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## Physiological Changes, Proline, Total Protein, Protein Analysis and Potassium of the Sugar Beet Plants in Response to Beet Cyst Nematodes, *Heterodera schachtii*

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**Abstract:** In this study, several physiological parameters including proline, total protein, protein analysis and potassium changes due to the biostress induced by different populations of the beet cyst nematode, 0 (control), 10 and 20 J2 L<sup>-1</sup>g soil on sugar beet plant were measured in the rhizosphere condition. Results of this bioassay indicated that infected plants showed higher foliar accumulation of proline as well as higher root accumulation of potassium in comparison to untreated control. Then, the leave's potassium decreased, as the beet cyst nematodes population increased from 10 to 20 J2 L<sup>-1</sup> g soil. The total protein in the leaves decreased when the population of juveniles was 10 J2 L<sup>-1</sup> g soil but it increased in 20 J2 L<sup>-1</sup> g soil which may be due to the stress caused by the higher population of nematodes. Protein analysis showed shifts in the number of protein bands which were almost similar in both leaves and roots as the population of the nematodes increased. The increased proline and total protein in the leaves due to the higher population of nematodes were proportional to Gel electrophoresis analysis using UV. Doc program. When the population of nematodes increased, the potassium decreased in the leaves and increased in the roots. These might be indication of adaptive osmuregulation or acclimations responses in plants to the nematodes biostress by increasing metabolites and solutes.

**Key words:** *Heterodera schachtii*, total protein, proline, gel electrophoresis rhizospher, potassium, biostress, sugar beet

### INTRODUCTION

Beet cyst nematode, *Heterodera schachtii*, (Schmidt) has been recognized as an important pest of sugar beet and 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 infested areas (Cooke and Scott, 1993).

One of the major problems among soil borne and plant pathogens in Iran which constitute the limiting factors for the successful sugar beet production is the sugar beet cyst nematode. *Heterodera schachtii*, is thought to be indigenous to Iran (Eshtiaghi, 1988; Schifer and Esmailpour, 1969). It is a devastating pest of sugar beet and possesses a potential to threat the sugar beet industries in the four main infested areas (Hojat-Jalali, 1998; Hojat-Jalali and coosemans, 1997; Julia and Grundler Florian, 2006).

It is well known that proline accumulates in plants during adaptation to various types of environmental stress such as drought, salinity, high temperature, nutrient deficiency and exposure to heavy metals and high acidity (Grote and Claussen, 2001). Also, free proline is produced and accumulated in response to biostress

(Ghasempour and Kianian, 2002; Ghasempour and Maleki, 2003).

The present study were conducted to evaluate several physiological parameters including proline, total protein, protein analysis and potassium changes due to the biostress induced by different populations of the beet cyst nematode infestation in sugar beet plants, cultivar's 7233 in rizosphere condition.

### MATERIALS AND METHODS

**Soil sampling and extraction of beet cyst nematode:** In this study ten soil samples were collected in late Dec. 2001 in sugar beet fields of Chenaran in Khorasan provience with different crop rotations. The time of sampling coincided with the third generation of the beet cyst nematode in the region (Kalali and Farivar-Mahin, 1977). Each sample consisted of eight cores from one hectare which were taken from a depth of 10 to 30 cm around the roots of sugar beet plants by means of an auger. A sub sample of 250 g of air dried soil was used in order to extract the cysts of nematodes by using Fenwick (1940) method. The extracted cysts were preserved in eppendorf micro tubes at 4°C until needed.

### **Conducting *in vivo* test**

**Sterilization of seeds of sugar beet plant cv. 7233:** Seeds of sugar beet plant cv. 7233 were surface sterilized by using a technique of Sijmons *et al.* (1991). Six sterilized seeds were placed aseptically on a petri plate (9 cm Ø) containing 0.7% water agar and replicated. The petri plates incubated at 25°C and checked regularly for development of any contamination. The uncontaminated seedlings with two cotyledon leaves were used as sugar beet seedlings in the rhizosphere bioassay.

**Preparation of nematode inoculum:** In this connection a bulk of cysts of *Heterodera schachtii* was used in hatching devices. The cyst were disinfected by 0.001 HgCl solution for five minutes and rinsed several times. The hatching device consisted of a 150 µm aperture nylon sieve stacked to the cut bottom of an eppendorf micro tube which was mounted into another eppendorf micro tube and sealed by a Parafilm. The device received 2 mL solution of zinc chloride (4 mM) as hatching agent of nematodes and incubated at 20°C in darkness. One week after hatching of nematodes a suspension of 100 juveniles of nematodes mL<sup>-1</sup> of sterile distilled water was prepared as nematode inoculum.

