

RELATION OF PLANT NUTRITION TO DISEASE DEVELOPMENT IN FUSARIUM WILT OF PIGEON PEA

By

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Effect of variation of nutrient concentration and balance on the expression of Fusarium wilt symptoms in pigeon pea plants was investigated. Disease development increased in a linear relation with increase in the concentration of balanced nutrient solution from 0.1 N to 3 N if the levels of micronutrients were kept constant. When the levels of micronutrients were also simultaneously altered, there was more wilting at the normal concentration (N) and its lower dilution (0.1N) than at the moderate dilution (0.3N). In unbalanced solutions, with the concentration of micronutrients remaining constant, significant inhibition to disease development occurred at low nitrogen, high phosphorus and high calcium levels. Most pronounced inhibitory effect was, however, recorded with high level of potassium. Only in the cases of calcium and potassium treatments, such inhibition of symptoms showed good correlation with the presence of fungus in the host vascular elements.

INTRODUCTION

Disease development in plants is influenced by many factors including those related to plant nutrition. Since nutrition affects growth and vigour of a plant more than any other factor, its role in symptom expression is of great importance and has been investigated for many host-parasite combinations. Vascular wilt diseases constitute good subjects for this aspect of plant disease study, because in such cases nutrient supply may directly affect not only the host but also the pathogen, which colonizes xylem vessels that are mainly responsible for upward conduction of nutrients absorbed from the soil. Studies on such diseases of fungal origin have shown that symptom expression is influenced as much by the concentration of balanced nutrients as by the

deficiency or excess of individual nutrient elements (Schroeder and Walker, 1942, Walker and Foster, 1946, Walker and Hooker, 1945, Keyworth and Hewitt, 1948, Walker et al., 1954). Wilt of pigeon pea (*Cajanus cajan* (L.) Millsp.) caused by *Fusarium oxysporum* f. sp. *udum* (Butler) Snyder and Hansen is a major disease of this crop under Indian conditions. Little is known about the role of nutrition on symptom expression in this disease except that manganese promotes (Sarojini, 1961, Subramanian, 1953). Aims of the present investigation were to study the effect of nutritional factors such as the concentration of balanced nutrients; availability of certain major elements, etc. on this disease. Plants used as test materials were grown in nutrient solution factor for an easier interpretation of the results.

MATERIALS AND METHOD

Materials included a wilt susceptible pigeon pea cultivar, T1 (Kanpur), and a virulent isolate of *F. oxysporum* f. sp. *udum*.

Seeds were germinated in soil in pots, and later one-week-old seedlings were transferred to various nutrient solutions in 180 ml capacity amber coloured bottles. Long Ashton nutrient solution (Hewitt, 1966) was used as the basal nutrient solution (Table 1). In some experiments, salt concentration in the basal solution (N) was increased two- or three fold (2N or 3N) and also reduced to half, three-tenth or one tenth or one tenth (0.1 N). In some other experiments, using the normal strength of nutrient solution (N) as the base, high and low levels of nitrogen, phosphorus potassium or calcium were obtained by increasing the amount of particular elements three-fold (as in 3 N) and reducing the amount to one-tenth (as in 0.1N). In each case other nutrient elements were kept at the normal level (as in N) by suitable modifications, through additions of sodium nitrate, potassium chloride or calcium chloride in amounts as required (Table 2). None of the variable elements were ever completely

lacking. Osmotic value of the different unbalanced solutions was kept uniform by addition of necessary amounts of sodium chloride. Different nutrient solutions were adjusted to a pH of 6.0 by addition of sodium hydroxide or hydrochloric acid as required.

The fungus was grown on sucrose—casamino acids agar medium and incubated at 27°C. Spore suspension obtained from 10–12 days old cultures was centrifuged, washed and finally made into a standardized suspension of 10^6 spores per ml to be used as inoculum. Plants were inoculated by trimming 4–5 lateral roots and then placing the root system in a spore suspension for 24 hr. Afterwards, inoculated plants were transferred to fresh nutrient solution, which was changed twice a week. At regular intervals after inoculation, symptoms were assessed for the individual leaves on the basis of a 0–4 scale, where 0 stands for no symptom and 4 for drying or shedding following wilting. Sum total of such values gave the wilt index per leaf was calculated. Each treatment had five replicates. On the last date of sampling, three inoculated plants from each treatment were removed and hand sections were prepared from them at a fixed point, 7.5 cm below the cotyledonary node. Sections were stained with safranin—picro aniline blue combination and then observed under microscope to determine the proportion of vessels that contained hyphae.

