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Response of tuberose genotypes to root knot nematode, *Meloidogyne incognita*: Biochemical, histological and nutritional characterization of host-pathogen interaction

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Abstract

Aim : The study was conducted to screen the tuberose genotypes for nematode resistance and to establish histopathological, biochemical and nutritional changes in resistant/tolerant genotype against root knot nematode, *Meloidogyne incognita*.

Methodology : Twenty seven tuberose genotypes comprising of two types viz., single (18 genotypes) and double (9 genotypes) were evaluated for root knot nematode resistance. Tuberose bulbs of uniform size were surface sterilized and planted in 9 inch plastic pots and inoculated with nematode juveniles (J_2) 15 days after planting. Gall Index was recorded on a 0-5 scale after 120 days of inoculation. Histopathological, biochemical (total phenols, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase) and nutrient estimation studies were conducted on the roots of resistant/tolerant genotypes against root knot nematode, *Meloidogyne incognita*.

Results : Among the twenty seven tuberose genotypes screened for nematode resistance, Suarna Rekha was categorized as resistant genotype and Arka Shirngar, IIHR-2, IIHR-4, Variegated, IIHR-12, GK-T-C4, Calcutta Double, Bidhan Rajani-2 and Bidhan Rajani-3 were categorized as tolerant genotypes. Histological characterization of resistant/tolerant genotypes revealed that giant cell formation was disrupted with higher number of phenolic cells and cells with lignified cell wall that prevented the successful establishment of nematode feeding site. The resistant and tolerant genotypes significantly recorded higher accumulation of phenols and defence enzymes viz., polyphenol oxidase (PPO), peroxidase (PO) and phenyl alanine ammonia lyase (PAL) and macro and micro nutrients as compared to susceptible genotypes.

Interpretation : Screening of tuberose genotypes for root knot nematode resistance in this study has led to the identification of several nematode resistant/ tolerant genotypes. This would serve as a valuable information for the breeders to utilize resistant/ tolerant genotypes as parents in their breeding programmes.

Key words: Floriculture, Gall index, *Meloidogyne incognita*, Root knot, Tuberose

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Introduction

Tuberose (*Polianthes tuberosa* L.) is a bulbous fragrant ornamental plant, native to Mexico. In India, tuberose occupies a prime position in floriculture industry. The major portion of tuberose flower consumption is in the form of loose flowers and cut flowers. The loose flowers of tuberose have high demand in the market for making garlands and other floral arrangements. The tuberose flowers are valued more because they impart sweet and lingering pleasant fragrance. The concrete content of highly fragrant single petalled flowers varies from 0.08 to 0.14% and is used in high grade perfumes. There is a good demand for tuberose concrete and absolute in the international market and fetches a good price. The of tuberose essential oil is exported at an attractive price to France, Italy and other countries. Tuberose is prone to attack by the root knot nematodes, *Meloidogyne incognita* (Kofoid & White) Chitwood. Melis (1959) was the first to report the infection of *Meloidogyne* spp. on *P. tuberosa*. Nematode affected crop shows chlorotic foliage, general stunting and heavy root galling below ground (Reddy, 2012). Root knot nematode menace in tuberose are wide spread and majority of the tuberose fields in North and South India are heavily damaged by this nematode (Rao et al., 2001). They widely occur in subtropical and tropical regions and are reported to cause 10-14% reduction in flower number and spike weight (Khan and Parvatha Reddy, 1992). Identification of nematode resistant genotypes and utilizing them for developing high yielding, resistant varieties will be a boon to tuberose growers for maximising the production of quality flowers.

