Morphological and Biochemical Host Response of Fifteen Indian Rice Cultivars to Rice Root-Knot Nematode, *Meloidogyne graminicola*

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ABSTRACT: The relative susceptibility and resistance reactions of fifteen rice cultivars were evaluated against rice root-knot nematode, *Meloidogyne graminicola* under pot conditions. The variation in the synthesis of total phenol (TP) and salicylic acid (SA) as influenced by host reaction and subsequently their impact on the disease etiology was assessed. The cultivars inoculated with 1000 second stage juveniles (J_2s) of *M. graminicola* expressed varied responses. The cv. Shanthi expressed resistance reaction, whereas the cvs. Pusa Basmati-6 and Pusa Sugandh-5 were found highly susceptible to the nematode. The greatest increase in the leaf contents of SA (9-15%) and TP (10-13%) was recorded in the cv. Shanthi, which also exhibited lowest root-knot index (0.8 at 0-10 scale) and decrease in the rice yield. The TP and SA contents of leaves increased up to 15 days of planting, and thereafter, decreased gradually. Increase in the TP and SA was negatively correlated with the root-knot index. Based on the morphological and biochemical host reaction, the relative susceptibility of the 15 cultivars was in the order; Pusa Basmati-6 > Pusa Sugandh-5 > Pusa Sugandh-4 > R-Dhan > Surya > Sharbati > Sadabhar > Virendra > Anjali > Swarna > CR-314 > PA6444 > JKRH401 > Vivekdhan 62 > Shanthi. The study revealed that the cvs. Vivekdhan 62 and Shanthi may be exploited for commercial cultivation of rice in the host zone of rice root-knot nematode, while the cultivation of cvs. Pusa Basmati-6 and Pusa Sugandh-5 should be avoided.

Keywords: Host resistance, salicylic acid, total phenols, rice, *Meloidogyne graminicola*

Rice is the world's most important staple food and globally it occupies 162 mha with an annual production of around 464 mmt (FAOSTAT, 2015). India is the second largest producer of rice after China and produced 104.32 million tonnes of rice during 2015-16 (Anonymous, 2017). Rice is quite susceptible to root-knot nematode and is attacked by *Meloidogyne incognita*, *M. graminicola*, *M. triticoryzae, M. javanica*, *M. oryzae* and *M. arenaria* (Bridge *et al.,* 2005). Amongst these species, *M. graminicola* is a primary pest of rice and unlike other *Meloidogyne* spp., it incites galls on the tip of lateral roots (terminal galls) which are spiral or hook like (Khan *et al*., 2014). *M. graminicola* attacks rice in the nursery as well as in the main field and possesses a substantial threat to rice cultivation in Southeast Asia, where around 90% of the world rice is grown and consumed (Dutta *et al.,* 2012). In India, this nematode is now an established problem and hampers the rice productivity up to 30% (Prasad *et al.,* 2010; Khan *et al.,* 2014); even the yield loss of 20 percent is significant, as it is sufficient to feed 120 million people for one year.

There are various methods available for the management of root-knot nematode in rice viz., flooding, deep plowing, summer fallowing, nematicide application, host resistance etc. Of all the strategies, chemical control has been most widely used by the farmers, but due to the high cost of chemical and its hazardous impact, farmers were compelled to look for more economic and eco-friendly approaches. Use of resistant cultivar is one of the best and safer alternatives for nematode management, without incurring any additional cost. However, in India, the majority of commonly growing rice cultivars have been found to be highly susceptible for this nematode (Devi and Thakur, 2007; Khan *et al.,* 2012; Mhatre *et al.,* 2015). Hence, it is indispensable to identify more resistance/ tolerance cultivars to combat this nematode problem in infested areas.