Other methods are described with the experiments.

RESULTS

Influence of balanced nutrient solution of different concentrations on disease development.

Five different concentrations of the balanced nutrient solution (N), varying between 0.1N and 3N, were used. Micronutrients were kept at the basal level, by using the same amount of minor element stock solution in each case, to avoid their excess or deficiency masking the effect of different strengths of balanced

nutrient solution, if any. Observations recorded at four different stages after inoculation are given in Table 3.

Table 1. Composition of Long Ashton nutrient solution used (to prepare stock solution for 100 litres of nutrient solution)

Chemical constituent	Weight (g)	Volume of stock soln. (ml)	Volume of stock soln. used for each litre of nutrient soln. (ml)	Final conc. in nutrient soln. (p.p.m.)
<i>Macronutrients</i>				
KNO ₃	40.4	1000	10	156 (K) 156 (N)
Ca (NO ₃) ₂	65.6	1000	10	160 (Ca) 112 (N)
MgSO ₄ · 7H ₂ O	36.8	1000	10	36 (Mg) 48 (S)
NaH ₂ PO ₄ · 2H ₂ O	20.8	400	4	31 (Na) 41 (P)
<i>Micronutrients</i>				
Ferric citrate 5H ₂ O	3.35	200	2	5.6 (Fe)
MnSO ₄ · 4H ₂ O	0.223	200	2	0.55 (Mn)
CuSO ₄ · 5H ₂ O	0.025	200	2	0.064 (Cu)
ZnSO ₄ · 7H ₂ O	0.029	200	2	0.065 (Zn)
H ₃ BO ₃	0.186	200	2	0.33 (B)
(NH ₄) ₆ Mo ₇ O ₂₄ · 4H ₂ O	0.0088	200	2	0.048 (B)

Table 2. Composition of Long Ashton nutrient solution as modified for studying the effect of host nutrition on the development of Fusarium wilt of pigeon pea

Chemical constituent	Ml of stock soln/5l. of nutrient soln.								
	Basal	High N	Low N	High P	Low P	High K	Low K	High Ca	Low Ca
KNO ₃	50	50	51	50	50	50	5	50	50
Ca (NO ₃) ₂	50	50	—	50	50	50	50	50	5
MgSO ₄ · 7H ₂ O	50	50	50	50	50	50	50	50	50
NaH ₂ PO ₄ · 2H ₂ O	20	20	20	60	2	20	20	20	20
NaNO ₃ b		300					45		90
KCib			35			100			
CaCl ₂ · 2H ₂ O _b			50					100	

- a) Ten ml each of six micronutrient stock solutions were added to the macronutrients to make 5l of nutrient solution.
- b) To prepare unbalanced solutions with low or high levels of different major elements, different volumes of NaNO₃, KCl or CaCl₂ · 2H₂O stock solutions, prepared by dissolving respectively 33.9, 29.8 and 58.8g of salts in 1000 ml of water, were added as replacements a; and (where required).

It appeared that disease did not develop in the same manner in plants growing in different concentrations of balanced nutrient solution. The onset of symptoms was earlier and the progress more rapid at higher concentrations. With time, however, plants in all the treatments became badly affected with disease. Fungal colonization of host vessels, recorded 24 days after inoculation was nearly of the same order in different treatments. Repetition of this experiment on two other occasions yielded essentially similar results.

