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Biochemical characterization of resistance to root-knot nematode, *Meloidogyne incognita* in sweet potato

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Abstract
Sweet potato is a perennial vine, usually cultivated as an annual crop. Its storage roots are rich in energy and are an important source of carbohydrates, vitamin A. Root-knot nematode (*Meloidogyne* spp.) causes galls or knots in the roots and considerable yield loses of 10.2 per cent. Seventeen sweet potato genotypes were screened against root-knot nematode, *M. incognita* and were subjected for biochemical estimation of enzymes content *viz*., PO, PPO, PAL and total phenols, in both susceptible and resistant varieties. The results revealed that, the genotype Sree Bhadra has recorded the maximum activity of different enzymes *viz*., Peroxidase (3.12 abs/min/g), Polyphenol oxidase (0.127 abs/min/g), Phenyl Alanine Ammonia Lyase (22.37 nmol/min/ml), and total phenol (447.00 micro gram/g). The genotype Kanhangad Local has recorded minimum levels of Peroxidase (1.02 abs/min/g), Polyphenol oxidase (11.03 nmol/min/ml), Phenyl Alanine Ammonia Lyase (11.03 nmol/min/ml) and total phenol (107.67 micro gram/g).

Keywords: Sweet potato (*Ipomoea batatas*(L.) Lam.,) root-knot nematode, biochemical characterisation

Introduction
Sweet potato, *Ipomoea batatas* (L.) Lam., is a dicotyledous plant that belongs to the family Convolvulaceae. It is originated from Central America and the North Western part of South America from where it was introduced to Europe by Columbus and to Asia, Africa and North America by Spanish and Portuguese explorers and traders. It is an herbaceous perennial crop with edible tuberous root that is usually long and tapered, with a smooth skin whose colour ranges between red, purple, brown and white. It can be cultivated throughout the tropical and warm temperature region wherever there is sufficient water to support its growth. It is a vegetable crop with great social, economic and nourishing importance, especially for the poorest regions of the planet, where it is one of the most important food sources (Oliveira *et al*., 2005) [7]. Sweet potato is a perennial vine, usually cultivated as an annual crop. Its storage roots are rich in energy and are an important source of carbohydrates, vitamin A and C, fibre, iron, copper, calcium and folic acid, especially the orange-fleshed sweet potatoes (Collins *et al*., 1999) [2]. Sweet potato is mainly grown in China, Nigeria, Tanzania, Ethiopia and Indonesia. The crop is grown in all states of India, except Jammu and Kashmir, Himachal Pradesh and Sikkim. It can be grown in different environments and it became an excellent supplement to staple foods. Biochemical studies in genotypes of sweet potato infested with *M. incognita* helps to know the differences in enzymatic activities *viz*., PO, PAL, PPO and total phenols. In future, the enhancement of respective enzymatic contents can be main objective for the resistance breeding in sweet potato against nematodes.

Material and Methods
A pot experiment was conducted in green house with seventeen sweet potato genotypes planted in plastic pots filled with sterilized soil. Thirty days after planting, the nematodes were inoculated at the rate of 6000 infective juveniles per 3000 g soil (two nematode per gram of soil), into four holes made in the soil around the base of each plant. Regular watering and weeding were followed. Three replications were maintained for each genotypes. The plants were carefully deported after completion of nematode life cycle.

Table 1: List of sweet potato genotypes used for screening against root-knot nematode, *M. incognita*

Treatments	Genotypes
T_1	Tsp 16-7
T_2	Kamala Sundari
T_3	Tsp 12-7
T_4	Tsp 12-12
T_5	44038
T_6	CIP-SWA
T_7	441027
T_8	Tsp 12-6
T_9	Tsp 12-4
T_{10}	Gouri
T_{11}	BSP-23
T_{12}	Vikram
T_{13}	SreeBhadra
T_{14}	Tsp 12-8
T_{15}	Tsp 12-9
T_{16}	HUB-98
T_{17}	Kanhangad Local

The screened genotypes were subjected for biochemical studies for estimation of enzymes content in both susceptible and resistant varieties. The content of the enzymes peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) and the content of phenols in the roots was determined for each replicate after four months.

The total phenol in the roots was estimated by using Folin Ciocalteau reagent and measuring absorption at 660 nm in spectrophotometer and was expressed as mg/g root (Spies, 1955) [10].

One gram of root sample per replicate was homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4 ^{0}C. The supernatant was used as crude enzyme extract for assaying peroxidase (abs/min/g) and polyphenol oxidase (abs/min/g) (Hammerschmidt, *et al*., 1982) [4]. The enzyme extracted in borate buffer was used for estimation of phenyl alalanine ammonia lyase (Meyer, 1965) [5].