In plants, phenolic compounds play very vital role in the induction of local and systemic disease resistance (Bari and Jones, 2009). Major players in systemic induced defense are the salicylic acid (SA), jasmonic acid (JA), ethylene (ET) and other phenolic compounds, which are produced in response to plant attack (Biere and Goverse, 2016). Root feeding by sedentary endoparasitic nematodes results in the production and accumulation of SA, JA, and ET at the site of infection (Kammerhofer *et al.*, 2015). Consequently, transcriptional reprogramming results in the activation of the core defense system involving SA/JA/ET-mediated pathways in plants including rice (Kyndt *et al.*, 2014). The outcome of these highly coordinated signaling responses ultimately determines the host susceptibility and resistance to the nematodes. This indicates that the phenol and SA play important role in host defense mechanisms but their synthesis varies with cultivars and etiology of the associated pathogen. In this context, the present study was carried out to evaluate the relative susceptibility and resistance reaction of fifteen rice cultivars against *M. graminicola*. The variation in the synthesis of total phenol and SA, as influenced by host reaction and subsequently their impact on the disease etiology was assessed under pot conditions.

MATERIAL AND METHODS

Seeds of fifteen cultivars of rice (*Oryzae sativa* L.) viz., Anjali, CR-314, JKRH 401, PA6444, Pusa Basmati-6, Pusa Sugnadh-5, Pusa Sugandh-4, R-Dhan, Sadabhar, Shanthi, Sharbati, Surya, Swarna, Virendra, and Vivekdhan-62 were procured from the International Rice Research Institute Centre, New Delhi, India.

Pure population of *Meloidogyne graminicola* Golden & Birchfield was maintained in a culture bed of 10 × 20 m in the experimental net house. Highly susceptible cv. Pusa 1121 (Kumari *et al.,* 2014) was continuously grown in the bed to support reproduction of the nematode and to maintain a high population of *M. graminicola* (4000-6000 J_2/kg soil). Ten root samples were collected randomly from the bed and association of *M. graminicola* with the galls on rice roots was ascertained by perineal pattern technique (Hunt and Handoo *et al.,* 2009). Juveniles of *M. graminicola* were used as the inoculum of the nematode for this experiment. The nematode juveniles were isolated from the soil using Cobb's decanting and sieving method followed by the modified Baerman funnel technique (Khan, 2008). The identity of the isolated juveniles was confirmed on the basis of morphological characters (Hunt and Handoo *et al.,* 2009). The population of *M. graminicola* obtained using the above process was standardized to 1000 J_2/5 ml of water and was used to inoculate the pots within 48 hrs of isolation.

Earthen pots of 25 cm diameter were filled with 2 kg autoclaved soil (sandy loam) and farmyard manure (FYM) in the ratio of 4:1. The soil and FYM were autoclaved at 15 kg/cm^2 pressure at 121°C for 15 min. Two sets of pots were maintained, one set was inoculated with the 5 ml suspension of *M. graminicola* (1000 J_2) a day before planting. The second set of pots were not inoculated and served as control (un-inoculated). Seedlings of all 15 rice cultivars were transplanted at the center of the pots. Seedlings were watered immediately after planting and the watering continued till harvest. For each cultivar, nine treatments with 3 pots were maintained. Hence, each cultivar had 27 pots. The pots were arranged in a completely randomized block in the net house receiving uniform sunlight. Plants from three pots were evaluated at 2, 5, 10, 15, 20, 30, 60 and 90 days after planting to determine total phenol and salicylic acid contents of the leaf. Additionally, root-knot and egg mass index, yield per plant and soil populations were determined in four months old plants (at harvest).

Leaf samples from each of the 15 rice cultivars were collected and processed separately for total phenol (TP) and salicylic acid (SA) estimation. TP was estimated using the method described by Sharma and Sain (2005). Catechol was used as a standard and the amounts of TP were expressed as µg catechol/g fresh leaf. SA content of leaf of all 15 rice cultivars was estimated using the procedure previously described by Pankaj *et al.* (2005). Standard curve of SA was prepared and the concentration of SA in each sample was calculated using standard procedures (Lowery *et al.,* 1951).

At harvest, roots were gently rinsed under a slow stream of water and stained with acid fuchsin (Byrd *et* *al.,* 1983). Gall (GI) and egg mass (EMI) indices were measured on 0–10 scales (Bridge *et al.,* 2005). Final soil population of *Meloidogyne graminicola* was determined at harvest.

The plants from a pot, 4 months after planting, were harvested, dried for 2 weeks and thrashed manually to determine the grain yield per plant (with seed husks, without grain milling).