**Rhizosphere bioassay:** Bioassay was conducted in autoclaved glass tube (20×3 cm Ø) containing of 30 mL sterile soil. The soil consisted of a mixture of 3:2 (clay, sandy-loam soil) and it was autoclaved twice in 121°C for 45 min. with a five day interval. Sterile seedling was planted immediately in a hole in the centre of soil. The plants were moistened and incubated in growth chamber condition under 16 h of artificial light at 25 and 20°C in day and night, respectively.

Three weeks later, the glass tubes with a seedling of four leaves stage were inoculated with three different populations of the second stage juveniles of nematodes, 0, 10, 20 J2 L<sup>-1</sup> g soil, respectively. Treatments were replicated six times and arranged in a completely randomized design in growth chamber conditions. The plants were watered regularly with a modified half Hoagland's nutrient solution.

After 45 days which coincided with development of the first generation of the beet cyst nematodes, the roots of sugar beet plants in tubes were washed completely with a vigorous water stream on to a 200 µm aperture nylon sieve. The cysts of nematodes were collected and recorded.

The leaves and roots of the sugar beet plant in different treatments were harvested and used as plant materials for other steps of studies.

### **Evaluation of treated and untreated plants**

**Assessment of proline in leaves:** Proline of leaves was determined by Bates *et al.* (1997) method data were measured at 520 nm by Bausch and Lomb spectrophotometer 70. A standard curve; 0, 1.9, 7.8, 15.62, 31.25, 62.5 and 125 µg of proline was prepared. Proline content of treated and untreated extracts was calculated by using the standard curve and recorded.

**Assessment of potassium in leaves and roots:** Fresh leaves and roots of treated and untreated sugar beets were powdered and processed as mentioned above. In this assessment a 0.1 g of each sample was treated with 10 mL of 3% sulfur salicylic acid and filtered through a whatman filter paper No.1 for 48 h. The solution was diluted with distilled water and potassium measured by using a flame photometer (Jenway model. PFP7). In order to prepare a standard curve of potassium, a 0, 0.1, 1, 10, 20 and 100 ppm of potassium chloride solution was used. The potassium content of treated and untreated extracts was calculated by using the standard curve and recorded.

**Measuring the total protein:** In order to quantify the total protein of leaves in each sample, a 0.05 g of dry weight of leaves was assessed by Lowry *et al.* (1951) method. The total protein was determined with Folin reagent and the color compared with Bovine Serum Albumin (BSA), serving as the standard for determining protein content, read at A660 (OD) and recorded.

**Protein SDS-PAGE and gel electrophoresis analysis:** Peterson (1997) method was used to determine the protein concentration of fresh leaves and roots of treated and untreated plants. For one-dimensional SDS-PAGE the supernatant of samples was diluted with UKS-buffer (9.5 M Urea, 5 mM K<sub>2</sub>CO<sub>3</sub>, 1.25% (W/V) SDS) (1:1). For each well 20 µL was applied, totally 6 wells. A Hoeffer SE 600 vertical unit was employed and coomassie blue used for staining.

In order to analyse molecular weight and mobility of proteins bands the UV. Doc program was used and molecular weight of marker bands entered to the program. The program gives the molecular weight of the bands on the stained gel.

## **RESULTS**

The cysts extracted from soil sample of Chenaran in Khorasan province was high enough, 14 L<sup>-1</sup> g and it was used as nematodes inoculum for bioassay.

Results of the bioassay indicated that the nematodes were multiplied properly on the roots of sugar beet plants

in glass tubes. The Populations of the beet cyst nematodes, *H. schachtii* collected around the roots were  $0.7 \pm 2$  and  $37 \pm 8$  which were inoculated with three different populations of the second stage juveniles of the beet cyst nematodes, 0, 10, 20 J2 L<sup>-1</sup> g soil, respectively.

The proline content in leaves of sugar beet plants was significantly different at 0.05 levels by using Tukey test. This result indicated that the stressed sugar beet plants by two different populations of the beet cyst nematodes, 10 and 20 juveniles L<sup>-1</sup> g soil accumulated greater amounts of proline than the control as nematodes population increased (Fig. 1).

