Profiling of defense responsive pathway regulatory genes in Asian rice (*Oryza sativa*) against infection of *Meloidogyne graminicola* (Nematoda: Meloidogynidae)

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Abstract

In the present study, 36 Asian rice cultivars/landraces were evaluated against *M. graminicola* under in vitro conditions using soilless Pluronic gel medium. The cultivars/genotypes Phule Radha, EK 70, LK 248 and Khalibagh showed significantly reduced nematode infection, endoparasitic development, and derived multiplication factor indicating the presence of resistance, while Halvi Sal 17 was found to be most susceptible. Performance of selected genotypes showing resistance/susceptibility under in vitro conditions was further confirmed in soil which also revealed Phule Radha to be highly resistant and Halvi Sal 17 as the most susceptible genotype. Further, expression profile of plant defense responsive genes related to MAPK pathway, phytohormones, PR-proteins and callose and lignin synthesis were quantified in Phule Radha (the most resistant) and Halvi Sal 17 (the most susceptible) at 2 and 6 days post nematode inoculation. Significant upregulated expression of several defensive genes was observed in the resistant cultivar Phule Radha in contrast to insignificant expression in the susceptible varieties. The resistant genotype identified in the present study will be highly promising for resistance breeding in rice against *M. graminicola*.

Keywords Rice · *Meloidogyne graminicola* · Plant defense · Resistance · Susceptible

Abbreviations

PPNs	Plant parasitic nematodes
SA	Salicylic acid
JA	Jasmonic acid
ET	Ethylene
J2s	Second stage juvenile
MF	Multiplication factor
MAPK	Mitogen activated protein kinase
PB	Pusa Basmati 1121

Introduction

Plant-parasitic nematodes (PPNs) cause an annual loss of approximately US\$ 173 billion to the world agriculture infecting cereals, vegetables, fruits, plantation crops etc. (Elling 2013). Rice (*Oryza sativa* L.) is one of the most important cereal crops and accounts for everyday meal of almost two-thirds of the world's population (Abodolereza and Racionzer 2009). Rice is attacked by several PPNS inflicting an estimated economic loss of almost 20%, coupled with substantial quality losses (Sasser and Freckman 1987; Kyndt et al. 2014). Amongst the major PPN species infecting rice, the root-knot nematode *Meloidogyne graminicola* (Nematoda: Meloidogyninae) causes considerable yield loss in the rice growing areas of Southeastern Asia and other countries (De Waele and Elsan 2007). The infective second-stage juveniles (J2s) of *M. graminicola* penetrate through the meristematic zone of root, move intercellularly within the root tissue to reach the vascular cylinder and develop specialized feeding cells, called “giant cells” (Kyndt et al. 2014). These modified feeding cells act as continuous source of nutrients for the nematode species to nurture them throughout their development and reproduction. A wide

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array of effector proteins of nematode oesophageal gland origin have been identified to interact with the host plant that help in maintaining the feeding cells and manoeuvring the plant defense responses (Chen et al. 2018; Naalden et al. 2018).

To date, almost all the rice varieties are known to be susceptible to root-knot nematode infection, and except for *O. glaberrima* and *O. longistaminata*, the reported resistance source against *M. graminicola* is very limited (De Waele and Elsan 2007; Kyndt et al. 2014). To meet the increasing demand of food for global population, rice varieties with superior yield potential and resistance/tolerance to biotic and abiotic stresses are being developed using molecular breeding approaches (Kumari et al. 2016). However, comprehensive knowledge about the molecular regulation of resistance/tolerance behaviour in rice—*M. graminicola* interaction is at a nascent stage (Kumari et al. 2016, 2017). It is a well-established fact that plants contain a nexus of defense responsive pathways which are activated on pest and pathogen attack (Liu et al. 2012), and the PPNs have also developed sophisticated methods to support their parasitism (Naalden et al. 2018). The resistant plants generally confront the pathogens (including nematodes) with activation of several basal and induced defense mechanisms (Li et al. 2015). The pathogen-host plant intercommunication is predominantly explained by ‘zigzag’ model (Jones and Dangl 2006), and similar understanding has also been established for PPN-host plant interaction (Choi and Klessig 2016; Ali et al. 2018).

