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ANALYSIS ON BIOCHEMICAL BASIS OF ROOT KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) RESISTANCE IN TUBEROSE GENOTYPES (*POLIANTHES TUBEROSA*)

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ABSTRACT
The study on biochemical constituents were carried out in the root of ten tuberose genotypes to select the genotype for hybridization which is resistance to root knot nematode infestation by inoculating the root knot nematodes artificially in the pot culture experiment. The study revealed that among the genotypes, the roots of Kahikuchi Single resulted with the highest level of phenol (19.84 mg g^{-1}), ortho-di-hydroxy phenol (15.84mg g^{-1}), peroxidase (3.65 OD min^{-1} g^{-1}), polyphenol oxiase (3.58 OD mg g^{-1}), phenylalanine ammonia lyase (16.10nmol of trans cinnamic acid min^{-1} g^{-1}) and acid phosphatase activity (131.88*m* moles p-nitrophenol min^{-1} mg^{-1} protein) at 96 hours after inoculation. Hence, it is suggested that the genotype Kahikuchi Single could be used as one of the parent to develop hybrids.

INTRODUCTION

Tuberose (*Polianthes tuberosa)* is one of the most important cut flower. It is an ornamental bulbous plant, native of Mexico and belongs to the family Amaryllidaceae. There are only two types of tuberose (Single and Double) cultivated in the world. Waxy white flowering spikes of single as well as double types of tuberose impregnate the atmosphere with their sweet fragrance (Sadhu and Bose, 1973; Benschop, 1993) and they are in great demand for making floral arrangement and bouquets in major cities of India. Tuberose plants have been reported to be severely affected by several plant parasitic nematodes, especially by the root knot nematodes, *Meloidogyne spp*. They have a wider occurance in the tuberose growing subtropical and tropical regions and reported to cause 10 and 14 per cent reduction in flower number and spike weight, respectively (Khan and Parvatha Reddy, 1992). *M. incognita* has become a major threat for tuberose cultivation. Hence, the use of resistant varieties is the most economical and easy practice. Identification of nematode resistant donors and utilization in development of F_1 hybrids by hybridization with high yielding lines coupled with quality parameters will be a boon to tuberose growers for maximising production of quality tuberose. The biochemical constituents like phenol, ortho-dihydroxy phenol, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, acid phosphatase possess nematode resistant properties. Phenolic compounds have been implicated as disease resistance factors in a number of host parasite combinations (Epstein, 1972; Pollock and Drysdale, 1976). Polyphenol oxidase and peroxidase, the enzymes involved in the oxidation of phenols to more toxic quinones, are known to increase in resistant plants (Yamamoto and Tani, 1978). Hence the study was conducted to screen the genotypes (Calcutta Single, Hyderabad Single, Kahikuchi Single, Mexican Single, Navsari Local, Phule Rajani, Prajwal, Pune Single, Shringar and Variegated Single) for resistance against root knot nematode to select the genotype for hybridization.

MATERIALS AND METHODS

An attempt was made to select the genotype which imparts resistance against root knot nematode (*Meloidogyne incognita*)to develop F_1 hybrids for high yield coupled with high concrete recovery and resistance to root knot nematode. With this aim, to assess the biochemical constituents in tuberose roots of ten single types were evaluated for root knot nematode resistance. Ten genotypes of diverse origin collected from distinct geographical regions were used for the study (Table 1).

Inoculation

The method of Sasser *et al.* (1957) was followed for inoculating nematodes. Infected roots from pure culture were cut into small pieces of about 2 cm long and placed in sodium hypochlorite (NaoCl) 0.5% solution. The container was shaken for about 3 minutes to dissolve the gelatinous matrix and freeing the eggs from the egg mass and incubated for 48

hours under laboratory condition. The inoculum concentration was adjusted to a known number by addition of water. The eggs were kept in petridishes and frequently aerated with the use of aerator to enable hatching. The nematode inoculums (J_2) were placed at a depth of 2 cm near to the rhizosphere and covered with sterile sand. Each pot was inoculated with J_2 of *Meloidogyne incognita* at the rate of two juvenile (J_2)/g of soil 15 days after planting.