The biochemical reactions stimulated by host parasite interaction lead to host cell necrosis in resistance genotypes that delay the nematode development (Giebel, 1982). Many phenolic compounds and enzymes are involved in defense reactions against plant parasitic nematodes (Ohri and Pannu, 2010; Chawla et al., 2013). Accumulation of reactive oxygen species lead to high resistance in host plants against phytonematodes. Antioxidant enzymes such as peroxidase, polyphenol oxidase, superoxide dismutase and catalase are the main protective enzymes involved in quenching of free radicals and activated oxygen species (Blokhina et al., 2003). These defense enzymes, functional in phenylpropanoid pathway, are induced in plants in response to nematode infection (Wyuts et al., 2006). As there are no reports available so far for resistance to root knot nematode in tuberose and documentation is lacking on establishing its mechanism of resistance towards nematodes, in view of the above, the present study was undertaken to screen the tuberose genotypes for nematode resistance and to establish the histopathological, biochemical and nutritional changes in resistant/tolerant genotype against root knot nematode, *M. incognita*.

Materials and Methods

The study was conducted at Division of Floriculture and Medicinal Crops, ICAR - Indian Institute of Horticultural Research (IIHR), Bengaluru, India. The collection of twenty seven tuberose

genotypes comprising two types viz., single (18 genotypes) and double (9 genotypes) were evaluated phenotypically for root knot nematode resistance. *M. incognita* was collected from the cultures maintained at Division of Entomology and Nematology, ICAR-Indian institute of Horticultural Research, Bangalore. The species identity was confirmed through characteristic perineal cuticular pattern of adult females (Sasser and Carter, 1985). The method of Sasser et al. (1957) was followed for preparing of nematode inoculum. Infected roots from pure culture were cut into small pieces of about 2 cm long and placed in 0.5% sodium hypochlorite solution. To dissolve the gelatinous matrix of egg masses, the container was shaken for about 3 min and freed eggs were incubated for 72 hr under laboratory condition. Eggs were kept in petri dishes and frequently aerated with an aerator to enable hatching. Hatched second-stage juveniles (J_2) were harvested from petri dishes after 72 hr and transferred into a beaker. After slight agitation for homogenised distribution of nematodes, the juveniles were pipetted into a counting dish and counted under a stereo zoom microscope (Motic SMZ 168 series). The average density of nematodes per ml of the concentration was calculated out of five counts. The inoculum concentration was adjusted to $500 \pm 20 J_2$ per ml by adding water.

Phenotypic screening for root knot nematode : Uniform sized tuberose bulbs (approx. 2.0 cm diameter) were surface sterilized with 70% ethanol and washed five times with sterile distilled water five times. The bulbs were then planted in 9 inch plastic pots filled with steam-sterilized potting mixture containing red soil, sand and farm yard manure in 1:1:1 ratio. Each of the test genotype consisted of ten plants, replicated five times, and the pots were arranged in a completely randomized design. The nematode juveniles (J_2) were inoculated in potting media in three places at a depth of 2 cm near the roots. Each pot was inoculated with *M. incognita* at 1500 J_2 per pot, 15 days after planting. The pots were kept in open field and watered as per requirement. After 120 days, the roots of uprooted plants were washed to remove the adhering soil. Gall Index was recorded on a 0-5 scale as per Taylor and Sasser (1978). A set of uninoculated pots with each genotype served as control. The whole experiment was repeated twice for confirmation of results. Histopathological studies were conducted on the roots of test plants following the method of Johanson (1940). The stained root sections were observed under a trinocular phase contrast microscope (Carl Zeiss Primo Star, Germany) attached to AxioCam ERc 5s Rev 2.0 with Zen image analyser to study the histopathological changes in host roots due to nematodes. Microphotographs were captured to reveal the variation in host reaction towards the pathogen.

Biochemical studies: Plants were uprooted carefully without damaging the root tissues 120 days after planting. Five plants from each variety were collected for analysis. After washing the fresh roots in running tap water, the samples were homogenized with liquid nitrogen in a pre-chilled pestle and mortar. It was then stored in a freezer (-70°C) for further biochemical analysis. Total phenols were estimated by Folin ciocalteau reagent method (Bray and Thrope, 1954). The defence enzymes viz. peroxidase,

