CHAPTER 2
REVIEW OF LITERATURE
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Mineral nutrition of plants</td>
<td>4</td>
</tr>
<tr>
<td>2.2 Role of calcium</td>
<td>5</td>
</tr>
<tr>
<td>2.3 Role of vitamins</td>
<td>26</td>
</tr>
<tr>
<td>2.4 Role of pyridoxine (vitamin B₆)</td>
<td>28</td>
</tr>
<tr>
<td>2.5 Concluding remarks</td>
<td>49</td>
</tr>
</tbody>
</table>
2.1 Mineral nutrition of plants

The use of farm yard manure is an old practice in agriculture adopted by farmers and even scientists until about the middle of the last century and it was believed that the entire nutritional requirements of crop plants were provided by applying adequate quantities of organic fertilisers to the soil before sowing. For the first time, de Saussure (1804) chemically analysed soils as well as the ash of the plants growing in them. He came to the conclusion that the composition of the plants varied with the soil, the plant part analysed and the age of the plant. Thirty to forty years later, other workers, including Boussingault in France, Liebig in Germany, and Lawes and Gilbert in England, confirmed these findings and elaborated other aspects of mineral nutrition of plants.

Sachs in 1860 and Knop in 1862 prepared solutions of salts supplying the major essential mineral nutrients and studied the importance of mineral nutrient for plant growth. Among the essential mineral nutrients, nitrogen, phosphorus, potassium, calcium, magnesium and sulphur are required in rather large quantities and have been named macronutrient elements. The elements that are required in small quantities by the plants are termed as micronutrient elements and
include boron, chlorine, copper, iron, manganese, molybdenum and zinc (Epstein, 1978). It may be added, however that various crops plants require different levels of essential nutrients depending upon their utilisation capacity and these are supplied mainly through chemical fertilisers.

Fertilisers are classified according to the particular nutrient element which forms their principal constituent. Thus, they may be grouped, for example, as nitrogenous fertilisers, phosphatic fertilisers and potassic fertilisers. The fertilisers added to the soil are not generally fully utilised by crop plants due to various factors (Donahue et al., 1990). Variation in the root system of plants is one of the main factors. Although nitrogen, phosphorus and potassium are of key importance in agriculture, other nutrients are in no way less important. Calcium is one of such essential elements. The significance of calcium is given in the following pages, keeping the importance of the present dissertation in view.

2.2 Role of calcium

A revolution is occurring in our thinking about the physiology and development of plants as we realise the importance of calcium ion (Ca$^{2+}$) which is involved in many different processes. The bulk of calcium ion in plant (vacoule of the cell) is found as salts of anions, including organic acids, sulphates and phosphates. The concentration of Ca$^{2+}$ in healthy tissue ranges from about 0.2% (dry weight basis) to several per cent. Calcium is a relatively large divalent
cation with a hydrated ionic radius of 0.412 nm and hydration energy of 1577 J/mol. It readily enters the apoplast and is bound in an exchangeable form to cell wall and at the exterior surface of the plasma membrane. Calcium can render some anions insoluble and thus reduces their activity. For example, oxalic acid is produced in potentially toxic quantities of some plants, but it is detoxified by the formation of insoluble calcium oxalate. Calcium also counteracts the effect of nutrient imbalances in the cell. An excess concentration of K⁺ or Na⁺ may injure the plant by promoting too much membrane permeability. The ill effect may be reversed by calcium (Streeter and Barta, 1988). In the absence of an exogenous calcium supply, root extension ceases within a few hours. Calcium is also involved in cell division (Burstrom, 1968; Schmit, 1981). In calcium deficient tissues, increased respiration rate has been observed and this is related to enhanced leakage of respiratory substrates from vacuoles to the respiratory enzymes in cytoplasm (Bangerth et al., 1972). Calcium stabilises cell membrane by bridging phosphate and carboxylate group of phospholipids (Coldwell and Hang, 1981) and protein preferentially at membrane surface (Legge et al., 1982). Calcium also plays a major role in membrane structure and plasticity. Calcium combines with pectins (long chain polymer carbohydrates). Its incorporation into cell wall with protein, serves as a "cement" and provides the strength to the structure and also gives it flexibility (Streeter and Barta, 1988).
There are increasing evidences for a close link between Ca$^{2+}$ and growth hormone action in plants. It has been shown that Ca$^{2+}$ plays an important role in auxin transport and secretion (Dela Fuente, 1984), ethylene synthesis (Lesham et al., 1984), secretion of enzymes (Gasper et al., 1983; Jones and Jacobson, 1983), senescence (Lesham et al., 1984) and gravitropism (Lee et al., 1983; Slocum and Roux, 1983). Calcium also acts as a second messenger in the response of roots to auxin and gravity (Evans et al., 1980; Moore and Evans, 1986). Calcium has a regulatory role in a protein, calmodulin, which plays an important role in plant cell for both the regulation of free calcium in the cytosol and enzyme activation (Marme, 1983; Dieter, 1984). Calcium increases the activity of enzymes like $\alpha$-amylase, phospholipases, and ATPase (Wyn Jones and Gunt, 1967). In case of $\alpha$-amylase, the effect of Ca$^{2+}$ depends on the presence of the phytohormone GA (Jones and Carbonell, 1984) and is more likely a reflection of a calcium/calmodulin induced enhancement of synthesis and secretion of this enzyme by the cell (Mitsui et al., 1984). In general, calcium stimulates membrane bound enzyme ATPase at the plasma membrane of roots of certain plant species (Kuiper et al., 1974). The mobility of calcium from cell to cell and in the phloem is very slow and it is the only mineral nutrient, other than boron, which functions mainly outside the cytoplasm in the apoplast region. Most of its activity is related to its capacity for co-ordination with
other metabolism by which it provides stable but reversible intermolecular linkage predominantly in the cell wall and the plasma membrane. Factors, such as humidity, root pressure and phytohormone activity influence the distribution of calcium in plant (Kirkby and Pilbeam, 1984).

The available literature regarding the effect of calcium in the form of different salts/fertilisers containing calcium on the growth, yield and quality of cereals, legumes, oilseeds and other crop plants is reviewed below.

Howard and Adams (1965) conducted a short term split-root experiment with cotton seedlings (Gossypium hirsutum) in which the upper portion of the root medium was a sandy loam surface soil and lower portion was either a nutrient solution or a sub-soil material, at various calcium levels. They found calcium as the only macronutrient ion required in the sub-surface of solution for primary root growth in the presence of other cations and that Ca was adequate at a concentration of 0.29 meq/l but was deficient at 0.15 meq/l. However, in the presence of Mg and K the requirement of Ca increased. It was observed that root growth was inhibited in solution with a Ca/total cation ratio in terms of chemical equivalent at approximately 0.1 or less. Regarding different pH, they observed that root length was inhibited only below pH 4.25 and pH range 4.3 to 6.5 was considered suitable for root elongation. The Ca required in sub-surface media for root penetration was
dependent upon Ca/total cation ratio rather than the Ca concentration of the medium. They also observed that the requirement of Ca was the same in soil solution and nutrient solution. Critical levels of exchangeable Ca were equal in Norfolk and Dickson sub-soil when Ca was expressed as a ratio of Ca to total exchangeable cations even though the clay fraction of Norfolk is kaolinite while the clay fraction of Dickson is vermiculite with some montmorillonite. Norfolk sub-soil at pH 5.0 and Dickson sub-soil at pH 4.6, as obtained from the field, contained adequate Ca for normal growth of primary cotton roots.

