Effects of different Trichoderma harzianum strains on growth and production of secondary metabolites in Trigonella foenum-graecum

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INTRODUCTION

Trigonella foenum graecum, commonly known as Fenugreek, is one of the most widely used medicinal plants in folk medicine and native to the mediterranean region, southern Europe, and western Asia (Baquer et al., 2011). Seeds of fenugreek have a wide range of non-therapeutic uses. It is used as a natural source to enhance flavor, color and texture of food (Yadav and Baquer, 2013). Resent researchers have identified a number of health benefits and physiological attributes in both experimental animals as well as clinical trials in humans.

OBJECTIVE

There is no report about use of Trichoderma as an elicitor to increase Fenugreek growth characteristics. The major objective of this study was to investigate the effect of two strains of Trichoderma added to plant cultures for finding possible plant-fungus interactions that may be effective on growth characteristics and trigonrllin accumulation of two Fenugreek genotypes.

MATERIALS AND METHODS

Plant material: Seeds of Fenugreek were supplied from two province of Iran (Hamedan and Bandarabbas). The seeds were cultivated at a depth of 2-3 cm in pots which were filled with Soil (50%) containing peat (25%) and perlite (25%). The pots were placed in a greenhouse for 10 days under partially-controlled temperature and relative humidity.

Fungal material: *Trichoderma harzianum* isolates (chit4215MK andT8-7MK) were used in this study. The isolates were obtained from Agricultural Biotechnology Research Institute (ABRII) Gene Bank. All of these isolates have been prepared from soil collected from different area of Iran. Trichoderma isolates were grown on potato dextrose agar medium (PDA) for five days at 25 ^{C} in the dark and then transferred to light conditions for two days. Following fungus colonies Extraction and quantitative analysis of trigonellin.

Determination of total soluble carbohydrate: For total soluble sugars content determination, 0.02 g of leaf tissue (15 leaves from the stem tip) was taken from plants. Soluble sugars content was measured according to Dubois et al. (1956).

Assay of antioxidant enzyme and protein: After Preparation of extracts with 0.05 g plant leaf tissue (15 leaves from the stem tip) or root, peroxidase activity was measured by method of Maehlyand Chance (1954). Also, total protein content of leaves was determined by Bradford method (1976), using bovine serum albumin (BSA) as a standard (Bradford 1976).

Determination of Chlorophyll content

Root colonization: At the end of the growing season, the roots were collected from a depth of 5 to 10 cm. The roots thoroughly rinsed with water. Then the roots were transferred to Falcon containing KOH (10 %) in a water bath at 70 ° C for 20 minutes. After that, the roots were washed and to coloring taken in a cotton blue solution container for 24 hours at room temperature. After coloring, the roots were washed again with distilled water and the longitudinal and transverse slices were prepared. Then microscopic slides prepared from them and were examined under a microscope (Nikon ECLIPSE E600, X20) (Gamalero et al., 2003).

RESULTS AND DISCUSSION

Effects of Trichoderma strains on growth characteristics: It is well documented that some strains promote plant growth, increase nutrient availability, improve crop production and enhance disease resistance (Harman et al., 2004). The results showed that, stem length of two ecotypes (Hamedan and Bandarabbas) were different in10-day non-treated plants. As shown in Fig. 1 Hamedan had more stem length after 10 days. There were no significant differences between root length and root and and shoot dry weight in two ecotypes. Fig. 1

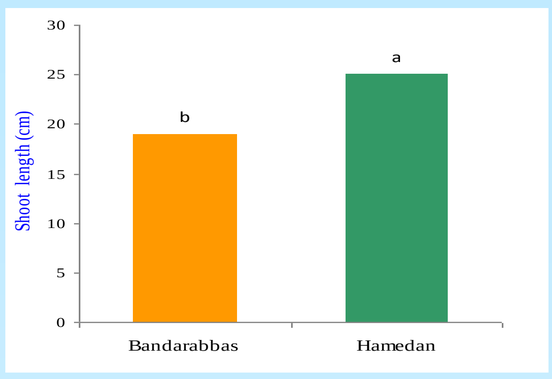

Fig. 1: The shoot length of two Fenugreek ecotypes (Bandarabbas and Hamedan) in 10-days non-treated plants. Values are means of triple results and same letters showed no significant difference.

