The Role of Heavy Metals on Metabolism of Polyamines in Bean Plants (VICIA FABA) and its Susceptibility to Fusarium Wilt

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Abstract: Faba bean plants (Vicia faba) showed remarkable resistant to wilt disease caused by Fusarium solani pv. Phaseoli when watered with a mixture of heavy metal at a concentration 10mg/L. However the use of Zn, Pb alone slightly increases the susceptibility of bean plants to Fusarium wilt. Plants exposed to Cd or Cu treatment accelerate the symptoms appearance in infected plant. The toxicity of a single heavy metal in watering bean plants was quite clear on the level of polyamines, in which polyamines reduced sharply in treated plants and nearly no effect on polyamines by the use of a mixture of heavy metal.

Key words: Heavy metals, polyamines, Fusarium wilt

INTRODUCTION

Higher or excess uptake of heavy metals may affect the natural resistance of plants to disease. Metal deficiency symptoms in crop plants or disease susceptibility due to specific metal deficiency have been reported earlier by Burdon.[2]

There is an evidence that application of iron to the soil reduced Verticillium wilt of peanuts but enhanced Fusarium wilt of tomato. Supplementation of copper to the soil significantly reduced take- all, ergot and powdery mildew diseases caused by Gaumannomyces graminis, Claviceps purpurea and Erysiphe graminis hordei, respectively.[3,4]

Forsyth,[5] reported that seedlings of two cultivars of Triticum durum (viz., Mindum and Spelmar) which were normally resistant to stem rust became susceptible after treatment with zinc.

Hydroxycinnamic acids (HCA) are formed of covalent bonding of polyamines (putrescine, spermidine and spermine) HCA are widespread in plants and constitutes the bulk of the acid- soluble polyamine pool[4]. They are known to accumulate in response to pathogen infection and indeed, levels of free polyamines and acid-soluble conjugated polyamines have been shown to undergo profound changes in leaves infected with fungal pathogens[14].

HCAs which are mainly acid-soluble polyamine conjugates[6], which are formed by the conjugation of polyamines to phenolic acids like caffeic, coumaric and ferulic acids. These compounds are known to be increased as a result of the plant defense mechanism[14].

Walters et al.,[15] found that three spermidine conjugates were shown to reduce mycelial growth of Pyrenophora avenae and powdery mildew infection of barley seedlings.

MATERIALS AND METHODS

Growing of Plants: Faba bean seeds (Giza 3) were sown under field condition. The seeds were disinfected soaked in water for overnight before sowing the seedlings were watered every 72h.

Two weeks post-emergence, the seedlings were divided into 6 groups, each group contains 15 seedlings.

A mixture of seven heavy metals Cu++, Ni++, Zn++ and Mn++ as sulphate, Cd++ as chloride, Pb++ as nitrate and Hg++ as chloride were supplied at the rate of 10 mg/L (sublethal dose).

Heavy metals were supplied separately or in mixture at the rate of 1mg of each heavy metal/100ml/day/seedling for 10 days. In case of combination of 2 heavy metals (1mg of each) of heavy metals/100ml/ day/seedling were supplied for two days. 30 days old plants were inoculated and disease intensity was measured at 5, 10 and 15 days after inoculation.

Culturing of the Pathogen: Fusarium oxysporum p.v. phaseoli was provided by MIRCEN, Faculty of Agriculture, Ain Shams University, Egypt.

The fungal inoculum was prepared by growing the organism on oat grains medium. 500 ml flasks, contained 100 gm. clean oat grain and 80 ml tap water, were autoclaved at 121°C for 15 minutes.
The autoclaved oat medium was inoculated with the fungus under aseptic condition, and incubated at 25°C for 21 days. The medium was mixed and rubbed together to release mycelium and spores from oat grains. The mycelium and spores were taken in sterile water and used for inoculation of bean plants. Control plants were inoculated with water.

**Extraction of polyamines**[16]: Samples about 0.3 gm were ground in 2 ml TCA (trichloro acetic acid), transferred to small plastic centrifuge tube, then left on ice for 30 min.

Samples were then extracted, centrifuged at 3000 rpm for 15 min. The supernatant was then removed and transferred to glass centrifuge tubes. The pellets were extracted several times using 0.25 ml of 5% TCA.

The supernatant, mixed thoroughly and centrifuged at 1500 rpm to separate phases, the upper phase was discarded.

**Dansylation**: The extract was dried down and then redissolved in 0.1 ml distilled water. To each sample 50 mg NaHCO₃, 25 µl Na₂CO₃ (saturated solution), mixed thoroughly and 0.2 ml of freshly prepared dansyl chloride (30 mg/ml acetone) were added. Dansylated samples were left in dark at room temperature, 0.1 ml of 150 mg/ml proline was added and then tubes were left for 30 minutes in dark.