polyphenol oxidase and phenylalanine ammonia lyase were estimated in selected tolerant and susceptible genotypes. One ml of cold 0.1M phosphate buffer (pH 6.5) was used to homogenize the root sample and centrifuged at 6000 rpm for 10 min at 4°C in a refrigerated centrifuge and supernatant was used for enzyme analysis. Peroxidase activity was assayed by the method of Srivastava (1987). Polyphenol oxidase activity was expressed as change in the OD of reaction mixture $\text{min}^{-1} \text{g}^{-1}$ of fresh weight of tissue (Srivastava, 1987). Phenylalanine ammonia lyase (PAL) activity was analysed and recorded as $\mu\text{mol trans-cinnamic acid min}^{-1} \text{g}^{-1}$ protein (Dikerson et al., 1984).

Estimation of nutrient content in leaf samples : Leaf samples were collected 120 days after planting. After washing with distilled water, these leaves were dried in an oven at 60°C for 72 hr. Dried samples were ground and powdered and used for further analysis of macro nutrients viz., N, P, K, Ca, Mg and micro nutrients viz., Fe, Mn, Cu and Zn with three replications. Nitrogen content was estimated by micro Kjeldahl's method (Piper, 1996). Phosphorus content in plant samples was determined by Vanadomolybdate method (Jackson, 1973) after digesting the sample with $\text{HClO}_4 + \text{HNO}_3$. The intensity of yellow colour was read at 430 nm. Potassium content was estimated using flame photometer (Jackson, 1973). Calcium and magnesium content was determined by Atomic Absorption Spectrophotometer after digesting the samples with $\text{HClO}_4 + \text{HNO}_3$. Micronutrient content viz. Fe, Mn, Cu and Zn was determined by Atomic Absorption Spectrophotometer. In the present study, 27 genotypes of both single and double tuberose were screened for their resistance to *M. incognita*.

Statistical analyses : Data were analyzed by using standard statistical methods; Analysis of variance (ANOVA) was determined by SPSS ver. 10.0 and critical difference was compared by Duncan's Multiple Range Test (Gomez and Gomez, 1984). Since similar results were obtained in both the pot experiments, pooled analysis was done and ANOVA results were furnished.

Results and Discussion

The results of the present study indicates clear difference in response of genotypes towards root knot nodule. The pooled analysis of both the experiments revealed that out of 27 genotypes screened for resistance, Suarna Rekha recorded the least gall index of 2.0 and was found to be resistant to *M. incognita*. It was at par with Calcutta Double followed by Bidhan Rajani-1, Variegated, IIHR-2, IIHR-4, GK-T-C4, Arka Shringar, IIHR-12 and Bidhan Rajani-2 which recorded lower gall indices (GI-2.33 to 3.33) revealing tolerance reaction towards *M. incognita*. Whereas, genotypes Arka Suvasini, IIHR-5, IIHR-6, Mexican Single, Pearl Double, 1x6 (1), 1x6 (2), IIHR-11, Arka Sugandhi, Hyderabad Single, Hyderabad Double, Arka Prajwal, Arka Vaibhav, Arka Nirantara, Phule Rajani, Calcutta Single, Bidhan Rajani-1 showed relatively higher gall index of 4.0 and were recorded as susceptible genotypes to *M. incognita*. The results were in close conformity with results obtained by Chawla and Singh (2002) who reported that tuberose genotypes Mexican

Single, Pearl Double, Arka Suvasini, Arka Shringar, Arka Vaibhav and Arka Prajwal had recorded nematode multiplication rates greater than 4 and concluded that all the genotypes were susceptible to *M. incognita*. Resistant and tolerant reactions might have resulted due to hypersensitive responses triggered against in host plants (Agrios, 2005). Inhibition of giant cell formation and hypersensitive responses-mediated cell death around nematode feeding site prevented the nematodes from feeding, thereby affecting thus further growth and development (Das et al., 2008; Ramesh kumar et al., 2012).