The experiment was conducted during two consecutive years (2013 and 2014). All the data were subjected to the analysis of variance (ANOVA) with the help of MINITAB 11.0 for Windows-7. The year differences in the data for plant yield were significant (*P* d" 0.05); hence the analysis was performed year wise separately. However, the year differences in the data for gall and egg mass indices, soil population and total phenol and salicylic acid assay were non-significant at $P \leq 0.05$, hence, data were pooled and are presented in the graphical form. Bars have been marked with standard errors in all figures. In figure 2 and 3 different alphabets have been assigned according to Tukey's Test at $P \leq 0.05$. The data on plant yield were analyzed using one factor ANOVA at probability levels of $P \leq 0.05$. Tukey's test was also employed to mark significance in the Tables. Correlation analysis was performed to determine the relationship between total phenol/salicylic acid concentration with gall index. The percent variation over the control was also calculated.

RESULTS

The severity of root-knot in terms of the gall index (GI; 0-10 scale) varied considerably with cultivars (Fig. 2). The highest GI was recorded on the cv. Pusa Basmati-6 (5.1) and Pusa Sugandh-5 (4.3). The cv. Sadabhar, Sharbati and Virendra showed GI in the range of 3.5-3.8, which was significantly less than the cv. R-Dhan ($P \leq 0.05$; Fig. 2). Four cultivars viz; CR-314, Swarna, JKRH401 and PA6444 developed 2.8-3.0 GI. The lowest GI was recorded on the cv. Shanthi (0.8). The overall susceptibility of the rice cultivars with regard to galling was Pusa Basmati-6 > Pusa sugandh-5> Pusa Sugandh-4 > R-Dhan > Surya > Sharbati > Sadabhar > Virendra > Anjali > Swarna > CR-314 > JKRH401 > PA6444 > Vivekdhan 62 > Shanthi (Fig. 2). The rice cultivars supported the reproduction of *M. graminicola,* and the order of egg mass production in term of egg mass index on different cultivars was more or less similar to GI (Fig. 2).

Plants grown in nematode inoculated soil exhibited a considerable degree of reduction in yield which varied with cultivars in both years (Table 1). The greatest decrease in the yield was recorded in cvs. Pusa Basmati-6 (44-49%) and Pusa Sugandh-5 (32-38%) over respective controls. The cvs. R-Dhan (20-23%), Surya (16-22%) and Sharbati (13-18%) also exhibited a significant decrease in the plant yield during two years of study (Table 1). The lowest decrease in the yield was recorded in the cv. Shanthi. The cultivars next to Shanthi were JKRH404 and Vivekdhan 62 and did not exhibit a significant decrease in the plant yield (Table 1).

Soil population of *M. graminicola* at the time of harvest (4 months after planting) showed a considerable degree of variation with regard to the cultivars (Fig. 3). Greatest soil population of *M. graminicola* was recorded in the root zone of cv. Pusa Basmati-6 and Pusa Sugandh-5. Nematode population in cvs. Vivekdhan 62 was significantly less than the cv. R-Dhan or Sharbati ($P \leq 0.05$). The lowest population of *M. graminicola* was recorded in the root zone of cv. Shanthi (Fig. 3).

Leaf phenol and salicylic acid

Total phenol content (TPC) of leaves of rice cultivars varied significantly ($P \leq 0.05$; Fig. 4). In general, the phenol contents were significantly higher in the nematode-inoculated plants than un-inoculated plants. In nematode-inoculated plants, TPC increased gradually and reached a maximum concentration on 10 or 15 days after inoculation, thereafter, it decreased and remained unchanged till 30 days after inoculation (Fig. 4). From 30 days onward, the TPC further decreased to a lowest at 90 days. Inoculation of *M. graminicola* resulted in a significantly higher increase in TPC of cvs. Shanthi (15%), Vivekdhan (12%) and JKRH404 (9%) compared to un-inoculated control. The lowest increase in the TPC was recorded in the cvs. Pusa Basmati-5 and Pusa Sugandh-5 (Fig. 4). The correlation analysis revealed higher TPC in the cultivars that developed a low level of GI and vice-versa (Fig. 6).