Results

The data on biochemical studies in sweet potato genotypes infested with *M. incognita* at 120 days after inoculation in pot culture, the content of the enzymes Peroxidase (PO), Polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL) and the content of total phenols in the roots was determined and the results are presented in Table 2.

Table 2: Biochemical parameters in different genotypes of sweet potato infested with root-knot nematode, *M. incognita*

Treatments	Genotypes	Peroxidase (abs/min/g)	Polyphenol oxidase (abs/min/g)	Phenyl - alanine ammonia lyase (PAL) (nmol/min/ml)	Total phenols (µg/g)
T_1	Tsp 16-7	1.91	0.083	16.47	244.33
T_2	Kamala Sundari	2.03	0.085	18.10	316.67
T_3	Tsp 12-7	1.93	0.064	17.27	318.33
T_4	Tsp 12-12	2.47	0.085	19.73	410.33
T_5	44038	1.80	0.045	12.00	124.67
T_6	CIP-SWA	2.20	0.069	16.90	151.00
T_7	441027	2.21	0.074	16.00	134.00
T_8	Tsp 12-6	2.17	0.090	14.40	161.33
T_9	Tsp 12-4	1.95	0.079	15.73	176.67
T_{10}	Gouri	2.18	0.081	15.43	276.33
T_{11}	BSP-23	2.59	0.110	20.73	405.00
T_{12}	Vikram	1.96	0.068	13.60	223.33
T_{13}	SreeBhadra	3.12	0.127	22.37	447.00
T_{14}	Tsp 12-8	2.67	0.125	21.67	432.00
T_{15}	Tsp 12-9	2.21	0.052	12.18	135.00
T_{16}	HUB-98	1.71	0.035	12.23	116.67
T_{17}	Kanhangad Local	1.02	0.033	11.03	107.67
S. Em±		0.10	0.005	0.45	10.44
C.D. at 5%		0.28	0.014	1.29	30.01

Peroxidase (abs/min/g)

For peroxidase enzyme extraction, one gram of root sample per replicate was homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4 ^{o}C. There was significant difference among the genotypes with respect to peroxidase activity. The maximum peroxidase activity was recorded in Sree Bhadra (3.12 abs/min/g) followed by Tsp 12-8 (2.67 abs/min/g), BSP-23 (2.59 abs/min/g), Tsp 12-12 (2.47 abs/min/g), 441027 (2.21 abs/min/g), Tsp 12-9 (2.21 abs/min/g) and CIP-SWA (2.20 abs/min/g). The minimum peroxidase activity was recorded in Kanhangad Local (1.02 abs/min/g), followed by HUB-98 (1.71 abs/min/g), 44038 (1.80 abs/min/g), Tsp 16-7 (1.91 abs/min/g), Tsp 12-7 (1.93 abs/min/g), Tsp 12-4 (1.95 abs/min/g) and Vikram (1.96 abs/min/g).

Polyphenol oxidase (abs/min/g)

Among the seventeen samples of different sweet potato genotypes homogenized for polyphenol oxidase estimation, genotype SreeBhadra (0.127 abs/min/g) has recorded maximum PPO activity followed by Tsp 12-8 (0.125 abs/min/g), Tsp 12-6 (0.090 abs/min/g), Kamala Sundari (0.085 abs/min/g), Tsp 12-12 (0.085 abs/min/g), Tsp 16-7 (0.083 abs/min/g) and Gouri (0.081 abs/min/g). The minimum polyphenol oxidase activity was recorded in Kanhangad Local (0.033 abs/min/g), followed by HUB-98 (0.035 abs/min/g), 44038 (0.045 abs/min/g), Tsp 12-9 (0.052 abs/min/g), Tsp 12-7 (0.064 abs/min/g) and Vikram (0.068 abs/min/g).

Phenylalanine ammonia lyase (nmol/min/ml)

Among the seventeen genotypes, SreeBhadra has registered maximum PAL content (22.37 nmol/min/ml) followed by Tsp 12-8 (21.67 nmol/min/ml), BSP-23 (20.73 nmol/min/ml), Tsp 12-12 (19.73 nmol/min/ml), Kamala Sundari (18.10 nmol/min/ml) and Tsp 12-7 (17.27 nmol/min/ml). The genotype Kanhangad Local has recorded minimum PAL

content (11.03 nmol/min/ml) followed by 44038 (12.00 nmol/min/ml), Tsp 12-9 (12.18 nmol/min/ml), HUB-98 (12.23 nmol/min/ml), Vikram (13.60 nmol/min/ml), Tsp 12-6 (14.40 nmol/min/ml) and Gouri (15.43 nmol/min/ml).

