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Table: 2, Figs : 2

Evaluation of tuberose (*Polianthes tuberosa* L.) genotypes against the nematode *Meloidogyne incognita* race 2

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ABSTRACT

Thirteen genotypes of tuberose (*Polianthes tuberosa* L.) were evaluated against root knot nematode (*Meloidogyne incognita*) to understand the relative resistance, phenol and polyphenol oxidase (PPO) activities. Results revealed that Shringar, Suvasini, Prajwal and Phule Rajani were found resistant against *M. incognita* race 2, had least galls and reduced the nematode population per 200 cm^3 soil + 5g root, while Calcutta single, Calcutta double, Vaibhav and Hyderabad double were susceptible. Estimation of phenol and (PPO) content in healthy and infected plant's roots of 13-tuberose genotypes revealed that nematode infected genotypes (Shringar, Suvasini, Prajwal and Phule rajani) contained high phenol and PPO activities than healthy ones. The high phenol and PPO activities imparted resistance against the *M. incognita* race 2. While infected Calcutta single, Calcutta double, Vaibhav and Hyderabad double showed low phenol and PPO activity, thus, were susceptible.

Key words: Genotypes, *Meloidogyne incognita*, nematodes, phenols, *Polianthes tuberosa* L., polyphenol oxidase, resistance, susceptible, tuberose.

INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) popularly known as *Rajanigandha* is important bulbous ornamental crop. It is cultivated widely for its various uses (decoration, floral ornaments, perfumes, beautification of home gardens and natural sweet fragrance and extraction of tuberose oil) mainly in West Bengal followed by Karnataka, Tamil Nadu, Maharashtra, Orissa, Assam and Uttar Pradesh. It is hardy plant, less infested by pests yet the phytonematodes [foliar nematode (*Aphelenchoides besseyi*), root knot (*Meloidogyne* spp.) and reniform (*Rotylenchulus reniformis*) nematodes] are reported in its rhizosphere. Root knot nematode (RKN) is threat to tuberose cultivation in nematode infested areas (6). The strategies to manage the RKNs involve use of nematicides, amendment with organic manure and growing resistant cultivars. The genetic resistance in tuberose against root-knot nematodes is most efficient to reduce nematode population in soil. The biochemical constituents in plants like phenol, polyphenol oxidase (PPO) etc. attribute to nematode resistant properties. Phenolic compounds have been implicated as disease resistance factors in numerous host parasite combinations (5,8). This study aimed to evaluate the Indian genotypes of tuberose to understand their relative susceptibility and biochemical resistance factors against *Meloidogyne incognita* race 2.

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MATERIALS AND METHODS

I. Experimental procedure

Experiment was conducted in the net-house of Directorate of Research, Kalyani, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Nadia, West Bengal during 2011 and 2012 (2-years). Genotypes were procured from All India Coordinated Research Project (AICRP) on Floriculture, Mohanpur Centre for evaluation. Experiment was carried out in earthen pots (15cm dia), filled with 3 kg sterilized soil mixture (sterile soil, FYM and river sand in 3:1:1). All the pots were arranged in a Complete Randomized Design (CRD). Thirteen test Genotypes were : Calcutta single, Calcutta double, Phule rajani, Swarna rekha, Shringar; Suvasini, Prajwal, Vaibhav, Hyderabad single, Hyderabad double, Rajat rekha, Sikkim selection and GK-T-C-4. Three bulbs of each genotype were transplanted per pot on 24th April 2011, adequate care for irrigation and other management practices were provided to establish the tuberose bulbs to grow normally. The nematode resistance was assessed based on nematode infestation (root galling on a 1-5 scale, nematode population (J2) per 5g root and plant parameters [spike length (cm), rachis length (cm) and number of florets]. The number of nematodes inoculated per pot was 254. At least three flower stalks were taken from each replication for observation. Root galling severity due to nematode infection was assessed on a 1-5 scale (1= no galls, 2=1-10 galls, 3=11-30 galls, 4=31-100 galls, 5= >100 galls per root system).

