Review

The potential roles of lime and molybdenum on the growth, nitrogen fixation and assimilation of metabolites in nodulated legume: A special reference to *Phaseolus vulgaris* L.

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Most soils in sub-Saharan Africa are acidic and have low contents of calcium (Ca^{2+}), magnesium (Mg^{2+}) and molybdenum (Mo). Higher concentrations and contents of hydrogen ion (H^+), aluminium (Al^{3+}) and manganese (Mn^{2+}) found in these acidic soils are known to be the major causes of poor plant growth due to their toxicity effects to plants and micro organisms such as N-fixing bacteria. Molybdenum is also a component of some bacterial nitrogenase and therefore, is especially important for plants that live in symbiosis with nitrogen-fixing bacteria such as *Rhizobium*. Calcium, magnesium and molybdenum deficient plants exhibit poor growth. The most common management practice to ameliorate these acidic soil problems is through the surface application of lime or molybdenum and/ or seed pelleting. The potential role(s) of lime and Mo in legumes with respect to growth, assimilation of metabolites, N-fixation and growth is given special attention in this review.

Key words: Anthocyanin, calcium, chlorophyll, flavonoid, growth, magnesium, nitrogen fixation, photosynthesis, phosphatase.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a major vegetable legume grown and consumed in Southern Africa. *Phaseolus vulgaris* L yields in Southern Africa are reported to be very low (Mukoko et al., 1995; Mloza-Banda et al., 2003) and the average yield for the African continent being only 650 kg ha^{-1} (Singh, 1999). The poor yields are partly due to infertility caused by acidic soils which have low nutrient contents including Ca^{2+} (Lunze et al., 2007; Wortman et al., 1995, 1998) and Mo content (Liebenberg, 2002). Research efforts at improving bean yields in Africa have increased over the past few decades, where the main emphasis focused on improving nitrogen and phosphorous nutrition (Anderson, 1974;

Choudhury et al., 1983; Ssali and Keya, 1986; Smithson et al., 1993; Amijee and Giller, 1998; Giller et al., 1998; Lunze et al., 2007). Therefore, it is important to assess effects of other limiting nutrients to plant growth such as Ca^{2+} and Mo.

Soil acidity may affect all stages of growth and specifically the legume-rhizobium symbiosis, from strain survival in soil and on the seed, to root-hair infection, nodule initiation and nitrogen fixation (Munns, 1978; Keyser and Munns, 1979; Graham et al., 1982; Wood et al., 1984). Higher concentrations and contents of hydrogen ion, aluminium and manganese in acidic soils are known to be the major causes of poor growth to plants due to their toxicity effects to plants and microorganisms such as N fixing bacteria (Munns, 1978; Graham et al., 1982, 1992; Peoples et al., 1995).

The most common management practice to ameliorate acid soils is through the surface application of lime (Bolan

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et al., 2003). The major influence of lime when applied in the soil is on its ability to supply Ca^{2+} which is essential for plant growth (White and Broadley, 2003) and neutralizing the toxicity effects of H^+, Al^{3+} and Mn^{2+} in the soil (Staley and Brauer, 2006). Lime may also increase soil pH resulting in negative charges on soil particles and therefore, decreasing the activities of iron and aluminium oxides which are good sinks for Mo in soils (Mandal et al., 1998). It is therefore justifiable to introduce lime in acidic soils with low Ca^{2+} levels such as those found in some parts of Southern Africa.

Molybdenum is a trace element found in the soil and is required for growth of most biological organisms including plants (Purvis, 1955; Anderson, 1956; Gurley and Giddens, 1969; Agarwala et al., 1978; Franco and Munns, 1981; Graham and Stangoulis, 2005). Similar to other metals required for plant growth, molybdenum has been utilized by specific plant enzymes as a co-factor that participate in reduction and oxidative reactions in plants (Mendel and Hansch, 2002; Williams and Frausto da Silva, 2002). Generally, molybdenum is an essential micronutrient for plants and bacteria. In some parts of Southern Africa, several cases of Mo deficiency have been identified in a variety of crops including maize, Lucerne, fruits, vegetables and other crops (Pienaar and Bartel, 1968; Tanner, 1978, 1982; Rhodes and Kpaka, 1982; Kang and Osiname, 1985; Thibaud, 2005).