Total phenols (micro gram/g)

The maximum levels of phenols has been recorded in the cultivar SreeBhadra (447.00 µg/g), followed by Tsp 12-12 (410.33 µg/g), BSP-23 (405.00 micro gram/g), Tsp 12-7 (318.33 µg/g) and Kamala Sundari (316.67 µg/g). The genotype Kanhangad Local has recorded minimum levels of total phenols (107.67 µg/g), followed by HUB-98 (116.67 µg/g), 44038 (124.67 µg/g), 441027 (134.00 µg/g), Tsp 12-9 (135.00 µg/g), CIP-SWA (151.00 µg/g) and Tsp 12-6 (161.33 µg/g).

As a whole, the genotype SreeBhadra has recorded the maximum activity of different enzymes *viz.*, Peroxidase (3.12 abs/min/g), Polyphenol oxidase (0.127 abs/min/g), Phenyl Alanine Ammonia Lyase (22.37 nmol/min/ml), and total phenol (447.00 micro gram/g). The genotype Kanhangad Local has recorded minimum levels of Peroxidase (1.02 abs/min/g), Polyphenol oxidase (11.03 nmol/min/ml), Phenyl Alanine Ammonia Lyase (11.03 nmol/min/ml) and total phenol (107.67 µg/g) and depicted in the Fig 1 and Fig 2.

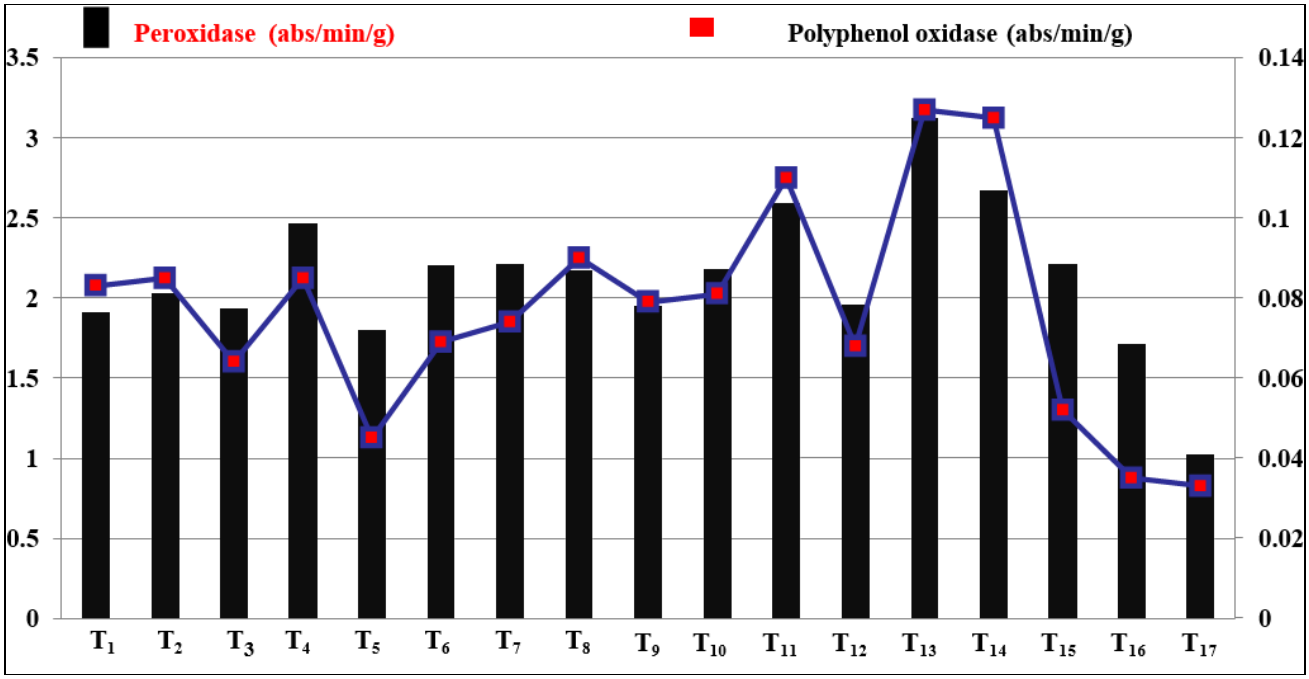

Fig 1: Peroxidase and Polyphenol oxidase content in different genotypes of sweet potato against root-knot nematode, *M. incognita*