II. Nematode extraction and estimation

Soil samples (200 cm^3) along with root (5 g) were collected from each pot. Soil was processed by Cobb's decanting and sieving (4) followed by modified Baermann's technique (11) to extract the second stage juveniles (J2). Root sample was carefully washed gently in running tap water to remove adhering soil particles from roots. Freshly collected galled roots (5 g) were placed over an assembly, fitted on a Petri plate containing water. The J2s hatched out from egg and settled in clear water below. Most of the J2s emerged from the egg mass within 4 to 6 days and collected in the water suspension. Nematodes in water suspension were killed by hot-water bath and fixed (3.0% formaldehyde) to determine population per 5 g root. The counting of nematode in nematode suspension was done with counting-disc under stereoscopic binocular microscope. The species of *Meloidogyn*e was identified based on perineal pattern study of mature female under OLYMPUS BX-51 compound research microscope.

III. Estimation of phenol

Phenol content in roots of tuberose was assayed as per Sadasivam and Manickam (10) and slight modification in our laboratory conditions. Roots (0.5-1 g) were cut into small pieces and ground with a pestle and mortar in 10 time volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant was used. The residue was further re-extracted with five times the volume of 80% ethanol, centrifuged and pooled the supernatant. The supernatant was evaporated to dryness using hot water bath, the beakers contained supernatant was placed on boiling water (80°-90°C) and dissolved the residue in 5 ml distilled water (10). The different aliquots (0.2-2.0 ml) were pipetted out into test tubes and made up the volume in each tube to 3 ml with water. Then 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% Na2Co3 solution was added to each tube. Mixed thoroughly and placed the tube in boiling water for one minute, cooled and measured the absorbance at 650 nm against a reagent blank. Finally, a standard curve was prepared using different concentration of Catechols.

IV. Estimation of polyphenol oxidase (PPO)

Polyphenol Oxidase (PPO) was also assayed as per Sadasivam and Manickam (10) with minor modification. At first 2.5 ml Phosphate buffer pH 6.5, 0.3 ml of Catechol solution (0.01 M) was added into corvette and set the spectrophotometer at 495 nm. Then 0.2 ml of enzyme extract was added and started recording the change in absorbance for every 30 seconds up to 5 min. For the above procedure, the enzyme extract prepared by grinding 5 g roots with a mortar and pestle in about 20 ml medium containing 50 mM Tris-HCL, pH 7.2, 0.4 M Sorbitol and 10 mM NaCl, centrifuged the homogenate at 20,000g for 10 min and used the supernatant for assay.

RESULTS AND DISCUSSION

Evaluation of genotypes against *M. incognita* race 2

Among the tuberose genotypes (Table 1), Calcutta double exhibited highest gall index (GI ~ 4.3) followed by Calcutta single (GI~ 4.0), Vaibhav (GI~4.0), Hyderabad double (single (GI~4.0) and GK-T-C-4 single (GI~4.0). However, the lowest gall index was noticed in Shringar (GI~2.0) and in Suvasini (GI~2.0). In Prajwal, low root galling (GI~2.0) followed by Phule Rajani (GI~3.0). The stalk length (cm) of RKN infected genotypes of tuberose (Figure 1a) was minimum in Calcutta double (52 cm), Calcutta single (55 cm), Vaibhav (58 cm) and in Hyderabad double (59 cm). While the highest stalk length (cm) was recorded in Shringar (70 cm), Suvasini (69 cm), Prajwal (67 cm) and in Phule rajani (66 cm). In the RKN uninfected and healthy genotypes, the highest stalk lengths was noticed in Sikkim selection (136 cm), Prajwal (121 cm) and in Hyderabad double (117 cm). Hence, there was considerable reduction in stalk length; the highest % reduction of stalk length over healthy ones was recorded in Sikkim selection (52%), Hyderabad double (49%) and in Prajwal (45%).