Upon entry of PPNs into the host roots, the constitutive and basal plant defense responses are triggered by major signaling pathways, including salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) mediated signals, pathogenesis-related (PR) proteins, and different plant transcription factors (Goto et al. 2013; Li et al. 2015). The SA signaling is considered to be most essential in *R*-gene mediated defense regulation against the sedentary endoparasitic nematodes (Branch et al. 2004; Uehara et al. 2010; Kandoth et al. 2011). Although, JA acts as an important signaling molecule in plant defense in wounding and pathogen infection, our current understanding of JA signaling in plant-nematode associations is less clear (Takahashi et al. 2004; Ozalvo et al. 2013). The ET biosynthesis and its signaling play an important role in susceptibility as well as resistance against sedentary nematodes which differ according to the infecting nematode species (Fujimoto et al. 2011). Role of several PR proteins and nematode associated molecular patterns (NAMPs) in relation with host resistance has also been reviewed earlier (Andrade et al. 2010; Manosalva et al. 2015). Although, the consolidated outcome of these coordinated signaling responses ultimately determine the plant susceptibility/resistance to PPNs, comparable articulation of these defense related genes in systemic and local defense in monocotyledons is an underexplored territory. In this

context, the present study was conducted to identify and determine the resistance/tolerance/susceptibility in different cultivars/landraces of Asian rice (*O. sativa*) under in vitro PF-127 medium and soil system. Additionally, our study also provides a comprehensive depiction of defense-related gene expression changes in resistant and susceptible rice genotypes upon *M. graminicola* infection. The extrapolation of the findings of the current investigation will help filling the knowledge gap of physiological and molecular interaction between rice and plant-parasitic nematodes.

Materials and methods

Culturing of nematodes

The pure culture of an Indian isolate of *Meloidogyne graminicola* (Golden and Birchfield 1965) was multiplied on rice (*O. sativa* cv. PB 1121) in glasshouse at ICAR- Indian Agricultural Research Institute, New Delhi, India. The second-stage infective nematode juveniles (J2s) were hatched via “modified Baermann’s technique” from the infected rice roots (Whitehead and Hemming 1965). The freshly hatched J2s were used for all experiments.

Screening of rice genotypes under in vitro condition

The seeds of rice cultivars/landraces, Heera, Halvi Sal 17, RDN185-2, Phule Radha, RTN1, Ratna, Phule Samruddhi, Jaya, Phule Maval, Pawana, Indrayani, Bhogavati, Pusa Basmati 1, Ghansal, Kalajirga, Vivek Dhan 82, Badshahog, Kothimbire, BPT 5204, RDN98-2-3-5-14, KJT2, EK 70, Patni, Diwani, Shyam Jeer, SD 17, Khalibagh, Champakali, Siddhagiri, RTN purple, Pavsai, Antersal, Nalabhat, Sonali, LK 248, Pomendi Local were procured from Agricultural Research Station, Radhanagari, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India. The pedigree of the genotypes is available at earlier publication of Kumbhar et al. (2015), and *O. sativa* cv. PB 1121 was used as a standard susceptible check.

The rice seeds were surface sterilized with 70% ethanol for 30 s–1 min, followed by rinsing with sterilized distilled water thoroughly until traces of ethanol vanished completely, and soaked overnight. The surface sterilized soaked seeds were placed in Petri dishes containing single layer of seed germination paper (SS Filters Pvt Ltd, Mumbai, Maharashtra, India) and incubated in a growth chamber, maintained at 28 °C, 70% relative humidity and 16:8 h light:dark photoperiod. The 3–4 day-old germinated seeds with 1.5–2 cm long radicals were used for experimental purpose. Seven seeds of each genotype were placed in a Petri dish, and screened (against *M. graminicola* infection) in vitro using Pluronic gel, PF-127 medium following standard protocols

(Wang et al. 2009; Kumari et al. 2016). The root tips of each germinated seeds were inoculated with approximately 30 nematode J2s, and three replicates (3 Petri dishes containing 7 germinated seeds each) were maintained. The Petri dishes, after inoculation, were left at room temperature (28 °C) up to the gel gets solidified, transferred to growth chamber, and maintained at aforesaid conditions for 15 days (Additional file 1: Fig. 1). Fifteen days post inoculation (dpi), the plants were harvested, roots were washed and stained by NaOCl–Acid fuchsin method (Byrd et al. 1983). Number of galls, endoparasites, and egg masses developed per plant, and average number of eggs per egg mass was counted for each of the plants by dissecting out the stained roots under stereo-binocular microscope. Nematode multiplication factor (MF) [(number of egg masses × number of eggs per egg mass)/initial nematode inoculum] was derived to assess the reproductive fitness. The whole screening assay was repeated four times for reproducible results.

Screening of selected rice genotypes under greenhouse condition

Selected rice cultivars/landraces showing high level of resistance and/or susceptibility under Pluronic gel assay, were further screened under greenhouse condition at ICAR-Indian Agricultural Research Institute, New Delhi, India. For this, 21 day-old rice seedlings (of Phule Radha, EK 70, Khalibagh and Halvi Sal 17) raised in 6 inch diameter pots were inoculated with *M. graminicola* with inoculum level of 2 J2s per g soil. The pots with seedlings were maintained at standard greenhouse conditions. Two months post nematode inoculation, plants were harvested, roots were washed, and stained as described above to confirm and quantify nematode infection. Number of galls, endoparasites, and egg masses developed per plant, and average number of eggs per egg mass was counted for each of the plants, and nematode MF was derived as stated above. The whole screening assay was repeated two times, and *O. sativa* cv. PB 1121 was used as a standard susceptible check.