Molybdenum deficient plants exhibit poor growth and low chlorophyll content (Gupta and Lipsett, 1981; Gupta et al., 1991; Marschner, 1995). Molybdenum is also a component of some bacterial nitrogenase and therefore is especially important for plants that live in symbiosis with nitrogen-fixing bacteria (Gupta et al., 1991; Hale et al., 2001) such as (*P. vulgaris* L) that is widely grown as a vegetable crop in Southern Africa. Experiments with soybean have shown that molybdenum fertilization in deficient soils enhanced nitrogen-fixation through increased nitrogenase activities and in nodule sizes (Parker and Harris, 1977; Adams, 1997).

In some parts of Southern Africa, reports indicate that soils are acidic and hence both Ca^{2+} and Mo are inadequate to support good plant growth (Pienaar and Bartel, 1968; Tanner, 1978, 1982; Rhodes and Kpaka, 1982; Ndakidemi, 2005; Thibaud, 2005; Lunze et al., 2007). In agricultural soils, molybdenum is strongly held into positively charged metal oxides in acidic soils of up to pH 5.5 (Smith et al., 1997). Research evidence indicates that as the soil solution becomes more alkaline, the MoO_4^- availability to plants and other forms of life increases (Lindsay, 1979; Brady and Weil, 2008). Consequently, the application of lime to agricultural soils may be an important tool to adjust soil pH and increase soluble molybdate (Kaiser et al., 2005). Therefore, it is important to understand the fundamental reactions of these important mineral nutrients (lime and molybdenum) at different stress levels. This review outlines how leguminous plants such as *P. vulgaris* L plants may respond and benefit to lime and Molybdenum in relation to growth, yield, N_2 metabolism and in the production of metabolites such as phenolic compounds and phosphatase enzymes in their tissues and the rhizosphere.

EFFECTS OF SELECTED MINERAL NUTRIENTS ON PHENOLIC COMPOUNDS METABOLISM

Phenolic compounds such as flavonoids and anthocyanins are diverse group of phytochemicals that are produced by various plants in high quantities (Dixon and Steele, 1999). They exhibit a wide range of biological activities mainly from their antioxidant properties and ability to modulate several enzymes or cell receptors (Hodek et al., 2002).

Flavonoids play an important role in plant growth and development and in defence of plants against microorganisms and pests serving as means of plant-animal warfare (Dixon and Harrison, 1990; Dixon and Steele, 1999; Ndakidemi and Dakora, 2003; Makoi and Ndakidemi, 2007). The pathway of phenolic compounds' biosynthesis in plant species is highly regulated (Hasegawa and Maier, 1981). For instance, constitutive levels of flavonoids are produced during normal growth and development, but additional formation of specific compounds can be induced by wounding, attack by pathogens and other mineral nutritional stresses (Stafford, 1990; Ndakidemi and Dakora, 2003; Makoi and Ndakidemi, 2007) such as those involving Ca^{2+}, Mg^{2+} and molybdenum.

Nutrient stress has a marked effect on phenolic levels in plant tissues (Rengel, 1999; Makoi and Ndakidemi, 2007). Phosphorus, sulphur, iron, calcium or magnesium starvation stimulates the production of phenolics in plant tissues (Gerschenzon, 1983; Ndakidemi and Dakora, 2003; Makoi and Ndakidemi, 2007). Therefore, phenolic compounds may be considered as an essential factor for a plant's adaptative success in diverse environments (Ndakidemi and Dakora, 2003, Makoi and Ndakidemi, 2007) such as those stressed with calcium or magnesium and molybdenum.

Phenolic compounds are known to prevent microbial degradation of ectoenzymes (phosphatases) and/ or organic acids released by the roots as the response to the nutritional deficiencies (Neumann and Romheld, 2001). The exudation of phenolic compounds from the roots of nutrient-starved plants seems to be an important way by which plants can respond to their environment. By modifying the biochemical and physical properties of the rhizosphere, plants increase nutrient availability and buffer the effect of hostile surroundings (Makoi and Ndakidemi, 2007). Although the fate of exuded phenolics in the rhizosphere and the nature of the reactions they are involved in within soils remain poorly understood, phenolics in the soil may clearly contribute significantly to plant growth and development. Nutrient deficiencies may

induce important modifications in several primary metabolic pathways such as in sugar metabolism and secondary metabolism (Gerschenzon, 1983; Ndakidemi and Dakora, 2003; Makoi and Ndakidemi, 2007) and finally influencing the final seed yield. The alteration of metabolism of phenolic compounds under nutrient stress seems to be a response that allows the plant to adapt and survive in harsh environments. Whatever physiological mechanism involved, the enhanced phenolic metabolism under nutrient scarcity may help plants to face the unfavourable environment (Aoki et al., 2000). As lime and molybdenum may play a crucial role in *P. vulgaris* grown in poorly depleted soils, their influence on the metabolism of phenolic compounds such as flavonoid and anthocyanin and the ultimate effects on plant growth needs to be further investigated and reported.