Similarly, reduction in the inflorescence length (cm) in RKN infected genotypes (Figure 2a) was also recorded; Shringar (30 cm), Swarna rekha (28 cm), Prajwal (27 cm), Rajat rekha (26 cm), Phule rajani (25 cm) and Suvasini (24 cm). The highest inflorescence length of healthy genotypes was in Hyderabad double (117 cm), Swarna rekha (44 cm), Vaibhav (41 cm) and Phule rajani (35 cm). Thus, the highest % reduction of inflorescence length occurred in Hyderabad double (50 %), Vaibhav (45 %) and in Swarna rekha (37 %). Thus infection of RKN reduced the stalk and inflorescence length in Shringar, Suvasini, Prajwal and in Phule rajani genotypes despite the root galling. Nematode infection in all tuberose genotypes caused variable reduction and stunting of stalk and inflorescence. Reproduction of RKN on different genotypes was also recorded (Table 1). The lowest reproduction factor (RF) was in Shringar (0.74), Suvasini (1.06), Prajwal (1.13) and Phule rajani (1.18).

The same experiment was repeated during 2012 (Table 1). The high infection of *M. incognita* was noticed in Calcutta double (GI~4.83) and Calcutta single (GI~4.67), followed by Vaibhav (GI~4.17), Hyderabad double (GI~4.00) and GK-T-C-4 (GI~3.83). However, the lowest gall index was recorded in Suvasini (GI~2.00), Prajwal (GI~2.00), Shringar (GI~1.83), Hyderabad single (2.00) and Phule Rajani (GI~2.17). Among the genotypes, the stalk length (cm) (Figure 1b) was relatively shorter in Calcutta double (55 cm), Calcutta single (58 cm), Vaibhav (59 cm), Hyderabad double (61 cm), Shringar (69 cm), Suvasini (67 cm), Prajwal (66 cm) and Phule rajani (66 cm). While the stalk length was maximum in Sikkim selection (161 cm), Prajwal (130 cm), Suvasini (124 cm) and Hyderabad double (119 cm). The genotypes with the highest (%) reduction in stalk length over healthy ones were: Sikkim selection (59 %), Prajwal (49 %), Calcutta double (47 %) and Suvasini (46 %). Inflorescence length (Figure 2b) in root knot nematode infected genotypes was recorded highest in Shringar (30 cm), Prajwal (29 cm), Suvasini (29 cm) and in Sikkim selection (27 cm). The highest inflorescence length of healthy germplasm was noticed in Shringar (47 cm), Suvasini (47 cm) and Prajwal (45 cm). There were also considerable reduction (%) of inflorescence length over healthy ones : Vaibhav (43 %), Swarna rekha (40 %), Shringar (38 %) and Calcutta single (38 %). During the second year also both stalk and inflorescence lengths were reduced due to the infection of RKN in Shringar, Suvasini, Prajwal and Phule rajani. However, nematode multiplication was relatively low (Table 1); the RF was low in Shringar (1.32), Suvasini (1.53), Prajwal (1.73) and Phule rajani (1.99). In both years, infection of RKN on genotypes of tuberose adversely affected the economic parts of tuberose (stalk as well as inflorescence lengths). However, there were considerable variations in magnitude of susceptibility among the genotypes to RKN and this study indicated the effects of nematodes on morphological and physiological parameters.

Table 1. Evaluation of tuberose genotypes against root knot nematode (*Meloidogyne incognita* race 2) during 2011& 2012

Genotypes	Gall Index (1-5)		RF= pf/pi	
	2011	2012	2011	2012
Hyderabad Single	3.0	4.00	1.34	3.75
GK-T-C-4	4.0	3.83	2.50	3.49
Hyderabad Double	4.0	2.00	2.65	2.60
Sikkim selection	3.0	2.33	1.70	2.56
Swarna rekha	3.0	2.17	1.13	2.86
Calcutta single	4.0	4.67	2.93	4.71
Suvasini	2.0	2.00	1.06	1.53
Vaibhav	4.0	4.17	2.73	4.03
Prajwal	2.0	2.00	1.56	1.73
Calcutta double	4.3	2.17	3.56	3.07
Phule Rajani	3.0	4.83	1.18	5.14
Shringar	2.0	2.17	0.74	1.99
Rajat rekha	3.0	1.83	2.13	1.32
CD (P=0.05)	2.09	4.00	-	-