Samples were dried down at 50°C on water bath, then redissolved in 0.1 ml water and 1 ml benzene was added. Each tube was shaken thoroughly, then centrifuged at 1500 rpm for 5 minutes to separate phases. 0.4 ml was taken of benzene phase as well as from standards.

40 µl samples of benzene layer were spotted on TLC plates. Plates were developed in the first direction in solvent system; Benzene: triethylamie acetone (10:2:1) and then developed in the second direction (vertical to the first direction (Benzene: triethylamine 5:1). The silica gel containing the dansylamine, was taken off, extracted with 3 ml of acetonitrile, centrifuged, the colour intensity was measured in the clear solution using spectrofluorometer.

**RESULTS AND DISCUSSION**

Polyamines are small, positively charged aliphatic amines at cellular pH values and therefore bind to negatively charged molecules, including nucleic acid, acidic phospholipids and proteins[9].

Consequently, they modulate DNA- protein[11] and protein-protein interactions[12]. Common natural polyamines include putrescine, spermidine and spermine, along with related minor compounds and conjugated forms. Their pathway of biosynthesis is well established[9].

In plants, polyamines are also thought to play important roles in growth, development and stress responses[13]. For example, the level of polyamines is reported to fluctuate during plant – microbe interactions[14].

Spermine accumulates in intercellular spaces and induces pathogenesis-related proteins during hypersensitive reaction[13].

Accumulation of polyamines has been observed in tobacco cultivars resistant to TMV, but not in TMV-susceptible counterparts[9].

These naturally occurring amines are distributed through out plant cells. Cell proliferation and differentiation appear to require their biosynthesis. Although most of the work on polyamines is incompatible interactions between plants and pathogens has focused on polyamines conjugated to phenolic compounds (hydroxycinnamic acid amides), changes in free polyamines and their catabolism have been shown to occur in such interactions. Common feature of these interactions is an increase in diamine oxidase activity and in some interactions, of polyamine oxidase. The activity of these two enzymes produces H₂O₂ which may act in structural defense, as a signal molecule, or as an antimicrobial compound in host resistance.

There are several possible roles for polyamines and polyamines catabolism in plant resistance to pathogen infection; H₂O₂ produced might trigger the hypersensitive response, thought to be a form of programmed cell death.

The polyamine spermine might act as an inducer of protein and as trigger for caspase activity and hence hypersensitivity. There is however, a need for more precise information on the timing and location of changes in polyamine metabolism in the development of resistance.

In the present results (Fig. 1-4) the level of polyamine in bean plants watered with a mixture of Pb, Zn, Cu and Cad has increased sharply accompanied with the increase in resistance of bean plants to fusarium infection, it seems possible that watering of bean plants with this mixture has increased the defense mechanism in bean plants through the increase in polyamine production, similar reports by Walters[14] in which the production of secondary metabolites including hydroxyl cinnamic acid, amides are formed from the covalent binding of polyamine to hydroxy cinnamic acids like caffeic acids and coumaric acid. Hydroxycinnamic acids are widespread in plants and constitute the bulk of the acid-soluble polyamine pool[9].

They are known to accumulate in response to pathogen infection and indeed, levels of free polyamine and acid-soluble conjugated polyamine have been shown to undergo profound changes in leaves infected with fungal pathogens[14].
Fig. 1: Putrescine level in bean plants treated with different types of heavy metals.

Walters, et al., confirmed that methyl jasmonate induces systemic protection in barley seedlings against powdery mildew through alteration of polyamine metabolism in treated leaves. Table (1) showed that symptoms of disease were clear on plants treated with toxic element such as Pb, Cu and Cad as well as on plant watered with water only; however no symptoms appeared on plants treated...
with Zn or a mixture of the used heavy metals. It seems possible that in bean plants that showed defense reaction against wilt disease as a result of treatment with Zn and mixture Table (1) as well as an increase in the level of polyamines, the use of the Zn and mixture of heavy metals may lead to an increase in polyamines (Figs. 1-4) which induce resistant against wilt disease in treated plants, as reported by[15].

Table 1: Response of faba bean plants to heavy metal treatment 10 mg/l against Fusarium wilt

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<thead>
<tr>
<th>Treatment mg/l</th>
<th>Days after infection</th>
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- = no wilt symptoms
+ = beginning of symptoms
++ = appearance of symptoms
+++ = totally wilted

REFERENCES

