Regulatory Role of Mineral Elements in the Metabolism of Medicinal Plants

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ABSTRACT

Minerals have a diversified role in medicinal plant metabolism. Severity or scarcity of these causes multifarious effects in plant metabolism. Each and every aspect of plant biochemistry, physiology, anatomy, etc. is affected due to mineral nutrient composition of soils. Medicinal plants inherit resistance due to biosynthesis of bioactive substances (secondary metabolites) against the various types of diseases caused due to fungus, bacteria, viruses, mycoplasmas, insects and pests. The concentration of these minerals of both group i.e. activators or inhibitors present in the soil play a vital role in secondary plant metabolism. Minerals also play a major role in the reproduction of these medicinally important plants. Bioactive molecules of medical relevancy such as alkaloids, flavonoids, lignans, lipids, carbohydrates, resins, glycosides, phenolic compounds, volatile oils, vitamins, tannins etc. produced through various biosynthetic pathways of plants are a boon to urban, hilly and remote population of each nation. However, soils with different compositions of mineral elements adversely influence the metabolic activities of such valuable medicinal plants. Various physiological activities are governed through important mineral elements present in soils from where these are transferred to area where their need arises. So, accumulation and biosynthesis of these bioactive molecules in a plant system are widely dependent on the availability of mineral elements in the soil. Different developmental stages of the medicinal plants need supplementation of different macro- and micro- elements during its various growth and biosynthesis steps. This review deals with regulatory role of various mineral elements in each biological activities of different medicinal plants viz. Papaver, Catharanthus, Withania, etc.

Keywords: alkaloids, bioactive molecules, growth, nutrition, pathways

INTRODUCTION

Minerals have a profound effect on the metabolic activities in medicinal and aromatic plants (MAPs) that represent a consistent part of the natural biodiversity endowment of many nations of the world (Okigbo et al. 2008). The role and contributions of various mineral elements can be revealed through their regulatory role played in metabolism of medicinal and aromatic plants. Innumerable substances of immense importance are synthesized such as sugars, carbohydrates, resins, glycosides, phenolic compounds, volatile oils, vitamins, tannins, etc. produced through various biosynthetic pathways of plants are a boon to urban, hilly and remote population of each nation. However, soils with different compositions of mineral elements adversely influence the metabolic activities of such valuable medicinal plants. Various physiological activities are governed through important mineral elements present in soils from where these are transferred to area where their need arises. So, accumulation and biosynthesis of these bioactive molecules in a plant system are widely dependent on the availability of mineral elements in the soil. Different developmental stages of the medicinal plants need supplementation of different macro- and micro- elements during its various growth and biosynthesis steps. This review deals with regulatory role of various mineral elements in each biological activities of different medicinal plants viz. Papaver, Catharanthus, Withania, etc.
starch, cellulose, acids, lignin, tannins, amino acids, proteins, amides, etc. during metabolic processes in plants (Soetan et al. 2010). Most of the medicinal plants produce secondary metabolites as for instance morphine in Papaver, withanolides in Withania, vincristine and vinblastine in Catharanthus, etc. which are a boon to human health. The utilization of MAPs as a source of fuel, building material, food, fodder and fibre, in every country has, however, led to a multiplicity of new natural product-based industries and pharmaceutical products. This has been spurred by the interests of the developed countries for traditional medicine and natural products (Okigbo et al. 2008). Today, there is an increasing requirement for plant-based medicines, health products, pharmaceuticals, food supplements, cosmetics, etc. in the international market. The international market of medicinal plants is over 60 billion US$ per year, which is growing at the rate of 7% per annum. The present export of herbal raw materials and medicines from India is about 100-114 million US$ per year approximately. India is one of the major exporters of crude drugs among six developed countries viz. USA, Germany, France, Switzerland, U.K. and Japan, and shares 75-80% of the total export market between them (Chatterjee 2001). Global market size for herbal and medicinal plants is projected to reach US$ 5 trillion by 2050 (Jay et al. 2010). During plant growth, there are continuous processes of building up of complex compounds of carbon (C) and nitrogen (N) and their breakdown into simple substances, in which water (H₂O) and oxygen (O) are intimately concerned. The chief processes involved in plant metabolism viz. absorption, C assimilation, photosynthesis, formation of protoplant, transpiration, respiration, translocation, storage, etc. are regulated by micro- and macro-elements readily available in different forms in the soil (Soetan et al. 2010). Supplementing nutrient requirement of medicinal crops through organic manures plays a key role in sustaining soil fertility and crop productivity (Patra et al. 2000). With growth cycles, there are certain well defined chemical cycles of nutrient elements and their elaborated products in the leaves, stems and roots, etc. Even the presence of adequate quantities of plant nutrients in the soil does not guarantee its absorption by the plant roots. For example, the latter condition is found in poorly aerated soils, where lack of O₂ near the roots may prevent them from being actively absorbed by the roots (Welch and Graham 2004; Soetan et al. 2010).

It is well known that certain elements are necessary for the healthy growth and proper functioning of plant metabolism (Ozcan 2003). They are sometimes referred to as essential. Indeed, there is no existence of some of them in larger quantities and while others in very small amounts. Thus, the former elements are referred to "major" elements and the latter ones as "minor" or "trace" elements or as "micro-elements" (Eruvbetine 2003). The terms "major" and "minor" do not refer to the relative importance of the functions of elements in plant growth, and for this reason the term "trace" element is preferable for the latter class. In addition, there is some other elements, such as sodium (Na), chlorine (Cl) and silicon (Si), which produce beneficial effects on the growth of certain plants but have not so far been shown to be absolutely essential to growth. The element aluminium (Al) is of general occurrence in plants, but it seems to be without direct nutritional value, although aluminum sulfate is used, because of its acidifying properties, to change the color of hydrangeas growing on alkaline soils to blue. Al may affect many nutritional processes as well (Mossor-Pietraszewska 2001). Other elements often occur in plants but they are not known to serve any useful function and are frequently referred to as poisons or toxins to the plant. The nutrient elements can only be absorbed by plants when present in certain definite forms, as for example N in nitrates and ammonium salts, phosphorus (P) in phosphates, calcium (Ca), magnesium (Mg) and potassium (K) in their salts as chlorides, sulphur (S) in sulphates, iron (Fe) in ferrous or ferric salts (more readily from ferrous salts), manganese (Mn) in magnaninisalts, boron (B) in borates, copper (Cu) and zinc (Zn) in their salts, and molybdenum (Mo) in molybdates (Soetan et al. 2010; Mazid et al. 2011). Among the various macro-elements required for plant metabolism, N is a major constituent of the most important substances found in plants. N constitutes 40 to 50% of the dry matter of protoplasm, proteins, chlorophyll, amino acids, nucleic acids (Oaks 1994; Lea 1997; Fuentes et al. 2001). P is closely concerned with the vital growth processes in plants. It is a major constituent of nucleic acid and nucleic acids (Soetan et al. 2010). In seeds, it is related to metabolism of fats. It is also required in root development and ripening of seeds and fruits. Ca and Mg are the constituents of cell wall and chlorophyll; and act as carrier of P element (Belakbir et al. 1998; Olaya 2006). K is required in the formation of carbohydrates and proteins. It is used in regulation of H₂O relations within the plant cell and H₂O loss by plants through transpiration. Besides, it is a catalyst and condensing agent of complex substances and an accelerator of enzyme action. S is a constituent of proteins and is also connected with chlorophyll formation (Hell 1997; Soetan et al. 2010; Waraich et al. 2011).

Micro-elements, required for plant metabolism, include Fe, B, Zn, Cu, Si, nickel (Ni), cobalt (Co) and Cl. Fe and Mn are used for chlorophyll formation (Rouault 2005). B is used as a catalyst or reaction regulator (Bolaos et al. 2000; Olivares et al. 2011); Zn and Cu are used as catalysts and regulators (Aziz et al. 2010); Si is used in deposition of cell walls (Sacala 2009); Mo is used as a co-factor to enzymes important in building of amino acids (Kaiser et al. 2005); Ni is used for activation of urease in higher plants and activates several enzymes involved in a plant metabolism. It is also a substitute element for Zn and Fe, which act as cofactors in several enzymes in the lower plants. Co has proven beneficial in some plants such as legumes, where it is required for N fixation (Aziz et al. 2007). Vanadium (V) is required by some plants at very low concentrations. Na is used as replacer of K for regulation of stomatal opening and closing (Pardo and Quintero 2002). The evidence of the role of Cl in plants is somewhat contradictory and no general statement can be made (Runguphan et al. 2010). In tobacco, it has been shown to increase H₂O content of the tissues and affect carbohydrate metabolism, leading to an accumulation of starch in the leaves. The element remains present in plants as chloride and is totally soluble. Toxic effects of metals in plants may be produced both by essential and non-essential elements. The major nutrients are less toxic than the trace elements. Indeed, for the major nutrients, the concentration of certain essential metals in plants is extremely toxic for excess or "luxury" consumption, but for the trace elements the margin is very narrow. Similar conditions exist in relation to non-essential elements; some plants tolerate fairly large amounts of elements such as Na or Cl, but are injured by relatively small amounts of elements like arsenic (As) or chromium (Cr) (Epstein and Bloom 2004). Thus, the present review explains regulatory role of each essential and non-essential mineral element in the common metabolic activities like photosynthesis, respiration, ion uptake, protein synthesis, alkaloid biosynthesis etc., occurring in medicinal plants with the emphasis given to selected medicinal plants such as Papaver somniferum, Catharanthus roseus, Withania somnifera, Nicotiana, etc.

REGULATORY ROLE OF MINERAL ELEMENTS IN PHOTOSYNTHESIS AND RESPIRATION WITH REGARDS TO MEDICINAL AND AROMATIC PLANTS

One of the most fundamental and least understood areas of plant physiology and biochemistry is the relationship between quantities of essential mineral nutrient elements and metabolic changes occurring in plants. A living plant is a complex biological system composed of several chemical constituents. C, hydrogen (H), O, S and N are the major
constituents of organic material, which are also involved in enzymatic processes. C and O have a significant role as components of carboxyl group. H and O are useful in oxidation-reduction processes and N is used in biological nitrogen fixation (Salisbury and Ross 2002).

**Role of macro-elements**

1. **Carbon, hydrogen and oxygen**

The assimilation of C in form of carbon dioxide (CO₂) takes place in plants and the process is called carboxylation, which is a basic mechanism by which CO₂ is fixed in photosynthesis. The first CO₂ acceptor is RuBP (Ribulose bisphosphate), a three C compound in C3 plants. This is one of the important reactions of Calvin Cycle (Soetan et al. 2010; Murchie and Niyogi 2011). In C₄ plants, CO₂ acceptor is phosphoenolpyruvate (PEP), a four C compound (Edwards et al. 2010; Gowik and Westhoff 2011). The reverse process by which CO₂ is liberated is known as decarboxylation; for example, the release of CO₂ from malic acid to form pyruvic acid is due to a decarboxylation reaction. The conversion of light energy into chemical energy is closely related to the conversion of CO₂ into organic compounds (Cruz et al. 2005).

\[
6 \text{CO}_2 + 6 \text{H}_2\text{O} \xrightarrow{\text{light energy}} \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2
\]

In modern terminology, the process of absorbing electromagnetic radiations by the pigment system and its conversion into chemical energy which can be made available for growth in a particular environment is termed as photosynthesis (Pottosin et al. 2005). In higher plants, light absorption is brought about by chlorophyll and carotenoids. Photosynthesis begins with the absorption of light by these pigments, which induces an electron flow that results in conversion of light energy into chemical energy. In higher plants, two photosystems are responsible for such energy conversion; photosystem I and II (Cruz et al. 2005). Each of these photosynthetic units consists of about 400 chlorophyll molecules along with additional pigments such as carotenoids. In photosystem I (PS-I) a special chlorophyll molecule is involved known as pigment 700 (P-700) (Yokthongwattha and Melis 2006; Murchie and Niyogi 2011).

Energy + P-700 $\rightarrow (P-700)^- + e^-$

This is the basic process that initiates electron flow. The electron ejected from P-700, thus, moves against an electrical gradient. Photosystem II (PS-II) functions in an analogous way to PS-I. In PS-II, the electron emitter is a chlorophyll molecule P-682 (Cruz et al. 2005; Kramer and Evans 2011).

\[
(P-682) \rightarrow (Q)
\]

These two photosystems function in series and constitute the components of electron transport pathway, transferring the electrons from H₂O to (Nicotinamide adenine dinucleotide phosphate) NADP⁺. Thus, H₂O being the ultimate electron donor and NADP⁺ the electron acceptor in the overall process. PS-II is closely associated with splitting of H₂O molecule, which serves as electron donor (Shikanai 2007).

\[
2\text{H}_2\text{O} \rightarrow 4\text{H}^+ + 4e^- + \text{O}_2
\]

Photolysis of H₂O i.e. splitting of H₂O molecule makes use of Mg (Mg²⁺) and Cl (Cl⁻) ions. The whole process does not seem to be legitimate to describe here in details. Plastocyanin (a Cu containing acidic protein) and ferredoxin (Fe-S protein) plays critical role in this electron transport system (Diner and Babcock 1996; Aubry et al. 2011). In photosynthetic energy conversion, light induces an electron flow that provides sufficient energy for the synthesis of adenosine triphosphate (ATP) and reduction of NADP⁺. In the whole process, another Cu containing compound plastoquinone and an Fe containing compound cytochrome play vital roles (Sacksteder et al. 2000).