Lowther and Loneragan (1968) studied the effect of ions on the nodulation of subterranean clover (Trifolium subterraneum) in culture solution. The data revealed that the growth of the host plant was not affected by the calcium concentration ranging from 246 to 720 \( \mu \)g. However, the number of nodules per plant increased under this range. On the other hand, lowering the calcium concentration from 246 to 4 \( \mu \)M progressively decreased both growth of the plant and number of nodules. The transferring of plants after 10 days in lower calcium concentration to 720 \( \mu \)M for 7 days did not increase nodule number above those on plants treated continuously at low calcium. Similarly, transferring of plant from 720 \( \mu \)M calcium to lower calcium treatment did not decrease nodule number much below those on plants grown continuously at 720 \( \mu \)M calcium even though growth was depressed. Nodule development after its initiation proceeded
at concentrations of calcium too low for plant growth. On the basis of these observations, it was suggested that nodule infection or initiation needed higher calcium concentration than the development of nodules. The high calcium requirement for root infection or nodule initiation was not thought to be due to the effect on survival or growth of \textit{Rhizobium} and could not be related to effect of calcium on tap root length, root hair development, or lateral root initiation.

Loneragan \textit{et al.} (1968) studied the requirement of calcium for growth of 30 grasses, cereals, legumes and herbs in solutions which were maintained at pH 5.7. They observed that some legumes and herbs grew much better at low concentration of calcium (2.5 and 10 \(\mu\text{M}\)) than many species of Gramineae. The minimum concentration required to produce maximum growth of plants and to eliminate calcium deficiency symptoms varied widely (2.5-1,000 \(\mu\text{M}\)) within each group. At 100 \(\mu\text{M}\), there were no calcium deficiency symptoms and all plants grew well. It was also observed that increasing the concentration of calcium to 1,000 \(\mu\text{M}\) enhanced growth of only a few species. The concentration of \(<1\ \mu\text{M}\) showed severe deficiency symptoms in the tops of all species. The shoot and root growth of legumes and herbs was affected more severely as well as quickly than that of Gramineae. The roots of Gramineae appeared healthy for some time in the same solution but their relative growth rate decreased rapidly. The higher concentration (2.5-1,000 \(\mu\text{M}\)) had marked
effect on Gramineae. They suggested that some distinct processes in the plants functioned differently at different range of calcium concentrations; for example, at extremely low concentration of calcium (<1 μM) to which legumes and herbs were found more sensitive than Gramineae, the dominant process might involve ion exchange equilibria between calcium in the environment and in cell wall or membrane. At higher concentration (2.5-1,000 μM), the processes of absorption of calcium from solution and translocation to plant tops were probably dominant.

Loneragan and Snowball (1969) estimated calcium concentration in tops and roots of 16 legumes, 7 cereals, 4 grasses and 3 herbs grown under a wide range of calcium concentrations (0.3 to 1,000 μM) which were maintained constant in flowing culture solution at pH 5.7. They found that increasing the calcium concentration from 0.3 to 2.5 μM increased the yield greatly, while the calcium concentration in the tops remained constant. On the other hand, increasing the concentration of calcium from 10 to 1,000 μM significantly increased the calcium concentration in tops but the yield of most of the species was not affected markedly. The concentration of calcium in tops of herbs and legumes was found very much higher than in grasses and cereals at luxury calcium supply. The increase in yield with constant calcium concentration in plant tops was believed to indicate the minimal functional requirement of the tops for
calcium. The average functional requirement of herb and legume tops was 0.1-0.2% of dry weight which was generally much lower and was about twice those of grasses and cereals (0.05-0.1%). On transferring the plant from higher to lower (1,000 to 0.3 μM) calcium concentration, it was observed that they developed calcium deficiency symptoms and grew less than plants which had one-third to one-tenth the calcium concentration in their tops but which had been grown continuously in solution of low and constant concentration (2.5-10 μM).

Munns (1970) studied the interaction effect between hydrogen and calcium ions on root nodulation in *Medicago sativa*. It was observed that lowering the pH from 5.6 to 4.8 increased the calcium concentration required to nodulate half of the plants from 0.1 mM to 6.0 mM. Below 0.2 mM calcium concentration or below 4.8 pH, nodulation was inhibited at all tested levels of the other variable. The extent of tap root was not affected by any of the pH treatment. However, below 0.2 mM calcium concentration, the growth of the roots became slow, stunted and stubby. The most acid-sensitive stage for the nodulation process was initiation of infection, which was also found the most calcium demanding one at pH 5.2. Once infection was initiated, infection threads still developed and nodules still grew despite transfer of plants to a solution too low in calcium to have permitted infection to begin. Treatment at 0.5 mM and 8 mM calcium at pH 5.2 before inoculation had
no significantly different effect on nodulation. Observation on root hair distribution suggested that developing nodules could suppress further infection by suppressing the emergence of root hairs on newly developing root.

Puntakar et al. (1972) conducted a field experiment to study the effect of gypsum and manure alone or in combination on the yield of wheat var. Sonora-64. Gypsum was applied at the rate of 5, 10 and 20 ton/ha and manure was applied at 15 and 30 ton/ha. The crop was irrigated with bicarbonate-rich water. Regarding the performance of crop, it was noted that gypsum at 20 ton/ha alone gave maximum yield, followed by gypsum at 20 ton/ha when applied in combination with 15 or 30 tons of manure. The combination of 20 ton/ha gypsum and 30 tons/ha manure was not found superior in comparison with highest dose of gypsum when applied alone. On the basis of the above observation, it was concluded that the application of gypsum at 20 ton/ha proved best for good harvest and to prevent the deterioration of soil irrigated with bicarbonate-rich water.

Volz and Jacobson (1974) studied potassium uptake by excised roots of vetch (Vicia dasycarpa) and barley (Hordeum vulgare) and found vetch roots more responsive to pH and requiring ambient calcium concentration 100 times greater to overcome the deleterious effect of low pH. At pH values above 7, the addition of very low concentrations of calcium decreased the uptake of potassium by vetch roots more markedly than those of barley. Potassium uptake by vetch
in the presence of calcium led to potassium being sequestered into a non-exchangeable form and the process was dependent upon aerobic metabolism. The data revealed that the degree of specificity for calcium varied between these two plants and it was higher for vetch roots than for barley roots. The difference in response to calcium for vetch and barley was probably due to the higher exchange capacity of vetch roots than those of barley.

Singh and Dahiya (1976) studied the effect of CaCO$_3$ and iron on the availability of calcium, phosphorus, iron and manganese in pea (Pisum sativum) crop grown on a light textured soil which was marginal in exchangeable calcium. The experiment was conducted in a green house. Soil was treated with 0, 2, 4 and 8% CaCO$_3$ which was free from soluble iron and manganese.

The iron treatment comprised 0, 5, 10 and 20 ppm ferrous sulphate. N, P and K were given basally, each at the rate of 15 ppm. Manganese was also added as manganese sulphate at the rate of 5 ppm. The sampling was done at 45 DAS and 75 DAS to record dry matter yield, calcium, phosphorus, iron and manganese. Addition of CaCO$_3$ caused significant increase in dry matter yield without iron at both the stages of crop growth; but 8% CaCO$_3$ decreased the dry matter yield at the later growth stage. Dry matter yield increased with the addition of iron upto 10 ppm at 45 DAS and upto 5 ppm at 75 DAS. The iron concentration and uptake
of iron decreased with the increase in CaCO$_3$ and increased with the application of iron at both the stages of crop growth.

Phosphorus concentration and uptake were decreased by the application of CaCO$_3$ and iron at both the stages. The concentration and uptake of manganese also decreased with the increase in CaCO$_3$ and iron levels at 45 and 75 DAS. The concentration of calcium increased with the addition of CaCO$_3$ and the increase was to the extent of 80% at 45 DAS and, 53% at 75 DAS with 8% CaCO$_3$, while the uptake of calcium increased more than 3 folds at 45 DAS and more than 2 folds at 75 DAS. The concentration of calcium decreased with the application of iron but the uptake of calcium at 45 DAS increased upto 10 ppm of iron sulphate and then decreased upto 5 ppm.

