

# UPTAKE OF IONS AND METALLIC CHELATION IN PLANTS\*

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THE essentiality of micronutrients for the normal metabolic functions of plants has been shown by many (Brenchley, 1936; Javillier, 1908; Hewitt, 1951), and there is no lack of experimental evidence to show that an altered nutrition of the host is mainly responsible for their susceptibility or resistance to disease, particularly to attack by root-disease pathogens (Walker and Hooker, 1945; Walker and Foster, 1946; Garrett, 1941, 1956). Although resistance or susceptibility has primarily a genetic basis in plants, their potentiality of becoming resistant or susceptible to an invading pathogen may be largely influenced by environment. The conditions prevailing before and after infection affect the physiology of the host and hence its capacity for excluding or for supporting development of the parasite. Much of the earlier postulates on the physiology of host-parasite interaction have largely been replaced in recent years by Brown's theory of enzyme-substrate interaction (Brown, 1934, 1936, 1955). In the course of a critical analysis of the conditions necessary for the establishment of true parasitism, Garrett (1956) has recently stressed the need for a full appreciation of the relative importance of the following factors: (i) the presence of a susceptible host, (ii) the presence of a parasite, and (iii) the occurrence of suitable environmental conditions for penetration and infection of the host. From a biochemical angle, it may be stated that the logical prerequisite for these conditions would be (a) a host substrate of composition suitable for the enzymatic activity of the parasite *in vivo*, (b) the presence of suitable enzyme systems in the parasite for the enzyme-substrate interaction during disease, and (c) the availability of optimal conditions for successful enzymatic breakdown. The important role of trace elements as bio-catalysts of enzyme action has been widely recognised. Most workers feel that like the vitamins, trace elements perform their important functions in the nutrition of plants by assuming a catalytic function in enzyme systems.

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Earlier work in this laboratory has indicated the role of micronutrients in altering host susceptibility, especially in the fusariose wilts (Sadasivan, 1950, 1951, 1952; Sadasivan and Subramanian, 1954; Sarojini, 1951; Sulochana, 1952; Varadarajan, 1953; Kalyanasundaram, 1954; Satyanarayana, 1955). The exact mechanism through which this is attained is variously described—increase in the antagonistic microflora in the rhizosphere, changes in the carbohydrate and protein metabolism representing unfavourable conditions for both pre-penetration and post-penetration phases of infection. It was also pointed out (Subramanian, 1956 *a*) that the quantum of available free trace elements in the host plants would determine susceptibility or resistance to wilt in cotton, in view of the important role of chelation of phytotoxins with heavy metals *in vivo* and their consequent potentiation. Kalyanasundaram and Saraswathi-Devi (1955) reported that an optimal concentration of zinc was essential for the production of fusaric acid and higher concentrations of the metal were inhibitory for its production and thus the concentration of available zinc in the host would appear to be an important factor which affects the production of this vivotoxin.

Gäumann (1951) has clearly indicated that resistance of the host depends on its ability to check the generalisation of the pathogen rather than its ability to counteract the toxic metabolites. In the resistant varieties the infection remains restricted to the primary focus and the fungus fails to develop and produce sufficient quantum of the toxin. It would be of interest to mention in this connection Lakshminarayanan's (1955) work on pectin distribution in cotton plants which indicated that the resistant roots recorded low pectin levels which resulted in non-secretion or secretion in low amounts of the adaptive pectic enzymes and consequent localization of the parasite. Secretion of suitable exo-enzymes by the pathogen that could attack the host far in advance of the mycelial growth would provide a plausible explanation of the mechanism of their vivotoxic action. The pectic enzyme secretion by plant pathogens like *Fusarium vasinfectum* fits in excellently well with the definition of vivotoxin (Waggoner and Dimond, 1953). Recently very considerable attention has been directed to the study of the role of these enzymes in fusariose wilts (Gothoskar *et al.*, 1953, 1955; Winstead and Walker, 1953, 1954; Waggoner and Dimond, 1955; Lakshminarayanan, 1955; Kamal and Wood, 1956). A detailed study of the conditions for the activity or synthesis of pectic enzymes by the pathogen would, therefore, seem imperative for understanding the physiology of parasitism. *In vitro* studies of this nature, though not strictly comparable to *in vivo* conditions, would, nevertheless, provide valuable information about the conditions offered by the resistant and the susceptible hosts.

