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Original Research Paper

Evaluation of F_1 hybrids against root knot nematode (*Meloidogyne incognita*) resistance in Brinjal (*Solanum melongena* L.)

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ABSTRACT

Brinjal is one of the most important commercial solanaceous vegetable crops. Root knot nematodes cause severe yield losses in brinjal both in open and poly house conditions. Host plant resistance is considered to be a viable solution and cost-effective method to manage the root knot nematodes. Ten genotypes along with their nineteen F_1 hybrids were screened for resistance to root knot nematode and biochemical basis of resistance was assessed. Two F_1 hybrids *viz.*, IIHR-824 x VI046101, IIHR-824 x IIHR-766, parents *viz.*, IIHR-824 and VI046103 were found to be resistance to *M. incognita* with gall index/ egg mass index of 1. Three F_1 hybrids, IIHR-824 x IIHR-356, IIHR-824 x IIHR-835, VI046103 x IIHR-834 showed moderately resistant reaction. The root biochemical compounds analysis indicated that, total phenolic content (67.05 mg/g) and flavonoids (121.32 mg/g) were found higher in the inoculated roots of resistant line *i.e.* IIHR-824 indicating their role in conferring resistance. The identified F_1 hybrids having resistance can be explored for commercial cultivation.

Keywords: Biochemical compounds, brinjal, F_1 hybrids, resistance root knot nematode

INTRODUCTION

Brinjal (*Solanum melongena* L.) originated in India, which is also considered as centre of diversity (Genebus, 1963). The fruits are popular in different cuisines and is a major element of many countries' diets, particularly in India, Bangladesh, and the Middle East. Globally it is grown in an area of 1.8 million hectares with a total production of 58.6 million tonnes (FAO, 2021). In India, brinjal is cultivated in an area of about 752.20 thousand hectares with a production of 13.08 million tonnes with productivity of 17.30 MT/ha (NHB 2021-22). Fruits are a good source of vitamin A, vitamin B, and vitamin C, as well as minerals such as calcium, phosphorus, iron, and proteins. Among various biotic stresses, parasitic root knot nematodes causes both primary damage and pre-disposes to other diseases such as bacterial wilt, collar rot, and fusarium wilt (Manjunatha et al., 2017). In India, plant parasitic nematodes reported to cause yield losses up to 1.25 million tonnes (Kumar et al., 2020) and economic losses up to 10-42 % in brinjal (Jain et al., 2007, Manjunath et al., 2017).

Second stage juveniles (J2s) of root knot nematodes (RKN) are motile and enter the root system, where they change host cells into feeding cells by modifying the expression of several genes (Hussey et al., 2002). Root knot nematodes penetrate easily into the root cells through hollow feeding stylet (Williamson & Hussey, 1996). Root knot nematodes, mainly depends on their compatible interaction with their host plants and because of their strong reproductive potential, short life cycle, and monoculture of host crops, they reproduce very quickly (Gawade et al., 2022), and cause formation of galls in the roots, preventing the plant from absorbing water and nutrients (Hussey et al., 2002; Karssen et al., 2013). Symptoms are characterized by galling on roots or root swellings which affect the translocation of water and nutrients to the above ground parts of the plants. Subsequently, plants exhibit stunting, wilting; chlorotic leaves, reduced fruit size and production is adversely affected causing significant economic losses. Though chemical measures are effective in controlling nematodes, excessive use of chemical/nematicides have negative impact on soil health, pesticide residues in produce and pollution in the environment (Devran et al., 2013). Hence, host plant resistance offers an eco-friendly and economically sustainable strategy to manage root knot nematodes (Williamson & Roberts, 2009; Devran et al. 2013). Exploring host plant resistance, breeding resistant varieties/hybrids and employing resistant sources as rootstocks is a cost-effective and environmentally safe nematode control strategy. Secondary metabolites, such as phenols and flavonoids, which are involved in plant defence against pests and diseases, are produced by plants in large quantities (Puja Ohri & Satinder Kaur Pannu, 2010). Understanding the biochemical basis of resistance will help in exploring host plant resistance in crop improvement programs. In the current study, ten parents and nineteen F_1 hybrids were evaluated for RKN resistance through artificial challenge inoculation to identify resistant F_1 hybrids for commercial cultivation. Further, biochemical basis of resistance was studied to identify potential candidate biochemical compounds conferring resistance.