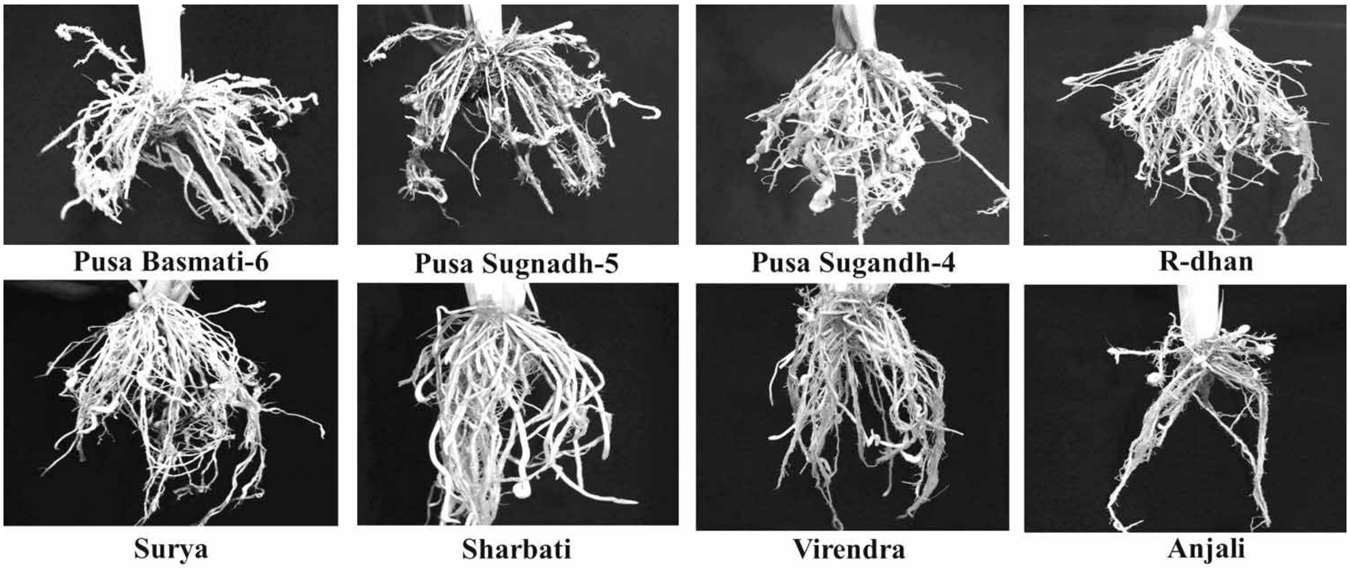

Fig. 1. Characteristic galling on roots caused by *Meloidogyne graminicola* on different rice cultivars

Table 1. Effect of *Meloidogyne graminicola* (1000 juveniles/kg soil) on the grain yield per plant of rice cultivars in inoculated and un-inoculated soil under pot conditions.

Rice cultivars	Grain yield per plant (g)			
	I year		II year	
	Un-inoculated soil	Inoculated soil	Un-inoculated soil	Inoculated soil
Anjali	23.2 ab	21.6 a	23.0 a	21.3 ab
Pusa Sugnadh-4	20.2 abc	18.1 a	20.4 ab	18.8 ab
JKRH 401	19.8 abc	18.7 a	19.6 ab	18.8 ab
PA6444	21.5 ab	19.4 a	21.3 a	19.2 ab
Pusa Sugnadh-5	24.0 ab	16.2 a	24.2 a	15.0 b
R-Dhan	23.7 ab	17.1 a	23.5 a	17.3 ab
Pusa Basmati-6	18.1 abc	9.2 b	18.5 b	10.3 c
Shanthi	16.9 bc	16.6 a	17.2 ab	17.0 ab
Sadabhar	25.3 a	22.8 a	25.1 a	22.7 a
CR-314	21.1 ab	19.3 a	21.6 a	19.7 ab
Sharbati	23.0 ab	20.3 a	23.2 a	20.5 ab
Surya	20.6 ab	16.9 a	20.3 ab	16.9 b
Swarna	23.2 ab	21.4 a	23.4 a	21.1 ab
Virendra	17.8 abc	16.2 a	17.7 ab	16.1 b
Vivekdhan 62	20.5 ab	18.3 a	20.2 ab	17.9 ab
LSD $P \leq 0.05$	4.49	3.88	4.69	3.17

Each value is mean of three replicates. Values within a column followed by different alphabets are significantly different at $P \leq 0.05$ according to Tukey's test.