Expression analyses of defense responsive genes in root and shoot tissues of rice

The root tips of selected rice genotypes (Phule Radha, Halvi Sal 17 and PB 1121) were inoculated with *M. graminicola* J2s in Pluronic gel medium as documented above. The plantlets were harvested at 2 and 6 dpi, washed thoroughly to wash away the gel material, and excised shoots and root tips were stored immediately at − 80 °C, separately, until further use. Total RNA was extracted separately from the frozen shoot and root tissues using NucleoSpin total RNA Kit (Macherey–Nagel, Düren, Germany) following manufacturer's instructions. Approximately 500 ng of purified RNA

was reverse transcribed using cDNA synthesis kit (Super-script VILO, Invitrogen, Carlsbad, CA, USA). Expression of different defense genes (Additional file 2: Table 1) was quantified using qRT PCR as described earlier (Kumari et al. 2016). Gene expression was normalized using constitutively expressed genes *Os18SrRNA* (acc no. AF069218) and *Osactin* (acc no. RAP-DB:Os03g0718100). Three biological and three technical replicates were maintained for each sample, data were analyzed by $\Delta\Delta C_t$ method (Livak and Schmittgen 2001) and results were expressed as log₂-transformed fold change values. Healthy rice plants were used as control. To represent the comparative gene expression profile of shoot and root tissues after nematode infection, heat map image was generated using programme “Heatmapper” (<https://www.heatmapper.ca>). The primer details for qRT PCR are provided in Additional file 2: Table 1.

Statistical analysis

The bioassay data were subjected to one way analysis of variance (ANOVA) and was conducted under completely randomized designs (CRD) with statistical significance determined at $P < 0.05$, $P < 0.01$. Data transformation was done by square root transformed method.

Results

Screening of rice genotypes against *M. graminicola* under in vitro condition (Table 1, Fig. 1, Additional file 1: Fig. 1)

Thirty-six rice genotypes were screened against *M. graminicola* under in vitro condition using PF-127 medium. The genotypes displayed large variability with respect to gall and egg mass formation when challenged against nematode J2s. Out of the 36 genotypes, least number of galls per plant was observed in EK 70 (0.29), LK 248 (0.43), Phule Radha (0.29), and Khalibagh (0.43). Corroborating this observation, both the number of endoparasites and egg masses per plant were also found very less in the aforesaid four genotypes, as compared to others and control. The average number of eggs per egg mass produced in EK 70, LK 248, Phule Radha, and Khalibagh were 8.00, 16.29, 6.57 and 11.43, respectively. On the other hand, highest number of galls were formed in Halvi Sal 17 (9.14), followed by PB 1121 (7.71). The derived MF for PB 1121 was found to be highest, followed by cultivars Bhogavati and KJT 2. The MF for EK 70 (0.08), LK 248 (0.39), Phule Radha (0.06), and Khalibagh (0.49) were found to be significantly ($P < 0.01$) less as compared to the susceptible cultivar PB 1121.

Table 1 Performance of rice genotypes by deliberate challenging by *Meloidogyne graminicola* in Pluronic gel (the value of the number of galls, total endoparasites, egg masses and average eggs/plant are the mean of seven replicates; figure in parentheses indicates $\sqrt{X+0.5}$ transformed value; values are significant at $P < 0.05$ and $P < 0.01$)