EFFECTS OF LIME AND MOLYBDENUM ON PHOSPHATASE ENZYME ACTIVITY

Soil enzymes serve several important functions. They are involved in the cycling of nutrients, affect fertilizer use efficiency, reflect the microbiological activity in soil and act as indicators of soil change (Dick et al., 2000; Ndakidemi, 2005, 2006). Enzyme activities in soil are known to serve as an indicator of soil health and to mediate and serve as a catalyst for soil functions such as organic matter decomposition, release of inorganic nutrients for plant growth, N_2 fixation, detoxification of xenobiotics, nitrification and denitrification (Dick, 1997; Nadiya et al., 2000; Makoi and Ndakidemi, 2008).

Phosphatase enzymes are believed to play a major role in transformation of organic phosphorous (P) into mineral P (Speir and Ross, 1978; Tabatabai, 1994; Makoi and Ndakidemi, 2008). They are produced when plants and soil micro organisms are subjected to stress such as P (Ndakidemi, 2005, 2006; Makoi and Ndakidemi, 2008) Ca^{2+} (Speir and Ross, 1978; Bremner and Mulvaney, 1978) and Mo (Sugiura et al., 1981; Gellatly et al., 1994; Guo et al., 1998; Bozzo et al., 2002; Lopez et al., 2007). Changes in soil pH can affect the activity of enzymes in the rhizosphere and plants (Dick et al., 2000). The pH can affect enzyme activity by influencing the concentration of inhibitors or activators in the soil solution and the effective concentration of substrate (Dick et al., 2000). From this background, the sensitivity of soil enzymes to pH should make it possible to evaluate the effective pH and the relative activity of phosphatase enzymes when lime and Mo are supplied in the cropping system.

Evidence in the literature shows that lime, organic and different forms and types of inorganic amendments added to soil influenced the levels of phosphatase enzyme activities (Bremner and Mulvaney, 1978; Speir and Ross, 1978; Ndakidemi, 2005). For instance, in acid soil, addition of lime generally increases sulfatase activities and decreases phosphatase activities in plants

whereas the addition of phosphate fertilizers decreases activities of phosphatase, sulfatase and urease (Haynes and Swift, 1988; Ndakidemi, 2005, 2006). It is worth investigating if a similar trend is observed in *P. vulgaris* plants supplied with lime.

Molybdenum has been described as a potent inhibitor of acid phosphatase activity (Lopez et al., 2007). Some studies carried out with phosphatases extracted from different species of plants showed an inhibition with the addition of Mo (Sugiura et al., 1981; Gellatly et al., 1994; Guo et al., 1998; Bozzo et al., 2002; Lopez et al., 2007). In a study involving tomato, the addition of small amounts of molybdenum (0.0028 mM of molybdate) significantly inhibited the activity of acid phosphatase (Bozzo et al., 2002). In another study involving a legume, Guo et al. (1998) reported an inhibitory effect of micromolar concentrations of molybdate on the activity of phosphatase from the cytosolic fraction of pea plumules. Other studies have observed a strong inhibition of acid phosphatase activity from Irish potato and sweet potato supplied with low levels of Mo (Sugiura et al., 1981; Gellatly et al., 1994).

Soil amendment through the addition of Mo and lime may result in the production of acid and alkaline phosphatases into ratios that will influence plant growth and development at different levels. Lack of adequate information on this aspect has prompted us to focus along this direction.

EFFECTS OF LIME AND MOLYBDENUM ON PHOTOSYNTHESIS AND CHLOROPHYLL FORMATION

Light is the environmental factor that has most influence on growth and yield quantity and quality of crops through its influence on photosynthesis and chlorophyll formation (Montanaro et al., 2007). If other factors are not limiting, high light intensity generally stimulates photosynthesis and hence plant growth (Sattelmacher et al., 1993). Other factors such as macro and micro nutrients may also affect the metabolic reactions in photosynthetic apparatus (Marschner, 1995). It is well established that inadequate levels of any mineral nutrient in the growth media may limit photosynthesis due to their involvement in carbohydrate synthesis (Lambers et al., 1998). Calcium (Ca^{2+}) and Molybdenum (Mo) are essential plant nutrients; whose role has been well documented (Marschner, 1995; White and Broadley, 2003). Ca^{2+} is involved in several biochemical and physiological processes in plants (Hepler and Wayne, 1985). The structural role of apoplastic Ca^{2+} is particularly important in cell wall and on the shelf-life of plant tissues (Bauchot et al., 1999).

