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Impact of Sugar Beet Cyst Nematode, *Heterodera schachtii*, on Some Physiological Aspects of Two Sugar Beet Cultivars, Nemakill and 7233, in the Rhizosphere Condition

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Abstract: The impact of beet cyst nematodes, *Heterodera schachtii*, on some physiological aspects including proline, potassium and sodium in two sugar beet cultivars, a susceptible 7233 and a resistance Nemakill was evaluated in the rhizosphere condition. In this study a bioassay was conducted with sugar beet seedlings in the presence of different populations of the beet cyst nematodes, 0, 10, 20 juveniles 1^{-1} g. soil as a biostressor in glass tubes (20×3 cm Ø) in growth chamber, totally 36 tubes. The tubes were placed in a growth chamber with 16-h artificial light, eight fluorescent 40 watts, at 25 and 21°C at darkness and moistened regularly with a half Hoagland's nutrient solution. The proline, potassium and sodium changes of treated and untreated plants were measured 45 days after nematodes' inoculation. In this stage, the root of plant was freed from soil and debrises by a fine jet of water stream and the number of developed females of nematodes counted on the root surface and recorded. Results of this study revealed that the proline in leaves, sodium in leaves and roots as well as potassium in roots were increased but K^+ decreased in leaves in both cultivars as the population of nematodes increased in the soil. Results of this study indicated that a few number of nematodes' females was developed on Nemakill's roots in comparison with the other treated and untreated susceptible cultivar in the rhizosphere condition.

Key words: *Heterodera schachtii*, proline, rhizospher, potassium, sodium, sugar beet cvs, nemakill, 7233

INTRODUCTION

Beet cyst nematode, *Heterodera schachtii* schmidt 1871, has been recognized as an important pest of sugar beet and some other crops throughout the temperate zones of the world for over 100 years. It causes serious stand and yield reductions wherever sugar beet is grown in the infested fields. Today, *H. schachtii* is present in 39 sugar beet growing countries throughout the world. It is the most serious nematode pest of sugar beet and capable of causing severe losses (Cook, 1984; 1987; 1992 a, b). In Iran, occurrence of this nematode on sugar beet has been first recorded from Khorassan province by Schaefer and Esmail-poor in 1969 (Hojat-Jalali and Coosemans, 1997).

It was suggested that proline accumulates in many plants species during adaptation to various types of environmental stress such as drought, salinity, high temperature, nutrient deficiency and exposure to heavy metals and high acidity. Proline is accumulated as osmotic regulators in many plants under water stress (Krauss, 1999; Rhodes *et al*., 1999; Brown and Simpson, 1972; Aspinall and Paleg, 1981). Also studies of Ghasempour and Kianian (2002) showed that free proline is produced and accumulated in response to the drought stress. Heber and Santarious (1964) suggested that in many plants species ions concentration increased to environmental stress such as water deficit. Nevertheless, little information is available on proline, potassium and sodium contents of sugar beet plants due to the different population of *Heterodera schachtii* as biostressor in the rhizosphere condition.

Aims of this study were: 1) To evaluate changes in some physiological aspects including proline, potassium and sodium contents in leaves and roots of two sugar beet cultivars, a sensitive cv. 7233 and a resistant cv. Nemakill, in soils infested with different populations of *Heterodera schachtii*. 2) To study the number of the beet cyst nematodes developed on the roots of two cultivars in the presence of three different populations of nematodes in the rhizosphere condition.

MATERIALS AND METHODS

Extraction of cysts of beet cyst nematodes: A soil sample of the village sultan-abade sarhang was used to extract

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cysts of *Heterodera schachtii* for trials. This sample was collected in late December 2003 from sugar beet fields of Chenaran, near Mashhad in Khorasan province (in eastern part of Iran), with different crops rotation. The sample was composed of eight cores from one hectare which were collected from depth of 10 to 30 cm around the roots of sugar beet plants using an auger. The time of sampling coincided with the third generation of the beet cyst nematode in the region (Hoja Jalali *et al*., 1998). A subsample of 500 g air dried soil was washed by using the Fenwick (1940) can method to extract cysts of nematodes. The number of cysts counted under a stereomicroscope and recorded. Then after the cysts were collected in an Eppendorf microtube and preserved at 4°C until it was required.