*RF: Reproduction Factor; pf: final population, pi: initial population

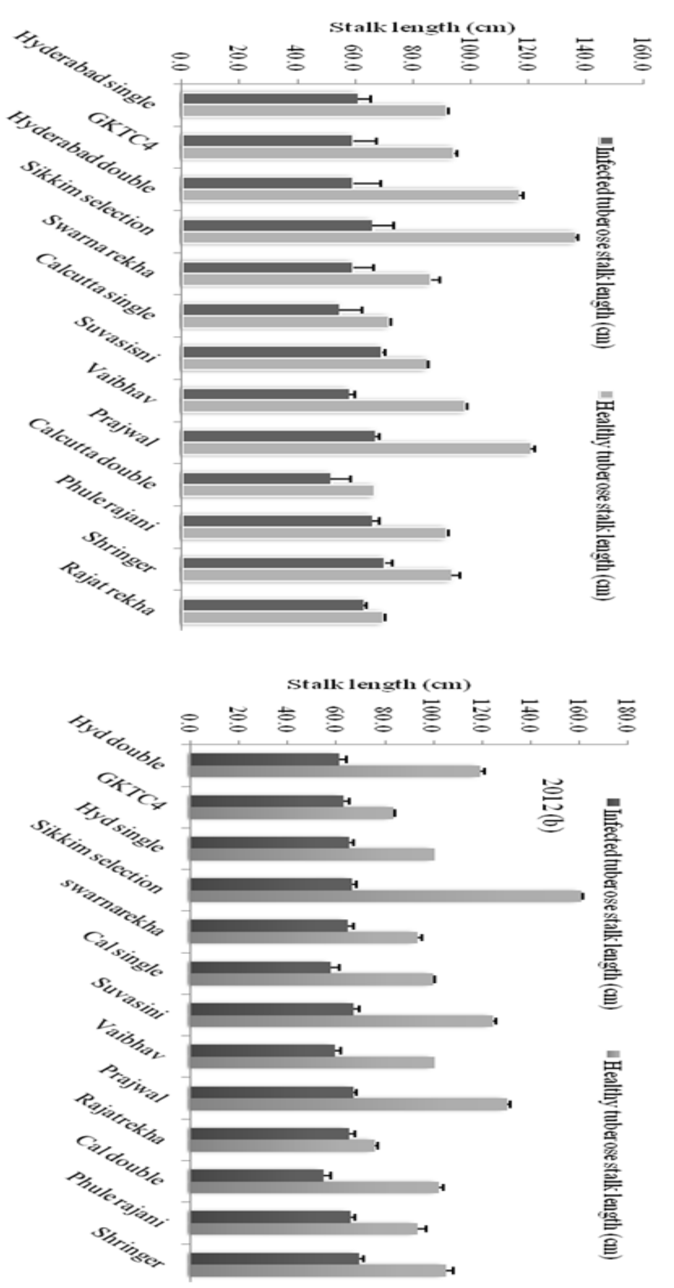

Figure 1. Stalk length (a-b) of nematode infected tuberose genotypes in comparison to healthy ones during 2011 and 2012

