DETERMINATION OF THE EFFECTS OF ENDOGENOUS HORMONE LEVELS ON RESISTANCE TO *Meloidogyne incognita* IN RESISTANT AND SUSCEPTIBLE TOMATO TYPES

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Abstract

The effects of abscisic acid (ABA), indole acetic acid (IAA) and gibberellic acid (GA_3) on resistance to *M. incognita* were investigated in roots of resistant and susceptible Fantastic F_1 tomato types. Hormone analyses were done with Reversed Phase High Performances Liquid Chromatography (HPLC) and bioassays. ABA and GA_3 were not detected with HPLC analysis while IAA was identified in both types. The level of IAA in the susceptible type was higher than that of the resistant type. ABA-like and IAA-like compounds also were found higher in susceptible type. The results indicate that there was relationship between endogenous hormones and nematode resistance.

Keywords: Tomato, *Lyoopersicum esculentum*, nematode, hormones

Dayanıklı ve Hassas Domates Çeşitlerinde İçsel Hormon Seviyelerinin *Meloidogyne incognita*'ya Dayanıma Etkisinin Saptanması

Özet

Dayanıklı ve hassas Fantastik F_1 domatesi çeşidi fidelerinin köklerinde saptanan absisik asit (ABA), indol asetik asit (IAA) ve gibberellik asit (GA_3) miktarlarının *M. incognita*'ya dayanıma etkisi araştırılmıştır. Analizler ters faz Yüksek Performanslı Sıvı Kromatoğrafi (HPLC)'de ve biyolojik testlerle yapılmıştır. ABA ve GA_3 HPLC'de saptanamazken, IAA her iki tipte de saptanmıştır. Hassas tipteki IAA seviyesi dayanıklı tipten daha fazla olmuştur. Aynı şekilde, hassas tipteki ABA ve IAA-benzeri madde miktarları daha fazla bulunmuştur. Sonuçlar, içsel hormon miktarlarıyla nematoda dayanıklılık arasında ilişkinin olduğunu göstermiştir.

Anahtar Kelimeler: Domates, *Lyoopersicum esculentum*, Nematod, Hormonlar.

1. Introduction

Tomato is a highly valuable vegetable crop in Turkey as in many countries in the world. It is grown over a wide range of climate both in the field and under protection.

Large differences exist between plant parasitic nematode communities of tropical and temperate regions. Four species of *Meloidogyne (M. incognita, M. javanica, M. arenaria* and *M. heplo)* are economically hazardous to tomato plants and their yield (Stevens and Rick, 1986). Conversely, root-knot nematodes, which are predominant in tropical regions, are not common in temperate regions (Taylor, 1976).

Damages caused by root-knot nematodes are high in subtropical and tropical vegetable-growing regions. Infestation with nematode causes plants to have shallow and defective root systems, with impairment to secondary root growth. Therefore, plants become more susceptible to stress (Stevens and Rick, 1986).

The presence of galls on root systems is the primary symptom associated with *Meloidogyne* infection. In cucurbits, roots react to the presence of *Meloidogyne* by the formation of large, flesh galls whereas in most other vegetables, galls are large but firm. Occasionally, very small galls develop and, in some cases, galls are not visible in plants (Netscher and Sikora, 1990).

There are few sources of resistance among crops, which are susceptible to *Meloidogyne*. Resistance has been found in some pepper cultivars and has been incorporated into tomato via an embryo culture of a hybrid created from a resistant line of *Lycopersicum peruvianum* and *Lycopersicum esculantum* (Smith, 1944). In most cases, the genetic basis for resistance is determined by one major gene (Gilbert and McGuire, 1956; Hare, 1957). However, Hendy et al., (1985) reported the presence of

five dominant genes present in one genotype resistant against *M. incognita, M. javanica* and *M. arenaria*. Similar work was done by Williamson et al. (1994).

There have also been studies, which indicated that ethylene production was increased in presence of free IAA through its stimulation ACC (1-aminocyclopropane-1-carboxylic acid) synthase activity (Cohen and Bandurski, 1982). Glazer et al. (1986) determined that ethylene-induced cell expansion in gall parenchyma by inhibiting fiber lignification, thereby allowing rapid expansion and swelling of the nematode body. It has been suggested that ethylene was closely associated with *M. javanica* infection (Glazer at al., 1985). Molinari (1991) indicated that the susceptible cultivar reacted to *M. incognita* infection with an increase in cytoplasmic PPD-PC oxidases activity, which presumably is involved in ethylene production; no changes in cell wall isoperoxidases were observed. IAA oxidase was inhibited in susceptible plants after nematode inoculation, whereas in resistant plants, this activity increased in the soluble fraction and decreased in the microsomal fraction.