Total phenol (roots)

Folinciocalteau reagent method was used for estimating the total phenol (Bray and Thrope, 1954). One ml of ethanol root extract was taken in a boiling tube to which one ml of Folinciocalteau reagent and 2 ml of 20 per cent sodium carbonate were added. This mixture was heated exactly for one minute on a water bath. After cooling 2 ml distilled water was added and the blue colour development was read at 660 nm.

Orthodihydroxy phenol

Arnow's method was followed for the estimation of Ortho-dihydroxy phenol (Malik and Singh, 1980). One gram of plant tissue was homogenized in a mortar and pestle with 10 ml methanol. The homogenized material was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected in a separate test tube. The sediments were reground in a mortar and pestle with 5 ml methanol, centrifuged as above and pooled together to form a total volume of 15 ml. One ml of alcohol extract was pipetted out and added with 0.5 N HCl, 1 ml Arnow's reagent (10 g $NaNO_2$ + 10 g Sodium molybdate in 100 ml of distilled water), 10 ml distilled water and 2 ml of 1N NaOH. Soon after the addition of alkali, pink color appeared. The absorbance of the pink colour was read at 515 nm and OD phenol content expressed as mg g^{-1}.

Peroxidase activity

Peroxidase activity was assayed, following the method described by Srivastava (1987). Reaction mixture consisted of 1.5 ml of Guaicol solution, 100 μl of enzyme preparation and 100 μl of 1 percent H_2O_2. At the start of the enzyme reaction, the absorbance of the mixture was set to zero at 420 nm and change in the absorbance were recorded at 30 seconds intervals. Boiled enzyme preparation served as control. Peroxidase activity was expressed as changes in absorbance/ minute/ gram fresh weight.

Polyphenol oxidase activity

Polyphenol oxidase activity was assayed using the method described by Srivastava (1987). Standard reaction mixture contained 1.5 ml of 0.1 M phosphate buffer (pH 6.5), 0.5 ml of enzyme preparation and 0.5 ml of 0.01 M catechol. At the start of the enzyme reaction the absorbance was set to zero at 495 nm. The changes in the absorbance were recorded at 30 seconds intervals and polphenol oxidase activity was expressed as changes in the OD of the reaction mixture per minute per 200 mg of fresh weight of tissue.

Phenylalanine ammonia lyase (PAL) activity

Plant samples (500 mg) were homogenized in 2 ml of ice cold 0.1 M sodium borate buffer at pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinylpyrrolidone. The extract was filtered through cheese cloth and the filtrate was centrifuged at 16,000 g for 15 min. The supernatant was used as enzyme source. PAL activity was determined as the rate of conversion of L-phenylalanine to transcinnamic acid at 290 nm (Dikerson *et al.,* 1984). Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM

L-phenylalanine in the same buffer for 30 min at 30°C. The amount of transcinnamic acid synthesized was calculated (Dikerson *et al.*, 1984) and enzyme activity expressed as n moltranscinnamic acid min^{-1} g^{-1} tissue.

Acid phosphatase activity

Powdered root sample was homogenized with 0.1 M sodium acetate buffer (pH 5.0) in a pre chilled pestle and mortar. The homogenate was centrifuged at 10000 rpm for 20 minutes in a refrigerated centrifuge. The reaction mixture, in a final volume of 2 ml contained 100 mM sodium acetate buffer (pH 5.0), 5 mMpNPP as substrate and enzyme. After 10 minutes of incubation at 37°C, the reaction was stopped by the addition of 1 ml of 1 N NaOH and the p-nitrophenol released. The released p-nitrophenol was yellow in colour in alkaline medium and was monitored at 405 nm and specific activity expressed as *m*moles of p-nitrophenol released $minute^{-1}$ mg^{-1} protein (Dikerson *et al.*, 1984).