Histological characterization of cross section of roots revealed active giant cells in susceptible genotype (Arka Prajwal) with dense, granular cytoplasm with multiple nuclei. The hypertrophied cells were initiated in both cortical and stelar region of the roots which then developed to abnormal xylem elements expanding into the cortex. Nematode within the vascular tissue (stele) induced giant cell development close to nematode heads (Fig 1a, b). The vascular strands adjacent to giant cells were dislocated and occurred as irregular patches. The susceptible reactions showed characteristic giant cells with dense cytoplasm and enlarged multiple nuclei and partial dissolution of cell walls, resistant/ tolerant reactions showed disruption of giant cell formation, lignification of cell walls and cell collapse. Similarly, giant cells of dissimilar sizes were noticed in galled roots of *Calendula*, *Centaurea* and *Papaver* with granular cytoplasm and hypertrophied nuclei (Siddiqui et al., 2014). Condensed cytoplasm, enlarged nucleus and retracted plasmatic membrane were noticed in nematode infected tissues upon establishment of feeding sites by J_2 (Anthony et al., 2005).

Whereas in resistant (Suarna Rekha) and tolerant genotypes (IIHR-4, IIHR-12 and Variegated), the giant cell formation was disrupted with lignified cell wall and higher number of phenolic cells which prevented the successful establishment of nematode feeding site (Fig. 1 c, d, e). The physiological and molecular events in resistant genotypes inhibited the formation of feeding site and delayed nematode development and reproduction (Anwar and McKenry, 2000). Due to subsequent cell collapse and necrosis in feeding site, the female nematodes could not receive adequate nourishment for reproduction that hindered the increase in nematode population.

Higher accumulation of total phenol content was recorded in nematode inoculated resistant/ tolerant genotypes viz., Suarna Rekha (1.78), IIHR-4 (1.597), IIHR-12 (1.59) and Variegated (1.48) compared to their respective uninoculated ones (1.33, 0.78, 1.26 and 1.37 mg g^{-1} , respectively (Fig 2a.). Similar findings earlier reported also indicate that phenolic compounds increased in *M. incognita* infected resistant varieties of chick pea (Chakrabarti and Mishra, 2002) compared to susceptible ones. Enhancement in phenolic levels at infection site interferes in the metabolic activities of pathogens, and thus involved in the defence mechanism of plants post infection (Kavitha et al., 2008; Ranchana et al., 2015). It is also possible that active phenols might have been released from glycosides by increased activities of β -glycosidases and later oxidized in resistant genotypes.

Fig. 1 a-e : Histological variations in tuberose root infected with *M. incognita*. Transverse sections on roots of susceptible tuberose cultivar Arka Prajwal (120 DAP) showing (a) juvenile nematode (N) with head localised among the giant cells with abnormal xylem, Scale: 50 µm; (b) Formation of dense giant cells cytoplasm with dark stained nucleus, Scale: 50 µm; (c) Disruption of giant cell formation, Scale: 50 µm; (d) Cells with lignified cell wall in tolerant tuberose cultivar IIHR 4, Scale: 50 µm and (e) Higher number of phenolic cells in tolerant tuberose cultivar variegated, Scale: 50 µm.

Table 1: Changes in nutrient content of *M. incognita* inoculated tuberose genotypes