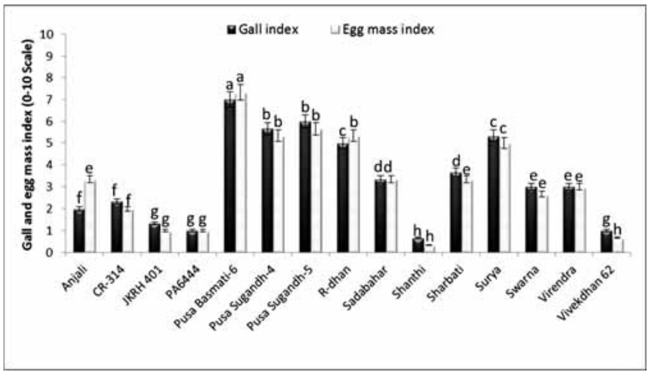

Fig. 2. Gall and egg mass indices (0-10 scale) of *Meloidogyne graminicola* on different cultivars of rice grown in the nematode inoculated soil (1000 J_2/kg soil). Error bars show standard error. Bars with different alphabet are significantly different at P≤ 0.05 according to Tukey's Test

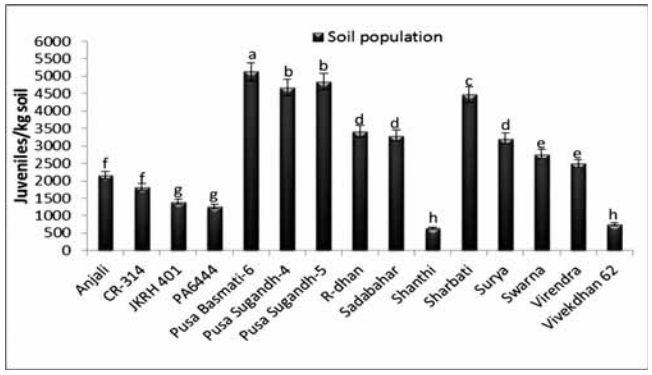

Fig. 3. Soil population of *Meloidogyne graminicola* on different cultivars of rice grown in the nematode inoculated soil (1000 J_2/kg). Error bars show standard error. Bars with different alphabet are significantly different at P≤0.05 according to Tukey's Test

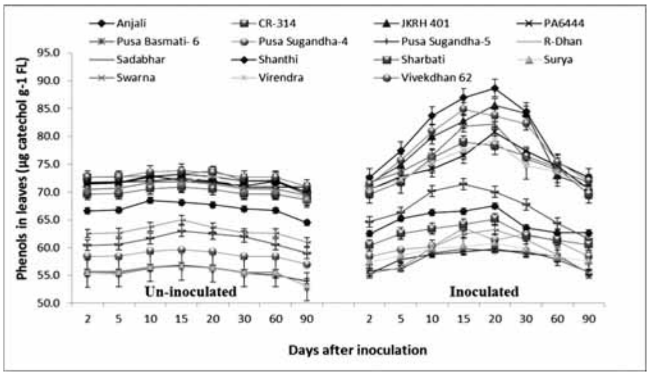

Fig. 4. Total phenols contents of leaves of rice cultivars grown in the soil un-inoculated or inoculated with *Meloidogyne graminicola* (1000 J2/kg) under pot condition. Error bars show standard error. FL= Fresh leaves