Zsoldos and Karvaly (1978) studied the differences in potassium uptake of different segments of excised roots of two thermophilic plants, i.e. rice (Oryza sativa Cv. Dunghan Shali) and cucumber (Cucumis sativus cv. Semega furtos) and a non-thermophilic plant, i.e. wheat (Triticum aestivum cv. Aurora) in the presence and absence of calcium at 0 and 25°C, using radiotracer potassium (86 Rb$^+$) technique. The calcium concentration used was 5x10$^{-4}$ M CaSO$_4$. In the case of rice and cucumber, an extra-ordinarily high potassium uptake was observed in the apical root portion at 0°C in the absence of calcium application. However, the presence of calcium lowered this abnormality. For wheat, normal pattern
of potassium uptake was observed under similar conditions. At 25°C, calcium-stimulated potassium uptake may appear in each root segment, depending upon the plant species and composition of the solution. The results indicated that there might be considerable differences in the composition of the cell wall and membrane of root cell of thermophilic and non-thermophilic plants, and in their ion-exchange properties, particularly in the apical region.

Dahiya and Singh (1980) conducted a pot experiment on loamy sand soil to find out the effect of different levels of CaCO₃ and farm yard manure (FYM) on the dry matter yield and nutrient uptake by oats (Avena sativa cv. HFO-114). They applied five levels of FYM (0, 0.5, 1, 2 and 4%) and four levels of CaCO₃ (0, 2, 4 and 8%) to each pot which contained 4 kg of soil. A uniform basal dose of N, P, K, Fe and Mn at the rate of 120, 60, 60, 5 and 10 ppm respectively was also applied to each pot. In general, application of CaCO₃ and FYM significantly increased dry matter yield of oats. The concentration and uptake of P, Fe and Mn decreased significantly with increasing levels of CaCO₃; but application of FYM resulted in a significant increase in the concentration and uptake of the nutrients. However, application of CaCO₃ significantly increased the concentration and uptake of calcium; but the effect of FYM application in the presence (as well as in absence) of CaCO₃ was found negative. Application of increasing levels of CaCO₃ and FYM significantly decreased the concentration as
well as uptake of Mn by the plants.

Krishnapillai and Pethiyagoda (1980) conducted two pot experiment to study the effect of CaCO₃ on efficacy of two sources of nitrogen, viz. ammonium sulphate and urea on young plant of tea (*Comellia sinansis*, cv. TRI-2025). In experiment one, the plants were divided into two groups of three each. One group of plants received ammonium sulphate with a suspension of 0.25% (w/v) calcium carbonate while the other group of plants received ammonium sulphate only as the source of nitrogen (105 ppm). In the other experiment, the plants were also divided into two groups of four each and one group of plants received urea as the source of nitrogen (105 ppm), while the other received urea with a suspension of 0.25% calcium carbonate. In both experiments, pots including blank received a daily application of 100 ml nutrient solution. It was observed that the addition of CaCO₃ to the nutrient solution containing ammonium sulphate, not only improved growth of the plants but also eliminated the toxic effect of ammonium ions. Nitrification also increased due to the inclusion of CaCO₃ in the nutrition solution. In the case where urea was used, only moderate (50%) nitrification was observed; but the nitrification was very rapid when CaCO₃ was added to the urea containing solution.

Sachan and Sharma (1981) performed a solution culture experiment on cucumber (*Cucumis sativus* cv. Sporu) to establish the absorption of calcium as an index for the
measurement of various root parameters. Absorption of calcium in shoot and various root parameters, viz. fresh weight, dry weight, length and surface area were assessed at weekly intervals. It was found that there was highly significant correlation between calcium uptake and various root parameters as well as among the root parameters. A continuous supply of calcium in the immediate vicinity of growing root apices was found to be the prime requirement for root growth. The calcium uptake by older root segments was negligible and stopped as soon as fresh root growth was checked. They suggested that reliable estimates of cucumber root parameters can very easily be made from the value of calcium uptake in the plant.

Ssali (1981) studied the effect of various levels of CaCO₃, inoculation and lime pelleting on nodulation, dry matter, yield and nitrogen content of bean plant (Phaseolus vulgaris cv. Canadian Wonder) in five acid soils in greenhouse condition. The soil represented a range of pH from 3.9 to 5.1 with exchangeable aluminium from 0.0 to 4 meq/100 g, exchangeable manganese from 0.35 to 2.32 meq/100 g and percent carbon from 6.69 to 5.60. This pH range was selected depending upon the buffering capacity of each soil. To achieve the required pH, CaCO₃ was applied.

It was observed that application of CaCO₃ increased soil pH and exchangeable calcium in all soils but exchangeable aluminium and manganese decreased with increasing amount of CaCO₃. Nodule weight decreased with
increasing percent carbon and at highest percent carbon no nodules were observed. Inoculation increased nodule weight, dry matter yield and percent nitrogen particularly at the low pH level. However, nodule weight and dry matter yield increased with soil pH where the seeds were not inoculated. At low organic matter content and with substantial amounts of aluminium and/or manganese, liming increasing nodule weight and dry matter yield and decreased exchangeable aluminium and/or manganese. At higher organic matter content, nodulation was very low or absent. However, low lime rates had little effect on exchangeable aluminium and calcium and dry matter yield; but higher lime rates decreased exchangeable aluminium and dry matter yield but increased exchangeable calcium.

Emanuelsson (1984) performed three pot experiments on barley (Hordeum vulgare cv. Nordal and in experiment 2 cv. Welam as well) to study the relationship between root growth and calcium uptake. The pots were filled with a sand-perlite mixture and the bottom of the pots was replaced by a layer of peat. The peat had been brought to equilibrium with a CaCl₂ concentration corresponding to the test medium concentration. The test medium consisted of solution of CaCl₂. The solution differed by a factor of 10 in concentration, in the range 10⁻⁶ to 10⁻² M CaCl₂ in experiment 1 and 10⁻⁵ to 10⁻² M CaCl₂ in experiments 2 and 3. In addition 0.5 mM NaNO₃ was included in the test solution of experiment 3. In order to increase the ionic
strength, nutrients were supplied to the top of the pots five times during the experimental period. The growth of the roots in the test medium was examined daily by counting the total number of roots and the number of roots with laterals. The development of the number of roots and on exponential course vs time at all calcium concentrations and the development was delayed by lowering the calcium concentration. At harvest, it was found that the size of the roots decreased with decreasing calcium concentration to a certain extent.

Golakiya and Patel (1986) conducted a pot experiment to study the effect of four levels of CaCO$_3$ (0, 10, 20 and 30%) and five levels of boron (0, 0.25, 0.50, 1.0 and 2.0 ppm) on the yield and its attributes in groundnut (Arachis hypogea, cv. GAUG 1). It was observed that application of CaCO$_3$ decreased flower number, total number of pegs, dry matter yield, shelling percentage and pod yield/pot, while the number of effective pages increased. As far as boron was concerned, it was noted that its application decreased dry matter yield, number of undeveloped pods, percentage of pops, number of effective pegs, total number of pegs, shelling percentage, pod yield and nodule count. Number of immature pods decreased at 0.25 ppm, increased at 0.50 ppm while the reverse was the case for number of mature pods; but, at 1.0 ppm, both mature as well as immature pods increased.