The next major metabolic process being the cellular respiration (oxidative metabolism) which is a set of metabolic processes and reactions are executed within the cell of an organism in order to convert biochemical energy, derived from the nutrients, to ATP (Soetan et al. 2010). The ATP is a major source of energy for cellular reactions taking place in all form of life. There are two types of cellular respiration: aerobic and anaerobic cellular respiration. Both animals as well as plants, execute cellular respiration to produce energy. They use nutrients such as glucose, amino acids and fatty acids to produce energy. The common oxidizing agent in this process is molecular O. Glycolysis being the first step of cellular respiration, is the process whereby the glucose is chemically modified to pyruvate. This process occurs in cytosol of the cell and is carried out in the absence of O. During this process, energy is released from glucose compounds in the form of 2 NADH molecules and 2 ATP molecules. Pyruvate decarboxylation is the second stage of cellular respiration. In this process, pyruvate is decarboxylated and added to coenzyme A (CoA) in order to form acetyl-CoA. It is an important stage in the cellular respiration process, which forms a link between metabolic pathways of glycolysis and the Kreb’s cycle.

The Kreb’s cycle comprises a series of steps, which oxidize acetyl-CoA molecule. Unlike glycolysis, the Kreb’s cycle requires O for its functioning and is catalyzed by various series of enzymes. Two complete turns of Kreb’s cycle produces 4 CO₂ molecules, 2 ATP molecules, 6 NADH; and 2 Flavine adenine dinucleotide (FADH₂) molecules (Soetan et al. 2010). The final stage of cellular respiration is electron transport chain (ETC), which produces the remaining 32-34 ATPs. The ETC comprises of electron-carrying proteins, present on the internal membrane of mitochondria. These proteins transfer electrons from one self to another. These electrons are finally added to O, which is reduced to form H₂O. In this process, ATPs are produced by pmf, a source of potential energy created by the gradient that is formed when protons move across the biological membrane. Simply the ETC triggers a pH gradient through which ATP is produced by the process known as chemiosmosis.

A short-term exposure to CO₂ enhanced the rate of photosynthesis and *vice versa*, while an intermediary exposure of CO₂ had variable effect on the rate of photosynthesis in C₃ plants (Makino and Mae 1999). The suppression of photosynthesis by CO₂ enrichment is always associated with decreases in leaf N and Rubisco in these decreases are not due to dilution of N caused by a relative increase in the plant biomass, but occur as a result of decrease in N allocation to leaves at the level of whole plant, and the decrease in Rubisco content is not selective. Accumulation of carbohydrates in the leaves may lead to the repression of photosynthetic gene expression. Besides, the excess starch hinders the CO₂ diffusion through the leaves. In *Papaver somniferum*, increasing CO₂ concentration (300, 400, 500, 600 μmol mol⁻¹) had positive effect on various morphological traits such as number of capsules, capsule weight and latex-yield, with an increase of 3.6, 3.0 and 3.7 times, respectively on per plant basis. The secondary metabolic products also responded positively to CO₂ enrichment. The concentration of alkaloids such as morphine, codeine, papaverine also increased in response to enhancement of atmospheric CO₂. The concentration of important alkaloids such as morphine was significantly increased by 10.4, 11.7, 12.9 and 12.4%, respectively for each dose (300, 400, 500 and 600 μmol mol⁻¹) of CO₂ (Ziska et al. 2008). Similarly, there was a positive effect of increasing atmospheric concentration of CO₂ (950-1050 μmol mol⁻¹) on hypericin and pseudo-hypericin content (14-fold increase) in *Hypericum perforatum* (Zobayed and Saxena 2004). There was an increase of 16.5% in digoxin yield per unit dry weight of plant and 75% increase in plant dry weight production per unit land.
area in *Digitalis lanata* by near-tripling of the air's CO$_2$ concentration (Stuhlfauth and Fock 1990). Similarly, the concentration of atrazine (11.9, 14.7, 18.9 mg) and scopo-lamine (12.9, 20.9, 25.4 mg, respectively) increased in *Datura stramonium* by varying CO$_2$ concentrations (294, 378 and 690 µmol mol$^{-1}$) (Ziska et al. 2005).

### 2. Nitrogen, phosphorus and potassium

N is a main constituent of all the amino acids, proteins and coenzymes (Mazid *et al.* 2011; Murchie and Niyogi 2011). P occurs as phosphate or orthophosphate and to a minor extent as pyrophosphate. The process by which plants convert inorganic N and S into organic forms is important for all the animals that depend on plants and microorganisms for their dietary source of organic N and S. N is one of the most widely distributed elements in nature that circulates in the atmosphere, lithosphere and hydrosphere. Many factors are involved in the N-turnover, some of which are physico-chemical and others biological. As such, dry plant material contains approximately 2 to 4% N. N is an indispensable elementary constituent of numerous organic compounds of general importance (amino acid, protein, nucleic acid, etc.). Both, NO$_3^-$ and NH$_4^+$ forms of N can be taken up readily by plants from the soil and are metabolized thereafter in the plant cells. The rate of uptake of NO$_3^-$ is generally very high as plants require large amount of N for their growth and development. It is now established that in this uptake process there are involved both influx and efflux components (Deane-Drummond and Glass 1983). Influx of NO$_3^-$ is an active process that involves the movement of NO$_3^-$ against the electrochemical gradient. The influx of NO$_3^-$ is also dependent on the concentration of NO$_3^-$ in the external medium (Lillo *et al.* 2004; Halkier and Gershenzon 2006; Soetan *et al.* 2010).

N fixation is the most important process, in which the molecular N of the atmosphere from its inorganic form is fixed and converted into an organic form. Only prokaroytes are capable of assimilating molecular N. In addition, prototrophy, ammonification, nitrification and denitrification are some other processes, which are controlled by microbial activity (Fuentes *et al.* 2001; Stitt *et al.* 2002).

**Soil organic N ---→ RNH$_2$ + CO$_2$ + additional products + energy**

RNH$_2$ + H$_2$O ---→ NH$_3$ + ROH + energy

In opium poppy (*Papaver somniferum* L.) suitable form, supply and date of application of N fertilizers play a decisive role in proper growth of poppy plants and its yield. The need of optimal supply of N for poppy plants begins shortly after seed germination and lasts till the stage of formation of the generative organs (Yadav *et al.* 1984; Pavliková and Tlustoš 1994). Yadav *et al.* (1984) suggested that slow release of N had a positive effect on the seed yield of opium poppy. Increase in the seed yield was due to enhancement in the process of photosynthesis, which was accelerated by the addition of N. In *Catharanthus roseus*, the growth of seedlings was improved by increasing N concentration in the pre-transplant fertilizer from 8 to 32 mM, when a peat-based growing medium was used in the experiment (Van Iersel *et al.* 1998). N supply in *C. roseus* also stimulated K and P uptake and their translocation to the leaves (Lata and Sadowska 1996). N is a major limiting nutrient for plant growth; it is assimilated as NH$_3$ by the converted action of glutamine synthase (GS; GS$_1$) and glutamate synthase (GOGAT). In tobacco (*Nicotiana tabacum*), Fuentes *et al.* (2001) over expressed GS$_1$, at the level of RNA and protein which increased the activity of GS up to six times in comparison to control plants which resulted in 70% higher shoot, 100% greater root dry weight and 50% more leaf area than controls. They further concluded that manipulation in GS activity has the potential to maintain crop productivity while reducing nitrogen fertilization and the concomitant pollution. Omer (1998) and Omer *et al.* (2008) noticed that nitrogen fertilizer was effective in increasing essential oil of *Origanum syriacum* and *Ocimum americanum*, respectively.

The organic forms of phosphate are the compounds in which, orthophosphate is esterified with hydroxyl group of sugars and alcohols or it is bound by a pyrophosphate bond to other phosphate group. A typical example of a phosphate ester is fructose-6-phosphate which is an intermediary meta-bolite of glycolysis. So phosphorus is found in various intermediary compounds of metabolism. Phosphate is also bound to lipophilic compounds particularly in phosphatidyli derivatives e.g. lecithin. Nagano and Ashihara (1993) revealed that the metabolic adaptation of inorganic phosphate (P$_i$) in respiratory pathways in plants was restricted to a limited level and that there were significant effects of long-term P$_i$ starvation on the activities of various enzymes related to respiratory metabolism. They found that only PEP-hydrolyzing enzyme and PEP carboxylase were in higher amounts in P$_i$-starved cells of *C. roseus*. Activities of other enzymes in the P$_i$-starved cells were lower than or similar to those of the control cells. Time-course studies indicated that PEP-hydrolyzing activity was inducible by starvation of P$_i$. However, fluctuations in the activity of P$_i$: fructose-6-phosphate i-phosphotransferase during starvation of P$_i$ to those of other P-degrading compounds (inorganic and 6-phosphoglucocone dehydrogenase (Williamson *et al.* 2001). In *Plantago ovata*, increasing concentration of P$_i$ plays a critical role in elevating total yield of the plant which was ascribed to the regulatory role of P$_i$ in metabolism (Tomar *et al.* 2010). Similarly, P deficiency prevented nodulation or stopped nodule growth and also decreased photosynthesis in *Trifolium repens* (Almeida *et al.* 2000; Asatsuma *et al.* 2003).

The most important compound in which phosphate groups are linked by pyrophosphate bond is ATP. The pyrophosphate bond is an energy rich bond, which on hydrolysis releases 30 KJ Mol$^{-1}$ energy. The energy produced or released in various metabolic processes is utilized in the synthesis of pyrophosphate bond of ATP. In the form of ATP, the energy can be conveyed to various endothermic processes such as active ion uptake and synthesis of various organic compounds. In the whole process, there is usually an initial phosphorylation reaction which involves transfer of phosphoryl group from ATP to another compound. Another organic compound containing P is phytin which occurs mainly in seeds. Phytin is synthesized during seed formation and occurs in the form of Ca and Mg salts of phytic acid in the seeds. Phytin is regarded as P reserve in seeds. Phytin is synthesized during seed formation and occurs in the form of Ca and Mg salts of phytic acid in the seeds. Phytin provides as P reserve in seeds. Phytin is synthesized during seed formation and occurs in the form of Ca and Mg salts of phytic acid in the seeds. Phytin is regarded as P reserve in seeds. Phytin is synthesized during seed formation and occurs in the form of Ca and Mg salts of phytic acid in the seeds. Phytin is regarded as P reserve in seeds. Phytin is synthesized during seed formation and occurs in the form of Ca and Mg salts of phytic acid in the seeds. Phytin is regarded as P reserve in seeds. Phytin is synthesized during seed formation and occurs in the form of Ca and Mg salts of phytic acid in the seeds.
other sources may provide a viable alternative to liming. Zaghloul et al. (2009) reported that the application of potassium humate (0.0, 0.5, 1.0, 1.5, 2.0 and 2.5% K-humate) leads to increase oil content in *Tithya orientalis*. Said-Al et al. (2009) concluded that foliar application of K-humate promoted growth and possessed the best oil percentage in *Tithya orientalis*. The increase in permeability of plant membranes due to humate application results in improved growth of various beneficial microorganisms, accelerate cell division, increased root growth and all plant organs for a number of horticultural crops and turf grasses, as well as, the growth of some trees (Russo and Berlyn 1990; Sanders et al. 1990; Poincelot 1993).

### 3. Sulphur, calcium and magnesium

S occurs in the soil in inorganic and organic forms. In most soils, organically bound S provides major S reservoir (Rennie et al. 1973; Scott and Anderson 1976). Soil organic S can be divided into 2 fractions: C bonded S, and non-C bonded S. The latter fraction is made up of phenolic and choline sulphates and lipids (Freney and Stevenson 1966). The inorganic form of S in soil consists mainly of SO$_4^{2-}$, which is mainly absorbed by plants. S taken up by plant cells, must be reduced as in majority of S containing molecules S is present in reduced form (Saito 2000; Mazid et al. 2011). The S containing organic compounds include the amino acids cysteine, cystine and methionine as well as the proteins containing these amino acids. The first step in S assimilation is the incorporation of S between H$_2$SO$_4$ (sulphuric acid) and ATP. The sulphuryl group of H$_2$SO$_4$ replaces the pyrophosphoryl group of ATP, thus forming adenosine phosphosulphate and pyrophosphate, which is catalysed by an enzyme ATP sulphurylase. The sulphuryl group of adenosine phosphosulphate (APS) is transferred to an SH-carrier complex which is then reduced to –SH group, the reducing power provided by the ferredoxin. In a further step, the –SH group of the carrier complex is transferred to acetyl serine leading to regeneration of original carrier SH-complex. Acetyl serine then split into cysteine and acetate. This splitting step requires two additional reducing equivalents, which also probably originate from ferredoxin (Saito 2000).

\[
\begin{align*}
\text{H}_2\text{SO}_4 + \text{ATP} + \text{SH} + \text{acetyl serine} \rightarrow \\
\text{cysteine} + \text{acetate} + 3\text{H}_2\text{O} + \text{AMP} + \text{P-P}
\end{align*}
\]

This equation shows that sulphate reaction needs energy in the form of ATP and reducing equivalents. Hence, the reduction process depends on photosynthesis and especially on the level of ATP (Schiff and Hodson 1973). Sulphate is mainly reduced during the light period because sulphate reducing enzymes are located in the chloroplast membrane. Whether other organelles are also capable of reducing sulphate, is not known. Modern agriculture requires adequate fertilization of S to achieve maximum crop yield and performances (Crawford et al. 2000). S$^2-$ a major form of SO$_4^{2-}$ in aqueous phase of apoplastic, may reduce photosynthesis rate and thereby crop yield through inducing reactive oxygen species (ROS), involved in S$^2-$-induced stress, and the S$^2-$-induced enhancements in levels of ROS (Li et al. 2007).