Sr. no.	Name of genotype	Number of galls	Total endoparasites	Egg masses	Average eggs/egg mass
1	Jaya	3.14 (1.89)	9.43 (3.11)	7.71 (2.82)	36.51 (5.93)
2	Bhogavati	3.14 (1.88)	14.57 (3.85)	10.29 (3.23)	63.81 (7.93)
3	RDN98-2-3-5-14	4.29 (2.14)	9.14 (3.05)	8.29 (2.92)	53.34 (7.30)
4	Kothimbire	4.57 (2.20)	12.43 (3.50)	11.43 (3.35)	46.36 (6.71)
5	PB 1	1.43 (1.33)	5.00 (2.13)	3.57 (1.80)	35.93 (5.21)
6	EK70	0.29 (0.86)	0.29 (0.86)	0.29 (0.86)	8.00 (2.01)
7	KJT2	3.86 (2.07)	11.86 (3.36)	11.71 (3.34)	53.67 (7.36)
8	Champakali	3.43 (1.93)	6.57 (2.62)	5.57 (2.43)	46.40 (6.79)
9	Siddhagiri	3.00 (1.85)	4.86 (2.25)	3.86 (2.06)	36.34 (6.03)
10	RTN Purple	3.57 (1.96)	9.86 (3.09)	9.57 (3.05)	49.58 (7.00)
11	Antersal	3.43 (1.97)	6.43 (2.60)	5.71 (2.45)	46.76 (6.84)
12	Pavsal	2.57 (1.64)	5.43 (2.36)	4.86 (2.24)	55.93 (7.45)
13	Nalabhat	2.71 (1.78)	6.71 (2.61)	6.71 (2.61)	52.68 (7.25)
14	Sonsali	1.29 (1.30)	3.57 (1.87)	2.14 (1.52)	29.14 (4.98)
15	LK248	0.43 (0.93)	0.71 (1.03)	0.71 (1.03)	16.29 (3.03)
16	Pomendi Local	1.71 (1.45)	4.00 (2.03)	2.00 (1.50)	33.54 (5.10)
17	Heera	4.00 (2.09)	12.14 (3.42)	10.00 (3.04)	45.84 (6.33)
18	HS 17	9.14 (3.04)	28.14 (5.14)	14.43 (3.48)	29.92 (5.13)
19	RDN 185-2	4.86 (2.25)	19.71 (4.10)	4.71 (2.08)	18.80 (4.05)
20	RTN-1	5.71 (2.44)	19.00 (4.18)	14.00 (3.51)	33.84 (5.38)
21	Ratna	8.29 (2.94)	29.14 (5.27)	8.57 (2.85)	17.03 (4.08)
22	Phule Radha	0.29 (0.86)	0.29 (0.86)	0.29 (0.86)	6.57 (1.86)
23	Pawana	3.00 (1.84)	12.71 (3.59)	11.57 (3.41)	49.11 (6.99)
24	Phule Maval	3.57 (2.00)	13.00 (3.59)	12.86 (3.57)	37.85 (6.11)
25	Indrayani	1.43 (1.31)	2.29 (1.49)	0.71 (1.05)	12.45 (2.99)
26	Phule Samruddhi	1.29 (1.33)	3.71 (2.01)	2.57 (1.61)	23.64 (4.24)
27	Ghansal	3.86 (2.08)	18.57 (4.15)	13.86 (3.62)	27.63 (5.25)
28	Kalajirga	3.57 (2.00)	15.71 (3.89)	6.43 (2.53)	14.56 (3.80)
29	Diwani	3.29 (1.88)	11.86 (3.43)	9.29 (2.99)	36.41 (6.01)
30	Badshabhog	4.71 (2.27)	13.71 (3.72)	7.86 (2.80)	28.94 (5.29)
31	Vivek Dhan 82	1.14 (1.28)	3.14 (1.77)	2.14 (1.42)	28.29 (4.64)
32	BPT5204	1.29 (1.27)	2.57 (1.60)	1.71 (1.38)	17.45 (3.30)
33	Khalibagh	0.43 (0.93)	1.43 (1.17)	1.29 (1.15)	11.43 (2.84)
34	Shyam Jeer	3.71 (1.99)	12.57 (3.59)	9.00 (2.97)	29.81 (5.33)
35	Patni	3.43 (1.96)	11.14 (3.23)	9.29 (2.88)	27.07 (5.11)
36	SD 17	3.57 (2.00)	11.43 (3.44)	11.14 (3.40)	36.64 (6.04)
37	PB 1121	7.71 (2.80)	28.86 (5.28)	26.14 (5.01)	78.20 (8.85)
	F	12.15	11.25	8.99	7.12
	CD ($P=0.05$)	0.36	0.82	0.76	6.14
	CD ($P=0.01$)	0.51	1.16	1.08	8.72

Screening of selected rice genotypes against *M. graminicola* under greenhouse conditions (Table 2, Fig. 2)

Selected resistant (EK 70, Phule Radha, and Khalibagh) and susceptible (Halvi Sal 17 and PB 1121) genotypes identified using in vitro assay were further screened under greenhouse condition to confirm their performance in the soil system. The average number of galls produced per

plant in Phule Radha (8.43), EK 70 (22.00), and Khalibagh (25.29) were significantly ($P < 0.05$) lower than PB 1121 (233) and Halvi Sal 17 (62.71). The number of endoparasites developed and egg masses formed per plant also followed the same trend. The derived MF was found to be lowest in Phule Radha (0.32), followed by EK 70 (1.41), and Khalibagh (1.94); as compared to Halvi Sal 17 (7.15) and PB 1121 (21.1).

