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PROLINE AND LEGHAEMOGLOBIN CONTENTS AND WATER ABSORPTION CAPABILITY OF COWPEA ROOTS AS INFLUENCED BY INFECTION WITH *ROTYLENCHULUS RENIFORMIS*, *MELOIDOGYNE INCOGNITA* AND *RHIZOCTONIA SOLANI*

by
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Summary. *Rhizoctonia solani* caused a greater accumulation of proline content than either *Meloidogyne incognita* or *Rotylenchulus reniformis*. However, all three pathogens, when present together caused the greatest accumulation. Proline content in the infected plants was higher in roots than in leaves. *R. solani* caused greater reduction of leghaemoglobin content in the nodular tissues than either *M. incognita* or *R. reniformis* but the presence of all three pathogens caused maximum reduction. Water absorbing capacity of roots was also significantly hampered by pathogenic infections.

Proline, a constituent of biological proteins, is reported to accumulate in various crops under stress conditions such as caused by disease, drought, environmental pollution, mineral (S and P) deficiency, salinity and sodicity (Thompson *et al.*, 1960; Seitz and Hochster, 1964; Erickson and Dashek, 1982). It is a natural compound with a greater solubility than other amino acids. Its accumulation, therefore, helps in increasing the amount of osmotically bound water, which in turn enables the plant to conserve water during drought stress caused by parasitism.

Leghaemoglobin is a protein which is synthesized exclusively in the nitrogen fixing root-nodules and is restricted to the rhizobia infected cells within these nodules where it constitutes 25-30% of the soluble protein of the cell. Several workers have suggested that leghaemoglobin is involved in nitrogen fixation (Virtanen *et al.*, 1947; Uheda and Syono, 1982). The fact that nodules lacking leghaemoglobin are unable to fix nitrogen and that a correlation exists between leghaemoglobin concentration and the rate of nitrogen fixation is apparent from the results of experiment by Virtanen *et al.* (1947). Leghaemoglobin acts as an oxygen carrier in the process of nitrogen fixation in nodules and has a role in protecting the oxygen sensitive nitrogen fixing enzyme from the effects of atmospheric oxygen.

This paper deals with investigations on the effect of *Meloidogyne incognita* (Kofoid *et* White) Chitw., *Rotylenchulus reniformis* Linford *et* Oliveira and *Rhizoctonia solani* Kühn on the proline and leghaemoglobin contents and water absorption capability of infected cowpea [*Vigna unguiculata* (L.) Walp cv. Pusa Barsati].

Materials and methods

Surface sterilized seeds were bacterized with a cowpea strain *Rhizobium* and then sown in 15 cm earthen pots containing 1 kg sterilized soil, river sand and farm yard manure (3:1:1) mixture. When the seedlings were one week old and of uniform size the pots were inoculated either individually or concomitantly in different combinations with *R. reniformis*, *M. incognita* (1000 nematodes/plant) and *R. solani* (1 g fungus/plant) by pipetting over the surface of carefully exposed roots which were then covered with soil. Uninoculated plants served as control. Each treatment was replicated five times and randomized on a glasshouse bench.

After 60 days from inoculation the plants were carefully removed from the soil and washed gently to avoid any damage to plant tissues. These plants were kept singly in 500 ml Erlenmeyer flasks containing 300 ml water and supported on cotton plugs. Flasks with only cotton plugs and water served as a control. The flasks were kept on a glasshouse bench at 30-34°C. After 24 hours the plants were taken out of the flasks and the remaining quantity of water was measured. The amount of water absorbed by the roots was calculated by deducting the amount lost from flasks with plants from that from flasks without plants.

Estimation of free proline from leaf and root tissues

was made by the Ninhydrine method (Bates *et al.*, 1973). The benzidine hydrogen peroxide method (Proctor, 1963) was used for the estimation of leghaemoglobin from the nodular tissue.