Calcium may function directly in several aspects of photosynthesis. It appears to modulate activity of the phosphatase enzymes in the carbon reduction cycle in the synthesis of different sugar components (Haupt and Weisenseel, 1976; Brand and Becker, 1984). Some

research evidence also supports a calcium function in the water-splitting complex and other evidence suggesting its role in a reaction centre in the photosystem II (Brand and Becker, 1984). As light is absorbed, this catalytic centre drives the photosynthesis process (Marschner, 1995). In the chlorophyll molecule embedded in a protein, there is a catalytic centre of photosynthetic water oxidation, which is composed of a Mn^4Ca cluster. It is obvious that supply of Calcium through lime may have a significant influence on the photosynthesis process at cellular level in *P. vulgaris* plants. In practice, calcium deficiency is corrected by supplying the agricultural lime or other sources of calcium. For instance, XiaoJun et al. (2004) showed that the photosynthesis and photosynthetic efficiency of the leaves of rice were significantly enhanced with the supply of Ca^{2+}. In this study, levels of net photosynthetic rate, stomatal conductance, the contents of chlorophyll and soluble sugar of the leaves increased more significantly than the control treatment without Ca^{2+}. Furthermore, addition of Ca^{2+} decreased the content of malondialdehyde and the permeability of cell membrane, but increased the superoxide dismutase activity (XiaoJun et al., 2004) and hence affecting photosynthesis. Molybdenum (Mo) is an essential micronutrient for plants. It plays an important key role in chlorophyll synthesis. In plants, it is absorbed as MoO_4^{2-}. In Southern Africa, several cases of Mo deficiency have been identified in variety of crops (Kang and Osiname, 1985; Pienaar and Bartel, 1968; Rhodes and Kpaka, 1982; Tanner, 1978 and 1982; Thibaund, 2005). Molybdenum deficiency of soil is a widespread agricultural problem that induces yield and quality losses in many crop species worldwide (Liu, 1991, 2001, 2002). Molybdenum deficient plants exhibit poor growth and low contents of chlorophyll and ascorbic acids and shows reduced leaf blade formation, inter-veinal mottling and chlorosis around edges and tips of older leafs (Marschner, 1995; Liu, 2002). It is generally accepted that legumes need more Mo than most of other plants (Mcbride, 2005) due to its key involvement in the Nitrogen-fixation process. Various studies have reported that application of Mo enhances the yield in crops that grow in deficient soil (Liu, 2001; Min et al., 2005; Xue-Cheng et al., 2006). Therefore, it is important to establish, verify, and quantify the influence of Ca^{2+} and Mo supplied as lime or Molybdenum salts or their interaction on different photosynthetic activities in *P. vulgaris* grown under different conditions in Southern Africa.

EFFECTS OF LIME AND MOLYBDENUM ON NITROGEN FIXATION

The mineral nutrient nitrogen is a constituent of all proteins, nucleic acids and many other biomolecules and it is essential in all living organisms (Marschner, 1995; McCammon and Harvey, 1987). In plants, nitrogen is the most limiting nutrient for growth (Verhoeven et al., 1996). Leguminous plants in partnership with *Rhizobium* have the ability to convert the atmospheric nitrogen into usable forms (Galloway et al., 1995). Nitrogen-fixation involving symbiotic association between rhizobia in legumes is influenced by several factors including Ca^{2+} and Mo (Kucey and Hynes, 1989; Bottomley, 1992; Graham et al., 1992; Tu, 1992; Banath et al., 1996; Andrade et al., 2002). According to established guidelines, some areas in the Southern Africa have been reported to be deficient in Ca^{2+} and Mo (Ndakidemi, 2005; Thibaund, 2005) and these may have N_2-fixation limitations.

In nutrient deficient soils, soil mineral distribution involving Ca^{2+} and Mo is mostly related to pH levels. For instance, calcium and molybdenum become scarce at acidic pH (Brady and Weil, 2008) and the exchange site are mainly dominated by aluminium (Al) and manganese (Mn) ions. At elevated levels, these ions may reduce N_2-fixation by injuring the host plant or interfering with nodulation or N_2-fixation processes (Kamprath and Foy, 1985). Under such circumstances, exogenous supply of Ca^{2+} and Mo into the growth media is important.