Seeds sterilization of sugar beet cvs. 7233 and Nemakill: The sugar beet cv. 7233 has polygerm seed and the Nemakill has monogerm seed, it is coated with fungicides, insecticides and colored. In this study seeds of Nemakill was donated by Swedish Hilleshog Company agent in Iran. Seeds of sugar beet cultivars, Nemakill and 7233, were surface-sterilized by using a technique of Sijmons *et al*. (1991). In this method seeds of sugar beet were shaken in 7% (w/v) calcium hypochlorite for 10 min and rinsed several times with the sterile distilled water. The seeds submerged in 70% (w\v) ethanol and subsequently rinsed three times with sterile distilled water.

Six sterilized seeds of each cultivar were placed aseptically on 0.7% water agar in a petri dish (9 cm Ø) and replicated ten times. The plates were incubated at 20° C in darkness and checked for development of any contamination of fungi and bacteria to be removed. The healthy seedlings of sugar beets were used as in the next step of the bioassay.

Viability test of nematodes and preparation of nematodes' inoculum: The viability of juveniles of nematodes was determined by using 30 randomized cysts on a sterile hatching apparatus devise containing 2 mL zinc chloride (4 mM) as an artificial hatching agent (Grok and Claussen, 2001) and the treatment was replicated six times. The hatching device consisted of a 150 μm aperture sieve stitched in the cut bottom of an eppendorf microtube which was mounted into another eppendorf microtube and sealed by a parafilm (Hojat-Jalali *et al*., 1998). The tubes were incubated at 20°C in the darkness. The number of second stage juveniles of nematodes emerged from cysts were counted by using a counting dish and recorded after one month. Finally, in order to have an appropriate number of viable juveniles to use them as nematodes' inoculum, a bulk number of cysts was used in hatching device as it was already described.

***In vivo* experiment**

Setting a rhizosphere bioassay: A bioassay was conducted in the disinfected glass tubes (20×3 cm Ø), totally 36 tubes. Each tube was filled with 30 g double autoclaved soils with 5 days interval. The soil was a mixture of loamy soil with the following properties: %saturation 39, electric conductivity 1.88, pH 7.6, %organic carbon 1.21, potassium average 490 ppm, phosphorus average 34.4 ppm, total nitrogen 0.21. Each tube received a sterile seedling of sugar beet with two cotyledon leaves in the center which was prepared from step 2.2.1. The tubes were moistened and placed in a growth chamber with 16 h artificial light, eight fluorescent 40 watts, at 25°C and 21°C at darkness.

Three weeks later, the sugar beet plants in four-leaf stage were inoculated with three different populations of nematodes, 0, 10 and 20 juveniles 1^{-1} g soil. Each treatment replicated six times and arranged in a randomized complete design in the growth chamber condition as it was already described (Fig. 2). The plants were moistened regularly with a half Hoagland′s nutrient solution.

The sugar beet plants were harvested after 45 days of nematodes′ inoculation. In order to extract the female of nematodes, the soil of each tube was washed from roots of plants with a vigorous fine jet of water stream on a 200 μm aperture sieve. The females of nematodes were collected manually with a fine-tipped forceps from roots and debris using a stereomicroscope (Olympus SZX12), recorded and preserved in appendorfe micro tubes. The roots and leaves of treated and untreated sugar beet plants were preserved at 4°C and used to measure different physiological aspects of plants as already described.

Measurement of cholorophll, proline, potassium and sodium in plants: Fresh leaves and tap roots of treated and untreated sugar beets were rinsed with distilled water several times, dried by filter papers and incubated in an oven at 70°C for three days until to be dried. The dried leaves and tap roots of plants were powdered by using a mortar and pestle separately.

The chlorophylls were determined by a method of Wellburn (1994) with a slightly modification. In this method a 0.2 g of powdered sugar beet leaves of each cultivar was mixed with 3 mL of 5% SDS (sodium dodecyl sulphate) and brought to 8 mL by adding distilled water.

The solution was centrifuged at 10000 rpm and the supernatant was read at 645 and 663 nm by using a Bausch and Lamb spectrophotometer mode l 70.