RESULTS AND DISCUSSION

Many biochemical factors are known to be associated with biotic resistance in crop plants. In many cases, it is obvious that the biochemical factors are more important than morphological and physiological factors in conferring non-preference and antibiosis (Prabhu *et al.,* 2008). Plants generally respond to nematode invasion by activation of a series of local and systemic defense mechanisms (Lindgren *et al.,* 1992; Trudgill, 1995). Inducible defense against nematodes included accumulation of peroxidase (Ibrahim, 1991), polyphenol oxidase and superoxide dismutase (Zacheo and Zacheo, 1995). In the present investigation, the biochemical mechanism in roots was established by analysing total phenols, ortho-dihydroxy phenol, ascorbic acid, protein and enzymes *viz.,* peroxidase, polyphenol oxidase, phenyl alanine ammonia lyase, IAA oxidase and acid phosphatase at different intervals after artificial inoculation in tuberose genotypes.

Phenolic compounds played a major role in the defense mechanism of the plants against various infectious agents. Total phenol content in roots is yet another indication of plant's resistance to root knot nematode. The accumulation of phenolic compounds in the injured area and the activation of associated oxidative enzymes have been reported by Balasubramanian and Purushothaman (1972). Acedo and Rohde (1971) also reported role of phenol contents towards the resistant mechanism against various nematode infections. In the resistant species, the non-toxic phenolic glycosides have been shown to be hydrolysed by β-glycosidase enzyme from the nematode and the resultant product might prevent localized parasitization or even cause the death of the nematode (Star, 1981; Hussey and Williamson, 1998). It is

Table 1: Peroxidase activity in tuberose genotypes (single) inoculated with root knot nematode

Genotypes	Peroxidase (changes in OD $min^{-1}g^{-1}$ of sample) Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Single	1.05	1.28	1.39	1.45	1.56	1.50	1.37
Hyderabad Single	1.06	1.30	1.41	1.44	1.53	1.51	1.38
Kahikuchi Single	2.49	3.01	3.14	3.30	3.65	3.54	3.19
Mexican Single	1.03	1.25	1.35	1.46	1.54	1.52	1.36
Navsari Local	0.65	0.74	0.81	0.86	0.89	0.84	0.79
Phule Rajani	0.61	0.72	0.84	0.87	0.89	0.85	0.80
Prajwal	0.63	0.74	0.82	0.86	0.88	0.85	0.80
Pune Single	0.64	0.73	0.84	0.87	0.89	0.81	0.81
Shringar	0.66	0.72	0.85	0.87	0.89	0.85	0.82
Variegated Single	0.65	0.76	0.83	0.85	0.89	0.83	0.80

Table 2: Polyphenol oxidase activity in tuberose genotypes (single) inoculated with root knot nematode

Genotypes	Polyphenol oxidase (changes in OD $min^{-1}g^{-1}$ of sample) Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Single	1.08	1.37	1.48	1.64	1.85	1.71	1.52
Hyderabad Single	1.06	1.42	1.51	1.65	1.89	1.78	1.55
Kahikuchi Single	2.57	3.04	3.18	3.27	3.58	3.45	3.18
Mexican Single	1.07	1.38	1.49	1.62	1.87	1.74	1.53
Navsari Local	0.79	0.84	0.89	0.90	0.91	0.90	0.88
PhuleRajani	0.72	0.82	0.87	0.94	0.92	0.91	0.86
Prajwal	0.74	0.83	0.88	0.94	0.92	0.91	0.85
Pune Single	0.72	0.84	0.87	0.91	0.92	0.90	0.86
Shringar	0.79	0.83	0.88	0.93	0.92	0.90	0.88
Variegated Single	0.78	0.85	0.87	0.91	0.93	0.91	0.88

Table 3: Phenylalanine ammonia lyase activity in tuberose genotypes (single) inoculated with root knot nematode