The role of Ca in membrane stability is by far the most important process but other metabolic processes are also dependent on Ca availability (Rengel 1992; Marschner 1995; Tuna et al. 2007). The feature includes a breakdown in the compartmentation of cell and an increase in cellular respiration, followed by the leakage of endogenous respiratory substrates from the vacuole to the respiratory enzymes in the cytoplasm (Bangerth et al. 1972). Most of the Ca present in plant tissues is located in the apoplast and vacuoles. The Ca concentration of cytoplasm remains low, ranging from 10$^{-6}$ to 10$^{-7}$ M (Marme 1983). The maintenance of this low cytoplasmic concentration of Ca is of vital importance for the plant cells (Hanson 1984), for there is now evidence that Ca may inhibit the activity of various enzymes located in the cytoplasm (Gavalas and Manetas 1980). The same is also true for the chloroplast enzymes. The low Ca concentration not only prevents inhibition of enzyme activity, but it also inhibits precipitation of P, as Ca-phosphate and the competition of Ca$^+$ with Mg$^{2+}$ for binding sites of the enzyme (Hepler and Sunkel 1978; Hepler 1985). In this regard, a low Ca concentration is achieved by the mechanisms which pump Ca$^+$ out of the cytoplasm into the apoplast or into the vacuole. Some of the Ca$^+$ is also sequestered by the endoplasmic reticulum and mitochondria, organelles relatively rich in Ca$^{2+}$.

Ca concentrations in the mitochondria are much higher than in the cytoplasm. Mitochondrial enzymes may be directly activated by Ca$^+$ without the help of calmodulin (Marme 1983). One such enzyme which occurs in the mitochondria is glutamate dehydrogenase, which brings about the deamination of amino acids. Franklin-Tong et al. (2002) demonstrated that increase in free cytosolic Ca$^{2+}$ could be triggered by self-incompatibility (SI) response in the pol-len of incompatible *Papaver rhoeas* (the field poppy). However, one key question that has not been answered is whether cellular Ca$^{2+}$ may be involved in this regard. To address this question, they used an ion-selective vibrating probe to measure changes in extracellular Ca$^{2+}$ fluxes around pollen tubes of poppy. They confirmed that there was an oscillating Ca$^{2+}$ influx directed at the apex of the pollen tube and also provided the evidence that CA$^{2+}$ influx occurred at the shanks of pollen tubes. Secondly, upon challenge with self-incompatibility (S) proteins, there was a stimulation of Ca$^{2+}$ influx along the shank of incompatible pollen tubes, approximately 50 mm behind the pollen tube tip. This demonstration of SI-induced CA$^{2+}$ influx suggested a role for influx of extracellular Ca$^{2+}$ in the SI response.

A major proportion of total plant Mg i.e. often over 70% is present in diffusible form and associated with inorganic anions and organic acid anions such as malate and citrate and also found associated with indiffusible anions including oxalate and pectate (Kirkby and Mengel 1976). The chief importance of Mg is due to its occurrence at the centre of the chlorophyll molecule. The fraction of the total plant Mg associated with chlorophyll, however, is relatively small, ranging 15 to 20% (Neales 1956). Besides its function in chlorophyll molecule, Mg$^+$ is required in other physiological processes. One major role of Mg$^+$ is that it acts as a cofactor in almost all the enzymes activating phosphorylation processes. Mg$^+$ forms a bridge between pyrophosphate structure of ATP or ADP and the enzyme molecule. The activation of ATPase by Mg$^{2+}$ is brought about by this bridging function (Balke and Hodges 1975). In addition to phosphokinases some dehydrogenases as well as enolase are also activated by Mg$^{2+}$. In these enzymes, however, Mg reaction is not specific and Mn$^{2+}$ is often a more efficient activator.

Mg$^{2+}$ also plays a vital role in the aggregation of ribosomes. Mg$^{2+}$ concentrations also modulate ionic currents across the chloroplast and vacuolar membranes and might, thus, regulate ion balance in the cell and stomatal opening. The significance of Mg$^{2+}$ in homeostasis has been particularly established with regard to the role of Mg$^{2+}$ in photosynthesis (Panayotov 2004, 2005; Panayotov et al. 2005; Kostova et al. 2008). Fluctuations in Mg$^{2+}$ level in the chloroplast regulate the activity of key photosynthetic enzymes. Relatively little is known of the proteins mediating Mg$^{2+}$ uptake and transport in plants. The plant vacuole seems to play a key role in Mg$^{2+}$ homeostasis in plant cells. Physiological and molecular evidences indicate that Mg$^{2+}$ entry into the vacuole is mediated by Mg$^{2+}$/H$^+$ exchangers (Shaull 2002; Hattori et al. 2007).

A key reaction of Mg$^{2+}$ is the activation of ribulose bisphosphate carboxylase. Light triggers the import of Mg$^{2+}$ into stroma of the chloroplast in exchange of H$^+$, thus providing optimum conditions for the carboxylase reaction.
The favorable effect of Mg$^{2+}$ on CO$_2$ assimilation and related processes, such as sugar and starch production, are probably due to the consequences of this activation of ribulose bisphosphate carboxylase. According to Barber (1982), Mg$^+$ is the most important cation species neutralizing the indiffusible anions of the thylakoid membrane. Skarpa et al. (2008) demonstrated the effect of Mg applied in the form of Mg nitrate to the soil supplemented with cadmium (Cd) on yield parameters as well as quantitative and qualitative parameters in Papaver somniferum L. During the vegetative period, Mg fertilization had a positive effect on Mg and Ca concentrations in the plant. Mg application also had a synergistic effect on N uptake and increased its content in the plant. In variants where Cd was supplemented, its content in the plants considerably increased. With Mg fertilization, the yield of poppy seeds increased when compared with the unfertilized variants; both in the variant with natural Cd content (3.6%) and the variant that was supplemented with Cd (19.9%). Cd had a synergistic effect, enhancing a better utilization of nutrients and resulting in the higher seed yield. As a result of Mg application the amount of morphine in poppy straw increased both in variants with natural and supplemented Cd content.

**Role of micro-nutrients**

**1. Manganese, iron, copper**

In its biochemical function, Mn$^{2+}$ resembles with Mg$^{2+}$ and both form an ATP bridge with the enzyme complex (phosphokinases and phosphotransferases). In the tricarboxylic acid cycle (TCA cycle), two of the functional enzymes i.e. decarboxylases and dehydrogenases are also activated by Mn$^{2+}$, although it appears that in most cases Mn$^{2+}$ is not specific for these enzymes and can be substituted by Mg$^{2+}$. For the Mn$^{2+}$ to be effective, it has to be utilized preferentially, as it occurs in the activation of chloroplast RNA polymerase. Activation is brought about by either Mn$^{2+}$ or Mg$^{2+}$ but at low concentrations (1 mM or less); however, the former is much more effective. The peroxidase enzyme activity is enhanced in contrast to catalase enzyme in Mn-deficient tissue (Husted et al. 2009). This increase in the activity of peroxidase is probably associated with the high activity of IAA oxidase, which is observed in Mn-deficient plants (Chatterjee et al. 1994). Peroxidases are most important constituents of IAA oxidizing system (Jansen et al. 2001). The most documented role of Mn in green plants is in the H$_2$O splitting and O evolution system in photosynthesis (Panayotov 2004, 2005; Panayotov et al. 2005). It has now been confirmed that Mn is required both by higher and lower plants, as it appears that a manganoprotein complex catalyzes the O$_2$ evolution (Papadakis et al. 2007). This tightly bound Mn complex has not as yet been isolated and its structure is until now unknown. The reaction has been depicted by Edwards and Walker (1983) with a minimum requirement of 4 Mn atoms at each reaction centre of photosystem II. From this scheme, it follows that when Mn is deficient the ETC in the light reaction is seriously disturbed resulting in the occurrence of other reactions including photophosphorylation and the reduction of CO$_2$ (Apel and Hirt 2004; Moller et al. 2007). As a consequence of absence of Mn, nitrite (NO$_2^-$) and sulphate are detrimentally affected. When NO$_2^-$ reduction is impaired the accumulated NO$_3^-$ can exert a feed back control on NO$_3^-$ reductase activity so that NO$_3^-$ is also accumulated. This is one reason why NO$_3^-$ accumulation is sometimes observed in Mn-deficient plants. Pande et al. (2011) investigated the effect of varying Mn concentration (0.0, 2.5, 5.0 and 10.0 mg/kg) on medicinally important plant Mentha spicata and concluded that optimal level required was 2.5 mg/kg, beyond which growth parameters showed a gradual and significant decline. Highest stem and leaf yield was noticed which may be attributed to proper root development as indicated by its higher root weight. Mn supply improves the root growth and its activity as reported by Mou et al. (2010).

The tendency of Fe to form chelate complexes and its ability to undergo a valency change are the two important characteristic which underlie numerous physiological effects of Fe (Cohen et al. 1998).

\[
\text{Fe}^{2+} \longleftrightarrow \text{Fe}^{3+} + e^-
\]

The most well known function of Fe relates to the activity of the enzymes in which haem functions as prothetic groups (Rouault 2005). Here, Fe plays a similar role as does Mg in the porphyrin structure of chlorophyll. These haem enzyme systems include catalase, peroxidase, cytochrome oxidase as well as various cytochromes. The detailed function of the cytochrome in electron transport and involvement of cytochrome oxidase in the terminal step of respiration chain does not seem to be legitimate to describe here. Misra et al. (2006), while evaluating the Fe-efficient genotypes of the medicinally important plant Ocimum, found an oxido-reducible reaction of peroxidase as well as high bands of peroxidase isoenzymes in OSP-6 genotype for the formation of monoterpene essential oil(s) and possibly the major constituents of eugenol through the high production of photosynthates. Pande et al. (2011) investigated the effect of varying Fe concentration (0.0, 5.0, 10.0 and 15.0 mg/kg) on medicinally important plant Mentha spicata and found that fresh weight, dry weight, oil yield and chlorophyll content increased significantly with increasing Fe concentration. The optimal dose was 10.0 mg/kg for maximum increment.

Plants require Cu in very less quantity for their proper functioning. The Cu content in most plants generally varies between 2-20 ppm in dry plant material. Enzymatically bound Cu participates in redox reactions which are mostly dependent on the valency change.

\[
\text{Cu}^{2+} + e^- \longrightarrow \text{Cu}^+ 
\]

In this respect, Cu is similar to Fe, although Cu$^+$ is much less stable than the corresponding Fe$^{2+}$. In protein complexes, Cu has particularly a high redox potential. Most Cu enzymes/compounds (plastoxygen, superoxide dismutase (SOD) and amine oxidases) react with O$_2$ and reduce it to H$_2$O$_2$ or H$_2$O. In Cu containing proteins, three different forms of Cu are distinguished (Sandmann and Boger 1983). Type I is the blue Cu protein (e.g. plastocynin), which functions without oxidase activity in one electron transfer. Types 2 are non-blue Cu proteins which are peroxide producing oxidases. Type 3 Cu-containing protein (e.g. phenolase) is non-blue and it functions as a two electron acceptor in oxidation process, which catalyses the oxidation of monophenols to ortho- and di-phenols and then further to o-quinones and H$_2$O. Cu proteins are also present in multi Cu enzymes containing all the above three types of Cu. These enzymes include ascorbic acid oxidase and laccase catalase (Navarro et al. 1997).

\[
2\text{AH}_2 + \text{O}_2 \longrightarrow 2\text{A} + 2\text{H}_2\text{O}
\]

Approximately 70% of the total Cu in leaf is bound to various organelles/components of the cell, of which more than half is bound to plastocynin, a component of ETC of photosystem I. The Cu is also a constituent of other photosynthetic enzymes, e.g. phenolase, SOD and fraction 1 protein, which has RuBP carboxylase and RuBP oxygenase activity (Navarro et al. 1997). However, a precise role of Cu in photosynthetic is still unclear. The most common of the three types of SOD isoenzymes contain Cu and Zn. This enzyme protein (Cu-Zn SOD) has a molecular weight of about 32,000 da and contains two Zn and two Cu atoms. SOD occurs in all aerobic organisms and is essential for their survival in O environment (Shikanai et al. 2003). They protect the organism from the damage of superoxide radicals, which can be formed when a single electron is transferred to O$_2$. The high proportion of SODs in leaves appears to be localized in chloroplast which indicates a role of Mn.
SODs in protecting photosynthetic apparatus but the details about how this is achieved are still lacking (Jackson et al. 1978). Cytochrome oxidase, the terminal oxidase in the mitochondrial transport chain, is one of the best studied Cu containing enzymes. This enzyme transfers electrons directly to molecular O2 which can be inhibited by CN−; however, when this reaction occurs, another Cu containing oxidase, the CN− resistant quinol oxidase comes into action. This relatively recently discovered enzyme, called alternative oxidase, thus, provides a second pathway of ubiquinone oxidation in the mitochondria (America et al. 1994). Electron are believed to be transported directly from quinol to molecular O2, avoiding the cytochrome. Cu influences both carbohydrate and N metabolism. In the vegetative stage of plants, Cu deficiency can lower the content of soluble carbohydrates as might be expected owing to the specific role of Cu in photosynthesis (Gupta et al. 2002). In Withania somnifera, due to the application of copper sulphate (CuSO4) to plants, there was observed accumulation of high amount of Cu in concentration manner; it also decreased the plant fresh weight, shoot and root length, chlorophyll content and carotenoid content. Similarly, few phenoic compounds were also increased due to CuSO4 application (Khatun et al. 2008). Santiago et al. (2000) analyzed the induction of phenolic compounds in leaves of Phyllanthus tenellus and their induction by CuSO4. They noticed that spraying plants with CuSO4 induced punctated lesions formed by groups of necrotic cells which accumulated brownish substances.