MATERIALS AND METHODS

Parent material and development of F_1 hybrids

Parental materials *viz.*, IIHR-824, IIHR-834, IIHR-835, IIHR-356, IIHR-B-BR-54, IIHR-766, VI046101, VI046103, VI045276 and VI034845 were used for development of nineteen F_1 hybrids by crossing in different combinations with other elite lines. A total of nineteen hybrids and their ten parents were screened for resistance to *Meloidogyne incognita* during *rabi* season *i.e.* December, 2022 to February, 2023 (temperature16.18°C to 28°C and average relative humidity 67.81%).

Artificial challenge inoculation

Pure culture of *M. incognita* was confirmed through female perineal cuticular pattern and culture was mass multiplied on susceptible tomato and brinjal cultivars, PKM 1 and Pusa Purple Long, respectively.

Infected roots were carefully cleaned, and fully mature egg masses were collected using forceps under a stereo zoom microscope. Egg masses were kept on wire mesh (blotted with sterilized filter paper) in petri dishes with distilled water for hatching. Juveniles were collected after every 24 hours up to 72 hours after hatching. Seven days after transplanting, each plant was inoculated with 1,000 second-stage juveniles of RKN per kg of soil.

Resistance scoring

Plants were uprooted after 90 days post inoculation and the roots were properly cleaned to remove soil. Galls and egg masses were counted as per the standard methodology (Table 1) with slight modifications (Taylor & Sasser, 1978). Reproduction factor was calculated according to the equation RF= PF/ PI, wherein PI indicates the initial population and Pf indicates the final nematode population being the sum of number of nematodes in soil (Benjamin et al., 2018; Sasser, 1984). Female population on the roots were counted according to standard procedure (Kirk Patrick et al., 1991). Soil nematode population (250 g of soil sample) was enumerated as described by combined Cobb's sieving and Baermann's technique (Ayoub, 1977) and nematode population was counted and expressed in cc^{-1}.

Table 1 : Score used for resistant screening for root knot nematode (Modified Taylor and Sasser, 1978)

Gall/egg mass index	No. of galls or egg masses per root	Host reaction
0	0.0-0.0	Immune
1	1.1-2.0	Highly resistant
2	2.1-10.0	Resistant
3	10.1-20.0	Moderate Resistant
4	>20.0	Susceptible

Biochemical analysis of roots

To understand the biochemical mechanism of resistance against *M. incognita*, root exudates from selected resistant, moderately resistant and susceptible lines were examined. Root exudates of selected resistant (IIHR-824), moderately resistant (VI034845) and susceptible (IIHR-766) lines were studied for total phenolic compounds and flavonoids.

Total phenols estimation

Total phenol content in root exudates was determined by spectrophotometric technique using Folin Ciocalteu Reagent (FCR) (Singleton & Rossi,1965). Total phenol content calculated using the following formula.

Total phenol content
(mg gallic acid equivalents/100g) =

$$\frac{OD_{700\ nm} \times \text{Std. value (mg/OD)} \times \text{Total volume of extract} \times 100}{\text{Assay volume} \times \text{Weight of tissue (g)} \times 1000}$$

Total flavonoids estimation

Total flavonoids in roots were estimated as per the spectrophotometric procedure (Dewanto et al., 2002). The following formula was used to calculate the total flavonoids content.

Total flavonoid content
(mg catechin equivalents/100g) =

$$\frac{OD_{510\ nm} \times \text{Std. value (mg/OD)} \times \text{Total volume of extract} \times 100}{\text{Assay volume} \times \text{wt. of sample (g)}}$$

Statistical analysis

The data were subjected to analysis of variance using the SAS Institute, Inc. (2012) and means were compared by analysis of variance and Duncan's multiple range test (DMRT) by using the SAS Institute, Inc. (2012).

RESULTS AND DISCUSSION

Reaction of parents and F_1 hybrids against *M. incognita*

Resistance or susceptibility nature of a plant to root knot nematodes depends on the penetration ability of nematode juveniles followed by formation of galls in the roots (Chen & Dickson, 2004). The galls formation is determined by the genetic make-up of the individual hybrid/cultivar (Jacquet et al., 2005). Tested genotypes and F_1 hybrids showed varied level of resistance to root knot nematodes (Table 2).