It need hardly be emphasized that heavy metals play a vital role in the physiology of the pathogen in controlling the key enzyme systems *in vitro* and *in vivo*. The important part played by the two pectic enzymes, pectin methyl esterase (PME) and pectin polygalacturonase (PG) in the fusariose wilt of cotton (Lakshminarayanan, 1955) necessitates a study of the influence of the heavy metal ions on these enzyme systems. Fe<sup>+++</sup> is found to favour the production of PME at an optimal level of 1 p.p.m. and inhibit its activity at higher concentrations (Subramanian, 1956 *b*). It has also been reported earlier (Lakshminarayanan, *loc. cit.*) that Fe<sup>+++</sup> significantly increased the toxicity of the culture filtrate of *F. vasinfectum* to cut shoots of cotton at a concentration of 10<sup>-3</sup> M. It is apparent from this that Fe<sup>+++</sup> not only accelerates the formation of pectic enzymes but also augments the toxicity of the products of host-parasite interaction. Other ions like Zn, Mn, Cu and Mo influence, in differing degrees, the production and activity of PME (which is the more important of the two enzymes), all the ions being inhibitory at higher concentrations. It appears, therefore, that the quanta of free heavy metals present *in vivo* in the host plants would largely determine the production of these enzymes in sufficient concentration for effective enzymatic degradation of the host substrate. Furthermore, the activity of these enzymes is influenced to a considerable extent by the presence of these ions in the enzyme-substrate complex. Zn and Mn inhibit enzymic activity, whereas Fe<sup>+++</sup> increases it (Subramanian, 1956 *b*). The exact manner in which the heavy metals bring about such an inhibition or activation is not clearly understood. It has been proposed by Hellerman and Stock (1938), Smith (1953) and Najjar (1948) that the enzyme activation brought about by trace elements takes place by the formation of metal chelates with the substrates resulting in the metallo-substrates suitable for enzymic action. Klotz (1951) proposed an alternative interpretation of the metal catalysis in enzyme reactions, emphasizing formation of metallo-enzymes. Similarly, the inhibitory effect may also be attributable to one of these reactions, *i.e.*, formation of metallo-substrate unsuitable for enzymic action or formation of metallo-protein rendering it inactive.

Recent work of Lakshminarayanan (*loc. cit.*) on the distribution of pectin in resistant and susceptible varieties of cotton revealed that the resistant varieties accumulated much less pectin in roots which form the primary focus of infection compared to susceptible ones, thereby providing unfavourable conditions for infection. High root pectin content was correlated with susceptibility and low root pectin content with resistance. The pectic enzymes which were also shown to be partly adaptive were secreted in sufficient quantities only in the presence of an optimal concentration of substrate. It

would be of particular interest to mention the work of Subramanian (1956 *b*) wherein amendment of the soil with various concentrations of zinc progressively reduced the root pectin content in the susceptible variety of cotton K 2, thereby affording protection against the disease. In addition to the change it produced in the substrate concentration at the focus of infection, namely the roots, zinc also had a marked inhibitory effect on the pectic enzymes of the pathogen as mentioned earlier. This is further borne out by the estimation of PME activity in roots, shoots and leaves of healthy and inoculated susceptible cotton plants grown at various concentrations of zinc. It has been observed that in the healthy cotton plants grown in unamended soil PME activity was almost none, especially in roots and shoots, but in the inoculated series there was a significant increase in the activity showing that this enzyme is operative during infection. As the concentration of zinc increases, there is a slight rise in the PME activity followed by a steep decline at 50 p.p.m. and thereafter. In other words, zinc seems to play a dual role in that it not only reduces the substrate concentration at the focus of infection but also inhibits its degradation by the enzyme. A study of the respiratory status of susceptible cotton plants in Zn-amended soil revealed the depressing effect of zinc on tissue respiration. In the inoculated series, however, the rise in the rate of oxygen uptake was counteracted and the rate closely followed the normal healthy controls. This was further confirmed by *in vitro* studies (Lakshmanan, 1956).