2. Silicon, chlorine and sodium

Si is found as a component of cell wall. Plants supplied with soluble Si produce stronger and tougher cell walls, providing a mechanical barrier against piercing and sucking the plant tissue by the insects. Si significantly enhances the tolerance of plant to heat and drought. Foliar sprays of Si have also shown benefits in reduction of populations of aphids in various field crops. Studies have also revealed that Si could be deposited by the plants at the site of infection by fungus to combat the penetration of cell walls by the fungi. Leaf erectness, stem strength and prevention of Fe and Mn toxicity have been improved by the application of Si. Though the Si is not essential for all the plants but can be beneficial for many plants (Epstein 1994; Hodson et al. 2005; Ma and Yamaji 2006). Silicic acid like boric acid, however, reacts with o-phenol such as caffeic acid, a precursor in the biosynthesis of lignin, to form mono-, di- or polymeric Si complexes (Sacala 2009). It has also been noted that a low level of Si in the transpiration in the plants. Ample evidence are there that Si, when readily available to plants, plays a large role in their growth, mineral nutrition, mechanical strength, and resistance to fungal diseases, herbivory, and adverse chemical conditions of the medium (Epstein 1994; Ma and Yamaji 2006; Epstein 2009; Sacala 2009).

Plant tissues usually contain substantial amounts of Cl, often in the range of 50-500 μmol kg−1 dry weight. These values are comparable to those of macronutrients. Though the demand of Cl for optimal growth is less but its deficiency symptoms occur when Cl content falls below 20 μmol kg−1 dry weight. This clearly established the role of Cl as micronutrient (Clarkson and Hanson 1980). Cl is required in Hill’s reaction and the H2O splitting reaction in photosystem II of photosynthesis (Kelly and Izawa 1978). Cl also enhances the photosynthesis in general but the evolution of O2 and photophosphorylation (Bove et al. 1963). In some plants, chloride ion influences photosynthesis indirectly via affecting stomatal regulation in the guard cells. During stomatal opening, inward flux of K+ into the guard cells must be accompanied by either accumulation of malate as a counter ion or by the inward flux of Cl. Cl sharply decreased NO3 uptake by the roots and facilitated the incorporation of N into organic compounds and translocation of N to the leaves in Catharanthus roseus (Flores et al. 2000). However, the studies depicting the role of chlorine and its compound in plant metabolism are very few, especially in medicinal and aromatic plants.

Na belongs to the group of micro elements and hence, it cannot be expected to have a specific role in the metabolic activities of plants. When Na produces significant effects in plant metabolism, it is often regarded as a replacer of K as it is partly able to replace K if present in excess. Na is ineffective as a substitute for K, even for Na-loving plants such as sugar beet (Beta vulgaris), marginol (Calendula officinalis) and barley (Hordeum vulgare). Na seems to affect the H2O relations of plants and often enables sugar beet and other crops to withstand drought conditions, which would otherwise produce severe adverse effects. In Cassia angustifolia, Arshi et al. (2005) found that sodium chloride (NaCl) decreased biomass of root, shoot and leaf significantly, while calcium chloride (CaCl2) increased the biomass of these plant parts. Combined doses of NaCl and CaCl2 increased the proline content 8 times, while NaCl alone increased it 5 times. Combined application of NaCl and CaCl2 decreased the biomass but this effect was less than that exhibited by NaCl application alone. K+ and Ca2+ concentration was inhibited with NaCl, while Na+ and Cl− concentrations were increased. CaCl2 and combined treatment (NaCl + CaCl2) increased the K+ and Ca2+ concentrations similarly, in some medicinal plants, they also found that NaCl decreased the biomass, length of root and shoot, photosynthetic rate, stomatal conductance, total chlorophyll content, protein content, nitrate reductase activity and reduced N content of leaves, while proline and nitrate content increased (Arshi et al. 2002). In Withania somnifera, different Na salts viz. NaCl, sodium sulphate (Na2SO4) and sodium carbonate (Na2CO3) decreased the germination percentage, shoot length, root length, plant dry weight and chlorophyll as well as carotenoids contents of the plants significantly (Jaleel et al. 2009).

3. Zinc, molybdenum and boron

Zn facilitates the binding between the enzyme and substrate, bringing about the conformational change in the system. A number of enzymes, including enolase, are activated by Zn2+ in more or less the same way they get activated by Mn2+ and Mg2+. Till now, the only authenticated enzyme that gets specifically activated by Zn2+ is carbonic anhydrase (CA). This enzyme catalyzes the following reaction:

\[
\text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{H}^+ + \text{HCO}_3^-
\]

It is generally accepted that CA is localized in the cytoplasm but there are also evidences to suggest its presence in the chloroplasts (Findene 1979). The enzyme promotes hydrolysis and hydration reactions involving Carbonyl groups (Sandmann and Boger 1983). Jacobson et al. (1975) reported its supposed role in mediating a short-term transient pH effect, thus acting as a buffer. Such a role of this enzyme was suggested because of its high concentration in chloroplast stroma. There it protects proteins from denaturation, which can occur as a result of local pH change associated with activation of H+ pump and the incorporation of CO2 into ribulose 1, 5 bisphosphate. The role of CA, however, is still a matter of speculation. Zn is involved in C assimilation, saccharide accumulation, free radical removal, antioxidant enzymes activation, C utilization in terpene biosynthesis and in overall growth of the plants. The requirement of Zn for Japanese mint (Mentha arvensis) and its limitation imposed on photosynthetic C metabolism and translocation in relation to essential oil accumulation were shown by Misra and Sharma (1991). Misra et al. (2005) showed that in geranium (Pelargonium graveolens L.), Zn acts as an antioxidant promoter, apart from its essentiality as micronutrient. Varying concentrations of Zn from 0–1.0 g m−2 (Zn0 to Zn1.0) affected net photosynthetic rate, contents of chlorophyll and essential monoterpene oil(s). Zn0.25 resulted in maximum total essential monoterpene oil(s) content i.e. 0.21%. However, Zn0.007-Zn0.25 significantly
affected the net photosynthetic rate, contents of chlorophyll and essential monoterpen oil(s). At Zn₉₅₂₅₀ concentration, maximum peroxidase activity was obtained with the production of biomolecule geraniol. Other enzymes containing Zn include alcohol dehydrogenase, SOD and RNA polymerase (Vallee and Wacker 1970; Sandmann and Boger 1983). Alcohol dehydrogenase catalyzes the reduction of acetaldehyde to ethanol, a reaction in higher plants largely conducted in peroxisomes and bacterial zones such as root apices. One of the three types of SODs, the metal prothetic group includes Zn associated with Cu (Cu-Zn SOD). The metal catalyzes the conversion of superoxide radical and O₂⁻ to hydrogen per oxide and O, promoting anaerobic organism to defend themselves from the damage caused by O₂. The function of Zn in this isoenzyme has yet to be established. Foliar fertilization of plants with Zn resulted in an increase of nutrient content in the dry matter of leaves, with the most intensely absorbed element being the Zn itself (Lata and Sadowska 1996).

Mo is an essential element of the respiratory and assimilatory nitrate reductase. The former is confined in denitrifying bacteria and catalyzes the reduction of nitrate to nitrite. Assimilatory nitrate reductase is present in different kinds of organisms such as bacteria, cyanobacteria, algae, fungi and higher plants (Kaiser et al. 2001). It is essential that in oxido-reduction process, Mo facilitates the transport of an electron. Three Mo-enzymes have so far been found in plants (Self et al. 2001). Nitrate reductase catalyzes the reduction of nitrate, the key step in inorganic N assimilation. Aldehyde oxidase catalyzes the last step of biosynthesis of phytohormones indole acetic acid and abscisic acid. Xanthine dehydrogenase is involved in the catabolism of purines (Mendel and Haensch 2002; Kaiser et al. 2005). With the exception of bacterial nitrogenase, Mo-enzymes share a similar heterocyclic compound (pterin) composed of a pyrazine ring and a pyrimidine ring at their catalytic sites, the Mo cofactor. Mo is biologically inactive unless it is complexed with its cofactor. Among the micronutrients essential for the growth of plants and microorganisms, Mo is required in minute amounts. In contrast to bacteria, no specific Mo uptake system is known in higher plants, but since molybdate (A molybate is a compound containing an o xoanion with molybdenum in its highest oxidation state of 6) and sulfate behave similarly and have similar structure, uptake of molybdate could be mediated unspecifically by one of the sulfate transporters. Transport of Mo into different plant organs occurs via xylem and phloem. Pterin-bound Mo constitutes the cofactor of important plant enzymes involved in redox processes e.g. nitrate reductase, xanthine oxidase, aldehyde oxidase and sulfite oxidase. Biosynthesis of the Mo cofactor (Moco) starts with a guanosine-X-phosphate. Subsequently, a S-free pterin is synthesized; then S is added and finally Mo is incorporated. In addition to the molybdoenzyme enzymes, small molybdoenzyme binding proteins without catalytic function are known to probably be involved in the storage of Moco (Williams and Frausto da Silva 2002; Sauer and Freiberg 2000). Molybdenum containing systems, especially those affecting the activity against O₂ damage. Boric acid may bind to cis-diols, i.e. the compounds that contain pairs of cis hydroxyl groups such as sugar and sugar alcohols. The effects of B on plant growth and development depend on its specific complexing with borate and polyhydroxy substrates, enzyme protein and the co-enzymes with proper configuration of OH groups (Blevins and Lukaszewski 1998; Herrera-Rodriguez et al. 2010). However, meager studies on the role of B in metabolism of medicinal plants have been conducted in recent years which need special attention to have a better understanding.

4. Cadmium and cobalt

Cd and Zn are chemically very similar. Cd is able to mimic the behavior of essential element Zn in its uptake and metabolic function. Unlike Zn, Cd is toxic both for plants and animals (Sandalio et al. 2001; Akguc et al. 2008). The basic cause of toxicity probably lies in its higher affinity with thiol group (SH) of enzymes and other proteins. In many plants, excess Cd may also disturb Fe metabolism and can cause chlorosis of plant parts (Kosma et al. 2004). Edelbauer and Stangl (1992) found a direct effect of Cd in reducing element uptake; the toxicity of this heavy metal affected various enzymes and membrane systems, but indirect effects are also conceivable. The reduction of micro-nutrient levels in the case of Cd exposition appeared not only to depend upon the applied Cd concentration to the substrate but also on the micronutrient status of the plant (Lux et al. 2011).