Jean and Allen (1987) observed calcium concentration in the tissue and severity of calcium deficiency in two
calcium efficient and three calcium inefficient tomato (Lycopersicon esculentum) lines. These lines were selected on the basis of dry matter production. The plants were grown in nutrient solution containing six levels of calcium, ranging from 15 to 365 mg/l, with 70 mg/l increments. It was observed that the concentrations of calcium in the shoot were 0.25% and 0.4% in calcium efficient and calcium inefficient lines respectively. The concentration of Ca, K, Mg, P and NO\textsubscript{3}\textsuperscript{-} were lower in shoots except for magnesium, which was lower in roots of efficient than in the inefficient plants. Moreover, all lines responded to increased supply of calcium by enhancing dry weight concomitant with the accumulation of calcium. The concentration of other nutrients decreased with the increase in calcium in shoot and roots as more calcium was available in the medium.

Grieve and Fujiyama (1987) studied the response of two cultivars ("M-9" and "M-201") of rice (Oryza sativa) to nutrient culture salinated at -0.4 MPa with varying ratio of sodium and calcium. They reported that at the highest Na/Ca ratio (198 on a molar basis) shoot exhibited calcium deficiency symptoms. These symptoms included tightly rolled emerging blades, tips withered and adjacent blades adhered to one another. Fresh and dry matter production was lowest at high Na/Ca. Maximum shoot growth of plants occurred when Na/Ca ratio was 18, and decreased with increasing calcium. The correlation between Na/Ca and seedling growth was highly
significant for "M-201", but marginally so for "M-9". Sodium concentration in the shoot decreased as the Na/Ca ratio in the external solution decreased and induced inhibition of calcium uptake and transport, which appeared to be more limiting to shoot growth of both cultivars than sodium toxicity per se.

Behling et al. (1989) studied the distribution and utilisation of calcium by two tomato (Lycopersicon esculentum Mill.) lines. One was calcium-efficient (113E) and the other calcium-inefficient (671), both grown under low calcium stress. The strains of tomato differed in the utilisation efficiency of calcium, expressed as CaER (mg of dry weight produced for each mg of calcium absorbed by plant). It was suggested that the calcium efficiency in strain (113E) had a slow movement of absorbed calcium, allowing for the continuous growth of the shoot apex and upper lamina under calcium deficiency condition. On the other hand, in calcium-inefficient strain (671), calcium was rapidly deposited in the lower leaves with little upward movements in the plants after absorption. The separation of tissue calcium into various forms suggested that calcium-inefficiency was associated with higher concentration of insoluble calcium in the shoot tissue. The calcium-efficient strain sustained growth at lower level of calcium and maintained a higher ratio of soluble to insoluble calcium in all shoot tissue. However in Ca-inefficient strain the calcium was concentrated in the lower plant tissues, which
limited its availability for continuous shoot growth. Autoradiographs suggested that the upward movement of calcium was sustained in line 113 (E), in spite of vastly reduced transpiration rates and a root system characterised by leakage of potassium ions from the roots back into the solution.

Pijenborg et al. (1990) conducted an experiment on lucerne (Medicago sativa cv. Resis) to study the effect of calcium on the nodulation using EGTA, [ethylene glycol-bis (α-amino-ethyl ehter) N,N,N',N'-tetraacetic acid], a calcium chelator. The plants were grown either in hydroponic culture or in rhizotron culture. The seeds were inoculated with Rhizobium meliloti strain K-24. It was observed that the number of nodules was optimum at 0.02 mM calcium given in the form of CaCl₂; but when calcium was not given the number of nodules decreased by 70% and similar decrease was observed with 0.2 mM calcium solution in the presence of 0.2 mM EGTA. At 0.4 M EGTA, root growth was reduced and nodule development was totally inhibited. However, nodulation was restored specifically by the addition of a higher dose of calcium, viz. 0.8M CaCl₂, to the 0.2 mM calcium solution containing 0.4 EGTA but not with MgCl₂. It was also observed that addition of chelator completely eliminated the nitrogenase activity and reduced dry weight by 50%. When the plants were grown in rhizotrons, 67% seedlings became nodulated when the soil around the seeds was changed from pH 5.2 to 6.1 and hence neutralised locally with 1.0 μM of
K₂CO₃ in drops of 12 μl volume. Removal of calcium by the application of 2 μmol of EGTA reduced nodulation by 12% and soil pH from 6.1 to 5.2 and under this condition even the application of phosphate buffer failed to keep soil pH stable. However, such a drastic decrease in pH was avoided by placing agar blocks containing 6 μM of EGTA for three hours on freshly developed roots. This treatment reduced nodulation from 87% to 32%, with soil pH getting lowered from 6.2 to 6.0 only. Like hydroponic system, here also nodulation was restored by adding 2 μM of CaCl₂ and the use of MgCl₂ had no effect. The calcium-sensitivity of nodulation with course of time was also studied. They observed that depletion of soil calcium could depress nodulation only during the first day after inoculation and concluded that calcium had different modes of action in the symbiotic process during the initiation and formation of the nodules.

Pinjenborg and Lie (1990) studied the effect of lime pelleting on the nodulation of lucerne (Medicago sativa) in an acid soil. The comparative study was carried out in the field, in pots and in rhizotrons. The seeds were either inoculated with Rhizobium meliloti (R) or inoculated and pelleted with lime (RP). In field condition, lime pelleting (PR) was found superior to inoculation (R) with regard to seedling establishment and nitrogen yield. This positive response of lime pelleting was the result of higher nodulation on the upper 10 mm of the top root of seedlings.
The number of seedlings carrying crown nodules increased from 18% (R) to 56% (PR) at 26 days after sowing. Like field conditions, in pots and rhizotrons also, lime pelleting increased crown nodulation. In pots, the increase was from 32% (R) to 60% (PR) and in rhizotrons from 5% (R) to 90% (PR). Rhizotron was found more suitable for following the early development of root. It was concluded that crown nodulation may be used to quantify the benefit of lime-pelleting.

Oyewole and Aduayi (1992) performed an experiment on tomato (Lycopersicon esculentum Ife Plum cv. 51691) grown in soil culture for 5 months and treated with calcium at concentrations of 0, 40, 80 and 160 ppm as Ca(OH)₂ and boron at 0.1, 2, 4, 8 and 16 ppm as H₃BO₃. It was observed that by the application of calcium alone from 0 to 160 ppm, leaf number decreased; but percent floral abortion increased at 160 ppm. When boron was applied upto 2 ppm without the addition of calcium, it increased floral number and stem diameter. Aborted flowers increased as boron supply increased consistently upto 4 ppm and decreased with higher rates of boron. Fruit yield increased with the combined application of 2 ppm boron and 160 ppm calcium, while at higher rates of boron, viz. 8 and 16 ppm yield decreased. It was also noted that application of calcium at 40 and 80 ppm suppressed the toxic effect of higher levels of boron on leaf number, stem diameter and floral number. However, the effects of the highest boron treatment (16 ppm) were not
reversed by calcium supply. Leaf N, K, Na and B was decreased and leaf calcium and magnesium increased with increasing application of calcium from 0 to 160 ppm. Increasing boron supply decreased leaf N, P and increased leaf K, Ca, Mg and Na. A highly significant increase in leaf boron was observed as boron fertilisation rates increased. Per cent citric acid and crude fibre increased and total chlorophyll content decreased. A decrease in total chlorophyll content was observed as boron increased from 4 to 16 ppm at all calcium levels. It was concluded that tomato yield of 13.8 ton/ha could be obtained by the combined application of 160 ppm (358.4 kg/ha) calcium and 2 ppm boron (4.401 kg/ha), corresponding to a B:Ca of 1:80. However, it was also observed that excessive calcium application in combination with less than 2 ppm boron decreased boron uptake and consequently boron deficiency symptoms developed in plants.