Table 3. Effect of different concentrations of balanced nutrient solution on foliar wilting and vascular colonization in pigeon pea plants infected with *F. oxysporum* f. sp. *udum*

Concentration of nutrient solution (Normal=N)	Mean wilt index per leaf Days after inoculation				Percentage of vessels with hyphae
	14	18	21	24	
0.1N	0.8	1.3	1.8	3.1	70
0.5N	1.2	1.9	2.1	3.3	75
N	1.3	1.9	2.3	3.7	68
2N	1.8	2.2	3.4	4.0	78
3N	1.9	2.8	4.0	4.0	6

In another series of three experiments, plants were grown in three different solution (N, 0.3 N and 0.1 N). Here, the concentration of micronutrients was also proportionately reduced. The two higher concentrations (2N and 3N) were not included in these experiments because too rapid disease development in such nutrient solutions, it was believed, might not allow any real assessment of the effect of micronutrients. With N, 0.3 and 0.1 N concentrations, mean wilt index per leaf in one experiment 18, 24 and 28 days after inoculation were respectively 1.6, 2.7 and 3.1, 1.1, 2.0 and 2.6 and 2.6 and 1.4, 2.2 and 3.1. There were variations in the results from one experiment to another. In the second experiment, most rapid development of symptoms was obtained with the higher concentration (N) and in the third experiment with the lower concentration (0.1N). One thing was common, however, in all the experiments i.e., symptoms were slow to develop at 0.3N than either at the higher concentration (N) or at the lower concentration (0.1N). Fungal colonization appeared

to be pretty extensive at the end of the sampling period in all three treatments. Differences between these treatments in the proportion of vessels with hyphae did not appear to be of much significance.

Influence of unbalanced nutrient solutions on disease development

Taking the normal concentration of nutrient solution as the basal dose, high and low levels of nitrogen, phosphorus and potassium were provided to the plants following the method described earlier. All other necessary elements including the micronutrients were made available in all the treatments at the basal level. Data were collected only once, 21 days after inoculation. Observations in respect of wilt development and fungal colonization of the vessels are given in Table 4.

Table 4 *Effect of different levels of some major nutrient element on foliar wilting and vascular colonization in infected pigeon pea plants recorded 21 days after infection with F. oxysporum f. sp. udum*

Concentration of ions in nutrient solution	Mean wilt index per leaf	Percentage of vessels with hyphae
Normal	3.0	67
Low Nitrogen	1.8	57
High Nitrogen	2.6	62
Low Potassium	3.1	2
High Potassium	0.8	12
Low Phosphorus	2.8	66
High Phosphorus	2.0	55

Results show that considerable reduction in symptoms occurred in plants receiving low level of nitrogen and high level of phosphorus, but more striking reduction was evident in plants receiving high level of potassium. Fungal colonization of host vessels was very remarkably inhibited in plants receiving high level of potassium. Other treatments had little effect.

The above experiment was repeated twice with basically similar results. Summarized observations in respect of the effects of different levels of nitrogen potassium and phosphorus are given below.

Nitrogen : In plants supplied with low level of nitrogen, wilt development was inhibited by 33-54 percent as compared to that obtained in plants receiving normal level of nitrogen. Where the level of nitrogen was higher than normal, there was always less wilting, but the reductions never appeared to be significant. Increase or decrease in the level of nitrogen had little effect on the fungal colonization of vessels.

Phosphorus : While increased supply of phosphorus inhibited wilt development moderately, by 24 to 38 percent, there was no significant effect when the supply was low. In the former case, the inhibitory effect on fungal colonization did not appear to be of much significance.

Potassium : At the higher level, potassium strikingly inhibited wilt development, by as much as 61 to 80 percent, and vascular colonization also equally remarkable. In one experiment, there was less wilting also in plants receiving lower level of potassium, but the reductions were only marginal in nature.

Calcium : The effect of different levels of calcium on disease development was investigated in a subsequent experiment run along the same lines. Results showed that plants receiving high levels of calcium developed 45 percent less symptoms and had hyphae in many fewer vessels as compared to those having a normal supply. But there was little effect on symptom expression where the level of calcium supply was low.

Influence of balanced and unbalanced nutrient solutions on fungal growth in vitro.