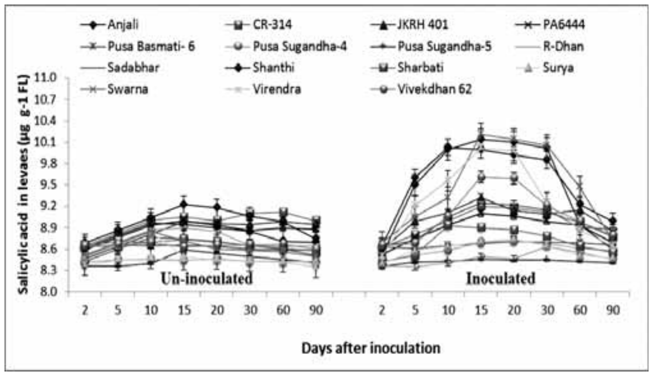

Fig. 5. Total salicylic acid contents of leaves of rice cultivars grown in the soil un-inoculated or inoculated with *Meloidogyne graminicola* (1000 J2/kg) under pot condition. Error bars show standard error. FL= Fresh leaves

The salicylic acid contents (SAC) of rice leaves increased in the nematode inoculated than un-inoculated rice plants (Fig. 5). Significantly greater SAC was recorded in the cvs. Shanthi (13%), Vivekdhan 62 (10%) and JKRH 404 (8%) compared to un-inoculated control (Fig. 5). The SAC in the leaves of cvs. Anjali and CR-314 were recorded highest at 10 days after inoculation, however, the concentration gradually and significantly decreased after 15 days of inoculation and retained a constant level till 30 days (Fig. 5). From 30 days onward, the SAC further decreased to the lowest level at 90 days. The correlation analysis has shown greater SA concentration in the cultivars that developed a lower gall index (Fig. 6).

DISCUSSION

All the tested cultivars express varied degree of susceptibility and resistance to the rice root-knot nematode. The highly susceptible cvs. Pusa Basmati-6 and Pusa Sugandh-5 showed a very high galling and egg mass index with 40-48% reduction in the plant yield. The cvs. Pusa Sugandh-4, R-dhan, Surya, and Sharbati were recorded to be moderately susceptible to the nematode with lower galling indices. However, the cv. Shanthi showed a very mild galling and the reduction in the plant yield was also not significant, hence, on the basis of the disease severity and yield performance, this cultivar can be categorized as moderately resistance/tolerance to *M.*

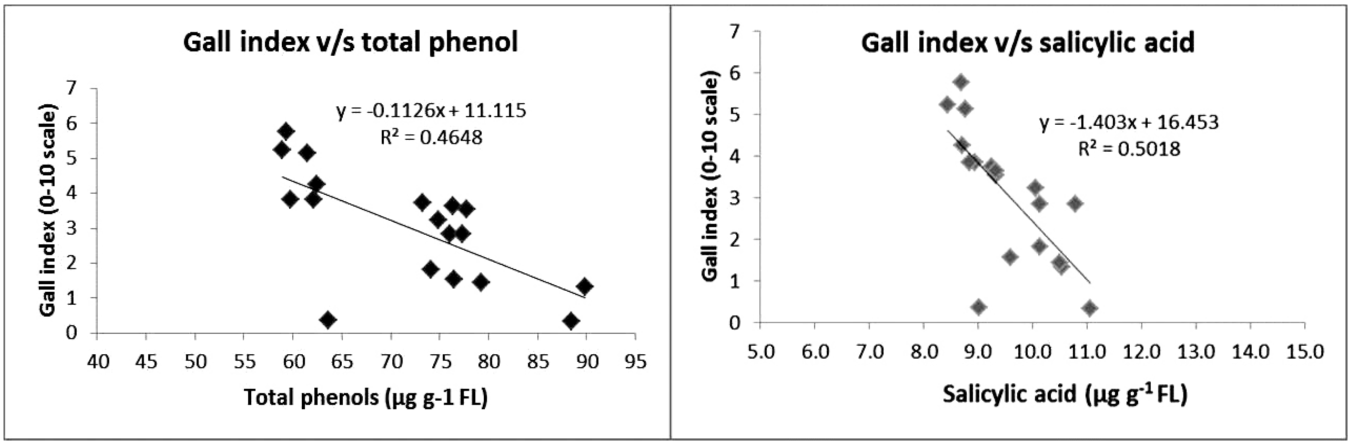

Fig. 6: Correlation between gall index and percent change in total phenol and salicylic acid of rice cultivars subjected to inoculation of *Meloidogyne graminicola* (1000 J_2/kg soil). FL= Fresh leaves

graminicola. The varied reaction of rice cultivars to *M. graminicola* has been reported in other studies also (Devi and Thakur, 2007; Kumari *et al.,* 2016). Many researchers have shown that the nematode may inflict yield loss to rice as high as 30-80% (Dutta *et al*., 2012), depending upon nematode population, soil condition and cultivar (Khan *et al.,* 2014).