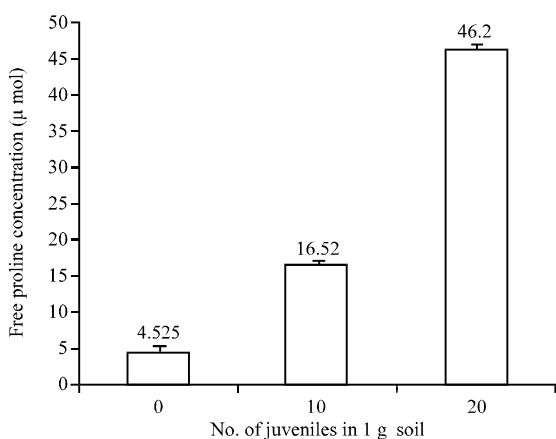


Fig. 1: Comparison of free proline contents in leaves of sugar beet plants infected with three different population of *Heterodera schachtii* 0, 10 and 20 J2 in L<sup>-1</sup>g soil in the rhizosphere area (the bars represent standard error)

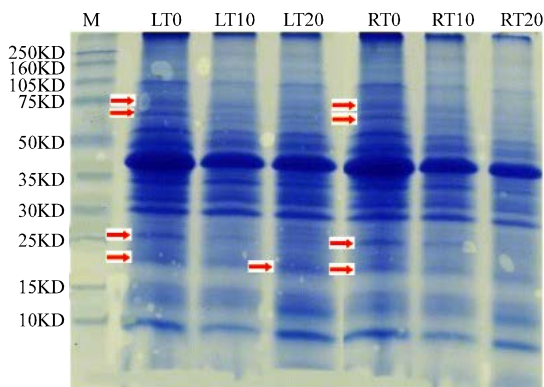


Fig. 2: SDS-PAGE gel electrophoresis of protein profile in leaves and roots of sugar beet plants infected with three different population of *Heterodera schachtii* 0, 10 and 20 J2 in L<sup>-1</sup> g soil in the rhizosphere area

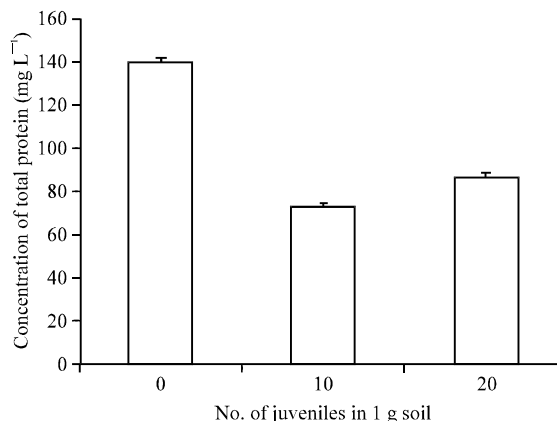


Fig. 3: Comparison of the total protein in leaves of sugar beet plants infected with three different population of *Heterodera schachtii* 0, 10 and 20 J2 in L<sup>-1</sup> g soil in the rhizosphere area

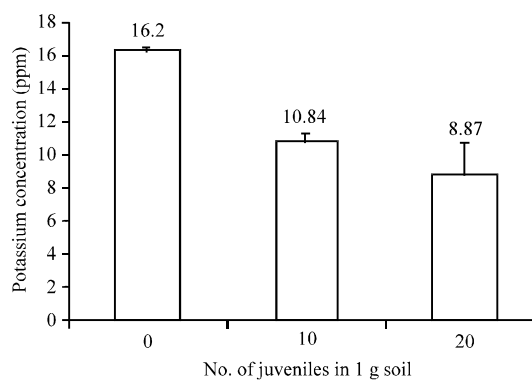


Fig. 4: Comparison of potassium contents in leaves of sugar beet plants infected with three different population of *Heterodera schachtii*; 0, 10 and 20 J2 in L<sup>-1</sup> g soil in the rhizosphere area

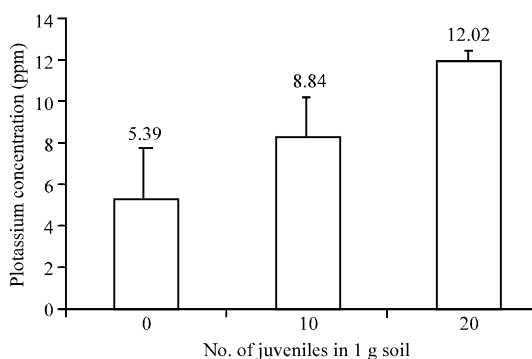


Fig. 5: Comparison of potassium contents in roots of sugar beet plants infected with three different population of *Heterodera schachtii* 0, 10 and 20 J2 in L<sup>-1</sup> g soil in the rhizosphere area

The data of potassium contents in roots and leaves of treated and untreated sugar beet plants was significantly different at 0.05 levels by using Tukey test. These results indicated that the roots of stressed sugar beet plants by two different populations of the beet cyst nematodes, 10 and 20 juveniles L<sup>-1</sup> g soil accumulated greater amounts of potassium than unstressed control as nematodes population increased (Fig. 5), in contrast the leaves potassium decreased (Fig. 4).