None of the parental lines and hybrids showed immune reaction to root knot nematodes. Among the parents, IIHR-824 exhibited the least number of galls per root (7.00), egg masses per root (6.67), females per root (9.00) and, J2 population per 250 cc soil (480.00), which was statistically at par with parent *i.e.* VI046103 (8.67 galls per root 7.00 egg mass per root, 13.33 females per root and 516.00 J2 population per 250 cc soil). VI034845 showed moderately resistant reaction with gall index/egg mass index of 3. Whereas, remaining parents showed susceptible reaction with gall/egg mass index of 4, and also with high reproduction factor (> 2.00). The parent, IIHR-766 recorded maximum number of galls per root (147.07) and egg masses per root (136.19), which was on par with IIHR-834 (galls per root:111.69 and egg mass per root: 111.57) and IIHR-356 (galls per root:107.00 and egg mass per root: 113.67). While, significantly higher number of J2 population (1872.00/250 cc soil) observed in IIHR-835, which was statistically at par with IIHR-356 (1862.67/250 cc soil), IIHR-834 (1813.33 J2 population/250 cc soil) and IIHR-766 (1790.67 J2 population). More number of females per root (93.33) recorded in IIHR-766, which was statistically on par with IIHR-835 (86.33) and IIHR-834 (82.00).

Out of nineteen F_1 hybrids evaluated for resistance to root knot nematodes, one hybrid *i.e.,* IIHR-824 x VI046101 (Fig. 3) showed resistance reaction to *M. incognita*, recorded lowest number of galls per root (6.33), egg masses per root (7.00), females per root (9.33), J2 population (529.40 / 250 cc soil), followed by IIHR-824 x IIHR-766 (Fig.3) with lowest infection (8.17 galls per root, 9.07 egg mass per root, 7.40 females per root and 558.40 J2 population per 250 cc soil). Moderately resistant reaction was observed in three F_1 hybrids *viz.,* IIHR-824 x IIHR-356, IIHR-824 x IIHR-835 and VI046103 x IIHR-834 with low gall/egg mass index of 3. The remaining fourteen F_1 hybrids showed susceptible reaction with gall/egg mass index of 4. The hybrid VI046101 x IIHR-766 showed susceptible reaction with a greater number of galls/root (106.67) and egg masses per root (98.70) and significantly differed with other hybrids with respect to galls per root but statistically on par to VI045276 x IIHR-835 and IIHR-B-BR-54 x IIHR-835 hybrids with regards to egg mass per root *i.e.* 96.67 and 102.17, respectively. In F_1 hybrids IIHR-B-BR-54 x IIHR-766 recorded higher number of J2 population (1945.33/250 cc soil) which was statistically similar with VI045276 x IIHR-766 (1908.00/250 cc soil). Maximum number of female populations (101.33) were observed in VI046101 x IIHR-766. The resistance/moderately resistance nature of these hybrids might be due to crossing between low × high *per se* values of root knot gall/egg mass indices, indicating dominant × additive type of gene expression that governs these traits in this cross, similar type of dominant × additive type of gene expression was also reported by Sundharaiya & Karuthamani (2018)

Table 2 : *Per se* performance of brinjal F_1 hybrids and its parents against *M. incognita*