Calcium supplied to plants through lime may perform multiple functions in plants. They are essential component in symbiotic N_2-fixation and nodule formation in legumes. Studies have indicated that Calcium deficiency in legumes depressed the calcium content of nodules, impairing nitrogen-fixation due to inadequate calcium for nodule structure and/or metabolism (Banath et al., 1996; Graham, 1992). In this context, Ca^{2+} deficiency in legume decreased the supply of fixed nitrogen from nodules to other organs, thus impairing plant growth. With regard to molybdenum, it is known to have a notable influence on nitrogen metabolism in N_2 fixing legumes (Franco and Munns, 1981; Prker and Harris, 1977; Marschner, 1995; Vieira et al., 1998). In nodulated legumes, Mo is necessary for the reduction of atmospheric nitrogen (N_2) to ammonia by nitrogenase enzyme. The symbiotic bacteria require about ten times more Mo for N_2-fixation than does the host plant (for protein synthesis). For this reason, Mo deficiency will commonly occur in legumes before it does in other plants when grown in the same soil (Thibaund, 2005). Molybdenum is also essential for nitrate reductase and nitrogenase enzyme activity (Westermann, 2005). The symbiotic bacterial enzyme nitrogenase is comprised of MoFe protein which is directly involved in the reduction of N_2 to NH_3 (Lambers et al., 1998) during fixation process. Supply of Mo to bacteroids is therefore an important process and most likely a key regulatory component in the maintenance of nitrogen-fixation in legumes that may influence plant growth (Kaiser et al., 2005). When leguminous plants are grown under molybdenum deficiency conditions, phenotypes with hindered and/or retarded plant growth characteristics may develop. Most of these phenotypes may be associated with reduced activity of molybdoenzymes (Agarwala and Hewitt, 1954; Spencer and Wood, 1954; Afridi and

Hewitt, 1965; Randall, 1969; Jones et al., 1976; Agarwala et al., 1978). These enzymes include the primary nitrogen assimilation enzymes such as nitrate reductase (NR) and the nitrogen-fixing enzymes (nitrogenase) found in bacteroids of legume nodules (Vieira et al., 1998). Other molybdoenzymes have also been identified in plants including xanthine dehydrogenase/oxidase involved in purine catabolism and ureide biosynthesis in legumes, aldehyde oxidase and sulfite oxidase (Mendel and Haensch, 2002; Williams and Frausto da Silva, 2002). Generally speaking, we can conclude that, Molybdenum deficiency is primarily associated with poor nitrogen health in plants and ultimately impaired growth.

Research reports have indicated the stimulating influence of Mo in N_2-fixation in legumes. In their research, Gurley and Giddens (1969), Franco and Munns (1981), Ishizuka (1982), and Brodrick and Giller (1991) showed that Mo supply in legumes increased molybdenum concentrations in nodules, improving N_2-fixation, development of seeds and other tissues. Experiments with soybean and common bean have also shown that molybdenum fertilization enhanced nitrogen-fixing symbiosis through increased nitrogenase activity rates and larger nodule formation (Parker and Harris, 1977; Adams, 1997; Vieira et al., 1998).

Despite of the existence of substantial evidence on the influence of lime and Mo on nitrogen-fixation in pasture legumes and other related crops in Southern Africa, their effects and interaction on N_2-fixation in *P. vulgaris* in some parts of Africa is not documented.

EFFECTS OF LIME AND MOLYBDENUM ON GROWTH AND YIELD OF LEGUMES

Plant needs some macro- and micronutrients for their normal growth. Some of these elements play vital roles in different growth process. For instance, research evidence suggests that Calcium and Mo deficiency in legumes can restrict plant growth through different mechanisms (Evans et al., 1950; Evans and Purvis, 1951; Marschner, 1995).

Calcium deficiency is known to restrict the amount of N_2-fixed in legumes, hence resulting into reduced plant growth due to inadequate nitrogen which is required as building blocks of proteins (Dutta, 2004). On the other hand, plants with severe Ca^{2+} deficiency have shown low levels of nitrogen in their tissues and this has always been associated with poor growth.

Studies by Lucrecia et al. (1987) demonstrated that supply of Ca^{2+} through lime significantly increased both nodule weight and plant productivity in *Pirulgaris*. In an experiment done by Hartley et al. (2004), lime supply increased nodulation and yield of Serradella (*Ornithopus compressus*). The beneficial effects of liming on nodulation and plant growth most likely resulted from the enhanced conditions for seedling growth and nodulation.