The sites of the proteins bands were obtained with UV. Doc program analysis. Results of electrophoretic analysis showed that 16 bands were exist in leaves and roots of untreated plants with the range of 370 to 9.7 kDa (Fig. 2). In leaves and roots of treated plants with 10 and 20 juveniles of nematodes, the numbers of protein bands were similar but in comparing with untreated plants the bands No. 5, 6, 13 and 14 were disappeared. The protein bands with molecular weights of 75.9, 66.5, 25.5, 21.2 kDa were present in leaves and roots of untreated plants but not in the treated plants. The exception band, No. 14, was present in leaves of treated plants with 20 juveniles of nematodes with molecular weight of 21.4 kDa. The shift of protein bands in treated plants compared with untreated plants also correlates with the amount of total protein obtained in this experiment (Fig. 3).

## DISCUSSION

Proline accumulation is a common metabolic responses of higher plants to water deficits, salinity and has been the subject of numerous reviews (Taylor, 1996; Rhodes *et al.*, 1999; Yan *et al.*, 2000). Accumulation of solutes in tissues in response to drought helps maintain turgor and facilitates physiological and biochemical processes. Amino acid proline have been particularly considered to contribute to osmotic adjustment in endophyte-infected grasses (Malinowski and Belesky, 2000; Kishor *et al.*, 2005).

In turgid leaves, oxidation of proline occurs to maintain low levels of cellular proline, but in stressed plants, proline concentration increases, because proline oxidation is inhibited or the rate of proline biosynthesis does increase (Stewart and Lee, 1974; Boggess *et al.*, 1976). It was suggested that the proline content of tomato leaves was a suitable marker for stress induced by both abiotic and biotic factors (Grote and Claussen, 2001).

This study indicates that there is a relationship between proline concentration and number of nematodes developed on the roots of sugar beet plants. In this case the damaged roots and root hairs of sugar beet plants by biostressor, *H. schachtii*, might cause a situation for

infected plants to act similar to the water stressed plants. Therefore, as the number of nematodes increased, the proline content of the sugar beet plants altered to higher amount in the rhizosphere condition.

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.

In K-sufficient sugar cane 29% of <sup>14</sup>C<sub>2</sub> fed to a leaf was incorporated into sugar and exported into the stalk below the leaf but less than 5% in K deficient plants. Potassium deficient leaf cells accumulate substantial quantities of low molecular weight organic compounds because they maintain osmotic balance in the absence of sufficient K (Krauss, 1999).

There is a severe positive correlation between root biomass production of sugar beet and amonical nitrogen, Na<sup>+</sup> and K<sup>+</sup>. In contrast, the percentage of recoverable sugar in plants has negative correlation with those three factors in roots (Alimoradi, 1987; De Lacerda *et al.*, 2003).

As potassium decrease in the leaves, its contemporaneous substantial accumulation in the roots might be an indication of its role as an osmoregulator in infected sugar beet plants. Potassium may help neighbouring cells to adjust the osmotic imbalances caused by nematode feeding from the damaged cells. Also, as an strategic ion for a biennial plant, potassium accumulation in root may be vital for completion of sugar beet plant life cycle. In this regard, potassium shifts from leaves to roots.

Results of SDS-PAGE are commonly employed in biological analysis to determine shifts in protein bands. These bands might be proteins or enzymes. Biostress, due to hormonal changes could cause protein synthesis and enzymatic shifts (Ghasempour *et al.*, 1998; Ghasempour *et al.*, 2001; Ghasempour and Kianian, 2002; Ghasempour and Maleki, 2003; Gianello *et al.*, 2000). Disappearance of protein bands in treated plants with different population of beet cyst nematodes might be due to heavier infestation and its consequent enzymatic changes in comparing to untreated plants. All these shifts and changes in protein bands might be harmonious with shifts in potassium and proline biosynthesis due to infection by sugar beet cyst nematodes.

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