The net effect of infection is an increased rate of oxygen uptake (Lakshmanan, 1955) but the effect of pure fusaric acid, a vivotoxin in the fusariose wilt of cotton (Laskhminarayanan and Subramanian, 1955; Kalyanasundaram and Venkata Ram, 1956), is one of inhibition. This indicates that fusaric acid is not the only factor responsible for the production of the disease syndrome. Many of the commercial chelating agents like 8-hydroxyquinoline and EDTA (ethylene diamine tetra-acetic acid) exert a similar influence on tissue respiration. This may be attributed to their ability to chelate with metallic ions like iron and copper which lose their co-enzyme function. Both the inhibitory action of the chelating agents and the acceleratory influence of the neat culture filtrate of *F. vasinfectum* were reversed by the addition of heavy metals.

Increased permeability of the host cells has been reported by many workers in response to an uptake of toxic metabolic products of fungi (Gäumann and Jaag, 1947; Gäumann *et al.*, 1947; Gäumann, 1951; Thatcher, 1942), but there is no suggestion of destroyed osmotic function. Davis and Dimond (1954) suggest that polyphenols like gallic acid may be responsible for the increased permeability. Recently we have observed

a significant derangement in the uptake of ions in infected cotton plants. Spectrochemical analysis using the standard Lundegårdh flame (spark-in-flame) method, of cotton plants infected by *F. vasinfectum* showed an increase in the magnesium, calcium, iron and manganese and a decrease in the potassium content over the healthy controls, suggesting a derangement in the selective absorption of ions (Sadasivan and Kalyanasundaram, 1956). An increased conductivity was also recorded in wilt-infected plants as compared to the healthy controls which may be attributed to the accumulation of electrolytes due to primary changes in osmoregulation of the host cells (Gnanam, 1956).

Kalyanasundaram and Subba Rao (1957) have recently obtained further evidence to show that with rise in temperature (over a range of 32.5° to 37.5° C.) of incubation of *F. vasinfectum* infected wilt susceptible cotton plants, grown in a thermostatically controlled,  $\pm 0.1^\circ$  C., miniature glass-house (Sadasivan *et al.*, 1956) there is an increased accumulation of fusaric acid, whereas there is a decreased disease syndrome as evidenced by a fall in wilt index. It seems, therefore, that the increased toxin content with rise in temperature (which is logical when one assumes higher transpiration and uptake of solutes) would presuppose an increased metallic accumulation and consequent *in vivo* chelation, a point that has to be proved by bioassay or spectrochemical evaluation of ash samples of infected plants grown under varying temperatures. This work of Kalyanasundaram and Subba Rao (*loc. cit.*) further showed the presence of cystine in plants grown at 37.5° C.; cystine, however, was absent at the lower temperature of 32.5° C. The spectrochemical estimations indicated a higher quantum of heavy metals (including Fe) alkali and alkaline earth metals other than K and as fusaric acid is known to be potentiated *in vitro* in bioassay in the presence of free Fe<sup>+++</sup>, it is presumed that the lower wilt index recorded by these workers at the higher temperatures may be due to (a) the presence of cystine which may be chelated with Fe making it unavailable for potentiation of the toxin, or (b) the adsorptive substances like proteins and carbohydrates being present in greater quantity in the host at higher temperatures.

In view of the fact that the heavy metals control several key metabolic functions and act as potentiators, activators or inhibitors of several enzyme systems (Foster, 1949; McElroy, 1953; McElroy and Nason, 1954; Saraswathi-Devi, 1956) of the host/parasite, it would be necessary to interpret carefully the important changes effected in the host, in the light of overall metabolic derangements during pathogenesis.

There seems little doubt that much work still lies ahead in the understanding of the intensely interesting host-parasite relationship of plants against a background of metallic chelation with vivotoxins and I naturally look forward to stimulating research in this fascinating field.

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