Co, a transition element, is an essential component of several enzymes and co-enzymes (Aziz et al. 2007). It has been shown to affect growth and metabolism of plants to different degrees depending on the concentration and status of Co in the rhizosphere and soil. Co interacts with other elements to form complexes. The cytotoxic and phytotoxic activities of Co and its compounds depend on the physico-chemical properties of these complexes, including their electronic structure, ion parameters (charge-size relations) and coordination. Thus, the competitive absorption and mutual activation of associated metals influence the action of plants for B is as little as 2 ppm and it may be present in plant tissue up to 75 ppm. It is required for translocation of sugars and also regulates flowering and fruiting, cell division, salt absorption, hormone movement, pollen germination, carbohydrate metabolism, H₂O use and N assimilation in the plants (Brown et al. 2002; Camacho-Cristobal et al. 2008). Evidence in the literature supports the idea that the major functions of B in growth and development of plants are based on its ability to form complexes with the compounds having cis-diol configurations (Hu et al. 1997). The formation of B complexes with the constituents of cell walls and plasma membranes as well as with the phenolic compounds seems to be a decisive step affecting the physiological functions of B. B seems to be of crucial importance for the maintenance of structural integrity of plasma membranes (Hu et al. 1997). This function of B is mainly related to stabilization of cell membranes owing to its association with the membrane constituents. B also protects plasma membranes against the oxidative damage caused by the ROS. In B-deficient plants, plasma membranes become highly leaky and lose their functional integrity. Enhanced oxidation of phenols is responsible for generation of reactive quinones, which subsequently produce extremely toxic O₂⁻ species, thus, increasing the risk of oxidative damage to various cell components such as membrane lipids and proteins. In B-deficient tissues, enhancement in levels of toxic O₂⁻ species may also occur as a result of impairments in photosynthesis and antioxidative defense systems. Recent evidence shows that the levels of ascorbic acid, non-protein SH-compounds (mainly glutathione and glutathione reductase, the major defense systems of the cells against toxic O₂⁻ species, are reduced in response to B deficiency. There is also increasing evidence that, in the heterocyst cells of cyanobacteria, B is involved in protection of nitrogenase activity against O₂ damage. Boric acid may bind to cis-diols, i.e. the compounds that contain pairs of cis hydroxyl groups such as sugar and sugar alcohols. The effects of B on plant growth and development depend on its specific complexing with borate and polyhydroxy substrates, enzyme protein and the co-enzymes with proper configuration of OH groups (Blevins and Lukaszewski 1998; Herrera-Rodriguez et al. 2010). However, meager studies on the role of B in metabolism of medicinal plants have been conducted in recent years which need special attention to have a better understanding.
of Co regarding various phytochemical reactions. Co is not found at the active site of any respiratory chain enzymes (Atta-Aly et al. 1991). There are two sites of action of Co\(^{2+}\) in mitochondrial respiration, since it induces different responses towards different substrates such as alpha-keto glutarate and succinate. In lower organisms, Co\(^{2+}\) inhibits hexokinase, but in higher plants it probably participates in chlorophyll \(b\) formation (Gad and Kandil 2009). The sum of the fixed metal can be transformed into plastids and brings about changes in the chlorophyll content (Gad and Kandil 2009; Tan et al. 2010). It also inhibits the differentiation of starch grains and alters the structure and number of chloroplasts per unit area in the leaf (Gad and Kandil 2009). The role of Co in photosynthesis is controversial. Its toxic effect results in the inhibition of PS-II. It inhibits either the function of reaction centre or that of component of PS-II acceptor by modifying the electron acceptor site of secondary quine (QB). Co\(^{2+}\) reduces the export of photoassimilates and the extent of dark fixation of CO\(_2\) (Kurosaki 1996). In \(C_4\) plants, it hinders fixation of CO\(_2\) by inhibiting the activity of the enzymes involved (Palit and Sharma 1994; Gad and Kandil 2009). Relatively higher concentrations of Co are toxic to the plants (Gal et al. 2008). Toxic effects of Co on plant morphology include leaf fall, inhibition of greening, discoloration of green tissues, premature leaf closure and reduction in shoot weight. Being component of vitamin B-12 and cobamide coenzyme, Co\(^{2+}\) helps in the fixation of molecular N in root nodules of leguminous plants (Yadav and Khanna 2002; Bolachander et al. 2003; El-Sheekh et al. 2003; Parmar and Chanda 2005; Gad 2006; Jayakumar et al. 2008). However, in cyanobacteria, CoCl\(_2\) inhibits the formation of heterocyst, ammonia (NH\(_3\)) uptake and nitrate reductase activity. The interaction of Co with other metals mainly depends on the concentration of the metals used. For example, high level of Co\(^{2+}\) induces Fe deficiency in plants and suppresses uptake of Cd by roots (Jayakumar and Jaleel 2009). It also interacts synergistically with Zn, Cr and Sn (Vinay et al. 1996; Tan et al. 2010). The beneficial effects of Co include retardation of senescence of leaf, increase in drought resistance in seeds, regulation of alkaloid accumulation in medicinal plants and inhibition of ethylene biosynthesis (Gad and Kandil 2009). The plant mechanism of resistance to toxic concentration of Co may be associated with its intracellular detoxification rather than the defective transport. Among higher plants, only few advanced Cu-tolerant families showed cotolerance to Co\(^{2+}\). Tolerance of plants against Co\(^{2+}\) may sometimes determine the taxonomic shifting of several members of the Nyssaceae. Due to the high Co content in serpentine soil, the uptake of essential elements by plants is reduced. This phenomenon is known as "serpentine problem," for New Caledonian families like Flacourtiaeae (Yang et al. 1985). A high amount of soil Ca may neutralize the toxic effects of heavy metals in the adaptable genera grown in this type of soil. The bio-magnification of potentially toxic elements, such as Co, coming from coal ash or H\(_2\)O into food webs, needs additional studies for effective biological filtering (Palit and Sharma 2003; Parmar and Chanda 2005). Co also shows a positive response to Co application; Fe, Mn, Cu and Zn also showed positive response to Co application, but at lower concentrations. Similarly, Co and Ca content in the soil were positively correlated with seed alkaloid content of Colchicum autumnale, which was probably due to enhanced uptake of various other minerals that significantly elevated metabolic activities in the plant (Poutaraud and Girardin 2005; Aziz et al. 2007).

5. Aluminum, nickel, chromium and selenium

More than 15% of the earth’s crust is made up of Al\(_2\)O\(_3\). The solubility of Al in soil is very low, due to which neutral and alkaline soils may not be toxic to plant growth. There are some evidences that Al in low concentration is beneficial for plant growth though the mechanism is not clear (Delhaize and Ryan 1995; Matsumoto 2000; Vardar et al. 2006). Higher plants usually contain Al up to 200 ppm in the dry matter. Generally, Al content in roots is much higher than in the upper plant parts. It is supposed that a substantial proportion of root Al is fixed in free space. The dissolution of Al hydroxy compounds in soil depends much on soil pH. Low soil pH values may result in high level of soluble Al, which is toxic to plants (Ozyigit and Akinci 2009). The first observable effect of Al on plants is limitation in root growth (Jones and Kochian 1995). Frequently, phosphate uptake and phosphate translocation to the upper plant parts are affected. In the plant cell, Al may interfere with the phosphate metabolism through the formation of stable Al-phosphate complexes. According to Siegel and Haug (1983), Al binds to calmodulins and may interfere with various enzymatic processes. The plasma membrane is also affected by high Al concentrations (Vardar and Unal 2007). Al may have a detrimental effect on ion uptake. Grimm (1983) noticed that Al especially retards the uptake of Mg\(^{2+}\) (Jones and Kochian 1995). Aluminum, nickel, chromium and selenium (Atta-Aly et al. 2003; Parmar and Chanda 2005; Gad 2006; Jayakumar et al. 2008). However, in cyanobacteria, CoCl\(_2\) inhibits the formation of heterocyst, ammonia (NH\(_3\)) uptake and nitrate reductase activity. The interaction of Co with other metals mainly depends on the concentration of the metals used. For example, high level of Co\(^{2+}\) induces Fe deficiency in plants and suppresses uptake of Cd by roots (Jayakumar and Jaleel 2009). It also interacts synergistically with Zn, Cr and Sn (Vinay et al. 1996; Tan et al. 2010). The beneficial effects of Co include retardation of senescence of leaf, increase in drought resistance in seeds, regulation of alkaloid accumulation in medicinal plants and inhibition of ethylene biosynthesis (Gad and Kandil 2009). The plant mechanism of resistance to toxic concentration of Co may be associated with its intracellular detoxification rather than the defective transport. Among higher plants, only few advanced Cu-tolerant families showed cotolerance to Co\(^{2+}\). Tolerance of plants against Co\(^{2+}\) may sometimes determine the taxonomic shifting of several members of the Nyssaceae. Due to the high Co content in serpentine soil, the uptake of essential elements by plants is reduced. This phenomenon is known as "serpentine problem," for New Caledonian families like Flacourtiaeae (Yang et al. 1985). A high amount of soil Ca may neutralize the toxic effects of heavy metals in the adaptable genera grown in this type of soil. The bio-magnification of potentially toxic elements, such as Co, coming from coal ash or H\(_2\)O into food webs, needs additional studies for effective biological filtering (Palit and Sharma 2003; Parmar and Chanda 2005). Co also shows a positive response to Co application; Fe, Mn, Cu and Zn also showed positive response to Co application, but at lower concentrations. Similarly, Co and Ca content in the soil were positively correlated with seed alkaloid content of Colchicum autumnale, which was probably due to enhanced uptake of various other minerals that significantly elevated metabolic activities in the plant (Poutaraud and Girardin 2005; Aziz et al. 2007).

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### REGULATORY ROLE OF MINERAL ELEMENTS IN PROTEIN SYNTHESIS

#### Major steps in protein synthesis

All the metabolic activities in living organisms are catalyzed by enzymes, which are proteins. Cells are capable of synthesizing the proteins, essential for the modulation and maintenance of cellular activities. Formation of proteins, using amino acids (building blocks), is based on the information encoded in the nucleic acids (Deoxyribonucleic acid (DNA) and Ribose nucleic acid (RNA)). Protein synthesis generally consists of two major steps: transcription
ous coenzymes. Glutamate and glutamine are the first two about 10% and soluble amino N about 5% of total N present in plants (Kusano 2000; Valadkhan and Manley 2001; Schmitt et al. 2002; Sonenberg and Hinnebusch 2009). The ribosome mediates the formation of a polypeptide sequence based on the mRNA sequence. During this stage, complexes, composed of an amino acid linked to tRNA, sequentially bind to the ribosome and are translated into complete polypeptide (protein) from the ribosome (Nissen et al. 2000; Valadkhani and Manley 2001; Schmitt et al. 2002; Mandal et al. 2003; Bruce 2008).

N is by far the largest N fraction and amounts to about 80-85% of the total N present in proteins present in green plants (Kusano et al. 2011). The N of nucleic acids makes about 10% and soluble amino N about 5% of total N present in plant material. In vegetative plant parts, the enzymes constitute the major portion of proteins, whereas in seeds and grains the major protein fraction is made up of storage proteins. Besides, N is also an essential constituent of various coenzymes. Glutamate and glutamine are the first two amino acids synthesized during the process of NH₃ assimilation. Glutamate and aspartate as well as their amides accumulate when high levels of inorganic N are applied to plants. Under such conditions the excess inorganic N is obviously used in protein synthesis (Coruzzi and Bush 2001; Coruzzi 2003; Schachtman and Shin 2007; Krouk et al. 2010).

S is essential for the conversion of N into amino acids and the linkage of these amino acids into complete proteins. S accounts for about 1% of the plant tissue. S deficiency causes accumulation of nitrates in plant tissue instead of forming amino acids and protein (Schnug and Haneklaus 1998; Kertesz and Mirleau 2004). Elemental S is oxidized into a plant-useable form of sulphate by an S-oxidizing microorganism as Thiobacillus thiooxidans Co 2+ is used to stabilize the matrix of the nucleus. Glutamate and aspartate as well as their amides accumulate when high levels of inorganic N are applied to plants. Under such conditions the excess inorganic N is obviously used in protein synthesis (Coruzzi and Bush 2001; Coruzzi 2003; Schachtman and Shin 2007; Krouk et al. 2010).

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noticed the dose-dependent effects of \( \text{La}^{3+} \) on soluble protein content, peroxidase activity and shoot differentiation. Yin et al. (2006) noticed that growth of Catharanthus roseus cells in suspension-culture ceased during phosphate starvation, but these cells grew up again upon addition of \( \text{P}_i \) even after long-term starvation. They studied the metabolic fate of \( ^{32} \text{P} \text{Pi} \) in 1-week-old stationary phase cells in ordinary culture and in 1- or 2-week-old \( \text{P}_i \)-starved cells. Immediate effects of \( \text{Pi} \)-starvation on the cell were inhibition of the uptake of nitrate, using both high and low affinity transporters (Chrispeels et al. 1999). Most plant species are able to absorb and assimilate nitrate \( (\text{NO}_3^-) \), ammonium \( (\text{NH}_4^+) \), urea and amino acids as \( \text{N} \) sources, but the response to a particular form of \( \text{N} \) varies from species to species. In general, most crop plants prefer a mixture of \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) and take up a higher proportion of \( \text{NH}_4^+ \) present in the soil solution only in specific cases, as in the case of \( \text{Papaver somniferum} \). Variation in soil nitrate level affects the rate of plant growth and concentration of \( \text{NO}_3^- \) stored in plants. In unlimited supplies of \( \text{NO}_3^- \), root and shoot concentrations of \( \text{NO}_3^- \) can reach up to 100 mM, most of which is stored within the vacuoles. However, the cytoplasm is a more important compartment for \( \text{NO}_3^- \) metabolism (Chrispeels et al. 1999). In soil, \( \text{NO}_3^- \) is carried towards the root by bulk flow and is absorbed into the epidermal and cortical symplasm. Within the root symplasm, \( \text{NO}_3^- \) has the four following fates: (1) reduction to \( \text{NO}_2^- \), by the cytoplasmic enzyme nitrate reductase; (2) efflux back across the plasma membrane to apoplasm; (3) influx and storage in vacuole; or (4) transport to xylem for long-distance translocation to the leaves. Following long-distance translocation, \( \text{NO}_3^- \) must leave the xylem and enter the leaf apoplasm to reach the leaf mesophyll cells, where \( \text{NO}_3^- \) is again absorbed and either it is reduced to \( \text{NO}_2^- \) or stored in the vacuole (Glass and Siddiqi 1995; Crawford and Glass 1998).