Genotypes	Phenylalanine ammonia lyase (nmol of transcinnamicacid $min^{-1}g^{-1}$ of fresh tissue) Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Single	9.04	9.38	9.65	9.84	9.98	9.89	9.63
Hyderabad Single	9.00	9.42	9.76	9.82	9.97	9.82	9.64
Kahikuchi Single	14.24	14.92	15.55	16.04	16.10	15.48	15.39
Mexican Single	9.03	9.31	9.64	9.89	9.92	9.84	9.61
Navsari Local	5.42	5.54	5.60	5.64	5.68	5.60	5.58
Phule Rajani	5.40	5.58	5.67	5.72	5.85	5.81	5.67
Prajwal	5.24	5.54	5.67	5.74	5.85	5.79	5.64
Pune Single	5.32	5.47	5.58	5.64	5.78	5.68	5.58
Shringar	5.41	5.52	5.61	5.66	5.72	5.69	5.60
Variegated Single	5.45	5.54	5.63	5.68	5.73	5.65	5.61

Table 4: Acid phosphatase activity in tuberose genotypes (single) inoculated with root knot nematode

Genotypes	Acid phosphatase (*m*moles p-nitrophenol min^{-1} mg^{-1} protein) Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Single	70.01	103.46	118.96	125.12	131.88	128.06	112.92
Hyderabad Single	69.02	102.54	117.92	126.21	132.86	125.62	112.36
Kahikuchi Single	95.42	103.46	118.96	125.12	131.88	128.06	117.15
Mexican Single	72.02	104.56	117.98	124.16	132.84	126.05	112.94
Navsari Local	43.54	51.94	55.18	63.11	67.13	60.37	56.88
Phule Rajani	42.53	54.42	55.21	64.15	68.17	61.38	57.64
Prajwal	44.56	52.98	56.35	62.17	69.18	62.37	57.94
Pune Single	45.54	53.94	57.18	64.11	69.13	60.37	58.38
Shringar	42.54	50.93	55.97	62.28	68.87	60.53	56.87
Variegated Single	43.45	51.49	55.81	63.11	67.31	60.73	56.98

also possible that active phenols might have been released from glycosides by increased activities of β-glycosidases and later get oxidized in resistant genotypes. Narayana and Reddy (1980) also inferred that an increased phenolic content was considered to be contributory factor in the resistance to various nematode infections.

The higher amount of total phenols (12.54, 14.68, 17.41, 18.59, 19.84 and 19.21) andortho-dihydroxy phenol (9.52, 10.04, 12.56, 14.21, 15.84 and 15.55) were observed in the Kahikuchi Single (Single type) might be responsible for

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	0	24	48	72	96	120	Mean
Calcutta Single	70.01	103.46	118.96	125.12	131.88	128.06	112.92
Hyderabad Single	69.02	102.54	117.92	126.21	132.86	125.62	112.36
Kahikuchi Single	95.42	103.46	118.96	125.12	131.88	128.06	117.15
Mexican Single	72.02	104.56	117.98	124.16	132.84	126.05	112.94
Navsari Local	43.54	51.94	55.18	63.11	67.13	60.37	56.88
Phule Rajani	42.53	54.42	55.21	64.15	68.17	61.38	57.64
Prajwal	44.56	52.98	56.35	62.17	69.18	62.37	57.94
Pune Single	45.54	53.94	57.18	64.11	69.13	60.37	58.38
Shringar	42.54	50.93	55.97	62.28	68.87	60.53	56.87
Variegated Single	43.45	51.49	55.81	63.11	67.31	60.73	56.98

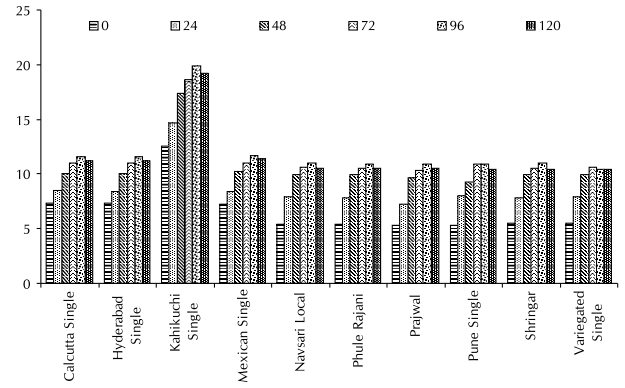

Figure 1: Total phenol content in RKN infected tuberose genotypes (single) after inoculation