Parent/hybrid	Gall index	No. of galls/root	Egg mass index	No. of egg masses/ root	No. of J2/250 cc soil	No. of females/ root	Rf*	Host reaction
IIHR-824	1.00^{e}	7.00^{k}	1.00^{e}	6.67^{l}	480.00^{h}	9.00^{j}	0.49^{g}	R
IIHR-356	3.00^{a}	107.00^{a}	3.00^{a}	113.67^{a}	1862.67^{ab}	76.67^{cde}	2.05^{a}	S
IIHR-766	3.00^{a}	147.07	3.00^{a}	136.19	1790.67^{abcd}	93.33^{ab}	2.02^{ab}	S
IIHR-835	3.00^{a}	65.00^{ef}	3.00^{a}	48.97^{ghi}	1872.00^{ab}	86.33^{bc}	2.00^{ab}	S
IIHR-834	3.00^{a}	111.69^{a}	3.00^{a}	110.57^{ab}	1813.33^{abc}	82.00^{bcd}	2.00^{ab}	S
VI046101	3.00^{a}	67.33^{e}	3.00^{a}	54.67^{gh}	1654.67^{cde}	72.67^{cde}	1.78^{cd}	S
VI045276	3.00^{a}	43.67^{hi}	3.00^{a}	33.03^{k}	1816.00^{abc}	72.00^{cde}	1.92^{abc}	S
VI046103	1.00^{e}	8.67^{k}	1.00^{e}	7.00^{l}	516.00^{h}	13.33^{j}	0.53^{g}	R
VI034845	2.00^{b}	18.67^{j}	2.00^{b}	19.07^{k}	240.00^{f}	31.00^{i}	1.01^{e}	MR
IIHR-B-BR-54	3.00^{a}	35.00^{ij}	3.00^{a}	32.33^{k}	1628.67^{cde}	45.00^{gh}	1.70^{d}	S
IIHR-824 x IIHR-766	1.00^{e}	8.17^{k}	1.00^{e}	9.07^{l}	558.40^{h}	7.40^{j}	0.57^{g}	R
IIHR-824 x IIHR-356	1.33^{d}	11.47^{k}	1.33^{d}	10.33^{l}	884.00^{g}	28.67^{i}	0.93^{f}	MR
IIHR-824 x IIHR-835	2.00^{b}	12.36^{k}	2.00^{b}	12.57^{l}	798.67^{g}	30.67^{i}	0.84^{f}	MR
IIHR-824 x VI0 46101	1.00^{e}	6.33^{k}	1.00^{e}	7.00^{l}	529.40^{h}	9.33^{j}	0.54^{g}	R
VI0 34845 x IIHR-766	3.00^{a}	51.67^{gh}	3.00^{a}	36.03^{jk}	1586.67^{e}	68.33^{de}	1.69^{d}	S
VI0 34845 x IIHR-356	3.00^{a}	56.00^{fg}	3.00^{a}	35.83^{jk}	1617.33^{de}	65.00^{ef}	1.72^{d}	S
VI0 34845 x IIHR-835	3.00^{a}	32.17^{j}	3.00^{a}	34.00^{jk}	1601.33^{de}	81.33^{bcd}	1.71^{d}	S
IIHR-B-BR-54 x IIHR-766	3.00^{a}	67.33^{e}	3.00^{a}	58.67^{efg}	1945.33^{a}	75.33^{cde}	2.08^{a}	S
IIHR-B-BR-54 x IIHR-356	3.00^{a}	68.40^{e}	3.00^{a}	56.23^{fg}	1709.33^{bcde}	63.00^{ef}	1.83^{bcd}	S
IIHR-B-BR-54 x IIHR-835	3.00^{a}	96.67^{b}	3.00^{a}	102.17^{bc}	1614.27^{de}	53.00^{fg}	1.77^{cd}	S
IIHR-B-BR-54 x IIHR-834	3.00^{a}	86.00^{cd}	3.00^{a}	76.17^{d}	1614.67^{de}	77.00^{cde}	1.76^{cd}	S
VI0 46101 x IIHR-766	3.00^{a}	106.67^{a}	3.00^{a}	98.70^{c}	1630.67^{cde}	101.33^{a}	1.83^{bcd}	S
VI0 46101 x IIHR-356	3.00^{a}	47.20^{gh}	3.00^{a}	45.97^{hi}	1521.33^{e}	80.33^{bcd}	1.67^{d}	S
VI0 46101 x IIHR-835	3.00^{a}	95.80^{b}	3.00^{a}	67.07^{de}	1573.33^{e}	71.67^{cde}	1.71^{d}	S
VI0 45276 x IIHR-766	3.00^{a}	78.67^{d}	3.00^{a}	43.87^{ij}	1908.00^{a}	68.00^{de}	2.02^{ab}	S
VI0 45276 x IIHR-356	3.00^{a}	67.00^{e}	3.00^{a}	65.07^{ef}	1600.00^{de}	63.33^{ef}	1.73^{cd}	S
VI0 45276 x IIHR-835	3.00^{a}	89.33^{bc}	3.00^{a}	96.67^{c}	1626.67^{cde}	79.73^{bcd}	1.80^{cd}	S
VI0 45276 x IIHR-834	3.00^{a}	65.00^{ef}	3.00^{a}	67.33^{de}	1568.00^{e}	53.67^{fg}	1.68^{d}	S
VI0 46103 x IIHR-834	1.67^{c}	12.00^{k}	1.67^{c}	13.33^{l}	835.73^{g}	38.67^{hi}	0.88^{f}	MR
S.E.±	0.09	3.18	0.09	3.19	57.66	4.35	0.06	
C.D. (P<0.05)	0.25	9.03	0.25	9.04	163.65	12.35	0.17	
C.V. (%)	5.94	9.52	6.00	10.61	7.03	12.88	6.64	