On reaching the root surface, uptake of \( \text{NO}_3^- \) into the cell requires energy, even though external \( \text{NO}_3^- \) concentration remains in low range. However, in spite of expenditure of energy on \( \text{NO}_3^- \) uptake, there appears to be a significant efflux of \( \text{NO}_3^- \) across the plasma membrane. The rate of efflux increases with increasing external \( \text{NO}_3^- \) concentration. The \( \text{NO}_3^- \) efflux is passive, and there is evidence that this process is \( \text{K}^+ \)-selective. Employing the use of inhibitors of RNA and protein synthesis has indicated that it is \( \text{NO}_3^- \)-inducible. However, nothing is known about the proteins involved in the efflux pathway (Chrispeels et al. 1999). The energy for ion uptake is provided by the proton gradient or pmf, which, based on typical cell parameters, is adequate to drive active \( \text{NO}_3^- \) uptake over a wide range of external \( \text{NO}_3^- \) concentrations. In addition, \( \text{NO}_3^- \) uptake is associated with depolarization (an increase in the positive charge inside the cell) of the plasma membrane. Physiological studies and molecular investigations in Nicotiana species and Arabidopsis indicated that several \( \text{N} \) forms, including \( \text{NO}_3^- \), \( \text{NH}_4^+ \) and amino acids, may participate in this down regulation, which can occur at the mRNA level. Such a regulation is important for coordinating the root uptake as per shoot demand for \( \text{N} \) during the growth cycle of the plant. A common explanation, given for the mechanism of rapid uptake of \( \text{NH}_4^+ \) and inhibition of that of \( \text{NO}_3^- \), is that \( \text{NH}_4^+ \) depolarizes the plasma membrane and, thus, reduces pmf for active \( \text{NO}_3^- \) uptake by \( 2\text{H}^-/\text{NO}_3^- \) symport mechanism (Taylor and Bloom 1998). This explanation is supported by the observations that indicate that \( \text{NH}_4^+ \) induces a sustained membrane depolarization and that the more depolarized the membrane potential is, the lower is the \( \text{NO}_3^- \) -induced current (Day et al. 2001; Roberts and Tyerman 2002; Kabala et al. 2003). However, since \( \text{K}^+ \) is also known to depolarize the membrane electrical potential without an equivalent reduction in \( \text{NO}_3^- \) uptake, the reduction in electrical potential alone seems insufficient to explain the short term inhibition by ammonium (García-Sánchez et al. 2000). An increase of \( \text{N} \) supply in Catharanthus roseus stimulated \( \text{K} \) and \( \text{P} \) uptake and their translocation to the leaves has been proven by the results of the chemical analysis (Lata and Sadowska 1996). Furthermore, the experiments demonstrated the effect of different doses of \( \text{N} \) in the form of compost on an important medicinal plant \( \text{Ruta graveolens} \) L. They found positive effect of the compost doses on the amount of essential oil, rutin, coumarin and other secondary metabolites. Pavlíková and Thustoš (1994) concluded that it is also important to choose a suitable form of \( \text{N} \) fertilizer, especially when the uptake of \( \text{N} \) from soil and the seed yields are concerned. The need for an optimal system of supply of \( \text{N} \) for poppy plants begins shortly after germination and lasts till the stage of formation of generative organs.

**Potassium channels**

The translocation of cations across biological membranes is an inherent feature associated with numerous physiological processes, including growth and development, signal transduction and cell homeostasis. It is well known that adeno-
K+ (K) is the most abundant cation in plants, whereas closely related Na (Na+) ion is toxic to most plants at high millimolar concentrations. Both K+ deficiency and Na+ toxicity are major constraints to crop production worldwide. In fact, K+ counteracts Na+ stress, while Na+, in turn, can alleviate K+ deficiency up to a certain degree (Pascal et al. 2002). The beneficial effect of K+ on photophosphorylation has been observed by Watanabe and Yoshida (1970), Hart (1972) and Hart and Wells (1975). Moreover, Weller and Hofner (1974) have reported that K+ stimulates photosynthetic O₂ production. These observations supported the view that K+ has direct influence on electron transport in the photosynthetic ETC. It might be supposed that the movement of K+ from the thylakoid spaces into the stroma of chloroplasts, which occurs in light, depolarizes the thylakoid membrane that, in turn, favours the electron flow in transport chain (Mitchell 1966). Depolarization of plasma membrane by K+ uptake has a direct influence on phloem loading. Numerous researchers have shown that K+ enhances the translocation of photosynthates. K+ not only promotes the translocation of newly synthesized photosynthates, but also has a beneficial effect on the mobilization of stored material. Liu and Zhong (1996) noticed the effects of initial K+ concentrations (0–60 mM) on the kinetics of cell growth, nutrients metabolism and production of ginseng saponin and polysaccharide in suspension cultures of Panax ginseng by altering KNO₃ and NaNO₃ concentrations at 60 mM of total NO₃. They showed that comparatively a higher concentration of soluble sugars was stored within the cells under K+ deficiency, and that there was a curvilinear relationship between initial K+ concentration and active biomass accumulation. The K+ consumption by the cells was dependent on the initial medium K+ concentration and there was a linear correlation between K+ consumption and nitrate rate consumption. Although K+ had a little effect on the content of ginseng polysaccharide, the saponin content was remarkably enhanced with the increase in initial K+ concentration within a range of 20–60 mM.

**Calcium gradient and oscillations in growing pollen tubes**

Pollen tube growth delivers two sperm cells to embryo sac; it is essential for sexual reproduction in higher plants (Feijó et al. 2001; Hepler et al. 2001; Cárdenas et al. 2008). The process has important and unique features and is extremely fast. For example, the rate of movement of sperm cells is 1 cm per hour in corn. The process is highly polarized and pollen tube growth is mainly confined to the tip ("tip-growth"). It possesses a guidance mechanism that determines the direction of growth of the tube (Parton et al. 2003; Wilsen and Hepler 2007). Several aspects of tip-growth of the pollen tube have been deciphered; for example, the Golgi apparatus produces vesicles containing cell wall precursor, and through cytoplasmic streaming the vesicles flow to the apex of the pollen tube, where they fuse with the plasma membrane and secrete their contents into the cell wall. It is related to the presence of K+ required to achieve the high degree of polarity during pollen tube growth, although recent work indicated that different ions, especially Ca ions and protons play an important role in this regard (Feijó et al. 2001; Hepler et al. 2001; Song et al. 2009).

It is well known that both Ca ions and protons are essential for growth (Hepler et al. 2001; Iwano et al. 2001; Song et al. 2009). The Ca ions must be available at the correct concentration in the growth medium at a concentration above 10 μM, but these ions have detrimental effect above 10 mM. A high proton concentration or low pH facilitates both pollen germination and pollen tube growth. Recent work, focused on the intracellular and extracellular status of Ca ions and protons has been conducted, using the technology that is available since the last decade. Studies on intracellular Ca, conducted by using fluorescent indicator dyes (Holdaway-Clarke and Hepler 2003), or the photoprotein and aequorin (Messerli et al. 2009), have shown that the intracellular Ca concentration is high, ranging from 100 to 1000 μM. The physiological role of intracellular Ca ions is still unclear, but it has been suggested that they play a role in the regulation of the plasma membrane Ca channel (PMCa) activity, which is essential for the regulation of the pollen tube growth. The role of extracellular Ca ions is not clear, but it has been suggested that they play a role in the regulation of the extracellular Ca channel (ECaC) activity, which is essential for the regulation of the pollen tube growth.
al. 2000), revealed that the growing pollen tube possessed a highly focused gradient of free Ca at its extreme apex (Song et al. 2009). The intracellular Ca concentration, immediately adjacent to the plasma membrane, remains 2,000 to 10,000 nM (Messerli et al. 2000), but it declines abruptly to a basal level i.e. 200 nM or less at 20 μm distance from the pollen tube tip. Recent studies showed that the apex of pollen tube contains an acidic region with a large alkaline domain towards the base of the clear zone (Feijó et al. 1999). The pH gradient is large and extends from 6.8 at the tube apex to 7.8 at 20–30 μm distances from the tip. The extracellular influx of Ca\(^{2+}\) and the intracellular Ca gradient, thus, mark the direction of pollen tube growth. Extracellular protons, like Ca ions, also show an influx at the apex of the growing pollen tube. However, in contrast to Ca ions, protons display a marked efflux along the side of the tube, close to the base of clear zone, in a position that corresponds to the location of the extracellular alkaline band (Feijó et al. 1999; Ikeda et al. 2003; Watahiki et al. 2004). Vernoica et al. (2002) have previously noticed that the increases in cytosolic free Ca\(^{2+}\) triggered by the self-incompatibility (SI) response in incompatible Papaver rheas pollens. In a recent study, they used an ion-selective vibrating probe to measure changes in the extracellular Ca\(^{2+}\) fluxes around poppy pollen tubes. They confirmed that there was an oscillating Ca\(^{2+}\) influx directed at the apex of the pollen tube and that Ca\(^{2+}\) influx also occurred at the shanks of pollen tubes. Additionally, as per the challenge with self-incompatibility (SI) proteins, there is a stimulation of Ca\(^{2+}\) influx along the shank of incompatible pollen tubes, approximately 50 mm behind the pollen tube tip. Thus they demonstrated that SI-induced Ca\(^{2+}\) influx played a role in influx of extracellular Ca\(^{2+}\) in the SI response. In a study on medicinally important plant Nicotiana tabacum, Tuna and Burun (2002) concluded that all heavy metals have negative effects on pollen characteristics; however, the damage to pollen varies with the dose. All plants showed some tolerance against different pollutants.

The genus Crataegus (hawthorn) includes some important species having medicinal, ornamental and sanitary properties. Among them, Crataegus douglasii (black hawthorn) and Crataegus oxyacantha (red hawthorn) are mostly used, having more nutrients, medical, ornamental and sanitary properties and used as a main dry tolerant rootstock recently. The fruits are the part having commercial value and the most important factors involved in fruit set. The pollens were cultured in different media and maintained about 24 h at 22°C but growth and tube growth were composed of 15% sucrose, 0.005-0.01 and 0.02% with (1.2%) agar and pollens were cultured in different media and maintained about 24 h at 22°C but growth and tube growth were composed of 15% sucrose, 0.005-0.01% boric acid and 1.2% agar and significant differences observed between various media in the studied pollens traits. Sharafi (2010b) also studied pollen germination and tube growth in some Hashtroud and Maragheh indigenous genotypes of Rosa canina as these were the main factors affecting fruit set. The pollens were cultured in an in-vitro medium containing 15% sucrose, 0.01% boric acid and 1.2% agar and maintained in 24°C in controlled condition. After 24 h pollens germination and growth were stopped with chlorophorm. The study concluded significant differences between germination percentage (PGP) and pollen tube length (PTL) of genotypes.

REGULATORY ROLE OF MINERALS IN ALKALOID BIOSYNTHESIS

Before discussing the regulatory role of minerals on alkaloid biosynthesis, it is necessary to describe the general information about alkaloids. Alkaloids are compounds of non-peptidic origin that typically contain N and have complex ring structures. They are produced primarily in higher plants, but also in lower organisms and in some animals having pharmacological importance. The name was derived from the word ‘alka line’ in 1819 by Carle Meissner, while originally the term was used to describe any N-containing base (an amine in modern terms) having similar properties as basic salts derived from the alkali ashes of plants. The first identified alkaloid was morphine, from opium poppy (Luch 2009). Evidence suggests that alkaloids have been used by humanity as medicines and poisons for thousands of years. The first civilization that used them was probably the ancient Sumarians and Egyptians (Kutchan and Dittrich 1995). However, it was not until early nineteenth century that these compounds were reproducibly isolated and analyzed. Advances in analytical separation techniques, such as chromatography and mass spectroscopy, led to elucidation of the chemical structure of alkaloids (Southon and Buckingham 1989). When separated, in general they are colorless, crystalline solids, basic in nature and have definite melting points. Some exceptions are, however, known. For instance, some alkaloids are not basic and others are brightly colored or liquid. Still it is unclear as to why the plants produce alkaloids. Several theories have been proposed explaining the reason; of them some suggest that alkaloids are byproducts of normal plant metabolism or are produced as a means of defense against insects and animals or may be the reservoirs for molecules that plants often use. It is likely that all of these theories are correct up to some extent.