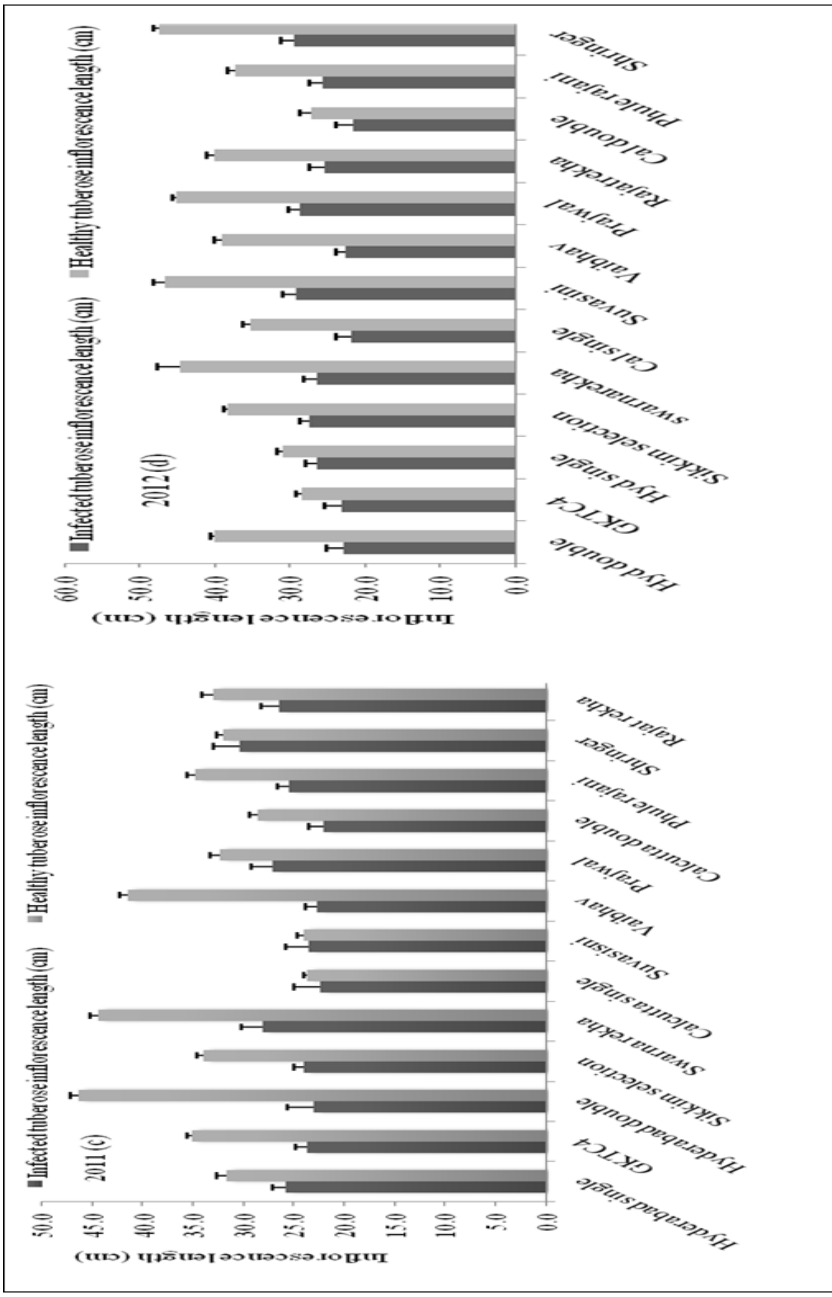

Figure 2. Inflorescence length (a-b) of nematode infected tuberose genotypes in comparison to healthy ones during 2011 and 2012

Our observations on incidence of *M. incognita* race 2 during 2011- 2012 revealed further that Calcutta double and Calcutta single were highly susceptible, because they were showing heavy infestation (gall severity) and supporting high reproduction factor. The genotypes, Shringar, Suvasini and Prajwal were found relatively less susceptible; they showed least root galling and reproduction factor. Therefore, Shringar and Suvasini were considered as least susceptible to *M. incognita* race 2. This observation was supported by the earlier observation of Nagesh *et al*. (7) who reported tuberose cultivars Shringar as resistant and Suvasini as tolerant to *M. incognita* of Bangalore population.

Phenol and PPO activities in tuberose genotypes

Estimation of phenol and polyphenol oxidase (PPO) content from healthy and infected plant's root of tuberose genotypes revealed that infected genotypes showed high phenol and PPO activity as compared to healthy one (Table 2).

Table 2. Phenol and PPO content in the root of tuberose genotypes infected by root knot nematode (means of three replications)