With regard to Mo, it is well known that leguminous plants are very sensitive to Mo deficiency, but excess Mo also may impair growth, decreases the biomass, seed yield and deteriorates the quality of production (Kevresan et al., 2001; Liu and Yang, 2000; Nautiyal and Chatterjee, 2004). During different growth processes in plants and in legumes in particular, Mo is involved in a number of different enzymatic processes (Marshner, 1995; Vieira et al., 1998). For example, molybdenum is a constituent of nitrogenase enzyme and *Rhizobium* bacterium fixing nitrogen needs molybdenum during the fixation process (Vieira et al., 1998). Therefore, with this task, molybdenum has a positive effect on growth, yield, N content of foliage and roots and nodule forming in legume crops (Kliewer and Kennedy, 1978; Togay et al., 2008). With regard to other aspects of plant nutrition, molybdoenzymes are involved in nitrogen metabolism, improving qualities of ascorbic acid, soluble sugar and chlorophyll concentrations (Zhao and Bai, 2001; Chen and Nian, 2004). Therefore, its deficiency may show overall reductions in plant growth and development, expose the plant to susceptibility to pest damage and poor pod and/ or grain development (Graham and Stangoulis, 2005).

Although there is considerable literature on the beneficial effects of liming and Mo on legume growth in other parts of the world (Staley and Brauer, 2006), site specific factors can yield different results. As Ca^{2+} and Mo or their interaction may play important role(s) in legume growth, these mineral nutrients warrant further investigations both to ascertain their effects on plant growth and development in common legumes grown by farmers such as *P. vulgaris*.

CONCLUSION

Lime and molybdenum are essential nutrient for legumes growing in acidic soils deficient in Ca^{+2}, Mg and Mo. Lime application in particular, is recommended for most legume species to counter deleterious effects of soil acidity and the availability of mineral elements such as Ca^{+2}, and Mg. Liming increases plant growth, accumulation of plant metabolites, nitrogen fixation, dry matter and final seed yield of legumes.

On the other hand molybdenum nutrition is an essential component in legumes. Molybdate which is the predominant form available to plants is required at very low levels where it participates in various redox reactions in plants. In symbiotic legumes, the enzyme nitrogenase is comprised of MoFe protein that is directly involved in the reduction of N_2 to NH_3 and finally to other available forms of N to plants. Much more research is required to ascertain the usefulness of these important mineral nutrients. It is also worth investigating how they may further be used in future to support the expanding legume cultivation in areas where soil Mo and Ca and or/Mg profiles limit plant growth and productivity, such as those found in acidic environments of Africa.

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REFERENCES

Adams JF (1997). Yield response to molybdenum by field and horticultural crops. In: Gupta UC, ed. Molybdenum in agriculture. Cambridge University Press.

Afridi MMRK, Hewitt EJ (1965). The inducible formation and stability of nitrate reductase in higher plants II Effects of environmental factors, antimetabolites and amino-acids on induction. J. Exp. Bot. 16:628-645.

Agarwala SC, Hewitt EJ (1954). Molybdenum as a plant nutrient: IV. The interrelationships of molybdenum and nitrate supply in chlorophyll and ascorbic acid fractions in cauliflower plants grown in sand cultures. J. Hort. Sci. 29:291-300.

Agarwala SC, Sharma CP, Farooq S, Chatterjee C (1978). Effects of molybdenum deficiency on the growth and metabolism of corn plants raised in sand culture. Can. J. Bot. 56:1905-1909.

Amije F, Giller KE (1998). Environmental constraints to nodulation and nitrogen fixation of *Phaseolus vulgaris* L in Tanzania I. A survey of soil fertility and root nodulation. African Crop Sci. J. 6:159-169.

Anderson GD (1974). Bean responses to fertilizers on Mt Kilimanjaro in relation to soil and climatic conditions. East Afr. Agr. Forest J. 39:272-288.

Andrade DS, Murphy PJ, Giller KE (2002). Effects of liming and legume/cereal cropping on populations of indigenous rhizobia in acid Brazilian oxisol. Soil Biol. Biochem. 34:477-485.

Aoki T, Akasaki T, Ayade S (2000). Flavonoids of leguminous plants: Structure, biological activity and biosynthesis. J. Plant Res. 113:475-488.