To measure proline, potassium and sodium contents, 0.1 g of dried leaves or roots of two cultivars, 7233 and Nemakill, was separatly added to 10 mL of 3%sulfu salicylic acid and after 48 h filtered through Whatman filter paper No. 1. The proline content of samples were assessed with a method described by Bates *et al.* (1973) with a slightly modification. In this step, the optical density of samples were measured at 520 nm by using a Bausch and Lomb spectrophotometer model 70 and recorded.

Potassium and sodium contents in leaves and roots of each sample were measured by a flame photometry method (Jenway PFP7 model) and recorded.

RESULTS

The soil sample of the village sultan-abade sarhang had a high number of cyst nematodes and it was 3 cysts L^{-1} g soil. The mean viability of juveniles emerged from cysts nematodes in hatching test was 400±99.

The chlorophyll contents, a and b, in both sugar beet cultivars in the presence of *Heterodera shachtii* decreased significantly as the nematode population increased (p = 0.01), while, the chlorophyll contents in susceptible cultivar was lowest than resistant ones (Fig. 1).

The proline content in leaves of sugar beet in resistant and susceptible plants cultivar was significantly different by using LSD (p = 0.01). This result indicated that the stressed sugar beet plants by three different populations of the beet cyst nematodes: 0, 10 and 20 juveniles 1^{-1} g. soil accumulated greater amounts of proline as nematode population increased (Fig. 2).

The results of potassium content in roots and leaves of treated and untreated sugar beet plants was significantly different by using LSD (p = 0.01). Potassium concentration in leaves of two untreated cultivars was the highest than treated plant with nematodes. There was a significant difference in potassium contents in leaves of two susceptible and resistance cultivars when the nematode population increased. The lowest concentration of potassium in leaves was in susceptible cultivar

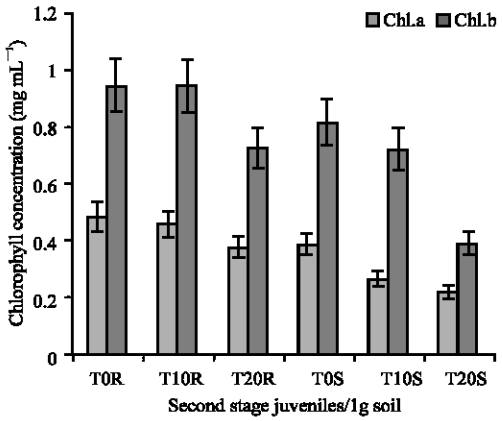

Fig. 1: Comparison of chlorophylls concentration in the leaves of two sugar beet plant cultivars, 7233 and Nemakill, treated with 0, 10 and 20 juveniles of sugar beet cyst nematodes, *Heterodera schachtii* 1^{-1} gr soil in the rhizosphere conditions. Number of replications = 6; R = resistance; S = susceptible; LSD (p = 0.01)

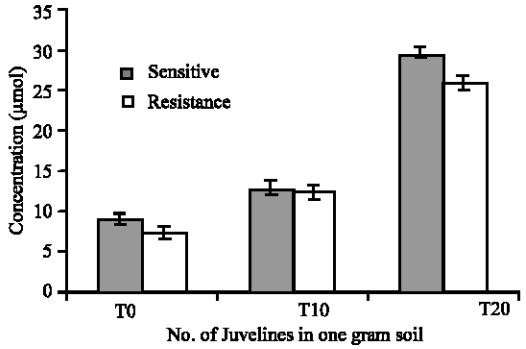

Fig. 2: Comparison of free proline concentration in the leaves of two sugar plant cultivars, 7233 and Nemakill, treated with 0, 10 and 20 juveniles of sugar beet cyst nematodes, *Heterodera schachtii* 1^{-1} g. soil in the rhizosphere conditions, Number of replications = 6; R = resistance; S = susceptible; LSD (p = 0.05)

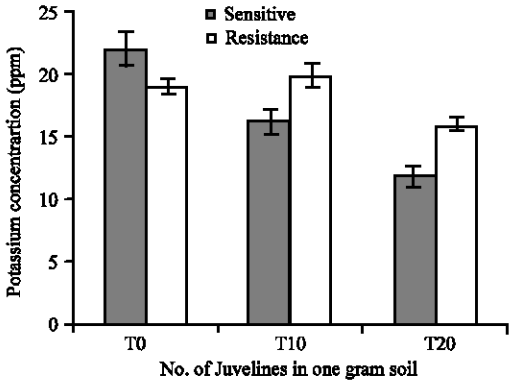

Fig. 3: Comparison of potassium contents in the leaves of two sugar plant cultivars, 7233 and Nemakill, treated with 0, 10 and 20 juveniles of sugar beet cyst nematodes, *Heterodera schachtii* 1^{-1} gr soil in the rhizosphere conditions, Number of replications = 6; R = resistance; S = susceptible; LSD (p = 0.05)