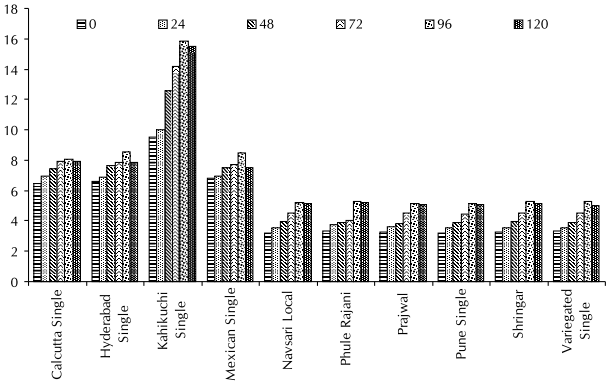

Figure 2: Ortho di-hydroxy phenol content in RKN infected tuberose genotypes (single) after inoculation

checking the infection of root knot nematode at different hours after artificial inoculation (Fig. 1 and 2). The accumulation of phenolic compounds in the nematode injured area and the activity of associated oxidative enzymes have been reported by Mountain (1965). Similar observation was made by Bajaj *et al.* (1983) in tomato and Sherly (2011) in brinjal. Nematicidal activity of phenolic compounds was also reported by Mahajan *et al*. (1985). Post infectional increase of phenols in the roots might be due to the tendency of phenols to accumulate at the site of infection, which is involved in the defense mechanisms of plants through the interference in the metabolic activities of pathogens that might have resulted in higher root length (Gopinatha *et al.,* 2002).

The enzymes polyphenol oxidase and peroxidase oxidizes the colourless dihydroxy phenols into coloured ortho quinines. While, certain dihydroxy phenols get conjugated with each other or with glucose hydroxyl groups to form tannins, both are constituents of plant melanins (Bell, 1981). Peroxidase played a vital role in alleviating free radical toxicity in plant tissues. Following the entry of nematode, an increased peroxidase level in the infested plant suggested a great *de novo* synthesis of peroxidases since nematode do not exudate peroxidate (Glasiov *et al.,* 1967). In the present study, the higher peroxidase activity was observed in the Kahikuchi Single (2.49, 3.01, 3.14, 3.30, 3.65, 3.54 and 3.19) at different hours of interval after artificial inoculation. These enzymes gradually increased when the nematode infestation started and it attained the peak at 96 hours after inoculation and then decreased gradually (Table 1). This falls in line with the findings of Indu Rani *et al.* (2008) and Sundharaiya (2008) in tomato; Janani (2009) in pepper and Sherly (2011) in brinjal.

Higher polyphenol oxidase activity was noticed in Kahikuchi Single (2.57, 3.04, 3.18, 3.27, 3.58, 3.45 and 3.18) at different hours of interval after artificial inoculation. These enzymes gradually increased when the nematode infestation started and it attained the peak at 96 hours after inoculation (Table 2). The role of PPO and ascorbic acid in impairing towards root knot nematode resistance had been well documented by several workers (Indu Rani *et al.,* 2008; Janani, 2009 and Sherly, 2011).

In the present study, higher phenylalanine ammonia lyase activity was noticed in Kahikuchi Single (14.24, 14.92, 15.55, 16.04, 16.10, 15.48 and 15.39) at different hours of interval after artificial inoculation. These enzymes gradually increased after inoculation and it attained the peak at 96 hours (Table 3). The activity levels of phenylalanine ammonia-lyase and anionic peroxidase induced early resistance response to many other pathogens and also increased in tomato (Brueske, 1980; Zacheo *et al.,* 1993), brinjal (Sherly, 2011) after nematode inoculation.

Acid phosphatase is yet another important enzyme closely related to nematode resistance. Increase in acid phosphatase activity in the roots was found to be a resistant mechanism to root knot nematode. In the study, increase in acid phosphatase activity after artificial inoculation was noticed in the moderately resistant genotype Kahikuchi Single. Increasing trend was observed in acid phosphatase activity from the day of

inoculation and it attained the highest at 96 hours and after which started to decline (Table 4).

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