Banath CL, Greenwood EAN, Loneragan JF (1996). Effects of Calcium Deficiency on Symbiotic Nitrogen Fixation. Plant Physiol. 41:760-763.

Bauchot AD, Hallet RJ, Redgwell RJ, Lallu N (1999). Cell wall properties of kiwifruit affected by low temperature breakdown, post harvest. Biol. Tec. 16:245-255.

Bolan NS, Adriano DC, Curtin D (2003). Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. Adv. Agron.78:215-272.

Bottomley PJ (1992). Ecology of *Bradyrhizobium* and *Rhizobium*. In: Biological Nitrogen Fixation (Chapman and hall, Stacey GS, Burris RH, Evans HJ. Eds), pp 293-348, New York.

Bozzo G, Raghothama K, Plaxton W (2002). Purification and characterization of two secreted purple acid phosphatase isozymes from phosphatase- starved tomato (*Lycopersicon esculentum*) cell cultures. Eur. J. Biochem. 269:6278-6286.

Brady NC, Weil RR (2008). The Nature and Properties of Soils. USA. 965p

Brand JJ, Becker DW (1984). Evidence for direct roles of calcium in photosynthesis. J Bioenerg Biomembr. 16:239-249.

Bremner JM, Mulvaney RL (1978). Urease Activity in soils. In: Soil Enzymes (Burns RG Ed.), pp 149-196. Academic Press, New York.

Brodrick SJ, Giller KE (1991). Root nodules of *Phaseolus*: efficient scavengers of molybdenum for N₂ fixation. J. Exp. Bot. 42:1339-1343.

Chen G, Nian FZ (2004). Effect of B, Mo on fatty acid component of *Brassica napus*. Chinese Journal of Oil Crop Science. 26:69-71.

Chowdhury MS, Msumali GP, Malekela GP (1983). Need for seasonal inoculation of soybean with rhizobia at Morogoro, Tanzania. Biological Agriculture and Horticulture 1:219-228.

Dick RP (1997). Soil enzyme activities as integrative indicators of soil health. In Biological Indicators of soil Health (Pankhurst CE, Doube BM, Gupta VVS. Eds), pp 121-156. CAB International: UK.

Dick WA, Cheng L, Wang P (2000). Soil acid and alkaline phosphatase activity as pH adjustment indicators. Soil Biol. Biochem. 32:1915-1919.

Dixon RA, Harrison MJ (1990). Activation, structure and organization of genes involved in microbial defence in plants. Adv. Genet. 28:165-234.

Dixon RA, Steele C (1999). Flavonoids and isoflavonoids- gold mine for metabolic engineering. Trop. Plant Sci. 4:394-400.

Dutta AC (2004). Botany for degree students. Oxford University Press. p. 708.

Evans HJ, Purvis ER (1951). Molybdenum status of some New Jersey soils with respect to alfalfa production. Agron. J. 43:70-71.

Evans HJ, Purvis ER, Bear FE (1950). Molybdenum nutrition of alfalfa. J. Plant Phys. 25:555-566.

.Franco AA, Munns DN (1981). Response of *Phaseolus vulgaris* L to molybdenum under acid conditions. Soil Sci. Soc. Am. J. 45:1144-1148.

Galloway JN, Schlesinger WH, Levy H, Michaels A, Schnoor JL (1995), Nitrogen Fixation: Anthropogenic Enhancement-Environmental Response, Global Biogeochem. Cycles. 9(2):235-252.

Gellatly KS, Moorhead GB, Duff SM, Lefebvre DD, Plaxton WC (1994). Purification and characterization of potato tuber acid phosphatase having significant phosphotyrosine phosphatase activity. J. Plant Phys. 106:223-232.

Gerschenzon J (1983). Changes in the level of plant secondary metabolites under water and nutrient stress. In Phytochemical adaptation to stress (Timmermann BN, Steelink C, Loewus FA. Eds), pp. 273-299. Plenum Press, New York.

Giller KE, Amijee F, Brodrick SJ, Edje OT (1998). Environmental constraints to nodulation and nitrogen fixation of *Phaseolus vulgaris* L. in Tanzania II. Response to N and P fertilisers and inoculation with *Rhizobium*. African Crop Sci. J. 6:171-178.

Graham P (1992). Stress tolerance in *Rhizobium* and *Bradyrhizobium* and nodulation under adverse soil conditions. Can. J. Microbiol. 38:475-484.

Graham PH, Viteri SE, Mackie F, Vargas AT, Palacios A (1982). Variation in acid soil tolerance among strains of *Rhizobium phaseoli*. Field Crop. Res. 5:121-128.