Fig. 1 Bar graph depicting the reproductive ability of the nematodes 15 days post inoculation on the rice genotypes (bar indicates standard error from seven replicates of each genotype)

Expression analyses of defense responsive genes in root and shoot tissues

Expression of MAPK-related genes (Fig. 3a)

The resistant cultivar Phule Radha showed upregulation of *OsMAPK5a*, *OsMAPK6* and *OsMAPK20* at 2 dpi in roots; but at 6 dpi, *OsMAPK6* and *OsMAPK20* were upregulated and *OsMAPK5a* was downregulated. On the other hand, *OsMAPK5a*, *OsMAPK6*, and *OsMAPK20* were found to be significantly upregulated in Halvi Sal 17 at 6 dpi in roots, while at 2 dpi only *OsMAPK20* was downregulated. Unlike

Halvi Sal 17, PB 1121 showed downregulation of all the three MAPK genes at 6 dpi in roots, however, *OsMAPK5a* was downregulated at 2 dpi, against upregulation of *OsMAPK6* and *OsMAPK20*.

Unlike the large variability of MAPK expression in roots, the shoots showed a general downregulated pattern of MAPK expression at both the time points, except upregulation of *OsMAPK6* and *OsMAPK20* in PB 1121 at 2 dpi. However, the resistant cultivar Phule Radha showed upregulation of *OsMAPK6* and *OsMAPK20* at 6 and 2 dpi, respectively.

Expression of SA-related genes (Fig. 3b, c)

The susceptible cultivar PB 1121 showed upregulation of *OsPAL1*, *OsICS1*, *OsEDS1*, *OsPAD4*, and *OsNPR1* at 2 dpi in roots; and Halvi Sal 17 showed upregulation of *OsICS1*, *OsEDS1*, and *OsNPR1* and downregulation of *OsPAL1* and *OsPAD4*. A large variability was observed in expression of these genes at 6 dpi for these two susceptible cultivars. On the other hand, the resistant cultivar Phule Radha showed upregulation of *OsPAL1*, *OsICS1*, *OsEDS1* and *OsNPR1*, and downregulation of *OsPAD4* at 2 dpi in roots. But, all the genes were upregulated in roots of Phule Radha at 6 dpi, except *OsPAL1*.

In shoots, the resistant cultivar Phule Radha showed upregulation of *OsPAL1* and *OsICS1*, and downregulation of *OsEDS1* and *OsPAD4* at both 2 and 6 dpi. But *OsNPR1* was downregulated at 2 dpi, and upregulated at 6 dpi in Phule Radha. Unlike the resistant cultivar, both the susceptible cultivars (PB 1121 and Halvi Sal 17) showed large variability in expression of all the SA-related genes at 2 and 6 dpi.

Expression of JA-related genes (Fig. 4a)

The JA biosynthesis and signaling related genes, *OsAOS2*, *OsJMT1* and *OsJAMYB* were mostly upregulated in the resistant (Phule Radha) and susceptible (Halvi Sal 17 and PB 1121) cultivars at both 2 and 6 dpi, except downregulation of all the three genes in PB 1121 at 6 dpi, in roots. Unlike the roots, shoots showed greater variability in expression of the JA-related genes in all the cultivars. Phule Radha showed upregulation of the genes at 2 and 6 dpi, except for downregulation of *OsJMT1* at 2 dpi. Both the susceptible cultivars (PB 1121 and Halvi Sal 17) showed mostly downregulation of *OsAOS2*, *OsJMT1*, and *OsJAMYB* at both the time points, except upregulation of *OsAOS2* and *OsJAMYB* in PB 1121 at 2 dpi.

Expression of ET-related genes (Fig. 4b, c)

Amongst all the ET-related genes, *OsACO7* was upregulated in roots of all the resistant and susceptible cultivars at 2 and

Table 2 Evaluation of the rice genotypes against *Meloidogyne graminicola* under greenhouse conditions (the value of the number of galls, total endoparasites, egg masses and average eggs/plant are the mean of ten replicates; figure in parentheses indicates $\sqrt{X+0.5}$ transformed value; values are significant at $P<0.05$ and $P<0.01$)

Sr. no.	Name of genotype	Number of galls	Total endoparasites	Egg masses	Average eggs/egg mass
1	Phule Radha	8.43 (2.94)	25.43 (5.04)	18.57 (4.31)	34.13 (5.88)
2	EK 70	22.00 (4.70)	67.71 (8.25)	55.57 (7.47)	50.72 (7.14)
3	Khalibagh	25.29 (5.04)	86.71 (9.33)	74.43 (8.65)	52.26 (7.26)
4	HS 17	62.71 (7.94)	258.00 (16.02)	232.86 (15.20)	61.43 (7.86)
5	PB 1121	233 (15.18)	547.71 (23.36)	529.57 (22.98)	79.69 (8.95)
	F	159.91	294.56	309.27	55.50
	CD ($P=0.05$)	1.10	1.23	1.22	0.43
	CD ($P=0.01$)	1.49	1.66	1.64	0.58