(*Reproduction factor= PF/PI) #The means with different letters as superscripts are significantly different (P <0.05)

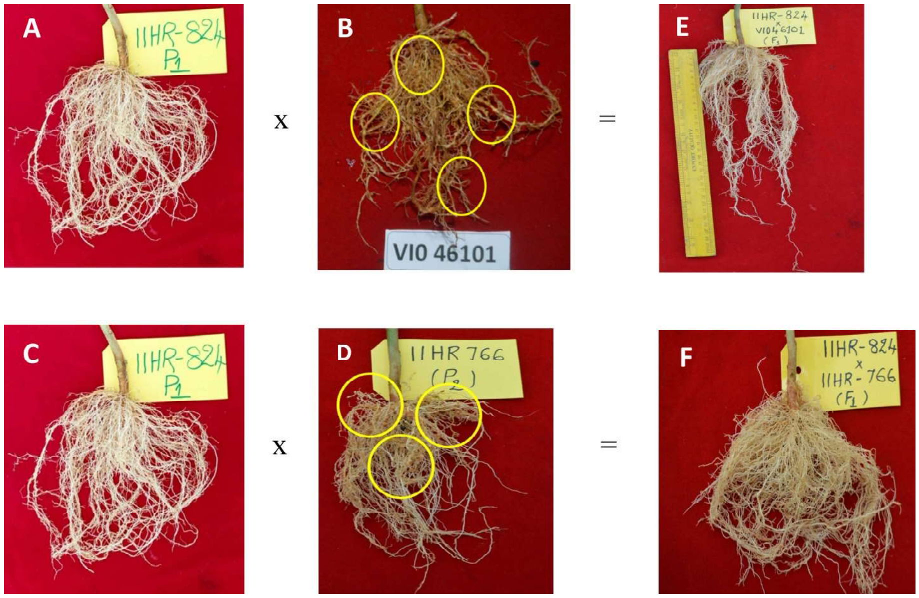

Fig. 3 : Brinjal F_1 hybrids resistance to root knot nematodes (*M. incognita*) E. IIHR-824 x VI046101, F. IIHR-824 x IIHR-766 and their parental lines *viz.*, A, C. IIHR-824 (Resistance) and susceptible lines *viz.*, B. VI046101, D. IIHR-766

whereas, Sindhu & Webster (1975) and Bost (1982) reported that one or more dominant alleles control genetic resistance to root knot nematode. The variance in pathogenicity among hybrids and of their parents may be related to differences in the genetic makeup of the tested hybrids and parents (Ullah et al., 2011; Devi et al., 2015). This could also imply that they have distinct genetic components that bestow different phenotypic characteristics.

The reproduction factor range in resistant hybrids and parents varied from 0.49 to 0.57, whereas, in moderately resistance category it varied from 0.84 to 1.01. High reproduction factor (1.67 to 2.08) was observed in susceptible F_1 (Table 2). Lowest reproduction factor was observed in the parent, IIHR-824, and which was statistically on par with VI046103, and F_1 hybrids *viz.*, IIHR-824 x VI046101 and IIHR-824 x IIHR-766. The resistance eggplant cultivar might have failed to produce functional feeding sites in the host after invasion due to hypersensitive responses facilitated by resistant genes that might have led to failure in nematode development. Once feeding sites are not produced in the host plant, *M. incognita* will not be able to access nutrients and as such will have their development and reproduction impaired as mentioned by Colak-Ates et al., (2018). The resistance/moderately resistant reaction could be the result of post-infection resistance, in which the nematodes penetrate the roots but failed to complete its life cycle, and this is linked to the early hypersensitive reaction, which could have resulted in the death of cells in root tissues around the root knot nematodes. This is owing to the presence of toxic or antagonistic compounds in eggplant roots, as mentioned by Tanimola et al. (2015).