Indian rice cultivars have been generally found highly susceptible to *M. graminicola* (Devi and Thakur, 2007; Mhatre *et al*., 2015) and support their reproduction. In the present study the cvs. CR-34, PA6444 and JKRH401 were found to be partially resistance/tolerance to *M. graminicola*, as evidenced by their lower soil population of nematode on these cultivars. Researchers have shown the existence of moderately resistance indigenous rice cultivars to *M. graminicola* (Das *et al.,* 2011; Kumari *et al.,* 2014). In the present study, none of the 15 cultivars were found completely resistant/tolerant to *M. graminicola*. However, the cvs. Vivekdhan 62 and Shanthi supported least proliferation of *M. graminicola* population.

Synthesis of phenols (TPC) and salicylic acid (SA) generally gets accelerated when plants are infected with the pathogens (Khan and Haque, 2013; Kyndt *et al*., 2014). The concentrations of these phenolic are found higher in the plants resistant/tolerant to the nematodes (Kammerhofer *et al*., 2015). In the present study, the increase in the TPC and SA contents of rice leaves was lesser in the cultivars that had a greater gall formation and decrease in the yield of the rice plant. For example in cvs. Pusa Basmati-6 and Pusa Sugandh-5 which exhibited greatest suppression in the plant yield, the lowest increase in the TPC and SA were recorded. Whereas, the highest concentrations of these phenolic were recorded in the cvs. Shanthi and Vivekdhan 62, which expressed moderately resistance/tolerance response. This indicates that the TPC (Meena *et al.,* 2000) and SA (Biere and Goverse, 2016) contribute in the self-defence of plants against nematode attack, including sedentary parasites (Khan and Haque, 2013; Kammerhofer *et al*., 2015). The greater accumulation of SA contributed towards a significant reduction in the infection of roots by *M. graminicola* (Nahar *et al.,* 2011). SA mediated tolerance reaction of cultivars was also evident from the correlation analysis between SA and root-knot index (Fig. 6).

Susceptibility of commonly growing rice cultivars to *M. graminicola* is of a serious concern and necessitates adopting appropriate management strategies in infested areas. Based on the morphological and biochemical host reactions, the cvs. Vivekdhan 62 and Shanthi may be exploited for commercial cultivation of rice in the host zone of rice root-knot nematode, after verification under multi-locational field trials. The cvs. Pusa Basmati-6 and Pusa Sugandh-5 should be avoided in the nematode-infested areas/field because of their higher degree of susceptibility to *M. graminicola*.

REFERENCES

Anonymous (2017). *Annual report 2016-17*. Department of Agriculture, Cooperation & Farmers Welfare. Government of India, New Delhi.

Bari, R. & Jones, J. (2009). Role of plant hormones in plant defence responses. *Plant Molecular Biology* **69:** 473-488.

Biere, A. & Goverse, A. (2016). Plant-Mediated Systemic Interactions Between Pathogens, Parasitic Nematodes, and Herbivores Above- and Belowground. *Annual Review of Phytopathology* **54:** 499-528.

Bridge, J., Luc, M., Plowright, R.A. & Peng, D. (2005). Nematode parasites of rice. In: *Plant parasitic nematodes in tropical and subtropical agriculture*. M. Luc, R.A. Sikora, J. Bridge (Eds.). Commonwealth Agricultural Bureau International, U.K., pp. 87"130.

Byrd, D.W., Kirkpatrick, T. & Barker, K.R. (1983). An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* **15:** 142-143.

Das, K., DuLe, Z., de Waele, D., Tiwari, R.K.S., Shrivastava, D.K. & Kumar A. (2011). Reactions of traditional upland and aerobic rice genotypes to rice root knot nematode (*Meloidogyne graminicola*). *Journal of Plant Breeding and Crop Science* **3(7):** 131-137.