It seemed important to investigate whether the increase or decrease in wilt development recorded with (a) different concentrations of balanced nutrient solution and (b) unbalanced solutions with different levels of nitrogen, potassium, phosphorus or calcium was due to a direct effect of such treatments on the mycelial growth or the pathogen or not. To assess this effect, the organism was grown in Erlenmeyer flasks in different nutrient solutions same as those used for growing pigeon pea plants but supplemented with 2% dextrose. There were four replicates for each treatment. After 10 days' incubation at 27°C

the mycelium was collected separately from each flask for dry weight determination. In balanced nutrient solution of five different concentrations varying between 0.1 N to 3 N, increased mycelial growth was recorded with increase in concentration. When the pathogen was grown in basal nutrient solution supplemented with three different levels of nitrogen, potassium, phosphorus and calcium, some correlation between the mycelial growth and wilt development could be noticed in the first two cases only.

DISCUSSION

Disease development showed good correlation with the concentration of balanced nutrient solution (0.1 N to 3 N) in which the plants were grown, provided the concentration of minor elements remained the same. Symptoms appeared earlier and developed rapidly in plants growing in the higher concentrations of balanced nutrient solution than in those growing in the lower concentrations. In the latter, foliar wilting was slow to start with but progressed steadily so that on the last sampling date, 24 days after inoculation, differences in disease development between different treatments were less pronounced. Disease development in various concentration of nutrient solution was well correlated with growth of the pathogen *in-vitro* in the same solutions supplemented with dex rose. This suggests the possibility that greater initial fungal growth *in-vivo* at higher concentrations may have resulted in early and more rapid development of symptoms as compared to those at lower concentrations. But with continued supply of nutrients over a period, differences in fungal growth *in vivo* and disease development between different treatments may have gradually levelled off. This is not unexpected. Little difference in the fungal colonization of vessels between the different treatments on the last date of sampling gives support to this view.

When the concentration of micronutrients was also changed along with that of the balanced nutrient solution, the results were a little different. Symptom expression was always slower at moderately dilute (0.3N) nutrient solution than

at the concentration(N) or at higher dilution(0.1.N). No explanation is available for such effects on disease development, particularly the increase in symptoms at 0.1 N over that at 0.3 N, unless it is assumed that at lower concentration of micronutrients the host is more adversely affected than the pathogen and becomes more prone to disease development.

Disease development in nutrient solution was affected by an increase or decrease in the concentration of some major nutrient elements. Significant reductions in symptoms were recorded at low level of nitrogen and high levels of phosphorus and calcium. Still more remarkable reductions were achieved at high level of potassium. Only in the cases of potassium and calcium nutrition, intensity of symptoms was well correlated with the spread of the pathogen in host vascular tissue. However, some degree of correlation between symptom expression in various unbalanced nutrient solutions and mycelial growth *in-vitro* in the same, when supplemented with dextrose, was noticed in case of nitrogen and potassium only. This indicates that while inhibition of symptoms achieved with high level of potassium can reasonably be explained on the basis of reduced *in-vivo* growth of the pathogen, some other explanations will be needed for the reduced symptoms recorded at high level of phosphorus and calcium and low level of nitrogen. There are reports to show that increased supply of calcium significantly reduces both vascular browning (Fisher, 1975) and foliar wilting (Edgington and Walker, 1958) in tomato plants infected with *Fusarium oxysporum* f. sp. *lycopersici*. Similar observations have been made for *Fusarium* wilt of watermelon (Everett and Blazquez 1967) and Vascular wilt of tomato (Hubbelling and Basu Chowdhury, 1967) also. Calcium is known to form salts with pectic acid which are rather insoluble in nature and less amenable to the action of pectic enzymes so abundantly produced by *Fusarium* spp. and other fungal vascular pathogens. On that basis, it has been suggested that an increased availability of calcium may increase resistance to *Fusarium* like vascular wilt pathogens and inhibit symptom expression through a modification of the cell wall pectic compounds by formation of salt bridges which interfere with their degradation under the influence of pectic enzyme action (Edgington, Corden and Dimond, 1961). This may be applicable in the present case also. Observations

do not indicate any possible mechanism which may help to inhibit symptom expression under conditions of nitrogen deficiency and excess of phosphorus.

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