Biochemical basis of resistance

Further, bio-chemical analysis of roots showed significant differences between resistant, moderately resistant and susceptible brinjal varieties in respect to the accumulation of total phenolic and flavonoid compounds. Resistance parent, IIHR-824 produced considerably more phenolic and flavonoid substances than the susceptible parent IIHR-766 (Table 3). The highest accumulation of total phenolic (67.05 mg/g) and total flavonoids (121.32 mg/g) were detected within inoculated resistant parent (IIHR-824), followed by inoculated moderately resistant parent (VI034845-total phenols-43.36 mg/g and flavonoids-93.98 mg/g). In contrary, the lowest concentration of total phenolic (32.59 mg/g) and total flavonoids (54.78 mg/g) were observed in roots of inoculated susceptible parent i.e. IIHR-356. Total phenols and total flavonoids content in roots in inoculated resistant parent (IIHR-824) increased by 34.90% and 33.12%, over moderately resistant (VI034845) and susceptible (IIHR-356) varieties, respectively (Table 3, Fig 1 & 2).

Table 3 : Phenolic and flavonoid compounds in root exudates of resistance, moderately resistance and susceptible parents

Germplasm	Host status	Total phenols (mg/g)		% increase over UI	Total flavonoids (mg/g)		% increase over UI
		UI	I		UI	I	
IIHR-824	Resistance	49.70	67.05	34.90	91.14	121.32	33.12
VI034845	Moderately resistance	39.38	43.36	10.11	79.28	93.98	18.54
IIHR-766	Susceptible	30.16	32.59	8.05	47.04	54.78	16.46
C.D. (P<0.05)		3.77	3.85		4.93	9.99	

UI = Un-inoculated; I = inoculated

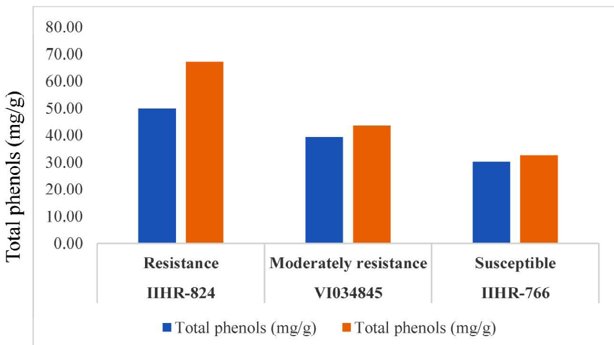

Fig 1 : Total phenolic compounds accumulated in root exudates of three eggplant varieties.

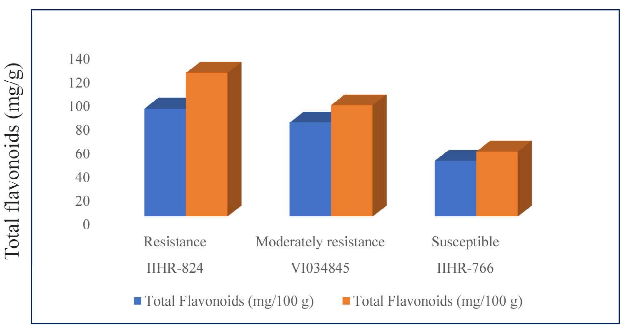

Fig 2 : Total flavonoid compounds accumulated in root exudates of three eggplant varieties.

A hypersensitive reaction (HR) is often the first sign of a pathogen infection in plants, which is followed by the development of a systemic resistance response (Keen, 1992). According to Sindhan & Parashar (1984), the general phenomena of resistance are the accumulation of phenolic compounds as a host parasite reaction, and the breakdown of these compounds determines the degree of resistance. Tayal & Agarwal (1982) and Sharama et al. (1990) have demonstrated biochemical alterations in tomato plants infected with *Meloidogyne incognita.* Nematode resistant plants contain higher constitutive levels of transcripts for important enzymes involved in the synthesis of flavonoids and phytoalexins, and are implicated in resistance to both stationary and migratory nematodes (Puja & Satinder, 2010, Selim et al. (2014) Mai et al., 2023**)**. Phenols are the major secondary metabolites present in plants and protect the tissues from diseases as well as insect attack. They also act as potential antioxidants and help in scavenging free radicals produced in the system (Nayak, 2015).

CONCLUSION

Screening of parents and F_1 hybrids through artificial challenge inoculation led to identification of two resistant F_1 hybrids *i.e.* IIHR-824 x VI046101 and IIHR-824 x IIHR-766 and three moderately resistant F_1 hybrids *viz.,* IIHR-824 x IIHR-356, IIHR-824 x IIHR-835, and VI046103 x IIHR-834 against root knot nematodes. Among the parents, IIHR-824 found to be good resistant source and can be explored as potential donor for resistance in crop improvement.

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