Genotype	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
Arka Shringar	2.90 ^a	0.40 ^d	2.59 ^d	3.62 ^b	0.80 ^{de}	175.56 ^{cd}	75.81 ^{bc}	14.49 ^e	34.34 ^d
IIHR-2	2.98 ^a	0.41 ^{cd}	2.62 ^{cd}	3.67 ^{ab}	0.84 ^{cd}	190.73 ^{bc}	78.02 ^{abc}	14.28 ^e	35.49 ^{ab}
ArkaSuvasini	1.91 ^{hjk}	0.32 ^a	1.71 ^{ik}	3.05 ^{cd}	0.65 ^{gh}	125.58 ^{efg}	58.01 ^{ghi}	10.14 ^{hijkl}	24.86 ^{efg}
IIHR-4	2.88 ^a	0.39 ^d	2.78 ^{bcd}	3.84 ^{ab}	0.89 ^{abc}	201.55 ^b	77.60 ^{abc}	15.96 ^{cd}	34.23 ^b
IIHR-5	2.16 ^{cdefgh}	0.27 ^{ijkl}	1.95 ^{fghi}	3.13 ^{cd}	0.69 ^{fg}	110.13 ^{ghi}	67.47 ^d	9.75 ^{ijklm}	19.60 ⁱ
IIHR-6	1.86 ^k	0.27 ^{ghijkl}	1.94 ^{fghi}	3.10 ^{cd}	0.63 ^{gh}	105.45 ^{ij}	58.60 ^{ghi}	11.31 ^{fgh}	22.82 ^{gh}
Mexican Single	2.13 ^{cdefghi}	0.31 ^{efg}	1.80 ^{hi}	2.86 ^{de}	0.72 ^{ef}	90.09 ^k	64.93 ^{defg}	10.92 ^{ghi}	27.69 ^{cde}
Pearl Double	1.88 ^{ik}	0.31 ^{ef}	1.89 ^{ghi}	2.98 ^{de}	0.68 ^{fg}	86.97 ^k	66.30 ^{def}	12.48 ^f	20.77 ⁿⁱ
1x6(1)	1.97 ^{ghijk}	0.31 ^{ef}	1.70 ^{ijk}	3.10 ^{cd}	0.70 ^{fg}	82.44 ^k	57.14 ^{hi}	12.2 ^{efg}	25.16 ^{efg}
1x6(2)	2.10 ^{cdefghij}	0.27 ^{ijkl}	1.85 ^{ghi}	3.21 ^c	0.70 ^{fg}	87.21 ^k	60.06 ^{efgh}	10.33 ^{hijk}	30.62 ^c
IIHR-11	1.80 ^k	0.28 ^{ghijk}	2.04 ^{efgh}	3.19 ^{cd}	0.66 ^{gh}	132.99 ^e	62.50 ^{defgh}	9.36 ^{ijklmn}	29.74 ^{cd}
Variegated	3.01 ^a	0.41 ^{cd}	2.85 ^{abcd}	3.85 ^{ab}	0.85 ^{cd}	225.17 ^a	80.75 ^{abc}	18.48 ^{ab}	37.17 ^{ab}
Arka Sugandhi	2.47 ^b	0.30 ^{cdefghi}	1.79 ^j	2.93 ^{de}	0.67 ^{fg}	123.53 ^{efgh}	59.77 ^{fgh}	11.31 ^{fgh}	29.93 ^{cd}
Hyderabad Single	2.16 ^{cdefgh}	0.30 ^{fgh}	2.19 ^{ef}	3.04 ^{cde}	0.71 ^{fg}	114.22 ^{ghi}	61.72 ^{defgh}	9.36 ^{ijklmn}	25.55 ^{ef}
Hyderabad Double	2.38 ^{bcd}	0.25 ^{klm}	1.75 ^{jk}	2.97 ^{de}	0.69 ^{fg}	82.05 ^k	62.79 ^{defgh}	6.63 ^p	20.87 ⁿⁱ
Arka Prajwal	2.47 ^b	0.24 ^{lmn}	1.94 ^{fghi}	3.11 ^{cd}	0.70 ^{fg}	86.73 ^k	63.08 ^{defgh}	6.05 ^p	19.21 ⁱ
Arka Vaibhav	2.03 ^{ghijk}	0.24 ^{lmn}	1.55 ^{jk}	3.10 ^{cd}	0.70 ^{fg}	105.45 ^{ij}	67.18 ^{de}	8.39 ^{mno}	26.13 ^a
Arka Nirantara	2.27 ^{bcddef}	0.30 ^{cdefgh}	1.80 ^{hi}	3.02 ^{cde}	0.59 ^h	133.38 ^e	59.87 ^{fgh}	10.72 ^{hij}	26.42 ^a
IIHR-12	2.97 ^a	0.44 ^{bc}	2.88 ^{ab}	3.76 ^{ab}	0.82 ^{cd}	164.12 ^d	75.18 ^c	14.28 ^e	34.44 ^b
Suarna Rekha	3.11 ^a	0.46 ^{ab}	3.04 ^a	4.00 ^a	0.92 ^{ab}	234.88 ^a	82.85 ^{ab}	18.90 ^a	36.44 ^{ab}
Phule Rajani	2.19 ^{cdefg}	0.28 ^{ghij}	1.90 ^{ghi}	3.03 ^{cde}	0.70 ^{fg}	124.02 ^{efg}	57.43 ^{hi}	12.28 ^{fg}	30.81 ^c
GK-T-C4	2.93 ^a	0.38 ^d	2.88 ^{ab}	3.61 ^b	0.84 ^{cd}	177.35 ^{cd}	77.39 ^{abc}	15.33 ^{de}	36.44 ^{ab}
Calcutta Single	2.24 ^{bcddef}	0.29 ^{cdefghi}	2.05 ^{efgh}	2.93 ^{de}	0.73 ^{ef}	115.83 ^{fghi}	59.87 ^{fgh}	9.36 ^{ijklmn}	21.26 ⁿⁱ
Calcutta Double	3.08 ^a	0.48 ^a	2.88 ^{ab}	3.59 ^b	0.97 ^a	232.52 ^a	83.79 ^a	17.22 ^{bc}	38.54 ^a
Bidhan Rajani-1	2.10 ^{cdefghij}	0.22 ^{lmn}	2.27 ^a	3.08 ^{cd}	0.71 ^{fg}	126.70 ^{efg}	62.01 ^{defgh}	7.02 ^{op}	22.04 ^{g^{hi}}
Bidhan Rajani-2	2.41 ^{bc}	0.25 ^{lm}	2.10 ^{efg}	3.07 ^{cd}	0.70 ^{fg}	126.17 ^{efg}	50.60 ^j	8.78 ^{lmn}	27.11 ^{d^e}
Bidhan Rajani-3	3.02 ^a	0.41 ^{cd}	2.86 ^{abc}	3.63 ^b	0.87 ^{bcd}	200.23 ^b	79.38 ^{abc}	16.38 ^{cd}	35.39 ^{a^b}
Mean	2.41	0.32	2.16	3.25	0.74	137.45	66.15	11.51	28.38
S.Ed	0.13	0.02	0.12	0.166	0.04	8.18	3.45	0.67	1.53
CD (p=0.05)	0.35	0.05	0.33	0.46	0.10	22.50	9.49	1.84	4.21

