Journal of Global Biosciences
ISSN 2320-1355
Volume 6, Number 4, 2017, pp. 4935-4939
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Research Paper

EFFECTS OF NEMATODE INFECTION ON SUGAR AND STARCH CONTENTS AS INFLUENCED BY ROOT-KNOT NEMATODE, *Meloidogyne incognita* IN SUSCEPTIBLE AND RESISTANT GREENGRAM CULTIVARS

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Abstract

A study was undertaken in greengram (*Vigna radiata* (L) Wilezek) varieties to assess biochemical changes of both resistant and susceptible cultivars due to the root-knot nematode infection. The variations in total sugar and starch contents in six greengram cultivars i.e 24 ML-233 (R), 7 GGG 10-14 (R), 17 IPM 9901-6 (R) ,8 GM 04-02 (R), 28 PM 10-12(S) and 29 PUSA 0672 (S) were studied 45 days after inoculation of *Meloidogyne incognita* . Total sugar content was maximum decreased (61.78%) in susceptible variety 28 PM 10-12 followed by 44.45% in susceptible variety 29 PUSA 0672 but increasing trend was observed in the variety 8 GM 04-02 (39%). Total starch content was maximum decreased in resistant variety 24 ML-233(93.55 %) and least decrease in the resistant variety 17 IPM 9901-6(9.67 %). The total sugar contents in the shoots of both healthy and resistant cultivars were higher compared to the susceptible cultivars.

Key words: Biochemical modifications, *Vigna radiata* ,cultivars, *Meloidogyne incognita*, total sugar and starch content.

INTRODUCTION

Plant response to the parasite depends not only on the quantitative and qualitative composition of the nematode secretion and excretion but also on the chemical composition of the plants or the tissues attacked. The infestation of plant parasitic nematode causes increased in sugar content and the increased sugar content is helpful for survival of nematode (Owensh Specht, 1966(7); Dropkin, 1969(1). They suggested the increased sugar contents in the infected leaves may be due to the degradation of starch or inhibition of starch synthesis from sugar. Farooqi *et al*.(1980)(2) confirmed there was increase in total as well as reducing sugar in root-knot infected tomato plants. In chickpea, (Upadhyaya and Banerjee, 1986(8) reported

the incidence of *M. javanica* resulted in the disturbance of metabolism of protein, carbohydrate as well as chloroplast pigment.

MATERIALS AND METHODS

Five to ten surfaced sterilized seeds each of the six greengram cultivars i.e 24 ML-233 (R), 7 GGG 10-14 (R), 17 IPM 9901-6 (R) ,8 GM 04-02 (R), 28 PM 10-12(S) and 29 PUSA 0672 (S) were sown separately in earthen pots filled with sterilized soil , sand and FYM mixture (2:1:1) @1 kg /pot along with NPK @ 20:40:40 kg/ha. Each variety was replicated thrice. Thinning to one healthy seedling per pot was done after 15 days of germination .Two weeks after seedling emergence axenised nematodes were released @1000 J2 per seedling in 10 ml sterile water in the root zone of the seedlings .The total sugar and starch contents in all the six varieties were estimated 45 after inoculation of the test nematode.

Procedure for estimation of total sugar and starch content:

Extraction of sugar in shoot

One hundred mg of ground seed samples were taken in 15 ml centrifuge tubes and 10 ml of 80 % ethanol was added to it. The mouth of the centrifuge tube was covered with polythene paper and kept in a water both at 80-85^{o}C for 30 minutes. Then it was cooled and centrifuged for 15 minutes at 2000 rpm. After centrifugation, the supernatant was decanted into a 25 ml. volumetric flask. This extraction procedure was repeated once again and the supernatant was collected in the previous 25 ml. volumetric flask. The final volume was made up to 25 ml with distilled water and was filtered through Whatman No.1 filter paper. This was the sugar extract kept for sugar estimation.

Estimation of total sugar

Two ml of sugar extract was transferred into a 50 ml volumetric flask and volume was made up to 50 ml in volumetric flask and volume was made up to 50 ml with distilled water. Five ml of this extract was taken in a 25 ml volumetric flask. Simultaneously standards of 0 ml, 1 ml, 1.5 ml and 2 ml. of 100 ppm glucose solution were taken in 25 ml volumetric flasks. Volume of these standards was made up to 5 ml with addition of distilled water and 2 drops of 80 per cent ethanol. Volumetric flasks containing samples and standards were kept in an ice-bath. To each volumetric flask, 10 ml of anthrone reagent (2 gm of anthrone in one litre of 95 % H_2SO_4) was added allowing it to run down the side of the volumetric flask. The contents of the flasks were shaken slowly by swirling the flask and then shaken thoroughly. The volumetric flasks were kept in boiling water bath exactly for 7.5 minutes. Then immediately the flasks were cooled in ice. After cooling, absorbance was measured at 630 nm and sugar content was calculated with the help of standard curve.

