 0.05$. Number of egg masses reflects the number of successfully reproducing females at 15 dpi. (B) Relatively more number of J2 had invaded, developed and reproduced in Pusa 1121 with higher galling intensity compared to Suraksha. At 10 dpi, J2s were developed to young females in Pusa 1121 and J3/J4s in Suraksha, suggesting the delayed RKN development in Suraksha than Pusa 1121. Nematodes were stained with acid fuchsin.Scale bar = 200 µm

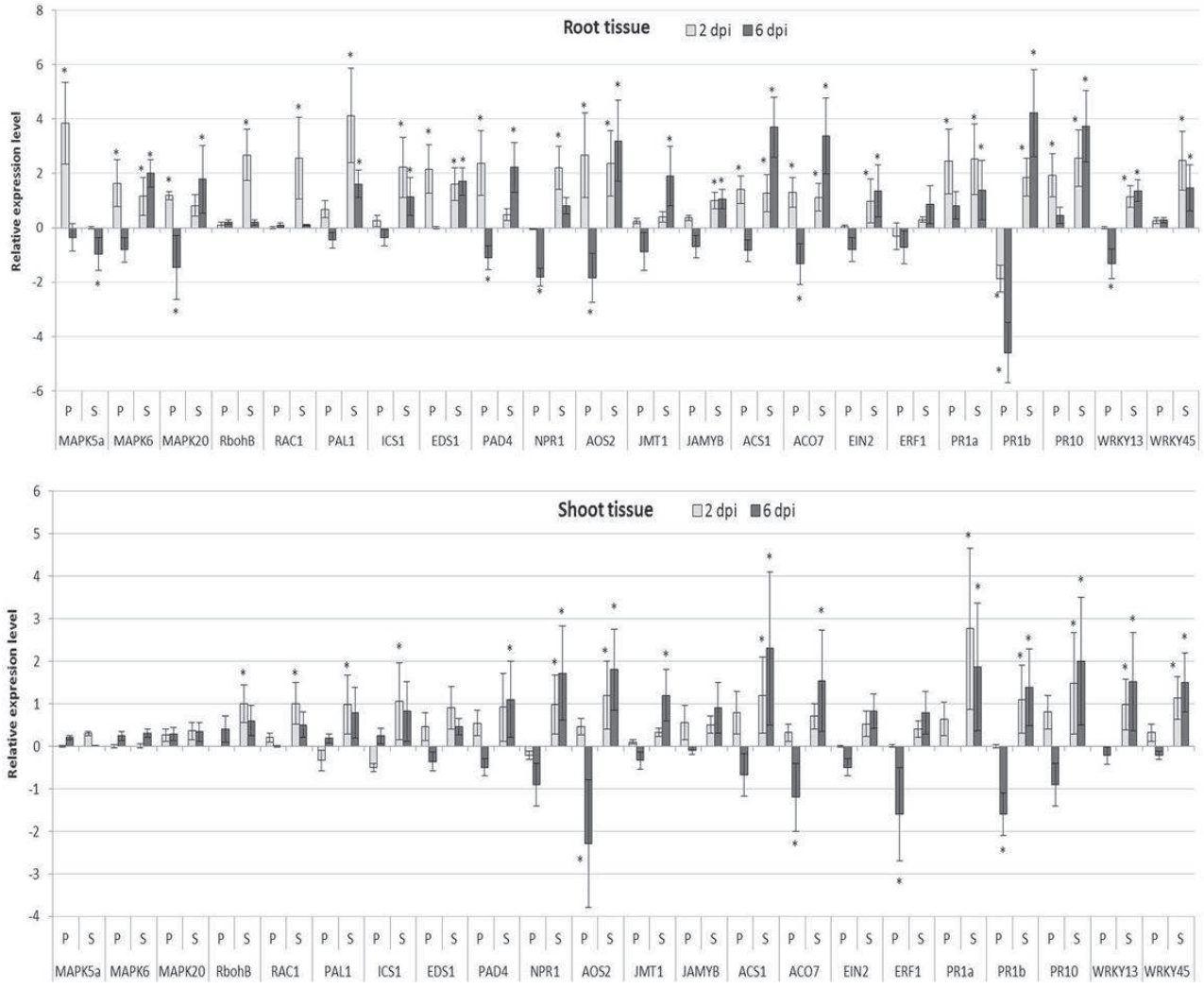
In order to understand the role of major hormone-regulated plant defence pathways in rice-RKN interaction, expression of some well-identified marker genes involved in those pathways were evaluated for their relative expression in the RKN-infected root and systemic shoot tissues of Pusa 1121 and Suraksha through qRT-PCR at 2 and 6 dpi (Fig. 3).