The purpose of this research is to determine the levels of ABA, IAA and GA_3 levels of the tomato types resistant and susceptible to *Meloidogne incognita*.

2. Material and Method

2.1. Material

The roots of 40 day old seedlings of resistant (RN) and susceptible "Fantastic" F_1 tomato types, grown in peat medium in a controlled plastic house, were used in this research. Seedlings of these varieties were provided by Antalya distributor of Hazera Seed Company. Fruits of "Fantastic" have a long shelf-life, they are firm, suitable for export, and it is a very productive cultivar. This plant is very hard and can grow in moderate saline soils. Fruit setting is very good under lower temperatures conditions, and it gives a good response to the application of growth regulators for fruit-setting.

2.2. Method

The roots were cut from the seedlings, washed under tap water, and homogenized in 70% methyl alcohol. Experiment was carried out with three replications, each containing 5 g fresh root. Detection and isolation of IAA, GA_3, and ABA in the samples were done according to Ülger et al. (1999). Crude extract of 100 µl samples were applied to TLC (Merck Silica Gel 60 F_{254}) plate for chromatographic detection. Isopropyl alcohol: ammonnia: water (84:8.8) solvent combination was used in the TLC tanks. Spots detected on TLC under UV light were dissolved in 1 ml methyl alcohol, and filtrated through the micropore filters. HPLC analyses of phythormones were performed on a Model Varian 9050 HPLC equipped with UV detector and model Varian 9010 pumps enabling the use of a concentration gradient of the mobile phase. Separation and determination were conducted on a Nucleosil C_{18} column (4.6 x 150 mm I.D.). IAA was analyzed using an isocratic 35% methanol mobile phase with a 1% acetic acid ion-pairing agent. GA_3 was resolved in a mobile phase of 30% methanol adjusted to pH 3 with (0.1 M) phosphoric acid. ABA was determined with 55% methanol containing 0.1 M acetic acid. Wavelengths with UV detector for detection were 208 nm, 265 nm and 280 nm for GA_3, ABA and IAA, respectively. Total run time for the separations was approximately 5 min. at flow rate of 1 ml/min.

The identifty of ABA and IAA-like compounds was verified by oat coleoptile test (Kaynak, 1992) while that of GA-like compounds by lettuce hypocotyle test (Kaynak, 1992).

3. Results and Discussion

Only IAA was detected with HPLC, and its level in the roots of the susceptible type was higher than that of the resistant type. The levels of IAA were confirmed with 1.50 and 0.5 $\mu g.g^{-1}$ fresh weights in the susceptible and resistant types, respectively (Fig. 1). The differences were found statistically significant ($p \leq 0.05$).

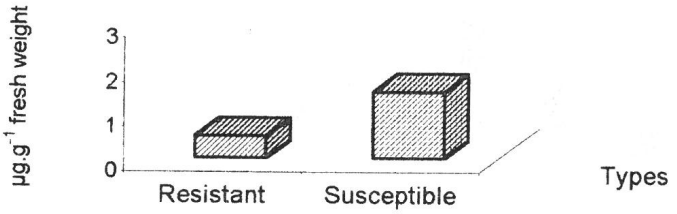

Figure 1. The amounts of IAA in resistant and susceptible Fantastic tomato types in HLPC.

IAA ($R_{f0.5}$) and IAA-like compounds in the susceptible type were found to be higher than in the resistant type (Fig. 2). High level2 of IAA in susceptible types were also confirmed with HPLC analysis (Fig. 1).

ABA-like inhibitors in the resistant type were detected in seven different R_f bands by the oat coleoptile test, whereas bands of IAA-like promoters were detected in the susceptible type in seven different R_f. The highest level of ABA-like inhibitors was confirmed in the $R_{f0.4}$ band, followed in decreasing order of activity by bands at $R_{f0.3}$ and $R_{f0.2}$. ABA ($R_{f0.7}$) was not found in the resistant type, although lower amounts of ABA in susceptible type was determined (Fig. 2).