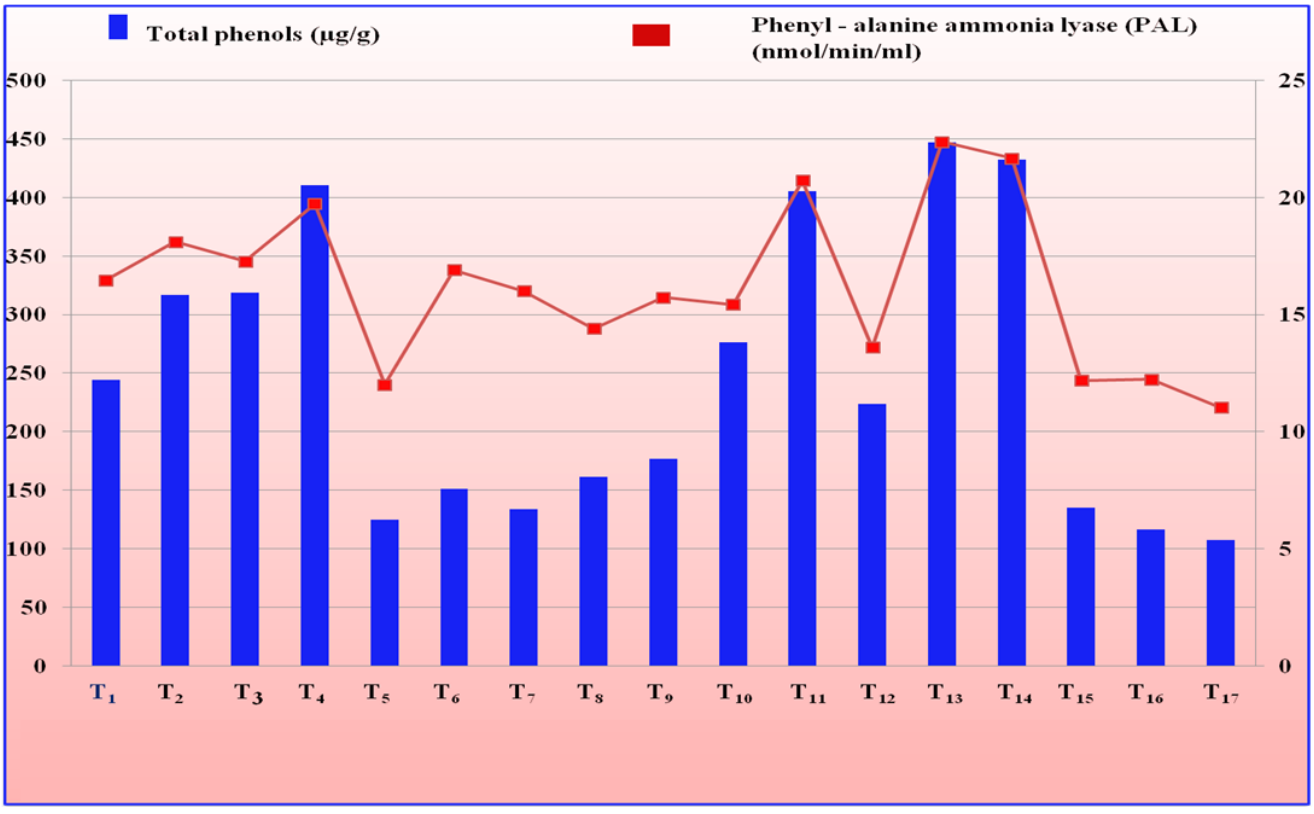

Fig 2: Peroxidase and Polyphenol oxidase content in different genotypes of sweet potato against root-knot nematode, *M. incognita*

Discussion

In these seventeen genotypes, SreeBhadra has recorded maximum peroxidase (3.12 abs/min/g), polyphenol oxidase (0.127 abs/min/g), phenylalanine ammonia lyase (22.37 nmol/min/ml) and total phenol (447.00 µg/g) activity followed by Tsp 12-8 (peroxidase 2.67 abs/min/g, polyphenol oxidase 0.125 abs/min/g, phenylalanine ammonia lyase 21.67 nmol/min/ml and total phenol 432.00 µg/g).

The present findings are in accordance with Anita *et al*. (2004) [1] who reported that activities of peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, were significantly higher in tomato root tissues challenged with the nematode and there was also a significant reduction in the root-knot nematode population in roots and soil of the resistant varieties. In both resistant and highly susceptible varieties sequential development of chitinase, peroxidase and acid phosphatase takes place but level of these compounds was much higher in roots of resistant variety compared to susceptible variety (Mohamed and Hasabo, 2005) [6].

Similar findings were observed by Shoba *et al*. (2017) who reported that the infested roots of variety ArkaSumeet (830 mg/g) in ridge gourd recorded significantly higher amount of total phenol. Phenol content and its enhancement during disease progress were least in susceptible varieties. The increase in phenolics in resistant plants was due to high activity of α-glucosidase, which converts non-toxic phenolic glycosides to toxic phenolics which are inhibitory to the pathogen.

The results were in agreement with the findings of Sireesha *et al*. (2015) [9] and Gopinath (2001) [3] who had reported phenolic content was higher in galled (infested roots) than in healthy tissue.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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