Fig. 2 Response of rice genotypes to the deliberate challenging by *Meloidogyne graminicola* under greenhouse conditions. **a** Root phenotype of the rice genotypes, Phule Radha (1), EK 70 (2), Khalibagh (3), Halvi Sal 17 (4), PB 1121 (5) 60 days post inoculation. **b** Assessment of the reproductive ability (multiplication factor) of *M. graminicola* on the challenged rice genotypes (bar indicates standard error from ten replicates of each rice genotype)

Fig. 3 Expression pattern of **a** MAPK-dependent and **b**, **c** SA-dependent responsive genes in root and shoot tissues of rice genotypes by attack of *Meloidogyne graminicola* at 2 and 6 days post infection (PB PB 1121, HS Halvi Sal 17, PR Phule Radha). Data are shown as the log 2-transformed values of the fold change level of infected root/shoot in comparison with the control root/shoot tissue (uninfected plant) (bar indicates mean expression level and SE from three biological and three technical replicates of each rice genotype)

6 dpi. However, the expression of *OsACS1*, *OsEIN2*, and *OsERF1* varied at both the time points in roots of Phule Radha (resistant), Halvi Sal 17 and PB 1121 (susceptible). The shoot tissues showed considerable variation in expression of these genes after nematode attack. *OsACS1*, *OsEIN2* and *OsERF1* were downregulated in Phule Radha at both 2 and 6 dpi, but *OsACO7* was upregulated. Expression of all the genes also considerably varied in shoot tissues for both the susceptible cultivars (Halvi Sal 17 and PB 1121).

Expression of PR-related genes (Fig. 5a–c)

The expression of *OsPR1a*, *OsPR1b*, *OsPR10*, *OsWRKY13*, and *OsWRKY45* mostly followed an

upregulated trend in the root tissues for all the resistant and susceptible cultivars, except downregulation of *OsPR1a*, *OsPR1b*, *OsWRKY13* and *OsWRKY45* in the susceptible cultivar PB 1121 at 6 dpi. Additionally, at 2 dpi, *OsWRKY13* and *OsWRKY45* were also downregulated in the resistant cultivar Phule Radha.

The shoots displayed greater variability of PR-gene expression than the roots. *OsPR1a*, *OsPR1b* and *OsPR10* were mostly upregulated in resistant cultivar Phule Radha at 2 and 6 dpi, except downregulation of *OsPR1b* at 2 dpi. However, *OsWRKY13* and *OsWRKY45* were also downregulated in the susceptible cultivars (PB 1121 and Halvi Sal 17) at both the time points except being upregulated in PB 1121 at 2 and 6 dpi. The expression of PR-genes

Fig. 4 Expression pattern of **a** JA-dependent and **b, c** ET-dependent responsive genes in root and shoot tissues of rice genotypes by attack of *Meloidogyne graminicola* at 2 and 6 days post infection

followed a variable trend for the susceptible cultivars Halvi Sal 17 and PB 1121.

Expression of callose and lignin-related genes

The expression of callose and lignin synthesis and hydrolysis genes (*OsC4H*, *OsCAD6*, *OsGSL1*, *OsGSL3*, *OsGSL5* and *OsGNS5*) in roots mostly followed an upregulated trend in the resistant and susceptible cultivars at 2 and 6 dpi, except for downregulation of *OsC4H* and *OsGSL5* in Phule Radha and Halvi Sal 17, respectively.

Discussion

In the present study, 36 genotypes of Asian rice including landraces, local selections, and improved varieties were screened and evaluated against *M. graminicola*, a serious root pest of rice. The screening was primarily done using the soilless Pluronic gel medium, and thereafter selected resistant and/or susceptible genotypes were further evaluated for their performance under greenhouse conditions. Additionally, our study also focused on profiling the expression of several key plant defense responsive genes

Fig. 5 Expression pattern of **a**, **b** general defense responsive genes in root and shoot tissues of rice genotypes by attack of *Meloidogyne graminicola* at 2 and 6 days post infection and expression pattern of **c** lignin biosynthesis, callose synthase and hydrolysing genes in root tissues of rice genotypes upon attack of *M. graminicola*

associated with major phytohormones (salicylic acid, jasmonic acid and ethylene), pathogenesis-related proteins (PR proteins), and lignin and callose synthesis and hydrolysis, against *M. graminicola* infection.

Here, we have evaluated the primary compatible/incompatible interaction of the rice genotypes with root-knot nematode *M. graminicola* using Pluronic gel PF-127 medium. Previously, this soilless medium was established as an appropriate matrix for monitoring the progression of nematode development and parasitism inside the plant roots in non-axenic environment (Kumari et al. 2016). Based on the in vitro screening assay, the genotypes Phule Radha, EK 70, Khalibagh and LK 248 were found to be highly resistant as compared to PB 1121 (an established susceptible cultivar).