2.3 Role of Vitamins

Vitamins are organic compound which are required in small quantities for normal growth and development of organisms. However, they do not furnish energy and are not utilised as building units for the structure of organisms. These compounds act as co-enzyme in a number of enzyme systems and thus take part in the regulation of metabolism. All known vitamins are synthesised by green plants with the exception of vitamin D. (Schopfer, 1949).
Hopkins in 1906 called these nutritional elements, required in addition to carbohydrates, fats, proteins, salt and water "accessory factors". Funk in 1912 was the first to isolate an amine from rice husks and polishings that alleviated the symptoms of the disease "beri beri". He called these compounds "vitamine" and finally, Drummond in 1920, dropped the terminal 'e' of "vitamine" and proposed the generic term "vitamin" which was accepted by later workers. Osborne and Mendal, and McCollum and Davis in 1915 distinguished two main types of vitamins on the basis of their differential solubility and called them fat-soluble and water-soluble. The fat-soluble vitamins include A, D, E and K while water-soluble vitamins cover B and C. Folker in 1969 gave the definition of vitamins. It reads as "an organic substance of a nutritional nature, present in low concentration as a natural component of enzyme systems and catalyses required reactions and may be derived externally to the tissue or by intrinsic biosynthesis" (Morton, 1974).

The role of B vitamins in plants has been elucidated to some extent during the first half of the present century (Bonner and Bonner, 1948). Vitamin B has been proved to be a mixture of compounds which is referred today as the vitamin B complex. The vitamins isolated and identified from this complex were designated as thiamine (vitamin $B_1$), riboflavin (vitamin $B_2$), niacin, pyridoxine (vitamin $B_6$), pantothenic acid, folic acid, inositol, biotin and cyanocobalamine. In
the following pages the properties and function of pyridoxine (vitamin $B_6$) and its effect on the performance of plants have been reviewed.

2.4 Role of pyridoxine (vitamin $B_6$)

Pyridoxine was isolated in crystalline form from yeast by Kuhn and Wendt in 1939 and Gyorgy and Eckhardt in 1939 and suggested the term pyridoxine. Harris and Folkers (1939) and Stiller et al. (1939) established that vitamin $B_6$ was a pyrimidine derivative, being methyl-3 hydroxy-4-5-di (hydroxy-methyl) pyrimidine. Kuhn and Wendt in 1939 proposed the following chemical structure of pyridoxine

\[
\begin{align*}
\text{H} & \\
\text{H-C-OH} & \\
\text{HO} & \\
\text{H}_3\text{C} & \\
\text{N} & \\
\text{CH}_2\text{OH} & \\
\end{align*}
\]

Pyridoxine is a colourless, crystalline powder, has a slightly bitter taste and melts at 160°C. It is crystallised in the form of various salts, for example, as hydrochloride, m.p. 204-206°C, empirical formula $C_8H_{11}O_3H$. HCl and is readily soluble in water (1 g in 4.5 ml water) and alcohol (1 g in 90 ml alcohol). Pyridoxine occurs in three forms, viz. pyridoxine, pyridoxal phosphate and pyridoximino phosphate. It serves as the tightly bound prosthetic group of a number of enzyme, such as decarboxylases, lyases, synthases and aminotransferases. In aminotransferase
reactions it serves as a transient intermediate carrier of the amino group from donor to acceptor (Lehninger, 1986).

It may be added that vitamin \( B_6 \) is necessary for all living organisms. It is proposed to review the literature pertaining to vitamins of B group as a whole in the following pages with particular emphasis on pyridoxine (\( B_6 \)) in relation to plants.

Bonner and Devirian (1939) cultured excised roots of various plants in nutrient media. Isolated pea roots grew for an unlimited period in a medium containing vitamin \( B_1 \), nicotinic acid, mineral salts and sucrose at the rate of 70-85 mm/week. This growth rate remained unaltered even though various other chemical compounds, including vitamin \( B_2, B_6, C, E, K, \) adenine, thelin, \( B \)-alanine, pantothenic acid and numerous amino acids were added. The rate of growth of isolated radish roots was 15 mm/week when they were grown through 15 passages in the presence of vitamin \( B_1 \) and nicotinic acid. These vitamins were found indispensable to maintain normal growth of the roots of these plants. The addition of other chemical compounds listed above was found ineffective. Similarly, excised flax roots responded only to vitamin \( B_1 \) and grew at the rate of 150 mm/week. On the other hand, excised roots of tomato showed a growth rate of 40 mm/week in the presence of vitamin \( B_1 \) and \( B_6 \). This rate was further enhanced up to 60 mm/week on supplementing the nutrient medium with nicotinic acid. According to the
authors, these studies revealed that excised roots of various plants required different growth factors in the nutrient medium.

Robbins and Schmidt (1939) found that addition of light brown sugar in nutrient medium was more beneficial for the growth of excised tomato roots than pure cane sugar. The growth of roots decreased when light brown sugar was replaced by its ash (treated with hydrochloric acid) or pure cane sugar, containing minerals, nicotinic acid, nicotinamide, thiamine and amino acids. However, addition of pyridoxine with pure cane sugar in the nutrient medium, promoted growth of roots. Addition of pyridoxine also induced the development of hooks and curls, indicating that it caused the elongation of cells. These observations showed that light brown sugar contains some growth factors.

Bonner (1940) investigated root growth factor requirements of several plants in vitro. Excised roots of alfalfa, clover and cotton needed vitamin B₁ and nicotinic acid for their luxurious growth. However, addition of vitamin B₆ was not found beneficial for further root growth. Regarding other plants, isolated roots of datura (Datura stramonium) and sunflower (Helianthus annuus) showed profuse growth in the presence of vitamin B₁, B₆ and nicotinic acid. Similarly, isolated roots of carrot required vitamin B₁ and B₆ but the addition of nicotinic acid was of no use. In the case of five different strains of tomato, vitamins B₁ and B₆
proved beneficial for root growth which was further promoted by the inclusion of nicotinic acid in the medium. At the same time, excised roots of clover and flax were found to synthesis vitamin \( B_1 \) in small amounts. Therefore, they maintained sub-optimal growth even in the absence of this vitamin.

White (1940) studied the effect of vitamin \( B_6 \), nicotinic acid and pyridine in the presence of sufficient thiamine in nutrient medium on the growth of excised roots of two tomato strains. Surprisingly, these strains of tomato did not significantly respond to vitamin \( B_6 \), nicotinic acid and pyridine. He, therefore, concluded that these strains of tomato contained adequate amount of these substances to support root growth under the experimental condition.

Day (1941) successfully cultured excised tomato roots in a nutrient medium containing 1.0% sucrose and 0.5% purified agar to which thiamine, pyridoxine, nicotinamide, neopeptone, glutamic acid and glycine were added in different combinations. It was observed that, with the addition of thiamine, root tips grew about 2.0 mm daily. In the same experiment, where pyridoxine was added to the agar medium containing, thiamine, similar root tips generally showed a daily increment of 5.0-6.0 mm, or in several passages as much as 8.0 mm. Supplementing this medium with nicotinamide had no effect on the rate of growth of roots. On the other hand, addition of neopeptone decreased the rate of growth to two thirds of that in the medium containing
thiamine and pyridoxine. Addition of glutamic acid to the agar medium containing thiamine decreased the growth of roots. The addition of glycine to the agar medium containing thiamine had no effect. On the basis of these observations, it was concluded that pyridoxine had a marked beneficial effect in promoting root growth.