The induction of plant basal defences involves the recognition of nematode-derived compounds, called nematode-associated molecular pattern (NAMP). Induction of effector-triggered immunity (ETI) due to NAMP recognition, leads to the activation of intracellular signalling pathways, which is known as MAPK (mitogen-activated protein kinase) cascades that restrict disease progression and promote host resistance (Holbein et al. 2016). In our study, early upon infection (at 2 dpi), the mRNA levels of *OsMAPK5a*, *OsMAPK6* and *OsMAPK20* were markedly up regulated followed by their downregulation at 6 dpi in the roots of Pusa 1121. On the contrary, transcript levels of *OsMAPK6* and *OsMAPK20* were strongly upregulated in the RKN-infected roots of Suraksha both at 2 and 6 dpi. However, in shoot tissues of either of the varieties MAPK marker genes were not differentially expressed (Fig. 3). Our results indicate that RKNs may suppress MAPK activation during later stage of infection in susceptible plants, which may be attributed to the increased susceptibility of Pusa 1121 to RKN invasion.

Accumulation of reactive oxygen species (ROS) in the form of hydrogen peroxide is indicative of early defence response of plants to pathogen invasion. ROS accumulation triggers hypersensitive cell death and acts a secondary messenger in plant systemic signalling network (Lamb and Dixon 1997, Shetty et al. 2003, Apel and Hirt 2004). In our experiments, increased levels of *OsRbohB* (Respiratory burst oxidase homolog - required for ROS accumulation; Yoshika et al. 2003, Torres et al. 2006) and *OsRAC1* (associated with oxidative burst in rice; Delteil et al. 2010) at 2 dpi in root tissues of Suraksha indicates the successful induction of ROS-mediated defence in resistant plants. By contrast, negligible induction of *OsRbohB* and *OsRAC1*were detected in Pusa 1121 (Fig. 3).

Downstream of ETI activation, expression of plant defence proteins is mainly regulated by phytohormones, such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET),that are known to play significant role in host innate immunity (Bari and Jones 2009, Li et al. 2015). In accordance with this presumption, we examined whether the overexpression or attenuation of SA/JA/ET-related genes in rice is an important determinant in conferring resistance or susceptibility to RKN. Endogenous mRNA levels of*OsPAL1* and *OsICS1* (involved in SA biosynthesis; Lee et al. 1995) were considerably elevated in the infected root and shoot tissues of Suraksha both at 2 and 6 dpi in contrast to non-infected tissue level expression of those genes in Pusa 1121. Similar expression profile was documented with *OsEDS1*, *OsPAD4* and *OsNPR1* (involved in SA signalling; Li et al. 2015) genes except the fact that *OsEDS1* and *OsPAD4* were initially upregulated in the root of Pusa 1121 at 2 dpi (Fig. 3). Considering the non-significant expression of *OsPAD4* (2 dpi) and *OsNPR1* (6 dpi) in the roots of Suraksha, it is assumed that SA-signalling genes may play indeterminate role in the incompatible rice-RKN interaction. Nevertheless, significant overexpression of *OsPAL1* (catalyses phenylalanine to transcinnamic acid that acts as a precursor for lignin biosynthesis; Dixon and Paiva 1995) at 2 dpi in Suraksha indicates pivotal role of this gene in protecting resistant plants to RKN penetration by strengthening the plant cell wall.