Additionally, Halvi Sal 17 was found to be a highly susceptible rice cultivar against *M. graminicola*. The superior performance of the resistant genotypes under Pluronic medium was further confirmed in soil system using threshold inoculum density (2 J2s per g soil) of *M. graminicola*. Although, previous studies have shown large variability while demonstrating the threshold density for *M. graminicola* infecting rice under different environmental conditions, the inoculum level of 2 J2s per g soil has been proved to cause substantial damage (Prasad et al. 1990; Dabur et al. 2004; Jaiswal et al. 2011).

Plants inhabit in a complex environment interacting with various abiotic (cold, drought, salt, heavy metal contamination etc.) and biotic stress (bacteria, fungi, insects,

nematodes, etc.) delimiting agents (Atkinson and Urwin 2012). Phytohormones play a pivotal role as signal molecules in plants, but exist in very low concentrations (Davies 2004). Upon biotic and abiotic stresses, the phytohormone concentration and/or sensitivity changes that arbitrate the range of adaptive plant response (Davies 2004). The role of SA, JA and ET, as primary signals in regulating host plant immune response, has been studied in detail (Pieterse and Dicke 2007; Pieterse et al. 2009). Subsequent to the activation of PTI (PAMP-triggered immunity) and ETI (effector-triggered immunity), a coordinated expression of SA/JA/ET-mediated signaling enables plants to effectively maneuver its local and systemic defense responses against pathogens (Bari and Jones 2009; Li et al. 2015). Additionally, SAR (systemic acquired resistance) response is generated in the infected tissues by pathogen attack, which is characterized by local and systemic buildup of SA and concomitant expression of PR (pathogenesis-related) proteins (Vlot et al. 2009). The involvement of SA, JA, and ET mediated defense signaling has also been established in rice against different nematode species (Pozo et al. 2004; van Loon et al. 2006; Loake and Grant 2007). The expression of different phytohormone related genes in both roots and shoots of the resistant and susceptible rice genotypes steadily shows the induction of local and systemic defense signaling after *M. graminicola* infection (Gheysen and Mitchum 2011).

The upregulation of *OsPAL1* and *OsICS1* in roots and shoots of resistant cultivar Phule Radha at very early stage of nematode infection possibly indicates activation of phenotypic resistance against the invasion of *M. graminicola*. However, upregulation of these genes were also noticed in the susceptible cultivars at 2 dpi, but at 6 dpi the expression showed downregulation indicating nematode mediated suppression of SA-mediated plant defense responses. Both the enzymes participate in salicylate biosynthesis (Li et al. 2015), and manifestation of apoptosis by coordinated activation of *PAL1* and *ICS1* through SA-mediated pathway has been observed in the dicots (Durrant and Dong 2004). Further, *EDS1* and *PAD4* are associated with SA production and signaling pathways responsible for activation of basal plant resistance against biotrophs and hemi-biotrophs (Li et al. 2015). Our data suggest that *M. graminicola* can actively induce the expression of *OsPAD4* and *OsEDS1* in resistant cultivar Phule Radha at later stage of infection. The engagement of *OsNPR1* for activation of SA signaling was found to be pivotal in rice against bacterial blight and fungal blast (Dong 2004; Yuan et al. 2007). Here, the induction of *OsNPR1* in resistant genotype indicates its possible involvement in activating the SA signaling for imparting resistance against *M. graminicola*. Jasmonic acid (JA) plays an important role for activation of SAR in rice than salicylic acid (Tamogami et al. 1997; Schweizer et al. 1998; Lee et al. 2001). The present experimental data shows

triggering of JA biosynthesis and signaling genes (*OsAOS2*, *OsJMT1* and *OsJAMYB*) in roots of both the resistant and susceptible genotypes of rice. But few of the JA-related genes were induced in the shoot tissues of resistant genotype Phule Radha, as compared to the susceptible genotype PB 1121 and Halvi Sal 17. The JA pathway is regulated by significant activation of ET biosynthesis and signaling to initiate defense response in rice against *M. graminicola* (Nahar et al. 2011). The complex expression pattern of the ET-related genes (*OsACS1*, *OsACO7*, *OsEIN2* and *OsERF1*) in the experimental genotypes indicates possible activation of similar defense responsive pathways in the resistant rice, upon invasion of *M. graminicola*.