Minnum (1941a) grew radish and cauliflower in sand supplied with Hoagland solution plus minor elements. He treated pots by weighing out the necessary number of mg of pure crystalline vitamins B_1, B_2 and B_6 and adding sufficient amount of water so that 1 ml solution contained 1 mg of vitamins. Vitaflor, a commercial compound containing 0.1% vitamin B_1, 0.5% nicotinic acid and traces of vitamin B_2, B_6 and pantothenic acid, was also included in the scheme of treatments. It was observed that none of the treatments had any influence on these two vegetables.

In a subsequent experiment, Minnum (1941b) treated roots of cauliflower, muskmelon, beets, sweetcorn, summer squash, potato, snap beans, peppers and radish with solution of pure crystalline vitaflor (vitamins, commercial compound containing vitamin B_1, B_2 and B_6 etc.) or brewer's yeast. It was found that none of the treatments effected the productivity of these plants significantly.

Robbins (1942) noted the effect of twelve analogues of pyridoxine on the growth of excised tomato roots in the presence of thiamine. Out of the twelve analogues tested,
nine were inactive. Acetylation of pyridoxine and substitution of an ethyl for the methyl group in the second position of the pyridoxine ring did not reduce the activity. He concluded that pyridoxine had a high degree of specificity for the growth of excised tomato roots.

Day (1943) transferred uniform pieces of inoculum from tomato roots grown for 57 or more passages in a mineral-sugar solution containing thiamine or its thiazole intermediate to petridishes, containing a modified Pfeffer's solution with 1% sucrose and 0.5% purified agar. To this medium thiamine, pyridoxine, nicotinamide, neopeptone, glutamic acid and glycine were added in various combinations. They were incubated in moist chamber at 25°C in the dark. In basal agar with no added growth substance, the roots seldom grew for more than two passages. With the addition of thiamine the root tips grew about 1.77 mm daily for an unlimited number of passages. When pyridoxine was added to the agar medium with the thiamine the daily rate was about 5.2 mm and the roots in this medium showed the characteristic hooks and curls. She further observed that supplementing this medium with nicotinamide had no appreciable effect on the rate of growth and the addition of neopeptone decreased it. However, further addition of glutamic acid to the agar medium containing thiamine had little or no effect.

Noggle and Wynd (1943) noted the effect of vitamins on germination and growth of orchid seeds. Orchids Cattleya
trianeae cv. mooreana and C. schrooderae seeds germinated and produced normal growth in an artificial nutrient medium when one lot of maltose was used; but there was no germination and seedling growth did not occur when a more purified maltose was used. This inhibitory effect was not overcome by the addition of thiamine hydrochloride, ascorbic acid (vitamin C) or calcium pantothenate (chick antidermatitis factor) when grown in the presence of purified maltose in the growth medium. However, a few seed germinated and slow development of seedlings occurred when riboflavin was present in the medium. Further, they observed that the presence of pyridoxine permitted good germination but poor seedling development, but good germination as well as excellent development of the seedlings occurred when nicotinic acid was supplied in the nutrient medium.

Almestrand (1950) tested the effect of thiamine, pyridoxine and niacin added to the solution medium on excised roots of wheat. He noted that pyridoxine alone had a marked effect on growth of wheat roots by promoting meristematic cell division. Optimum growth of the roots was obtained in pyridoxine concentrations ranging from 0.5 to 1.0 mg/l at 27-28°C.

Later, Almestrand (1951) observed the effect of pyridoxine and its derivatives (pyridoxal phosphate and pyridoxamine) on the growth and metabolism of strains of wheat, barley, oats and rye. The pyridoxine sensitive wheat variety Eroica showed marked increase in root length,
whereas other varieties were not affected by the application of the vitamin. In order to study the metabolism, two wheat strains, namely Eroica-pyridoxine sensitive and Virtus-pyridoxine insensitive, were selected. It was noted that the absorption of glucose phosphate and nitrate uptake in Eroica wheat responded significantly to the applied pyridoxine concentration, while Vitrus did not show any response to pyridoxine treatment with regard to absorption of glucose and the ions. It was further noted that the effect of pyridoxine derivatives, i.e. pyridoxal phosphate and pyridoxamine, was similar to that of the mother compound. However, barley, oats and rye strains did not show any response to either pyridoxine or to its derivatives.

Lee and Whaley (1953) studied the effect of individual or combined vitamin supplement of thiamine, niacin and pyridoxine to culture medium for 4 weeks on the growth of excised tomato roots. The roots were grown in culture media (a) containing no vitamins and (b) containing thiamine, niacin, and pyridoxine alone or in combinations. They noted that growth in all media was about the same during the first week. Little additional growth was obtained from roots grown in media containing pyridoxine or niacin alone. In media containing thiamine alone or combinations of any two or three of the vitamins, growth of roots was significantly higher than in the other media. Further, in the medium containing all the three vitamins, growth of roots was significantly more than in any other medium.
However, between the third and fourth week, growth in culture was markedly decreased. They concluded that the best period for investigation of the effect of growth substances under their experimental conditions was during the second and third week.

Boll (1954) observed that a clone of excised tomato roots required thiamine, pyridoxine and niacin for optimal growth and could also be maintained in a medium supplemented only with thiamine and pyridoxine. It was also found that pyridoxine was replaceable by pyridoxal or pyridoxamine. The order of activity was pyridoxal > pyridoxine > pyridoxamine. Likewise, niacin was replaceable by niacinamide. However, niacinamide was, in general, more active. He further noted that pyridoxine could be replaced with glycine. This replacement was more pronounced in the presence than in the absence of niacin. In basal medium containing thiamine, pyridoxine and niacin, the addition of glycine at certain concentrations of pyridoxine increased the level of growth to that obtained with optimal concentration of pyridoxine. It was also observed that glycine and pyridoxine had similar morphogenetic effect on the growth of roots. Moreover, glycine seemed to exert an independent effect upon the initiation of laterals. It was concluded that the morphology of root was controlled by balanced growth factors given in the medium.
Fujiwara and Ojima (1954) studied the effect of thiamine, pyridoxine and niacin on the growth of excised root tips of rice and wheat in liquid medium. The root tips of rice responded positively to thiamine or pyridoxine application. However, in case of wheat, pyridoxine gave better results. They further observed that the excised plant roots attached to their scutella grew much longer than their root tips and showed no reaction to vitamins.

Fries (1955a) compared the biosynthetic capabilities of decotylised pea seedlings grown in the dark with those of excised roots of same plant. The decotylised pea seedlings required a mixture of water-soluble vitamins and various amino acids in sucrose-mineral salt medium for their optimum growth. Excised roots, on the other hand, remained unaffected by the application of these substances and attained a length of 150 mm even in the absence of the vitamins. From this, he inferred that excised roots had adequate reserve of vitamins. However, the roots ceased to grow after one or two transfers; but the growth was again maintained on supplying thiamine and niacin. In case of pea seedlings, the growth diminished very soon in a medium lacking the vitamins and the main root did not grow beyond 100 mm. The growth of the seedlings was further maintained by inclusion of thiamine and pyridoxine. However, niacin showed either poor or no effect. These studies showed that shoots failed to produce vitamins in the dark and consumed the vitamin reserves of the hypocotyl and young roots for
their growth.

Fries (1955b) also worked out the doses of thiamine and pyridoxine which could support the growth of decotylised pea seedlings in the dark on an agar-nutrient medium containing niacinamide and various amino acid. 10 g of thiamine and 100 \( \mu \text{g} \) of pyridoxine /l were required to maintain normal growth of the seedlings. However, the growth and development of these seedlings was regulated independently by thiamine, pyridoxine and niacin. It was also observed that, even in light, the added thiamine alone controlled the growth rate considerably, suggesting that the synthesis of thiamine in light could not keep pace with the optimum requirement of the seedlings.

