CHAPTER IX

GENERAL DISCUSSION

The last two decades have witnessed new developments in the concepts and application of plant nutrients, unprecedented in the history of plant nutrition. The establishment of the dual mechanisms of ion uptake by Epstein group (43) followed by a spate of modifications and extensions of the concept of ion transport (45) marks the beginning of the transformation of our earlier views on ion uptake. The theory of dual mechanisms of Epstein has been useful in interpreting ion uptake data in terms of kinetics and ion competition.

Parallel to the development of ion uptake concepts which related to nutrient elements like K, Ca, P, Na, Cl and of Sc4, several groups/scientists were engaged in studying the physiology of micronutrient absorption and utilization (4, 5, 6, 16, 20, 106, 111, 165, 166). The extensive work by J.C. Brown over 3 decades, has led to a number of theories relating to micronutrients like Fe, Mn, Zn, B and Mo. The most important and the most recent one relates to the discovery of an Fe-stress tolerant mechanism present in Fe-stress tolerant crop cultivars (16, 20). The group developed a method of identifying cultivars which possess genetic capability to tolerate deficiencies and excesses of several micronutrient elements.
For the purpose of the investigations described in the thesis, two elements Zn and Mo were chosen. It is needless to state that these 2 elements play a very critical role in crop growth. A number of aspects of Zn and Mo uptake was examined. Zn and Mo uptake mechanisms were investigated using excised roots and also intact seedlings. Absorption by leaf cell systems was also studied with narrow leaf slices and leaf discs. Foliar uptake and transport were measured by a leaf immersion technique. The results revealed many features of Zn and Mo absorption and transport. There were cultivar differences in these patterns. Detailed studies were made to induce Zn deficiency stress and to identify tolerant cultivars. Further, the influence of Mo on nitrate reductase activity and also chlorophyll synthesis was investigated. Some of the important findings are discussed herein.

1) Mechanisms of uptake and transport of Zn and Mo:

A number of theories on the mechanisms of ion absorption have been discussed in the introductory chapter and the studies are restricted to the carrier model of absorption. Excised roots and intact seedlings of different cultivars of sorghum, maize (monocotyledons), tea, groundnut and bean (dicotyledons) were used for the experimentation. It was found that the amount of Zn absorbed by the dicotyledonous roots (bean) were twice than that of sorghum or maize. It was also found that the ion transport to the shoot was much less compared to the absorption by
roots. It has frequently been observed that the dicotyledonous plants generally contain a larger proportion of divalent to monovalent cations than monocotyledons. The cation exchange capacity of the roots of dicotyledons is also usually higher than that of monocotyledons (38, 114). It is now believed that cation exchange effects of roots are of little significance in regulating cation uptake and the cation composition of plants (115).

ii) Mechanisms of uptake of Zn and Mo by intact leaf or leaf slices:

Ion absorption by a leaf starts from cuticular surface, followed by diffusion through cuticular membranes and then through the leaf cells below the cuticles. Foliar absorption and translocation have been discussed by Kannan (82) recently. The mobility of Zn was examined by immersing the leaf in the labelled ZnCl₂ solution. It was found that a greater translocation occurred towards the lower leaf compared to the upper leaf in tea seedlings. Mechanisms of foliar uptake of ⁶⁵Zn and ⁹⁹Mo was further investigated using either the leaf discs of tea leaves or leaf slices of maize leaves. It is interesting to note that age of the leaf is an important factor affecting absorption of nutrient elements. The leaf slices obtained from younger leaves showed greater absorption of Mo than those from the older leaves. This shows that, absorption is dependent upon the greater physiological activity of the leaves and the younger leaves are more active than the older ones.
The absorption and transport of Zn and Mo are found to be through an active process. Moore (118) reviewed the broad aspects of micronutrient uptake by plants. Considerable controversy exists as to whether Zn uptake by roots is active or passive. Much of the controversy arises because early workers did not differentiate between passive exchange adsorption and active accumulation by cells (48). Passive absorption arises from electrostatic absorption on cell walls and other surfaces within the apparent free spaces of roots. This binding is nonselective and nonmetabolic. In contrast, active accumulation is highly selective and metabolically mediated (145). In short-term uptake experiments, exchangeable Zn may constitute 90% or more of the total Zn held by the roots. Since exchangeable Zn reaches saturation readily, it constitutes a smaller percentage of the total Zn uptake as absorption continues. As a result, many of the short-term uptake experiments in which exchangeable Zn was not removed (15, 138) must be discounted as proof that Zn uptake is passive. Other studies in which sufficient calcium was not used in the medium to maintain cell integrity must also be discounted.

Tiffin (158) and Ambler et al. (8) reported Zn concentrations in xylem exudates from decapitated tomatoes and soybeans that were considerably higher than the nutrient solution thus showing accumulation against concentration gradient. This would suggest active absorption only if the internal Zn is not chelated or present in forms different from that in the external solution.
The requirement of Mo by plants ranges among the lowest of the essential microelements (73) and there is no direct evidence that Mo is taken up actively. The results on the absorption and transport of Mo in sorghum and maize using different concentration, time-course or interaction with Zn showed that Mo absorption is through an active process. There are reports showing that Mo uptake is reduced by competitive effects of SO$_4$ (141, 154) and PO$_4$ ions enhanced Mo transport to the tops in short-term experiments (154). Moore (118) considered on the basis of above reports, that Mo is actively absorbed. The concentrations used in the ion uptake experiments were low and the rate of absorption increased with increasing external concentration of Zn and Mo but at progressively higher concentration, each added increment adds less and less of an increment in absorption rate. Many experiments on the relation between the concentration of an ion and the rates of its absorption have yielded results which conform to Michaelis-Menten kinetics.

iii) Ion interactions:

The effects of different levels of PO$_4$ on Zn absorption and translocation and vice versa were studied. It was found that there was a mutual interaction between Zn and PO$_4$ and presence of one element inhibits the absorption and transport of other element in general, both in M-35 and M-47 cultivars. The interaction between Zn and P has been studied by many workers, and high levels
of P supply are well known to induce Zn deficiency. Three major causes for this effect have been considered by Olsen (127). These are: a slower rate of Zn translocation from root to tops i.e., zinc accumulation in roots or low Zn uptake (155); a simple dilution effect on Zn concentration in the tops owing to a growth response of P; a metabolic disorder in plant cells relating to an imbalance between Zn and P, as for example, an excessive concentration of P interfering with the metabolic function of Zn at specific sites in cells. Support for all three effects has been obtained by different reports. The literature on Zn/P relationship in plants has been well reviewed by Olsen (127). The interaction of Zn and Fe, Cu or FeO₄ was presented in figure 8 and it was found that all these elements inhibited Zn absorption by excised roots of 36A, 168 and CSH-7. The inhibition of Zn absorption by Fe was of a competitive nature, while that of Cu and I were non-competitive. Burleson et al. (29) suggested the possibility of a Fe-Zn antagonism within the root. Fe interferes with the uptake of Zn and, Fe and Zn interactions has been reported by Schrer and Höfner (146) in maize seedlings.

iv) Response of genotypes:

When plants of different varieties or species are grown side by side under identical conditions, and analysed for their content of nutrient and other mineral elements, large differences
often become apparent. It has been reported by Lee (95) and others (135), that the variation in the mineral content may be due to the difference in the root growth. Nevertheless there are many instances which suggest that such variation in the uptake of nutrients reflect genetically controlled differences in mechanisms of mineral nutrition, especially those concerned with absorption and translocation of a given element. These criteria have been well discussed by Burkholder and McVeigh (28) in their report on genetic effects in the nitrogen nutrition of inbred lines and hybrids of corn. Further examples for several elements have been given by Epstein and Jefferies (47) and many other scientists also (8, 9, 16, 166).

The results on the absorption and transport of Zn and Mo in 5 cultivars of sorghum showed not only differences in their absorption capacity but also in their Zn deficiency stress conditions and their recovery from chlorosis (Figure 34 a, b, c). The $V_{\text{max}}$ and $K_m$ values also differ among cultivars (Table III). Only rarely have genetically controlled quantitative differences in mineral nutrition been examined in detail to determine what aspects of nutrition was affected. The genetic control may govern the initial absorption of an element by the roots, its subsequent translocation into and through the xylem, the degree of its retention in tissues adjacent to the conducting elements, its mobility in the phloem, the efficiency of its metabolic utilization and others (43, 45). Sorghum cultivars consisting of hybrids and parents differed in
their root and shoot growth when grown in nutrient medium consisting of different levels of Zn. The seedlings were found to be normal in the low concentration of Zn treatment. However, they showed toxic symptoms with high Zn. Further, when the seedlings grown with high Zn, showing toxic symptoms were transferred to normal nutrient medium, the toxic symptoms disappeared and they became normal. This seems to be due to the different capacity of the seedlings in the utility of nutrient and their interaction. Munns et al. (121) reported on the usefulness of the genetic approach to problems of mineral nutrient especially on the uptake and distribution of manganese in oat plants. In one of the recent reviews, Hageman et al. (57) analysed the problem involved in the genetic control of heterosis and its effective manipulation in plant breeding programs. Genetic variability has been made use of by plant breeders with spectacular results in terms of increased yields, better resistance to disease and more advantageous chemical composition.

v) Nitrate reductase activity:

It was the first enzyme in higher plants found to be inducible by its substrate nitrate (144, 157). The assimilation of nitrate in higher plants is an important metabolic process and has therefore been the subject of extensive physiological and biochemical investigation (13, 64). However, the genetic control of nitrate assimilation in higher plants is still relatively unclear.
Experiments were carried out in 3 cultivars of sorghum to find out the differences in the NRA among them. Since the development of NRA in many plant tissues is dependent on nitrate (13), experiments were carried out in the absence and presence of \( \text{NO}_3^- \) in maize seedlings (Figures 62, 63 & 64). The NRA was found to be increased with the addition of exogenous \( \text{NO}_3^- \). The enhancement was more conspicuous with the presence of Mo.

Nitrate reductase is the most well studied Mo containing enzyme. It was isolated from soybean by Evans and Nason (53). The activity of the enzyme in cauliflower is enhanced by increasing levels of Mo supply (31). Mo deficiency thus leads to a decrease in the activity of nitrate reduction. NRA in plant roots is generally much lower than activity in leaf extracts (144). This shows the similarity with the results on the less NRA in maize roots compared to the shoots. The amount of nitrate reduced in the root, relative to that exported to shoot differs greatly among plant species (130). The ratio of root to shoot nitrate reduction also depends upon the plant's environment, especially the nitrogen nutrition of the plant (13, 69, 124, 130). In addition, the regulation of NR may differ somewhat between the species and even between the root and shoot of the same species (69, 124, 130).

Hence the cultivars showed differences not only in their absorption capacity of Zn and Mo but also in their utilization. The variability is common among individuals in a local population. However,
a necessary prerequisite of the breeding will be the determination of genetic differences with regard to specific ionic stresses and the yield and nutritional quality of the plants produced (124, 130, 152). Efficiency in the determination of such differences will require cooperation among several disciplines. Sprague (152) states, "Information on the underlying causes of such differences must come from detailed studies combining biochemistry, or physiology and genetics. This field has been relatively neglected in crop plants and it merits greatly increased attention and support. There is little question that increased activity in this area would be highly productive".