During compatible interaction between root-knot nematodes and their host (e.g. *Arabidopsis* and tomato), several pathogenesis-related (PR) genes are repressed in shoot tissues (Sanz-Alferez et al. 2008; Hamamouch et al. 2011). Significant differences in expression of PR genes were observed in shoot tissues in compatible and incompatible interaction of *M. incognita* and tomato (Molinari et al. 2014). At later stage of *M. graminicola* infection, the expression of *OsPR1a* was upregulated in roots of resistant genotype Phule Radha, but *OsPR10* was upregulated only at early infection point against insignificant expression in susceptible genotypes. In the shoot, higher expression of *OsPR1a* and *OsPR10* was recorded in Phule Radha at both early and later stages of infection, while expression of *OsPR1b* was upregulated at later stage only. Similar upregulation of these PR genes were also found in the root tissues. This suggests the activation of PR genes in rice plants probably enabling them to encounter nematode attack. Additionally, the WRKY transcription factors are constitutively associated with resistance and/or susceptibility of plants after attack of nematodes (Li et al. 2015). In the present study, it has been found that the expression of *OsWRKY13* and *OsWRKY45* was upregulated in roots of susceptible cultivar Halvi Sal 17, while downregulation was observed in resistant genotype Phule Radha. This possibly indicates the nematode induced perturbation of the genes in both the resistant and susceptible rice cultivars.

Deposition of lignin and callose in plant cell wall restricts the effect of cell wall degrading enzymes released by plant-parasitic nematodes during root cell perforation and movement within roots (Gheysen and Jones 2006). However, in the present study, no definite correlation was observed between the resistance and susceptible rice cultivars, with respect to callose and lignin synthesis. The upregulation of majority of the related genes indicates a possible generalised plant defense response against the nematode invasion.

The host plant recognizes the pathogen associated molecular pattern (PAMP) and induces effector-triggered immunity (ETI) upon pathogen attack (Jones and Dangl 2006). The intracellular signaling molecules, such as mitogen-activated protein kinase (MAPK), help in limiting the disease

Fig. 6 Heat map envision of expression pattern of defense related genes in the root and shoot tissues of rice genotypes by invasion of *Meloidogyne graminicola* at 2 and 6 days post inoculation (PB PB 1121, HS Halvi Sal 17, PR Phule Radha, dpi days post inoculation)

progression leading to host resistance (Holbein et al. 2016). The explicit role of MAPKs to induce defense responses in monocyledonous plant upon challenging with nematodes is scant (Kumari et al. 2016). In present study, expression of MAPK genes was analysed during compatible and incompatible interaction of rice genotypes (resistant and susceptible) and *M. graminicola*. The expression of *OsMAPK5a* was downregulated in roots of Phule Radha at 6 dpi, probably demonstrates the role of MAPK in inducing late resistance response in rice against *M. graminicola*. However, *MAPK6* and *MAPK20* showed variable expression in the resistant and susceptible rice cultivars, when challenged against the nematode species. Notably, downregulation of *OsMAPK* in rice imparts resistance against fungal and bacterial pathogens (Xiong and Yang 2003), and negative correlation of *OsMAPK* has been observed with the PR genes in resistant rice plants (Seo et al. 2001; Xiong and Yang 2003). The differential expression of the MAPK genes possibly indicates the activation of the ETI pathway in the plant upon infection of *M. graminicola*.

Conclusions

Our study reveals distinct variation in expression of the plant defense genes between the resistant (Phule Radha) and susceptible (PB 1121 and Halvi Sal 17) rice genotypes. Categorically, amongst all the selected plant defense genes, the expression of *MAPK20*, *ICS1*, *NPR1*, *PAD4*, *AOS2*, *JAMYB*, and *ACO7* involved in several pathways was upregulated in Phule Radha possibly rendering resistance against *M. graminicola*. This observation can be further strengthened by their insignificant expression in the susceptible genotypes (PB 1121 and Halvi Sal 17) (Fig. 6). Additionally, it was also observed that the invasion of *M. graminicola* triggers the biosynthesis phytohormones to activate plant defense signaling pathways in rice. The activation of all the defense signaling might collectively regulate the compatibility/incompatibility status of the rice—*M. graminicola* interaction.

In the present study, we have evaluated resistance response of 36 Asian rice cultivars and landraces grown in Indian subcontinent. To date, information regarding

resistance sources in Asian rice is largely scant as compared to *O. glaberrima* and *O. longistaminata*. The present study adds substantial information about resistance sources of Asian rice that can be exploited for breeding purposes. Further any of the landraces studied here have not been evaluated in detail earlier, especially with reference to the underlying molecular signalling and pathways. The study also strengthens the knowledge gap regarding involvement of several defense responsive pathways in susceptible and resistant rice-*M. graminicola* interaction.

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Author contributions UR and BH conceived and designed the study. BH and DS performed the experiments. BH analysed the data. SK supplied the seeds on requirement. BH, VP and UR wrote and revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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