Genotypes	Phenol cat/g (mg/ml)			PPO EU/g tissue		
	Healthy	Infected	change over control (%)	Healthy	Infected	change over control (%)
Calcutta double	2.24	2.14	- 4.46	0.21	0.13	-38.10
Calcutta single	2.16	2.07	- 4.17	0.17	0.08	-52.94
Hyderabad double	2.31	2.23	- 3.46	0.24	0.16	- 23.81
Hyderabad single	2.36	2.28	- 3.39	0.21	0.14	- 33.33
GK-T-C-4	2.62	2.53	-3.44	0.23	0.15	- 34.78
Vaibhav	2.30	2.22	-3.48	0.25	0.17	- 32.00
Prajwal	2.89	2.98	+ 3.11	0.43	0.48	+11.63
Suvasini	2.96	3.04	+ 2.70	0.28	0.33	+17.86
Shringar	3.09	3.17	+ 2.59	0.26	0.30	+15.38
Phule rajani	2.79	2.86	+ 2.51	0.28	0.33	+17.86
Swarna rekha	2.54	2.62	+ 3.15	0.32	0.39	+ 21.88
Sikkim selection	2.69	2.77	+ 2.97	0.29	0.36	+ 24.14
Rajat rekha	3.03	3.10	+ 2.31	0.32	0.37	+ 15.63
CD (P=0.05)	0.74	0.82	-	0.14	0.26	-

*PPO: Polyphenol oxidase

The genotypes responded as resistant against *M. incognita* were containing high phenol and PPO activities; Suvasini (3.04 mg/ml, 0.33 EU/g tissue), Prajwal (2.98 mg/ml, 0.48 EU/g tissue), Shringar (3.17 mg/ml, 0.30 EU/g tissue), Phule rajani (2.86 mg/ml, 0.33 EU/g tissue), Swarna rekha (2.62 mg/ml, 0.39 EU/g tissue), Rajat rekha (3.10 mg/ml, 0.37 EU/g tissue) and Sikkim selection (2.77 mg/ml, 0.36 EU/g tissue). Relatively reduced phenol and PPO activity over healthy one were recorded in Calcutta single (2.07 mg/ml, 0.08 EU/g tissue), Calcutta double (2.14 mg/ml, 0.13 EU/g tissue), Hyderabad single (2.28 mg/ml, 0.14 EU/g tissue), Hyderabad double (2.23 mg/ml, 0.16 EU/g tissue), Vaibhav (2.22 mg/ml, 0.17 EU/g tissue) and GK-T-C-4 (2.53 mg/ml, 0.15 EU/g tissue); these genotypes showed low phenol and PPO activities and responded as susceptible. The infection of RKN increased the Phenol and PPO activities in Prajwal (+3.11), Suvasini

(+2.70), Shringar (+2.59), Phule rajani (+2.51), Swarna rekha (+ 3.15) and Sikkim selection (+ 2.97), and decreased in Calcutta double (- 4.46), Calcutta single (-4.17), Hyderabad double (- 3.46), Hyderabad single (-3.39) and in Vaibhav (- 3.48). The genotypes with higher phenol contents and PPO activities proved resistant to RKN nematode. In fact, phenol compounds are largely considered as resistance factors in a numerous plant pathosystems. Similar results were also known in the roots of resistant tomato cultivars against root knot nematode; they contained substantially higher phenol than that of the susceptible one (2). Phenolic contents were higher in infected roots of resistant barley and wheat cultivars than those of susceptible cultivars and control (9). Bhau *et al*. (3) also demonstrated the increased phenolic content in patchouli plant acted a means of defence against *M. incognita*. PPO, the enzymes involved in the oxidation of phenols to yield more toxic quinones, is known to increase in plants infected with fungi (1,12).

CONCLUSIONS

The Prajwal, Suvasini, Shringar, Phule rajani and Swarna rekha genotypes of tuberose proved tolerant to *M. incognita* race 2. They showed least nematode infection (low galling severity) and restricted multiplication for reduced nematode population in soil and root. This observation has also been supplemented with biochemical data; high phenol and PPO activity recorded in root knot nematode infected plant over healthy one. Susceptible genotypes were Calcutta single, Calcutta double, Hyderabad single, Hyderabad double, Vaibhav and GK-T-C-4; they showed low phenol and PPO activities over healthy ones, infested heavily (high gall index), allowed nematode to multiply on the host and increased number of nematode in soil and root. We concluded that Shringar, Suvasini, Prajwal and Phule rajani are resistant to *M. incognita* race 2 and high phenol and PPO activities elicited in response of nematode infection, thereby reduced the nematode infestation.

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