Classification of alkaloids

Alkaloids can be classified in three ways based on their chemical structure (nucleus containing N) or based on their biosynthetic pathway (the way they are produced in the plant) (Trease and Evans 1959). According to first type of classification as proposed by Hegnauer (1986), alkaloids are divided into three groups:

a. True alkaloids: The basic unit in biogenesis of true alkaloid is amino acid. They are poisonous and found in plants as salts of organic acids having heterocyclic ring with N. The non-N containing rings or side chains are derived from terpene units or acetate, while methionine is responsible for the addition of methyl groups to N atoms, e.g. morphine, codeine, etc. (Trease and Evans 1959; Aniszewski 2007).

b. Proto alkaloids: The basic unit in biogenesis of a proto alkaloid is also amino acid but they do not have heterocyclic ring with N. Instead, they may have N atoms outside the ring systems e.g., colchicine and ephedrine (Aniszewski 2007).

c. Pseudo alkaloids: The basic unit in biogenesis of proto alkaloid is terpenoids or purines instead of amino acids. They also have heterocyclic ring with N, e.g. caffeine, theobromine, and theophylline (Aniszewski 2007).

On the basis of biosynthetic pathways, alkaloids can also be divided into three groups (Trease and Evans 1959; Aniszewski 2007). Group I liberates amino N from the precursor during the biosynthesis of its alkaloids. Alkaloids belonging to this group are synthesized from diamino acid family, phenylalanine-tyrosine family and tryptophan family. They have a higher C:N ratio than their immediate precursors. In this process, amino acid is converted into alkaloids, liberating one molecule of ammonia. Group II includes those alkaloids, which have the same number of N atoms as
their precursor do. Group III includes those alkaloids, which have a lower number of C:N ratio compared to their precursor. This group is sometime called as the group of pseudo alkaloids e.g., steroid alkaloids, isoprene derivatives, etc. (Waller and Nowacki 1978).

The third type of classification system to be considered was offered by Manfred (2002). This classification concentrates on the N atom present within the chemical structure of the alkaloid, and classifies the alkaloids based on the environment of that N atom. If there are present multiple N atoms in the chemical structure of the alkaloid, the priority is given to the N atom present in the predominant ring. The following five groups of alkaloids may be framed based on this classification of alkaloids:

**Heterocyclic alkaloids**

The majority of the alkaloids occur in this group. These alkaloids have an N atom in a heterocyclic ring, or in some cases part of two heterocyclic rings (Trease and Evans 1959; Joule and Mills 2000).

**i) Pyrrolidine alkaloids**

Alkaloids of this group contain five N rings and are predominantly synthesized from ornithine and lysine, e.g. hygrine, isolated from the leaves of *Erythroxylum coca* (coca plant); it is an important precursor to hyoscymamine and scopo-alamine (Trease and Evans 1959; Joule and Mills 2000).

**ii) Terpenoid indole alkaloids**

The indole alkaloids comprise a large family of alkaloids. Many of these natural products are physiologically active in mammals. For instance, the antimalarial drug quinine extracted from *Cinchona officinalis*, the antineoplastic drug camptothecin extracted from *Camptotheca acuminata*, the rat poison and homeopathic drug strychnine extracted from *Strychnos nux-vomica* and the antineoplastic drug vincristine extracted from *Catharanthus* (periwinkle) belong to this group of alkaloids. In fact, the chemical synthesis of these complex alkaloids would be of academic interest, but due to their low yields they are not likely to be applied commercially. The indole alkaloids have indole as their structural backbone and are derived from tryptophan. The indole system can be considered to consist of a pyrrole ring and a benzene ring fused together to form the two isomeric benzopyroles: indole and isoindole (Trease and Evans 1959; Joule and Mills 2000).

**iii) Pyrrolizidine alkaloids**

A common example is ergine, one of the ergot alkaloids, isolated from many plants and fungi. Ergine is a hallucinogenic alkaloid having similar effects like indole alkaloids but has an overall lower level of visual stimuli (Laing 2003). Strychnine is another indole alkaloid, isolated from the *Stychnos nux-vomica* (strychline tree) in Southeast Asia. It is used as a pesticide for killing small birds and rodents, as it is a very potent neurotoxin (Brams et al. 2011). The alkaloid of *Rauwolfia* and *Papaver* also belong to this group. For centuries, the natives of the Himalayan foothills are using the leaves of *Rauwolfia* for healing affictions ranging from snake bites to insanity. Reserpine is the most important constituent of *Rauwolfia*. Reserpine depletes adrenergic vesicles of their transmitters, thereby, reducing the amount of neurotransmitter released. This causes hypotension, bradycardia and increased gastrointestinal motility and increased the rate of defecation (Liles et al. 2006). The widespread reference in folklore of the oral hypoglycemic properties of leaf extracts from the *Catharanthus roseus* prompted investigations which led to the discovery of dimeric indole-dihydropyridine alkaloids i.e. vinblastine and vincristine. These alkaloids produced severe leukopenia in rats suggesting an anti-cancer potential especially for the treatment of leukemia (Zhigaltsev et al. 2005).

The indole alkaloids derived from tryptamine and secologanin can be classified according to the structural characteristics of their skeletons into the following eight types (Trease and Evans 1959; Joule and Mills 2000).

1. Corynanthean or C-type (sarapine, yohimbine, ajmaline)
2. Vincosan or D-type (vincoside)
3. Vallesiachotonam or V-type (vallesiachotamine)
4. Strychnan or S-type (vomicine)
5. Aspidosperman or A-type (condylocarpine)
6. Eburnan or E-type (vincamine)
7. Plumeran or P-type (kopsine)
8. Ibogan or J-type (voaluteine)

**iii) Pyrrolizidine alkaloids**

The pyrrolizidine alkaloids have pyrrolizidine rings as their back bone, e.g. retronecine. Its ester derivatives are used as sex pheromones in lower animal species (Xiong et al. 2009).

**iv) Pyridine alkaloids**

Pyridine alkaloids have pyridine in their basic structure, e.g. nicotine. Nicotine, the addictive compound found in tobacco, can also be isolated from several other plants including *Solanum seafordthium* (nightshade) (Lindvai and Vizi 2008). Nicotine interacts with nicotine acetylcholine receptors throughout the nervous system. In brain, by interfering with these receptors, nicotine increases the concentrations of several neurotransmitters including dopamine, which induces the feeling of relaxation (Dani and Bertrand 2007). Anabasine, an alkaloid found in *Nicotiana glauca* (tobacco tree), produces the same effects as nicotine. It also interferes with the nicotine acetylcholine receptors throughout the body (Lindvai and Vizi 2008).

**v) Piperidine alkaloids**

The piperidine alkaloids have a piperidine ring in their chemical structure, e.g. Conine. In the history, the alkaloid conine was used to kill Socrates. This alkaloid is found in *Cinchona* and has been used in treatment of malaria for centuries (PrayGod et al. 2008). It also has antipyretic properties. Its mechanism of action is by prevention of hemoglobin cosesitated inside the plasmid, thus, causing a buildup of cytotoxic heme groups (Jones et al. 2007).

**vi) Quinoline alkaloids**

The quinoline alkaloids have quinoline rings in their chemical structure, e.g. quinine. It isolated from cinchona bark and has been used in treatment of malaria for centuries (PrayGod et al. 2008). It contains formic acid and has an overall lower level of visual stimuli (Laing 2003). It also has antipyretic properties. Its mechanism of action is by prevention of hemoglobin cosesitated inside the plasmid, thus, causing a buildup of cytotoxic heme groups (Jones et al. 2007).

**vii) Iso-quinoline alkaloids**

The iso-quinoline alkaloids contain an iso-quinoline ring in their structure, e.g. papaverine found in *Papaver somnife-
rum (Shukla et al. 2010). Papaverine is a compound used in treatment of spasms in gastro-intestinal tract and bile ducts as well as it is used as vasodilator (Mishra et al. 2010a). It acts by inhibition of a phosphodiesterase, which is responsible for degradation of cyclic AMP, a common second messenger in the cell (Mishra et al. 2010b).

viii) Tropaeone alkaloids

Tropaeone alkaloids are alkaloids with nitrogenous bicyclic rings. The tropaeone class of alkaloids are found mainly in Solanaceae family containing the anticholinergic drugs hyoscyamine and scopoline. Scopolamine isolated from Duboisia and is commonly used as transdermal patch to combat motion sickness (Chan et al. 2006; Patel and Ezzo 2009). Cocaine is yet another famous tropaeone alkaloid. It is isolated from the coca plant and works as a stimulant and appetite suppressant by blocking dopamine reuptake in the brain. Cocaine was actually marketed as a children’s toothache medicine in the early 1900s (Dickerson and Janda 2005; Favrod-Coune and Broers 2010).

Atropine, which is isolated from Atropa belladonna (deadly nightshade), is another example of tropaeone alkaloids. Atropine lowers the activity of all the muscles and glands controlled by parasympathetic nervous system, as it is a competitive inhibitor for muscarinic acetylcholine receptors in synapses of parasympathetic nervous system (Takeda et al. 2003).

Alkaloids with exocyclic N and aliphatic amines

In their chemical structure, these alkaloids have N outside the cyclic structures (Trease and Evans 1959; Joule and Mills 2000). This category is further subdivided into the following three main categories:

1) Erythrophleum bases

Classification of these alkaloids is based on the appearance of an aminoethanol group. An example of this category of alkaloids is cassaine, which is a cardiotoxic alkaloid that can cause anorexia, defective vision, increased heart sounds, and dyspnea (Phoenix et al. 2008).

2) Phenylalkylamines

The main structural component of these alkaloids is an alkylamine attached with two phenyl group (Awasthi and Yadav 2007). This group tends to have hallucinogenic properties and is, therefore, one of the major groups in psychodelic drugs. Most of these psychodelic drugs are used for recreational purposes. For example, mescaline, a phenylalkylamine, is more commonly known as ecstasy. Alkaloids from this category have also been used in order to block voltage-gated Ca channels by binding within the intracellular mouth of ion conducting pore. There are several naturally occurring phenylalkylamines in human body such as dihydroxyphenylalanine (DOPA), dopamine, tyrosine, and adrenaline. Some of these naturally occurring molecules are used as protein building blocks (amino acids) and also for the execution of motive, cognitive, and neuroendocrinic functions. Several of the molecules like dopamine are also involved in pathogenesis of multiple neuropsychiatric diseases such as Parkinson’s disease, Alzheimer’s disease, and schizophrenia. The Ca channel blocking molecules have antiarrhythmic and antihypertensive properties and can be highly toxic if they bind too strongly to the ion channel preventing the inflow of Ca for an extended period of time (Awasthi and Yadav 2007).

3) Alkaloids having an exocyclic N

Alkaloids like colchicine have an exocyclic N, are normally more biogenetically related to alkaloids with an isoquinoline group. Colchicine itself inhibits the polymerization of tubulin protein, which is required for mitosis; so it can be used as an antitumor drug, as cancer cells rapidly go through mitosis (Pelletier 1999).

Putrescine, spermidine and spermine alkaloids

Putrescine, spermidine and spermine bases are part of a group of natural products known as biogenic amines; thus, they are not considered as alkaloids (Karovicova and Kohajdova 2005). This class of alkaloids arises because they are derived using these three bases as building blocks. A classic example from the spermine sub group is Oncinotine, the main alkaloid of Oncinotis nitida (Apocynaceae).

Diterpene and steroidal alkaloids

Terpenes are another form of naturally occurring products that are derived from isoprene subunits (Mishra et al. 2009). Terpene is a 10-C molecule; thus, diterpenes are two terpenes put together creating twenty C molecules. Although terpenes do not have a Nous base within their isoprene subunits, basic N can be added to the molecule, classifying certain terpenes as alkaloids. The diterpenes can be subcategorized into the following two major groups:

1) Diterpenes with a 20-C skeleton

These alkaloids belong to several plant families such as Rubunculaceae, Garryaceae, Compositae, and Rosaceae (Mishra et al. 2009). The 20-C backbone can be arranged into two major forms:

A) Veatchine skeleton (e.g. veatchine, cuauchichicine, and songorine);

B) Atisine skeleton: This arrangement differs from the veatchine skeleton by having a six-membered ring instead of a five-membered ring, e.g. atisine, atidine, hetiesine, ignavine, and kobusine.

2) Diterpenes with a 19-C skeleton

The diterpenes, which have lost a C molecule during their synthesis, belong to this group. They are most commonly found in Aconitum and Delphinium species (Diaz et al. 2005; Forgo et al. 2011). Due to the 20-C backbone, diterpene skeletons have two different arrangements:

A) Lycoctonine skeleton, e.g. aconitine, hyaconitine, delphinine, and lycoctonine;

B) Heteratisine skeleton: This arrangement differs from lycoctonine due to addition of an O functional group to produce a lactone.

Peptide alkaloids

These alkaloids are derived from short peptide chains or the chains, which resemble with the peptides. Most of these alkaloids have already been classified under previous categories as many alkaloids are derived from amino acids. The alkaloids considered in this category contain an obvious peptide backbone. For example, celenamide-E is a tripeptide alkaloid in Patagonian sponge, Cliona chilensis (Sugumar and Robinson 2010). This molecule shows antibiotic activity against Gram-negative bacteria.

The chemical classification of alkaloids is universally adapted and depends on the type of heterocyclic ring structure present. It is usual to classify alkaloids according to amino acids (or their derivatives) from which they arise. Thus the most important classes are derived from the amino acids ornithine and lysine; or those derived from aromatic amino acids phenylalanine and tyrosine; or those derived from tryptophan and a moiety of mavelonoid origin. Besides, a number of such compounds are also derived from anthranilic acid or from nicotinic acid.