Values in each column followed by same letter are not significantly different ($P < 0.05$)

Higher peroxidase activity was observed among the nematode inoculated tolerant genotypes which ranged from 0.50 (IIHR-4) to 0.76 OD min⁻¹g⁻¹ (Variegated). Among the nematode inoculated susceptible ones, the maximum activity of 0.42 OD min⁻¹g⁻¹ was noticed in Arka Prajwal and minimum in Mexican Single (0.27 OD min⁻¹g⁻¹). In uninoculated tolerant genotypes, higher activity was recorded in IIHR-12 and lower in Suarna Rekha (Fig. 2b). Similar findings for PO activity was reported in resistant banana (Dhar *et al.*, 2016) and resistant cotton (Mandhania *et al.* 2018). PO has diverse function in plant systems, often related to stress. It protect the cells from reactive oxygen species generated by various stresses. It plays a vital role in alleviating free radical toxicity in plant tissues. PO is functional in a broad range stress-related physiological processes like lignification and suberisation (Passardi *et al.*, 2004). Higher polyphenol oxidase content was noticed in Suarna Rekha followed by Variegated, IIHR-12 and IIHR-4 in inoculated tolerant

genotypes and among the uninoculated tolerant genotypes, the maximum activity was observed in Suarna Rekha followed by Variegated, IIHR-12 and IIHR-4 (Fig 2c). Similar results of increased PPO activity due *M. incognita* infestation were observed in sugarbeet (Korayem *et al.*, 2012) and tomatoes (Patel *et al.*, 2018). Phenolic compounds serve as substrates for many antioxidant enzymes and, hence, increase in their quantity decreases damage due to stress (Khattab, 2007).