According to Pieterse et al. (2009), JA biosynthesis and signalling are presumably modulated by the strong induction of ET. In addition, wound-responsive JA pathway, rather than SA, plays decisive role in systemic acquired resistance (SAR) in rice (Tamogami et al. 1997 Schweizer et al. 1998; Lee et al. 2004; Nahar et al. 2011). In light of this knowledge, our observations revealed the enhanced accumulation of transcripts of *OsAOS2* (a key enzyme in JA biosynthesis; Mei et al. 2006) in the root and shoot tissues of Suraksha during both early and later stage of RKN infection compared to early induction followed by repression of *OsAOS2* in Pusa 1121. Almost identical response was documented for transcripts of *OsJMT1* (involved in MeJA biosynthesis; Seoet al. 2001) and *OsJAMYB* (JA-inducible Myb transcription factor; Lee et al. 2001). Concomitantly,*OsACS1* and *OsACO7* (involved in ET biosynthesis; Iwai et al. 2006) were consistently upregulated in the RKN-infected tissues of Suraksha both at 2 and 6 dpi compared to downregulation of those genes in Pusa 1121 at 6 dpi. In corroboration, *OsEIN2* (associated with ET signalling; Jun et al. 2004) and *OsERF1* (ET-inducible gene; Hu et al. 2008) were also differentially expressed in Suraksha and Pusa 1121 (Fig. 3). Taken together, a consistent induction of JA and ET responsive genes



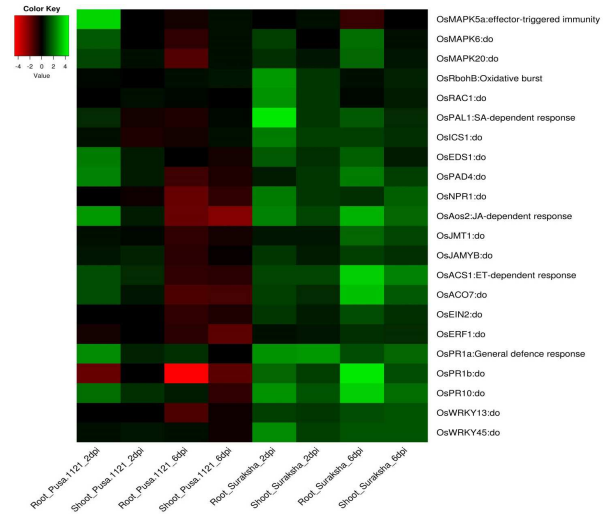


Fig. 3. (A) Bar graph representation and (B) Heat map visualization of defence-related genes differentially expressed in the root and shoot tissues of Pusa 1121 (P) and Suraksha(S) infected with *M. graminicola* at 2 (early response) and 6 (late response) dpi. Measured by qRT-PCR, differential expression pattern is based on the $\log_2$ fold changes of mRNA levels of candidate genes post normalization with two reference genes, *Os18srRNA* and *Os-actin*. Bars represent mean expression levels ± SE. Asterisks indicate significant differential expression ($P < 0.05$) in comparison with uninfected plants. Heat map was generated using R statistical package. Green boxes, gene is upregulated in infected tissue vs the corresponding healthy control; red, downregulation. MAPK: mitogen-activated protein kinase, Rboh: respiratory burst oxidase homolog, RAC: small RhoGTPase, PAL: phenylalanine ammonia lyase, ICS: isochorismate synthase, EDS: enhanced disease susceptibility, PAD: phytoalexin deficient, NPR: nonexpresser of pathogenesis-related (PR) gene, AOS: allene oxide synthase, JMT: JA carboxyl methyltransferase, JAMYB: JA-inducible Myb transcription factor, ACS: 1-aminocyclopropane-1-carboxylate synthase, ACO:1-aminocyclopropane-1-carboxylate oxidase, EIN: ethylene insensitive, ERF: ethylene response factor

throughout the course of RKN infection in the local and systemic tissues of resistant plants suggests a positive correlation between JA and ET-inducible gene expression in rice and induced resistance to *M. graminicola*.

Considering the ultimate importance of pathogenesis-related (PR) proteins in eliciting the general defence of plants under biotic stress, three candidate PR genes (*OsPR1a*, *OsPR1b*, *OsPR10*; Agrawal et al. 2000; Mitsuhara et al. 2008) were assessed for their differential expression in the present study. Additionally, *OsWRKY13* and *OsWRKY45* (transcriptional regulator of defence genes; Shimono et al. 2007, Qiu et al. 2009, Liu et al. 2013) were also included for the expression analysis. An increased and consistent transcript abundance of all the PR genes were recorded in both root and shoot tissues of Suraksha at 2 and 6 dpi. By contrast, except *OsPR1a* and *OsPR10* at 2 dpi, all the PR genes were either down regulated or returned to basal expression levels in Pusa 1121. Likewise, WRKY genes were over expressed in Suraksha compared to non-infected tissue level expression in Pusa 1121 (Fig. 3).