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1) Alkaloids derived from lysine

Such alkaloids, e.g. pelletierine and pseudopelletierine, are found in the bark of pomegranate trees (Khare 2004). Another example is sedamine that is one of the 600 alkaloids isolated from the genus Sedum, a common garden plant, while halosalone, a minor constituent, is obtained from Haloxylon salicornicum (Cossy et al. 2002). Lycopodine is obtained from various species of the moss Lycopododium.

The comparison of structures of pelletierine, sedamine, and halosalone suggests that each of these molecules arises from the reaction of a common intermediate with a different Δ-ketoacid. The most likely intermediate is thought to be Δ-piperideine.

2) Alkaloids derived from phenylalanine and tyrosine (benzylisoquinoline)

The simplest members of this group of alkaloids are compounds that contain β-phenylethylamine, C₆H₅CH₂CH₂NH₂, skeleton, e.g., morphine, codeine, thebaine, etc. (Ziegler et al. 2005).

3) Alkaloids derived from tryptophan

These are quinine type alkaloids derived from tryptophan. Quinine skeleton includes atoms derived from non-amino acid sources, in particular the terpene geranium. However, feeding experiments, using isotopically labelled geranium and tryptophan, showed involvement of molecular rearrangements (Dewick 2011).

BIOSYNTHESIS OF ALKALOIDS

Amino acid, the building blocks of alkaloids, are modified by decarboxylation, aldol condensation, reductive amination or methylation. Successive alterations using these relatively few biosynthetic transformations lead to formation of specific alkaloids.

Biosynthesis of terpenoid indole alkaloids

It begins with the conversion of tryptophan to tryptamine and secologanin and ends with the synthesis of terpene indole alkaloids (Facchini 2001).

Biosynthesis of benzylisoquinoline alkaloids (BIA)

The BIA biosynthesis begins with decarboxylations, ortho-hydroxylations, and deaminations of tyrosine that convert it into both dopamine and 4-hydroxy phenylacetaldehyde (Rueffer and Zenk 1987; Facchini 2001).

Biosynthesis of tropane alkaloids

Biosynthesis of tropane alkaloids starts with ornitine and finally ends with the synthesis of tropane alkaloid (Facchini 2001).

Biosynthesis of purine alkaloids

Purine alkaloids, such as caffeine, theobromine, and acrine, are widely distributed in plant kingdom. Recent metabolic studies on tea and coffee have elucidated the biosynthesis of caffeine. The major route begins with xanthosine and proceeds through three N-methylations via 7-methylxanthosine, 7-methylxanthine and theobromine (Suzuki et al. 1992; Ashihara et al. 1996, 1997) although a number of minor pathways have also been suggested (Kato et al. 1996; Schultless et al. 1996; Facchini 2001).

REGULATORY ROLE OF MINERALS IN ALKALOIDS

An alkaloid is a naturally occurring nitrogenous organic molecule that has pharmacological effect on humans and other animals. Being a nitrogenous compound, availability of N directly influences the level of alkaloids (Demeyer and Dejagere 1998). Biosynthesis of alkaloids, although controlled genetically, could be affected by different environmental factors, such as light, high temperature, stress and nutrients (Andrijany et al. 1998; Chatterjee et al. 1988; Lovkov and Buzuk 1998). The mode of action of various groups of alkaloids varies depending on the level of N available. Several decades back, Mothes (1928) conducted a study to look into the effect of different levels of added N on the plant of Nicotiana and noticed that initially alkaloid content increased rapidly upon addition of first level of N but additional level of N decreased the total amount of alkaloids. Previously it was noted by geneticist and chemist that type of fertilizer does not affect alkaloids. Later on, Marten et al. (1974) studied that ammonium source of N caused greater increase in total alkaloid content than did a nitrate source in Canary grass. However, later on Pavlikova and Tlustos (1994) confirmed the findings of Marten et al. (1974). Similar study was also conducted by Yadav et al. (1984) on poppy and suggested three important factors, i.e. supply, form and date of application of N in the form of fertilizer, play decisive role in alkaloid production. They suggested that application of N increased the alkaloid content in the strains in which alkaloid content was already high. Similarly in Catharanthus, N in NO₃ form enhanced the alkaloid content up to 50% (Miranda-Ham et al. 1996), while application of a mixture of NO₃ and NH₄ decreased the alkaloids by 45%. It was reported in opium poppy that direct dose of N as well as the split doses not only affected the alkaloid concentration but also affected the habits of poppy and seed yield (Kharwara et al. 1988; Jain et al. 1990; Pavlikova and Tlustos 1994).

Not only N, other macro- and microbiogenous elements also modulate the formation and accumulation of isoquinoline (papaverine) alkaloids in opium poppy (Lovkov et al. 2006). In their experiment, they took 12 elements and clustered them into three groups. Group I included molybdenum, cobalt, tungsten, and Cr, group II had Cu and nickel and group III had boron, Fe, vanadium, Mn, zinc and Ca. Group I elements stimulated the formation of isoquinoline alkaloids. The elements of group III inhibited the formation and accumulation of alkaloids at various concentrations. Mo, Cr, and W has probably related to the inhibition of nonspecific phosphatases, which contributed to a more pronounced retention of phosphorylated intermediates during the biosynthesis of amino acids, precursors of alkaloids (tyrosine and phenylalanine) and other intermediate substances.

Co has stimulatory effect on isoquinoline alkaloids by activating the biosynthesis enzymes of the initial stage of aromatic amino acids, including 3-deoxy-D-arabino-hexulosonate-7-phosphate synthase (DAHP synthase, EC 2.5.1.54) and 3-dehydroquinate synthase. Unlike Co-dependent isoform, Mn dependent DAHP synthase is located in the plastids and are characterized by feedback inhibition. The Mn blocks the formation of precursor amino acids by feedback mechanism, which results in the inhibition of isoquinoline accumulation. The difference in the effect of Co and Mn may be related not only to the regulatory activity of DAHP synthase isoforms, but also to the variations in intracellular localization of the isoform (cytosol and plastids, respectively). Lovkov et al. (2006), in an experiment on opium poppy with glyphosate, showed the effect of Co. The glyphosate specifically inhibits two enzymes in the biosynthesis of aromatic amino acids and Co-dependent DAHP synthase, but does affect the Mn-dependent isoform. Iron (Fe) has inhibitory effect, which provides the evidence for the hypothesis that Co has a role in formation and accumulation of isoquinolines. Fe at various concentrations suppresses different steps of biosynthesis, but does not
have an effect on the growth and formation of seedlings. The antagonistic relationship between Co and Fe suggested that effect of Fe is realized via the displacement and substitution of Co in the enzymes catalyzing the biosynthesis of primary alkaloid precursors (aromatic amino acids).

Another microelement Cu also has stimulatory effect on isoquinoline alkaloids by activating Cu-containing enzyme, polyphenol oxidase located in the plastids and alkaloid accumulation. Formation of latex in the plants catalyzes the major early stages of biosynthesis of isoquinoline alkaloids, including tyrosine hydroxylation (with the formation of DOPA), transformation of 4-hydroxyphenyl-acetaldehyde into 3,4-dihydroxyphenyl-acetaldehyde, and conversion of N-methylcoaquaronine to 3-hydroxy-N-methylcoaquaronine. Vanadium and Ca is associated with the inhibition of isoquinoline synthesis by inhibiting protein ATPases, which determines the transmembrane electrochemical gradient. Vanadium inhibits the active transport by suppressing the supply of energy required for isoquinoline synthesis. Ca strongly inhibits the biosynthesis of isoquinoline alkaloid of Papaver somniferum (Buzuk and Lovkov 1985). Ca modulates the passive transport by stabilizing membranes and decreasing the availability of substrates for the enzymes. Due to high degree of compartmentalization of alkaloid biosynthesis and the decreased active transport, passive transport has a major role in the formation and accumulation of isoquinolines. The element boron stimulates consumption of N, which increases synthesis of various N-containing compounds (e.g. amino acids). The above changes have a positive impact on alkaloid formation in opium poppy. In the poppy capsules, there is positive relationship between boron and C and the negative one between papaverine and zinc and codeine and zinc (Lachman et al. 2006). It was also found that atmospheric CO2 influences the alkaloid content in the case of morphine, codeine noscapine and papaverine. Morphine had maximum response to CO2 and as a fraction in total alkaloid percentage (Ziska et al. 2005, 2008).

In Catharanthus, Zn and N play an important role in the synthesis of alkaloids. Zn is indispensable for the synthesis of tryptophan, which is the precursor of indole alkaloids; whereas N is a constituent of the alkaloids. It was reported previously that K had inhibitory role in the synthesis of alkaloids. Depletion of K usually results in an increase in the percentage of alkaloids, while the total percentage of alkaloids is decreased sometimes. In Lupins, K-depleted plants had a higher alkaloid level (Mironenko 1975). Gentry et al. (1969) found that P and K together greatly reduced the periwinkle biosynthesis in tall Festuca arundinaea trees, whereas N and P alone increased the periwinkle levels. Other element such as P had significant positive effect on the alkaloid production (Mironenko 1975; Dovrat and Goldschmidt 1978; Chandra 1981), while there was a negative effect in the case of Trifolium repens (Jose et al. 2000). Ca activates the accumulation of tropane and indole alkaloids in Hyoscyamus niger and Catharanthus roseus.

In Datura stramonium, high amount of Co decreases the yield of all plant parts and alkaloid content of leaves; while in roots, alkaloid content rose with increasing Co rates (Tammuru 1980). However, in Atropa belladonna, there was a significant relationship between Co supply and alkaloid content (Petrishek et al. 1983). The level of NO3 decreased the alkaloid content in young plants of D. stramonium due to its association with vegetative growth. However, increase in N2 uptake increases alkaloids in mature plants due to its association with generative parts (Dermeyer and Dejaegere 1998). In mature plant, N2 increased alkaloid content as a result of high metabolic activity and an increase in amino acid. In Ricinus communis, the content of high NH4NO3 showed an increase in ricine concentration.

In Colchicum autumnale there is significantly positive effect of Co and Ca elements on the alkaloid synthesis (Poutaraud and Girardin 2005). A sufficient supply of N, K, Mg, B, Mo, Zn, Fe and Cu increased the galanthamine content in Leucojum aestivum.

Tso et al. (1973) tested the effect of 54 rare elements on nicotine level of Nicotiana tabacum. Be, Cu, Pd, Pt, and Sm increased the nicotine yield over 25%, whereas Bi, Co, Ho, Pb, Ni, Rh, Ag, TI, Sm, U V and Zr decreased the nicotine yield. The elements Cs, Er, Li, Rh, Ru, Se, Sr, Ti, and Yb possibly increased the nicotine level less than 25%, whereas As, Ce, Cr, Dy, Gd, I, Mo, Nd, Re, Ta, and Th possibly decreased it. Other elements including Al, Ge, Au, Hf, In, Ir, La, Lu, Hg, Os, Pr, Sc, Te, Th, Tb, W, and Zn showed no significant effects on nicotine level. This increase in nicotine level in tobacco plants was as a result of the interaction of the elements applied together.

CONCLUSION

Minerals have a diversified role in medicinal plant metabolism. The regulations of metabolic activities remain under strict control of mineral nutrients that are solely provided by soil. Severity or scarcity of minerals causes multifarious effects on plant metabolism. The mineral nutrients are classified as macro and micronutrients depending upon their requirement to the plants. Among different macro elements, N is a major constituent of protoplasm, chlorophyll, amino acids, amides and alkaloids. P is essential to the vital metabolic processes in plants. Co and Mo are the constituents of cell wall, chlorophyll as carrier of P element, K is used in the formation of carbohydrates and proteins and S occur as a constituent of amino acids. Micro elements, required for plant metabolism, include Fe and Mn for chlorophyll formation, boron as a catalyst, and Zn and Fe as a co-factor in some enzymes. Co is required for N fixation in lower plants, while Na as a replacer of K in the regulation of stomatal opening and closing. Though minerals have various regulatory roles in different metabolic processes occurring in plants, the present chapter deals only with their roles in photosynthesis, respiration, protein synthesis, ion uptake and alkaloid biosynthesis, especially in medicinal plants. Both macro and micro elements play major roles in catalytic and anabolic activities in the processes of photosynthesis and respiration, working as a driving force of plant life cycle. Proteins, which are required for structural integrity of plants, are constituted by twenty amino acids; they are also solely dependent on minerals for their structure formation, action and behavior. Different ionic forms of minerals also play regulatory roles in translocation of various solutes and ionic uptake occurring through the biological membranes. Apart from being involved in physiological activities, different minerals also have regulatory roles in alkaloid biosynthesis in different plants. Minerals in the form of fertilizers directly enhance the alkaloid content in medicinal plants. A number of studies have been carried out regarding various aspects of plant metabolism and interactions of different minerals on various plants, but still a gap in literature exists with respect to specific roles of minerals in medicinal and aromatic plants, which needs further researches to be conducted in depth.

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