R_f bands, which have GA-like activities, are more numerous in the susceptible type but total band counts of both types are approximately the same found in by the lettuce hypocotyle test. The detected level of GA_3 ($R_{f0.6}$) in the resistant type was considerably higher than the susceptible type (Fig. 3).

HPLC and bioassay results clearly showed that the levels of IAA in the susceptible type were higher than in the resistant type. Similarly, Kochba and Samis (1972) found that when resistant seedlings of peach were wick-fed with kinetin or NAA, the development of a normal nematode population became visible. The levels of both free and conjugated IAA were higher in infected tissues than uninfected ones, and nematode-infected roots contained the highest amounts of endogenous IAA on the tenth day after inoculation (Glazer et al., 1986). Sawhney and Webster (1975) indicated that NAA and kinetin in combination increased the susceptibility of the susceptible cultivar. When treated with a combination of NAA and kinetin, the resistant cultivar produced galls but only a few larvae developed to maturity. It has been suggested that plant growth hormones are not the only factors, which determine the host response of tomato to *M. incognita* (Glazer et al., 1986). On the other hand, Molinari (1991) observed that IAA oxidase was inhibited in susceptible plants after nematode inoculation, whereas in resistant plant this activity increased in soluble and

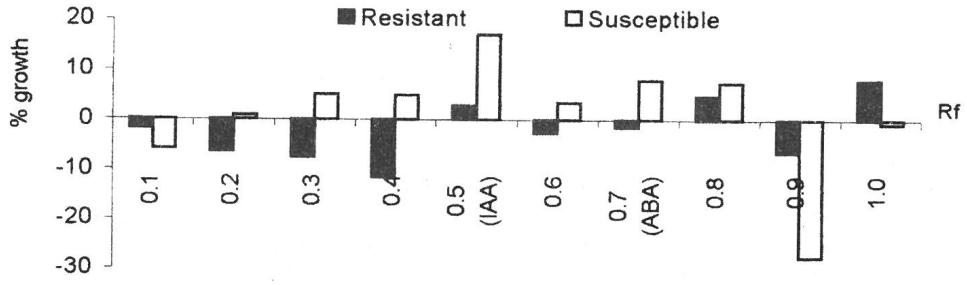

Figure 2. The level of ABA and IAA-like compounds obtained by oat coleoptile test in both resistant and susceptible type of Fantastic tomato roots.

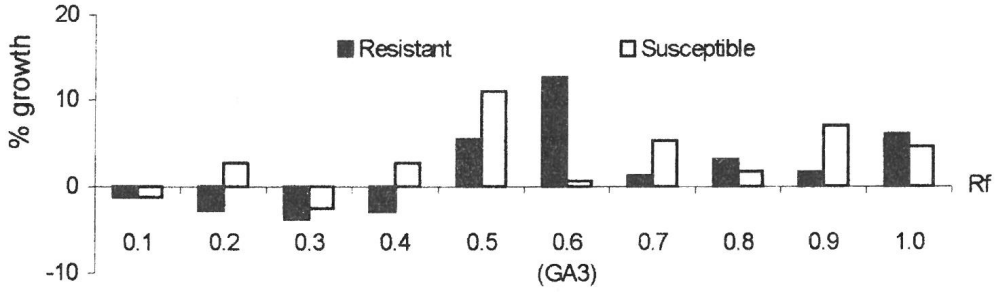

Figure 3. The level of GA-like compounds detected by lettuce hypocotyle test in roots of both resistant and susceptible Fantastic tomato types.

decreased in the microsomal fractions. Hangarter and Good (1981) proved that IAA in the conjugated form served as a mobile storage compound and could be hydrolyzed enzymatically to form active free IAA molecules. The reason why ABA and GA_3 were not identified in HPLC and instead were observed in bioassay was probably that biological tests were possibly more sensitive in hormonal detection and also that ABA and GA_3–like activities are present as well, even if ABA and GA_3 are not present themselves.

This report along with similar studies has shown that there is a relationship between endogenous hormones and nematode resistance. It is likely that either endogenous auxin and cytokinin levels are increased to higher levels when there is sensitivity to nematode infection, or that auxin, cytokinin and ethylene levels (Cohen and Bandurski) are increased during nematode infection. Sawhney and Webster (1975) have indicated that endogenous hormone levels should not be thought as the only factor involved in resistance to nematode.

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