Results

From the data presented in Table I it is evident that all the pathogens, whether present singly or in their various combinations, significantly reduced the water absorbing capacity of the plants. *R. solani* caused greater reduction (32.4%) than either *M. incognita* or *R. reniformis*. The greatest reduction (80.4%) was, however, caused by the simultaneous inoculation of *R. reniformis*, *M. incognita* and *R. solani*, followed by inoculation of *R. solani* with either *M. incognita* or *R. reniformis*.

On the other hand, the infection of all three pathogens, singly or in various combinations, caused significant increase in the proline content of leaves and roots. *R. solani* caused a greater increase in proline concentration (17.75 µmoles/g leaf, 13.04 µmoles/g root) than either *M. incognita*, or *R. reniformis*. Maximum accumulation of proline (26.10 µmoles/g leaf, 20.26 µmoles/g root) was found in plants concomitantly inoculated with all three pathogens followed by plants inoculated with *M. incognita* and *R. solani* or *R. reniformis* and *R. solani*.

Data presented in Table I show that all three pathogens, singly or in various combinations, significantly reduced leghaemoglobin concentration of root nodules. *R. solani* caused greater reduction (4.3 mg/g nodules) than either *M. incognita* or *R. reniformis*. The greatest reduction (1.4 mg/g nodules) in leghaemoglobin content was, however, caused by the simultaneous inoculation of all three pathogens, followed by combined inoculation of *R. solani* with either *M. incognita* or *R. reniformis*.

Discussion

The water absorption capability of cowpea roots was adversely affected by the individual as well as combined inoculations of all the three test pathogens. Each of the pathogens caused damage to the roots and thus as expected the simultaneous inoculation of these pathogens in different combinations showed more pronounced inhibitory effect on water absorption because of greater damage caused to the shoot and root system by their interaction (Khan, 1986).

Greater accumulation of proline in response to nematode and/or fungus infection of plants has been reported by several workers (McCombs and Winstead, 1964; Howell and Krusberg, 1966; Sharma *et al.*, 1980).

Since proline is a readily available storage compound in moisture deficient tissues, the hydrolysis of plant proteins by enzymes secreted by nematode and/or fungus might have resulted in the greater accumulation of proline in the diseased plants, or the nematodes by themselves might have contributed to the increase in proline content as was earlier observed by Krusberg (1971). Proline accumulation in the infected plants was higher in roots than in leaves probably because of increased downward translocation of proline to the infected site, increased rate of synthesis *in situ*, decreased rate of translocation out of the infected roots and/or decreased rate of breakdown (Howell and Krusberg, 1966).

An adverse effect of nematode or fungus infection on leghaemoglobin concentration was also reported by Orellana and Worley (1976) and Chahal and Singh (1984). This impairment in the leghaemoglobin concentration as a result of pathogenic infection may either be due to suppression of nodulation, premature decay of nodules, or interference with the development of bacteroides. However,

TABLE I - *Effect of individual and concomitant inoculations of* Rotylenchulus reniformis, Meloidogyne incognita *and* Rhizoctonia solani *on the proline and leghaemoglobin contents and water absorption capability of cowpea plants.*

Treatment	Water absorbed (ml)	Percentage reduction over control	Proline (µmoles/g fresh weight materials)		Leghaemoglobin (mg/g nodules)
			Leaf	Root	
Control (Uninoculated)	14.8		12.2	8.8	6.8
R. reniformis (Rr)	11.7	20.9	14.7	11.0	5.6
M. incognita (Mi)	10.8	27.0	16.2	12.3	4.9
R. solani (Rs)	10.0	32.4	17.7	13.0	4.3
Rr + Mi	8.4	43.2	18.5	14.0	3.9
Rr + Rs	6.4	56.7	20.8	15.5	2.4
Mi + Rs	4.6	68.9	23.7	17.6	2.0
Rr + Mi + Rs	1.9	80.4	26.1	20.2	1.4
L.S.D. at 5% level	1.510		1.295	0.764	0.613
L.S.D. at 1% level	2.096		1.798	1.060	0.851

the basis of a possible decrease in the leghaemoglobin concentration of the nodules of infected plants needs further investigation.

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