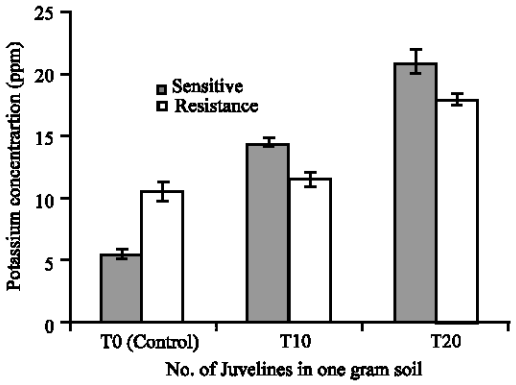

Fig. 4: Comparison of potassium contents in the roots of two sugar plant cultivars, 7233 and Nemakill, treated with 0, 10 and 20 juveniles of sugar beet cyst nematodes, *Heterodera schachtii* 1^{-1} g. soil in the rhizosphere conditions, Number of replications = 6; R = resistance; S = susceptible; LSD (p = 0.05)

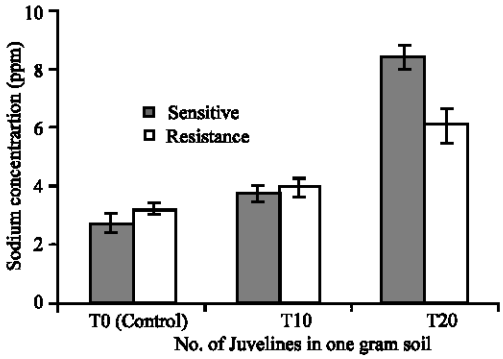

Fig. 5: Comparison of sodium contents in the leaves of two sugar beet plant cultivars, 7233 and Nemakill, treated with 0, 10 and 20 juveniles of sugar beet cyst nematodes, *Heterodera schachtii* 1^{-1} g soil in the rhizosphere conditions, Number of replications = 6; R = resistance; S = susceptible; LSD (p = 0.05)

than resistant plants (Fig. 3). In contrast, the potassium content of roots in both cultivars increased as the population of beet cyst nematode became higher (Fig. 4).Data of sodium contents in roots and leaves of two cultivars was significantly different by using LSD (p = 0.01). In leaves or roots of sugar beet plants, the sodium concentration in two cultivars was increased when the nematode population reached to the highest number, 20 juveniles 1^{-1} g soil (Fig. 5 and 6). Although, the sodium concentration in leaves and roots differed between two cultivars, but the highest concentration appeared in the susceptible cultivar.

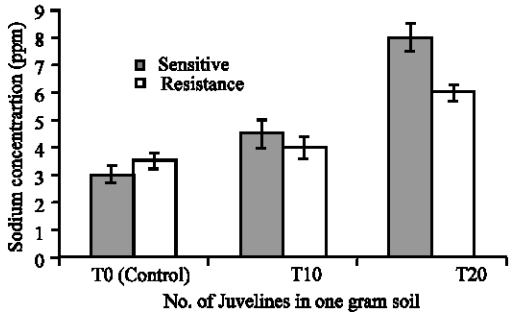

Fig. 6: Comparison of sodium contents in the roots of two sugar beet plant cultivars, 7233 and Nemakill, treated with 0, 10 and 20 juveniles of sugar beet cyst nematodes, *Heterodera schachtii* 1^{-1} g. soil in the rhizosphere conditions, Number of replications = 6; R = resistance; S = susceptible; LSD (p = 0.05)

Table 1: Number of developed females of sugar beet cyst nematode, *Heterodera shachtii*, on roots of two cultivars, Nemakill and 7233 on the rhizosphere condition

	Cultivars	
	Nemakill	7233
Treated No. of juveniles in (1^{-1} g soil)	Mean of developed females	Mean of developed females
10 J_2	2.33 ± 2.06	8.66 ± 4.54
20 J_2	6.66 ± 2.73	25.66 ± 6.43

Results of this experiment revealed that there were significant difference in developed females on roots of two cultivars, nemakill and 7233. The highest number of females of nematodes developed on the roots of susceptible cultivar when it was treated with 20 juveniles 1^{-1} g soil (Table 1).