Accumulation of lignin and callose confer mechanical strength to plant cell wall, which contributes to basal defence against phytonematode penetration and migration in plant root (Gheysen and Jones 2006). This apprehension prompted us to assess the relative expression of two lignin biosynthesis genes (*OsC4H*, *OsCAD6*; Portillo et al. 2013), three callose synthase genes (*OsGSL1*, *OsGSL3*, *OsGSL5*; Hao et al. 2008) and one callose hydrolysing gene (*OsGNS5*; Hao et al. 2008) in roots of Suraksha and Pusa 1121 during early RKN attack (at 2 dpi). Results indicated that lignin and callose biosynthesis genes were quantitatively greatly induced in Suraksha compared to Pusa 1121, whereas comparatively greater induction of callose-degrading gene was documented in Pusa 1121 than Suraksha (Fig. 4) possibly because of the genotype-specific mechanism of resistance in Suraksha. This exemplifies the significance of lignin- and callose-mediated basal defence in resistant plants to *M. graminicola* invasion.

From the present study, we conclude that the basal as well as systemic defence is quickly activated in rice upon *M. graminicola* infection. As the infection advances nematodes can effectively suppress those defence responses in susceptible plants. Conversely, JA and ET pathway, PR proteins and WRKY genes may play major role in arresting infection progression

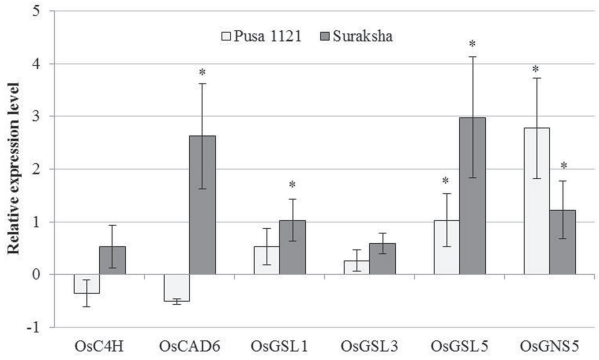


Fig. 4. Differential expression patterns of genes involved in lignin biosynthesis and callose deposition in the root tissues of Suraksha and Pusa 1121 infected with *M. graminicola* at 2 dpi. Measured by qRT-PCR, differential expression pattern is based on the $\log_2$ fold changes of mRNA levels of candidate genes post normalization with two reference genes, *Os18srRNA* and *Os-actin*. Bars represent mean expression levels and SE from two biological and three technical replicates each containing a pool of ten plants. Asterisks indicate significant differential expression ($P < 0.05$) in comparison with uninfected plants. C4H: cinnamate 4-hydroxylase, CAD:cinnamyl alcohol dehydrogenase,GSL: glucan synthase-like, GNS: β-1,3-endoglucanase

in resistant plants. In addition, activation of MAPK cascades and ROS accumulation was evident in resistant plants compared to susceptible ones upon RKN infection. This interesting observation was not documented in RKN resistant cultivar Vandana in our earlier study (Kumari et al. 2016). The outcome from this study can be extrapolated to decipher the molecular determinants that govern resistance/susceptibility of plants to phytonematodes. Further, the role of manipulated phytohormone pathways on RKN infectivity may be investigated by generating JA/ET/WRKY/PR gene-deficient *O. sativa* mutants. Exogenous application of synthetic analogues of JA/ET/PR genes may also protect the rice plants to RKN infection.

Authors' contribution

Conceptualization of research (UR, TKD); Designing of the experiments (CK, TKD); Contribution of experimental materials (CK); Execution of field/lab experiments and data collection (CK); Analysis of data and interpretation (TKD, SG); Preparation of manuscript (UR, TKD).

Declaration

The authors declare no conflict of interest.

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