Brusca and Haas (1957) studied the effect of several chemically pure salts of organic compounds on citrus grown in sand culture. Addition of vitamin \( B_6 \) (0.01 and 0.2 g/plant) and vitamin \( B_{12} \) (0.02 g/plant) to the nutrient solution was found to stimulate the growth of citrus plants.

Barbieri (1959) performed a pot culture experiment on pea, broad bean, beet and wheat and observed that application of vitamin \( B_1 \) and \( B_6 \) enhanced plant height, leaf number, fresh weight and dry weight. The effect of both the vitamins was most pronounced on beet and poorest on pea. For example, each of these vitamins at 0.01 mg/l increased leaf number by 5\% in beet seedlings compared with 4\% in pea.
Vergnano (1959) tried vitamins $B_1$ and $B_6$ for improving the rooting in cuttings of some plants in sand culture. Each vitamin was added to the nutrient solution at the rate of 0.01 mg/l. Vitamin treatment did not improve rooting in *Colutea arborescens* but *Hedera helix* and *Rosa* showed good response. Treated plants of *Rosa* also produced greater number of buds and leaves with broader leaf blades than the controls.

Kudrev and Pavlov (1965) applied vitamin $B_6$ on the leaves of flooded wheat crop. The foliar application of this vitamin averted the ill effect of flooding at tillering, shooting and heading stages. The spray of the vitamin solution also corrected disturbed nitrogen metabolism, particularly when very little damage had been done, and consequently grain yield was enhanced.

Das and Das (1966) observed the growth of excised pea roots as influenced by thiamine and pyridoxine treatments. They reported that these two vitamins showed similar growth promoting activity. However, the optimum dose of thiamine and pyridoxine differed, being 0.1 and 0.01 ppm respectively. It was pointed out that vitamins might become more effective in the presence of mineral salts.

Ovcharov and Kulieva (1968) soaked cotton seeds in 0.01% pyridoxine solution for 1-3 h. After soaking, the seeds were sown in nutrient culture with different fertiliser sources of nitrogen and phosphorus. The vitamin
slightly promoted germination and increased the area of first leaf two- to three-fold. It was observed that the effectiveness of the vitamins depended upon the form of fertiliser used. Root length of treated seedlings in the presence of ammonium sulphate was 49% more than in controls (untreated) and was 14% less with calcium nitrate. Nitrogen and phosphorus content of 2 day old seedlings was increased by the vitamin and the content was more in the presence of potassium dihydrogen orthophosphate than in that of superphosphate.

Dimistrova-Russeva and Lilova (1969) performed an experiment in nutrient solution and in soil to study the effect of the application of thiamine, pyridoxine and nicotinic acid on the uptake of nitrogen and phosphorus by *Mentha piperata*. It was found that uptake of these two major nutrients was increased by the application of these vitamins. Phosphorus uptake was increased by a single application of nicotinic acid and double application of others. Nicotinic acid also enhanced the yield of essential oil as did the two application of thiamine whereas pyridoxine reduced it.

Zavenyagina and Bukin (1969) studied the effect of application of an antagonist of pyridoxine on the germination and viability of seeds, development of root system and on the above ground portion of pea and wheat seedlings in water culture. They observed that the
pyridoxine antagonist, introduced into Knop's nutrient medium at $10^{-3}$ to $10^{-5}$ M lowered the germination rate and suppressed the growth of roots and above ground portion of the seedlings of both genera. The appearance of symptoms of $B_6$ avitaminosis was partially or entirely prevented by the addition of pyridoxine to the culture medium. They also observed that vitamin $B_6$ stimulated the growth of normal wheat and pea seedling and increased chlorophyll content in the leaves.

Kozhim and Kravtsov (1973) added various concentration of pyridoxine (0.001, 0.01, 1.0 and 10 mg/l) in a medium to study the effect on isolated pear and apple embryos at different stages of ripeness. It was observed that application of pyridoxine increased germination, accumulation of chlorophyll and differentiation of embryos into seedlings. The embryos from unripe seeds were more affected by pyridoxine than those from ripe seeds.

Gopala Rao et al. (1974) observed high succinic dehydrogenase activity in root and shoot, and enhanced respiration and protein synthesis in 4 day old seedlings of *Phaseolus radiatus* in a water culture experiment as a result of supplying the plants with biotin, pyridoxine, niacin, and thiamine in the nutrient solution.

Kulieva et al. (1976) investigated the response of melon and watermelon to vitamin treatments in laboratory and field conditions. Seeds of these plants were treated with
various concentrations ranging from 0.01 to 0.0001% of thiamine, cyanocobalamine, nicotinic acid, pyridoxine or ascorbic acid. The effect of these compounds on stem and root development as well as on number and weight of fruit was studied in 45 and 90 days old plant respectively. Generally, the best results were obtained by treating the seeds with thiamine (0.001%), cyanocobalamine (0.0001%) or nicotinic acid (0.0001%). However, beneficial results were obtained by spraying the plants with cyanocobalamine (0.001%) or ascorbic acid (0.01%) respectively.

Afridi et al. (1979) tested a number of common vitamins and phytohormones with respect to seed germination and radicle growth of barley and noted vitamin B\textsubscript{6} to be the most effective. On the basis of this preliminary trial, they performed an experiment on barley var. K 672/28 in sand culture. Seeds of barley were soaked for 24 h in 0.1, 0.3 and 0.5% aqueous pyridoxine solution and thereafter sown in pots. Treatment with pyridoxine benefited most of the root, shoot and ear characteristics as well as grain yield and quality. Generally, soaking in 0.3 and 0.5% proved equally beneficial. Root length and number of lateral roots as well as leaf number were found to be 66, 19.0 and 13.5% more respectively in 0.3% treatment than in the water-soaked control. Tiller number/plant was at par in 0.3 and 0.5% pyridoxine treatments, being enhanced by 13% over the control. Treatment with 0.3% pyridoxine increased shoot length by 5.8% over the control, with 0.5% treatment
following closely behind. Both these treatments consequently resulted in 15% more dry weight than in control. Similarly, other characteristics, including seed yield and seed carbohydrates were enhanced by 9.0 and 1.4% respectively by 0.3% treatment over the control; but straw yield was 12.7% more in 0.5% treatment (at par with 0.3%) than the control.

Ahmad et al. (1981) studied the effect of pre-sowing soaking treatment of grain with pyridoxine on the growth of five varieties of barley, namely NP 3, NP 21, K 572/10, K 572/28 and Clipper. The seeds of these varieties were soaked for 24 h in different concentrations of aqueous pyridoxine solution, viz. 0.0, 0.002, 0.1 and 0.5% before sowing in the field. Pyridoxine treatment significantly affected tiller number, leaf number, shoot length and fresh and dry weight at tillering, heading and milky grain stages. Seed treatment with 0.1% solution proved optimum at all stages of growth. Tiller and leaf number were maximum in Clipper and minimum in K 572/28 but the later produced the tallest plants. NP 21 had the maximum fresh and dry weight, followed by K 572/28. Treatment x variety interaction effect was also found to be significant. The response to various combinations varied from character to character and stage to stage but the combination of 0.02% pyridoxine and K 572/28 proved best.

Ahmad et al. (1982) reported the response of grain and straw yield of the same five barley cultivars under the same pyridoxine treatment as mentioned above. Maximum grain
yield was recorded in 0.1 pyridoxine treatment, whereas maximum straw yield was found in 0.02% treatment. Regarding varieties, K 572 28 and NP 21 out-yielded all others in grain yield and straw yield respectively. The combination 0.1% x K 572/10 gave maximum grain yield while 0.02% x NP 13 produced maximum straw.