DISCUSSION

In this study, the results of chlorophylls' measurement in two sugar beet cultivars, 7233 and Nemakill, indicated that as the nematode population increased in the roots, the chlorophyll concentration declined in susceptible cultivar more than that of the resistant ones, 45 days after nematodes inoculation. These results confirmed the results achieved by Melakeberhan *et al*., (1985a) and Melakeberan and Feris (1988) in *Phaseolus vulgaris* and *Vitis vinifera* cultivars, in which a significant decline of chlorophyll begun at seven days after nematodes' inoculation.

Increase in proline concentration of leaves was proportional to the nematode populations in two cultivars but it was higher in susceptible sugar beet plants. Proline accumulation is a common metabolic responses of higher

plants to water deficits and salinity and has been the subject of numerous reviews (Taylor, 1996; Rhodes *et al*., 1999). Amino acid proline have been particularly considered to contribute to osmotic adjustment in endophyte-infected grasses (Malinowski and Belesky (2000). Hanson *et al*. (1977) reported that, the proline increased more in a drought-sensitive than in a resistant cultivar of water stressed barley. It was suggested that the proline content of tomato leaves was a suitable marker for stress induced by both abiotic and biotic factors (Grok and Claussen, 2001). According to our results, in the presence of sugar beet cyst nematode, proline increased more in the leaves of susceptible cultivar, 7233, than in resistant nemakill.

Potassium is involved in numerous functions in the plant such as in enzymes activation, cation/anion balance, stomatal movement, phloem loading, assimilate translocation and turgor regulation to name only a few. There is a severe positive correlation between root biomass production of sugar beet and ammoniacal nitrogen, Na^+ and K^+. In contrast, the percentage of recoverable sugar in plants has negative correlation with accumulation these three factors in roots (Aspinall and Paleg, 1981). Potassium deficient leaf cells accumulate substantial quantities of low molecular weight organic compounds because they act as an osmoticum in the absence of sufficient K (Krauss, 1999). Also, our results indicated that, as the number of nematodes increased in sugar beet plants, the proline content altered to higher amount and potassium and sodium of the leaves declined and K shifted to the roots, but the decline of potassium in nemakill was lower than that of the 7233 cv. Therefore, the increase of K in the root and its contemporaneous substantial accumulation along with sodium in roots might be an indication of its role as an osmoregulator in infected sugar beet plants by nematodes. Also, potassium and sodium may help neighbouring cells to adjust the osmotic imbalances caused by nematode feeding from the syncetium. Sijmons *et al*. (1991) calculated that developing juveniles of *Heterodera shachtii* withdrew daily from syncytium an amount equivalent to four times the total volume of their syncytia. Thus, the juvenile demand for water and the solutes is high and their permanent feeding from syncytium induces strong metabolic sink in the host plant. Syncytia associated with female nematodes are connected to the phloem by functional and GFP-permeable plasmodesmata. Accordingly, it is an important physiological implication that phloem-mobile nutrients are unloaded symplasmically into syncytia of females. Plasmodesmata to surrounding root cells are closed by deposited secondary material and therefore restrict nutrient efflux. Supported by sugar transport proteins this system maintains high solute concentration within the syncytium and supplies the necessary nutrients for female development (Hofmann and Grundler, 2006). Syncytia were found to have higher concentrations of solutes and water compared with other root tissue as revealed by their higher turgor pressure and their low water potential (Bockenhoff and Grundler, 1994; Melakeberhan, 2004).

The influence of nutrition on resistance and tolerance of host plants to pests and diseases is very complex. Therefore, in this study, the accumulation of proline in leave tissues, potassium in roots and sodium in both roots and leaves of sugar beet plants in response to biostressor, H. schachti, might help to maintain turgor and facilitates physiological and biochemical processes of damaged plants. Also, Nemakill is a resistance cultivar to the sugar beet cyst nematodes. Results of this study revealed that this cultivar could only support the developmental of a few number of nematodes in the rhizosphere condition.

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