Devi, G. & Thakur, N.S.A. (2007). Screening of rice germplasm/ varieties for resistance against root-knot nematode (*Meloidogyne graminicola*). *Indian Journal of Nematology* **37(1):** 86.

Dutta, T.K., Ganguly, A.K. & Gaur, H.S. (2012). Global status of rice root-knot nematode, *Meloidogyne graminicola*. *African Journal of Microbiology Research* **6(31):** 6016-6021.

FAOSTAT (2015). *FAO Statistical Year Book 2015 world food and agriculture*. Food and Agriculture Organization of the United Nations, Rome.

Hunt, D.J. & Handoo, Z.A. (2009). Taxonomy, identification and principal species. In: *Root-knot nematodes*. R.N. Perry, M. Moens, J.L. Starr (Eds.). CAB International, Wallingford, U.K., pp. 55-97.

Kammerhofer, N., Radakovic, Z., Regis, J.M.A., Dobrev, P., Vankova, R. (2015). Role of stress-related hormones in plant defence during early infection of the cyst nematode *Heterodera schachtii* in Arabidopsis. *New Phytologist* **207:** 778-89.

Khan, M.R. (2008). *Plant Nematodes: Methodology, Morphology, Systematics, Biology and Ecology*. Science Publishers, New Hampshire.

Khan, M.R., Haque, Z. & Kausar, N. (2014). Management of the root-knot nematode *Meloidogyne graminicola* infesting rice in the nursery and crop field by integrating seed priming and soil treatments of some pesticides. *Crop Protection* **63:** 15-25.

Khan, M.R. & Haque, Z. (2013). Morphological and biochemical responses of five tobacco cultivars to simultaneous infection with *Pythium aphanidermatum* and *Meloidogyne incognita*. *Phytopathologia Meditterenea* **52(1):** 98-109.

Khan, M.R., Ashraf, T. & Shahid, S. (2012). Evaluation for relative susceptibility of rice against field population of *Meloidogyne graminicola*. *Indian Journal of Nematology* **42(1):** 46-52.

Kumari, C., Dutta, T.K., Banakar, P. & Rao, U. (2016). Comparing the defence-related gene expression changes upon root-knot nematode attack in susceptible versus resistant cultivars of rice. *Scientific Reports* **6:** 22846. DOI: 10.1038/srep22846

Kyndt, T., Fernandez, D. & Gheysen, G. (2014). Plant-parasitic nematode infections in rice: molecular and cellular insights. *Annual Review of Phytopathology* **52:** 135-53.

Meena, B., Radhajeyalakshmi, R., Marimuthu, T., Vidhyasekaran, P., Doraisamy, S. & Velazhahan, R. (2000). Induction of pathogenesis-related proteins, phenolics and phenylalanine ammonia lyase in groundnut by *Pseudomonas fluorescens. Journal of Plant Disease and Protection* **107:** 514-527.

Mhatre, P.H., Pankaj, Malik, S.K., Kaur, S., Singh, A.K., Mohan, S. & Sirohi, A. (2015). Histopathological changes and evaluation of resistance in Asian rice (*Oryza sativa* L.) against rice root-knot nematode, *Meloidogyne graminicola* Golden & Birch. *Indian Journal of Genetics* **75(1):** 41-48.

Nahar, K., Kyndt, T., Vleesschauwer, D., de Hofte, M. & Gheysen, G. (2011). The jasmonate pathway is a key player in systemically induced defense against root-knot nematodes in rice. *Plant Physiology* **157(1):** 305-316.

Pankaj, Chawla, G., Shakil, N.A., Kishor, V. & Rohatgi, D. (2005). Estimation of salicylic acid and its role in resistance mechanism in chickpea against *Meloidogyne incognita. Indian Journal of Nematology* **35(2):** 160-162.

Prasad, J.S., Somasekhar, N. & Varaprasad, K.S. (2010). Nematode Infestation in Paddy. In: *Nematode infestations, Part I- Food Crop.* M.R. Khan, M.S. Jairajpuri (Eds.). Indian Academy of Sciences, India. pp. 17-71.

Sharma, P. & Sain, S.K. (2005). Use of biotic compounds against damping off of cauliflower by *Pythium aphanidermatum. Indian Phytopathology* **58(4):** 395-401.