PAL activity was recorded higher in inoculated tolerant genotypes IIHR-12 (10.06), Suarna Rekha (9.63), IIHR-4 (9.63) and Variegated (8.58) than their respective uninoculated ones which recorded 9.20, 8.75, 9.30 and 5.78 n mol of trans-cinnamic acid min⁻¹g⁻¹, respectively (Fig 2 d). PAL is primarily involved in phenyl propanoid metabolism, and is responsible for the production of phytoalexins and phenolics that prevents establishment of pathogen (Mariutto *et al.*, 2011). It is correlated

Fig. 2 (a-d) : Biochemical changes (a) Total phenols; (b) Peroxidase activity; (c) Polyphenol oxidase and (d) Phenylalanine ammonia lyase activity in nematode inoculated and nematode uninoculated tuberose genotypes.

with defence reactions against plant pathogens in tuberose (Ranchana *et al.*, 2015). Under biotic stress condition due to *M. incognita* infection in tomato, maximum PAL activity was observed in the susceptible cultivar AT 3 (Patel *et al.*, 2018).

A significant difference was observed in nutrient accumulation in nematode resistant/ tolerant and susceptible genotypes. Higher content of major and minor nutrients viz., N, P, K, Ca, Mg, Fe, Mn, Cu and Zn were found in nematode tolerant / resistant genotypes than susceptible ones. Among the nematode inoculated genotypes, nematode tolerant/resistant genotypes viz., Suarna Rekha, Calcutta Double and Variegated recorded maximum nutrient content as they revealed resistance/ tolerance to root knot nematode (Table 1). Suarna Rekha, the resistant genotype, recorded the highest amount of nitrogen (3.11%), potassium (3.04%), and calcium (4.00%). The maximum phosphorus content was observed in Calcutta Double (0.48%)

and Suarna Rekha (0.46%) which were at par with each other. Magnesium was recorded relatively higher in Calcutta Double (0.97%) and Suarna Rekha (0.92%). Higher manganese content was recorded in Calcutta Double (83.79 ppm) and Suarna Rekha (82.85 ppm); iron (234.88 ppm) and copper (18.90 ppm) in Suarna Rekha and zinc in Calcutta Double (38.54 ppm). The lowest nitrogen content was observed in IIHR-11 (1.80%) which was recorded as susceptible to *M. incognita*. Minimum micronutrients were also recorded in susceptible genotypes. These findings are in close conformity with the results of Devarajan *et al.* (2002) who concluded that *M. incognita* infestation reduced N, P, K and Mg levels in banana leaves. Gauo and Wang (2004) also reported reduction in total boron, iron, copper and zinc contents in root-knot nematode infected ginger roots owing to its weakened capability to absorb nutrients. Nematode infection causes nutrient deficiency in plants by disrupting the regular physiological processes such as

photosynthesis, respiration and transpiration (Melakeberhan and Webster, 1993). However, in resistant hosts, inhibition in formation of specialized feeding sites or giant cells enable the plants for normal absorption of nutrients.

Hence, it can be concluded that, biochemical analysis of root knot nematode inoculated and uninoculated genotypes resulted in higher accumulation of total phenols, peroxidase, polyphenol oxidase and PAL activity in resistant/tolerant genotypes. Nutrient analysis also showed similar trend and revealed higher nutrient accumulation in resistant/ tolerant genotypes and lower nutrient contents in the susceptible ones. The results showed marked difference in resistant and susceptible genotypes for biochemical, nutrient and histopathological studies towards the response of root knot nematode. Identification of nematode resistant/ tolerant genotypes through this study provides valuable information for the breeders to utilize them in their breeding programmes. The development of resistant varieties using selected genotypes as parents will be a green and viable option for managing nematode problems in tuberose cultivation. Elucidation of mechanism of resistance through histopathological, biochemical and nutritional changes throws more light on the host-pathogen interaction in tuberose which is necessary for successful nematode management.

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