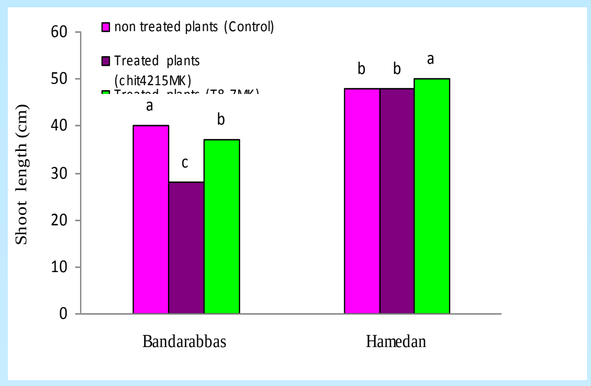

Fig. 2: Effects of two Trichoderma strains (T8-7MK and Chit4215mk) on shoot length of two Fenugreek ecotypes (Bandarabbas and Hamedan) in flowering stage (after 60 days). Values are means of triple results and same letters showed no significant difference.

The results demonstrated that, the shoot length in Hamedan (treated and non-treated) was higher than Bandarabbas ecotype. As can be seen from the Fig. 2 the highest shoot length was observed in plants (Hamedan) treated with T8-7MK strain (50 cm) that was 1.18- fold that of the control (non-treated plants)(48 cm) and chit 4215MK treated plants (48 cm).

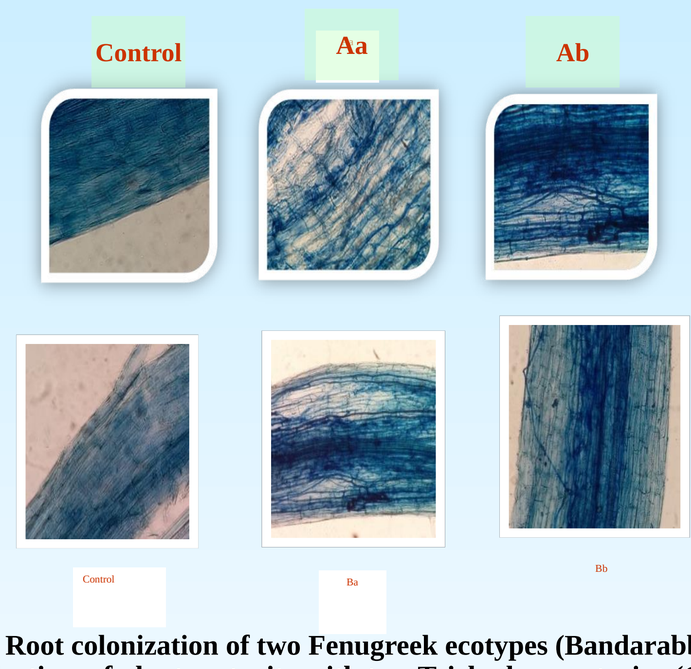

Fig. 3: Root colonization of two Fenugreek ecotypes (Bandarabbas (A) and Hamedan (B)) at the time of plant maturity with two Trichoderma strains (Chit4215mk (a) and T8-7MK (b)) and control (non-treated).

Table 1: The growth characteristics and yield of two Fenugreek ecotypes (Bandarabbas and Hamedan) at the time of plant maturity. Values are means of triple results and ± SD

Ecotype	number of lateral branches	number of pods on lateral branches	number of pods on main shoot	pod length (cm)	Length of lateral branches(cm)	weight of thousands seeds (g)
Hamedan	1.2±0.3	3.7±0.11	1.5±0.10	7±0.08	1.3±0.08	14±0.10
Bandarabbas	1.4±0.25	4.5±0.18	1.9±0.09	5±0.11	2±0.09	11±0.11

CONCLUSIONS

Certain Trichoderma specious have beneficial effects on plant growth and enhance resistance to both biotic and abiotic stresses. The results of the study various strains of Trichoderma and medicinal plant species can be very useful for introduction of new biofertilizers to increase the production of secondary metabolites. Also, a better understanding of the principles regulating the interaction between Trichoderma and plants would enhance the practical application of these beneficial microorganisms for increasing production of plant secondary metabolites.

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