Kodandaramaiah and Gopala Rao (1984) observed the influence of B vitamins on photosynthesis of isolated chloroplast of *Cyamopsis tetragonoloba* CL. Taub. They noted that thiamine, riboflavin, niacin, pyridoxine, pantothenic acid and folic acid, at concentration varying from 50-200 mg/l, significantly increased photosynthetic carbon fixation by isolated chloroplasts. However, direct action of the vitamins above 5 mg/l concentration in vitro diminished carbon fixation. The maximum promotion was noticed in niacin (51.0%), followed by pyridoxine (44.9%).

Raghava Reddy and Gopala Rao (1984) noted the influence of pyridoxine and citric acid on flowering in *Brassica nigra* Kock. and found that these two induced early flowering in black mustard. They concluded that this was due to the increase in the leaf area and height of the treated plants.

Gopala Rao and Raghava Reddy (1985) observed the effect of B vitamins on the uptake of sodium, potassium, calcium and phosphorus in one week old *Vigna radiata* seedlings. Treatment with the vitamins promoted the uptake
of these elements variably. Thiamine and biotin were found ineffective in the uptake of phosphorus, but riboflavin, pyridoxine and pantothenic acid increased the uptake of sodium, potassium and calcium in addition to that of phosphorus by the seedlings. Application of pyridoxine, pantothenic acid and nicotinic acid particularly showed more influence on potassium and phosphorus uptake than the other vitamins included in the study.

Samiullah et al. (1985) conducted two simple randomised field experiments to compare the efficacy of method of application of pyridoxine to moong (Vigna radiata cv. K-851). In one experiment seeds of moong were soaked in 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5% aqueous pyridoxine solution for 4 h. In the other experiment, the same concentrations of pyridoxine solution were sprayed on the tops of the crop at 35 and 45 DAS. The parameters studied at 20, 30, 40 and 50 DAS included root length, root nodule number and NRA. Root length, root nodule number and NRA in foliar spray experiment were recorded at 45 DAS. In both the experiments, seed yield was measured at harvest and the correlated between NRA and yield determined.

In the seed soaking experiment, 0.3% pyridoxine solution, in general, gave optimum value for root length and nodule number at all sampling stages and increased NRA by 29.7, 7.1, 11.8 and 15.6% over the respective water-soaked controls at 20, 30, 40 and 50 DAS respectively. Spray of
0.1% pyridoxine solution at the pre-flowering stage (35 DAS) and of 0.2% at the post-flowering stage (45 DAS) proved best for NRA and the activity was increased by 49.2 and 29.6% respectively over the water-sprayed controls. Spray of 0.1% and soaking in 0.3% pyridoxine solution gave 33.7% and 44.9% more seed yield respectively compared with the controls. A highly significant and positive correlation was found between NRA and seed yield in both the experiments. From the study, they concluded that leaf NRA levels at early stages of growth could be utilised for predicting crop productivity. It was also inferred that soaking treatment has superiority over spray treatment.

Ansari et al. (1990) studied the effect of pre-sowing soaking of seeds with 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5% aqueous pyridoxine solution for 12 h on growth parameters (root length, root nodule number, dry weight of root and whole plant, and leaf area), leaf NRA and NPK as well as on yield parameters (pods/plant, seeds/pod, single seed weight, and seed yield) and on protein content of seeds of lentil (*Lens culinaris* cv. T 36). Unsoaked and water-soaked controls were also included in the scheme of treatments. The growth parameters, leaf NRA and NPK were studied at 60, 90 and 120 DAS. NAR was computed for the periods 60-90 and 90-120 DAS. Yield parameters and seed protein content were studied at harvest. 0.3% pyridoxine treatment proved optimum for growth parameters. NAR, NRA and leaf NPK at all sampling stages. All yield parameters were found to be significantly
affected by pyridoxine treatment. The maximum number of pods/plant and seeds/pod was in the 0.5% treatment. Pyridoxine at 0.3% concentration gave highest seed yield and seed protein content, the increase being 21% and 12.5% respectively over the control.

In a field experiment on mustard (Brassica juncea cv. Varuna) Samiullah et al. (1991) studied the effect of pre-sowing seed soaking treatment for 4 h with 0.0125, 0.025, 0.05 and 0.10% aqueous pyridoxine solution with three basal doses of N and P, viz. N$_{60}$P$_{20}$, N$_{90}$P$_{30}$ and N$_{60+30}$P$_{30}$, alone as well as in combination. The parameters studied included root length, leaf number, dry weight and leaf NPK at 50, 70 and 90 DAS and NAR for the period 50-70 and 70-90 DAS. Two controls (unsoaked and water-soaked) were maintained for comparison. At harvest, pods/plant, seeds/pod, hectolitre weight of seeds, oil content of seeds, seed yield and oil yield were determined. Soaking the seed with 0.025% pyridoxine solution gave maximum value for all parameters at various stages, except hectolitre weight which was more in 0.0125% pyridoxine treatments. 0.025% pyridoxine increased seed oil content by 18.0%, seed yield by 14.9% and oil yield by 14.9% in comparison with the water-soaked control. N$_{60}$P$_{20}$ fertiliser level proved best for all parameters. The interaction 0.0125 x N$_{60}$P$_{20}$ gave maximum values for various parameters. However, it effect was at par with that of 0.025 x N$_{60}$P$_{20}$ and 0.025 x N$_{90}$P$_{20}$. It was concluded that soaking the seeds of mustard in 0.0125% pyridoxine solution and
giving a basal fertiliser dose of $N_{60}P_{20}$ only would give optimum results, thus saving 30 kg N and 10 kg P/ha compared with the recommended dose of basal fertiliser, viz. $N_{90}P_{30}$.

Samiullah et al. (1992) studied the effect of pre-sowing seed treatment for 12 h with 0.02, 0.3 and 0.4% pyridoxine solution on various parameters of lentil (*Lens culinaris* L. Medic cv. T 36). The crop was grown with 15, 30, 45 and 60 kg P/ha. The parameters studied included NAR at 60-90 and 90-120 DAS intervals, NRA at 60, 90 and 120 DAS, pods/plant, length/pod, seeds/pod, 1,000 seed weight, seed yield and seed protein content at harvest (140 DAS). Seed treatment with 0.3% pyridoxine proved optimum and increased NRA by 23.3% at 60 DAS, 42.7% at 90 DAS, 87.4% at 120 DAS, NAR by 56.2% at 60-90 DAS and 43.5% at 90-120 DAS. At harvest, pods/plant, length/pod, seeds/pod, 1,000 seed weight, seed yield and seed protein content were noted to be increased by 70.6%, 24.0%, 72.9%, 6.4%, 29.1% and 12.8% respectively. Basal application of 30 kg P/ha gave maximum value for all parameters. The interaction, 0.2% pyridoxine x 30 kg P/ha showed maximum value for most of the parameters studied, except 1,000 seed weight. NRA and NAR showed strong correlation with seed yield and seed protein content, while yield attributes showed a similar correlation with seed yield only. Thus, 0.3% pyridoxine in combination with 30 kg P/ha ensured higher yield and improved quality of lentil.
2.5 Concluding remarks

The foregoing review clearly established that pyridoxine (vitamin B₆) is a potent root growth promoting substance for many crops. In some cases, pyridoxine has been shown to stimulate nutrient uptake. However, the information regarding to interaction effect of mineral nutrients and vitamin on the performance of crops is meager. The work on the interaction effect of calcium and pyridoxine (vitamin B₆) on the performance of important crops in particular, seems to be neglected. Therefore an in depth study on the physiological response of pulse, oil and cereal crops to calcium and pyridoxine (vitamin B₆) alone and in combination is highly desirable. The research work reported in the subsequent chapters is related to this aspect in order to fill the existing lacuna in our understanding of pyridoxine-calcium relationship in augmenting the performance of said crops.