Estimation of total starch content

1 ml of starch extract was taken in a 100 ml volumetric flask and diluted to 100 ml with distilled water.5 ml of the above extract was transferred in a 50 ml test tube. Then all the standards and sample test tubes were kept in ice bath for cooling, and 10 ml of anthrone reagent was added to each test tube, allowing the reagent to run down the side of the flask. It was stirred slowly with a glass rod and then shaken thoroughly. The flask was kept in boiling water bath exactly for 7.5 minutes. Then the test tube was immediately cooled in ice-bath. After cooling, the O.D. at 630 nm was measured and the starch content was calculated with the help of standard curve, which was multiplied by 0.91 to get the exact value of the starch.

RESULTS AND DISCUSSIONS

In order to know the chemical and genetic basis of resistance, six varieties were grown with utmost care, both in inoculated and control condition.

Chemical analysis of plant samples

Total sugar in seeds

The amount of sugar present in the seeds of the inoculated plants were recorded as 8.12 , 9.37,6.25,9.37,6.25 and 6.95 per cent in the varieties of 28 PM 10-12, 29 PUSA 0672, 24 ML-233,7 GGG 10-14, 17 IPM 9901-6 and 8 GM 04-02, respectively on dry weight basis (Table 1). Conversely this amount was decreased in all cases than healthy plants by 61.78, 44.45, 41.14, 34.79, and 1.57 but the increase in resistant variety 8 GM 04-02 was 39 percent due to root-knot infection (Fig.1).

Table 1 Percentage increase /decrease in total sugar content in healthy (H) and root-knot infected (I) plants

Sl. No.	Variety	Total sugar content mg/g on fresh weight basis			
		Infected seed	Healthy seed	Mean seed	% increase(+)/ decrease(-)over control
1	28 PM 10-12	8.12	21.25	14.68	-61.78
2	29 PUSA 0672	9.37	16.87	13.12	-44.45
3	24 ML -233	6.25	10.62	8.43	-41.14
4	7 GGG 10-14	9.37	14.37	11.87	-34.79
5	17 IPM 9901-6	6.25	6.35	6.3	-1.57
6	8GM 04-02	6.95	5	5.97	39
	SEM(±)	**2.16**	**3.64**		
	CD(0.05)	**6.81**	**11.47**		

Total starch in seeds

The amount of starch present in the seeds of the inoculated plants were recorded as 6.25 , 7.5, 2.5, 12.5, 35, and 35 per cent in the varieties of 28 PM 10-12, 29 PUSA 0672, 24 ML-233,7 GGG 10-14, 17 IPM 9901-6 and 8 GM 04-02, respectively on dry weight basis (Table 2). Conversely this amount was decreased in all cases than healthy plants by 83.87, 75, 93.55, 37.5, 9.67 and 71.13 per cent in the seeds due to root-knot infection (Fig.2).

Table 2 Percentage increase /decrease in total starch content in healthy (H) and root-knot infected (I) plant

Sl. No.	Variety	Total starch content mg/g on fresh weight basis			
		Infected seeds	Healthy seeds	Mean seeds	% increase(+)/ decrease(-) over control
1	28 PM 10-12	6.25	38.75	22.5	-83.87
2	29 PUSA 0672	7.5	30	18.75	-75
3	24 ML -233	2.5	38.76	20.63	-93.55
4	7 GGG 10-14	12.5	20	16.25	-37.5
5	17 IPM 9901-6	35	38.75	36.88	-9.67
6	8GM 04-02	35	121.25	78.12	-71.13
	SEM(±)	**3.87**	**25.87**		
	CD(0.05)	**12.19**	**81.52**		

CONCLUSIONS

Sugar is the prime source of metabolic energy in all living organisms. Perusal of data clearly envisaged the significant increase in the quantity of total sugars in the roots of nematode inoculated plants but the same was decreased in the shoots of nematode infected plants. Increase in sugar contents following nematode infection is in confirmation with the findings of earlier workers (Ganguly and Dasgupta, 1983(3), Mohanty & Pattnaik, 1993(4). The increased sugar content in the infected samples might be due to movement of various metabolites towards the infection site from other parts of plants. Alternatively more of these metabolites are produced by cell at the infection site as a result more of carbohydrates are required for respiration and metabolism. The total sugar in the shoots of the healthy plants increased but reducing sugars decreased. Increase in total sugar due to higher metabolism in the hypertrophied cortical and endodermal cells of infected plants (Nayak, 2006(5) and Nayak et al.,2016(6).

ACKNOWLEDGEMENT

Authors are thankful to those who helped in the chemical analysis of plant samples for different parameters ,Project co-ordinator and Principal Scientist(Nematology) IIPR, Kanpur for providing seed materials to conduct this investigation.

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