Graham RD, Stangoulis JRS (2005). Molybdenum and disease. In: Mineral nutrition and plant diseases (Dantoff L, Elmer W, Huber D. Eds) St Paul, MN: APS Press.

Guo Y, Terry ME, Roux SJ (1998).Characterization of a cytosolic phosphatase from pea plumules having significant protein tyrosine phosphatase activity. Plant Physiol. Biochem. 36:269-278.

Gupta G, Grund B, Radhika Narayanan R (1991). Photosynthesis and nitrogenase activity in soybean treated with sulphur dioxide and molybdenum. Plant Sci. 79 (2):157-161.

Gupta UC, Lipsett J (1981). Molybdenum in soils, plants and animals. Adv. Agron. 34:73-115.

Gurley WH, Giddens J (1969). Factors affecting uptake, yield response and carryover of molybdenum in soybean seed. Agron. J. 61:7-9.

Hale KL, McGrath SP, Lombi E, Stack MS, Terry N, Pickering IJ, Graham, NG, Pilon-Smits, EAH (2001). Molybdenum Sequestration in *Brassica* Species. A Role for Anthocyanins? Plant Physiol. 126:1391-1402.

Hartley E, Greg-Gemell L, Herridge FD (2004). Lime pelleting inoculation serradella (*Ornithopus* spp.) increases nodulation and yield. Soil Biol. Biochem. 36:1289-1294.

Hasegawa S, Maier VP (1981). Some aspects of Citrus biochemistry and juice quality. Proceeding of International Society. 2:914-918.

Haupt W, Weisenseel MH (1976). Physiological evidence and some thoughts on localized responses, intracellular localization and action of phytochrome in light and plant development (Smith H. Ed) pp. 63-74. London: Butterworths.

Haynes R, Swift RS (1988). Effects of lime and Phosphate additions on changes in enzyme activities, Microbial Biomass and Levels of Extractable Nitrogen, Sulphur and phosphorus in an Acid Soil. Biol. Fert. Soils. 6:153-158.

Hodek P, Pavel T, Marie S (2002). Flavonoids potent and versatile biologically active compounds interacting with cytochromes. Chem. Biol. Interact. 139(1):1-214.

Ishizuka J (1982). Characteristics of molybdenum absorption and translocation in soybean plants. J. Soil Sci. Plant Nutr. 28:63-71.

Jones RW, Abbott AJ, Hewitt EJ, James DM, Best GR (1976). Nitrate reductase activity and growth in Paul's scarlet rose suspension

cultures in relation to nitrogen source and molybdenum. Planta. 133:27-34.

Kaiser NB, Gridler KL, Ngaire BJ, PhillipsT, Tyerman SD (2005). The role of molybdenum in agricultural plant Production. Ann. Bot. 96:745-754.

Kamprath EJ, Foy CD (1985). Lime- fertilizer- plant interactions in acid soils. In: Fertilizer Technology and Use, 3rd ed. (Engelstad OP Ed.), Pp. 91-151. Soil Sci. Soc. Am. J. Madison WI, USA.

Kang BT, Osiname OA (1985). Micronutrient problems in tropical Africa. Fert. Res. 7(1-3):131-150.

Kevresan S, Petrovic N, Popovic M, Kandrac J (2001). Nitrogen and protein metabolism in young pea plants as affected by different concentrations of nickel, cadmium lead and molybdenum. J. Plant Nutr. 24:1633-1644.

Keyser HH, Munns DN (1979). Tolerance of rhizobia to acidity, aluminium and phosphatase. Soil Sci. Soc. Am. J. 43:519-503.

Kliewer WM, Kennedy WK (1978). Studies on response of legumes to molybdenum and lime fertilization on mardin silt loam soil. Soil Sci. Soc. Am. J. 24:377-380.

Kucey RMN, Hynes MF (1989). Populations of *Rhizobium leguminosarum biovars phaseoli* and *viciae* in fields after bean or pea in rotation with non legumes. Can. J. Microbiol. 35:661-667.

Lambers H, Chapin FS, Pons TL (1998). Plant Physiology Ecology Springer. New York. 540 p.

Liebenberg AJ (2002). Dry bean Production. Printed and published by Department of Agriculture, Resource Centre, Directorate Agricultural Information Services, Private Bag X144, Pretoria, 0001 South Africa. p. 27. Available online on 15/09/2009: http://www.nda.agric.za/docs/drybeans2.pdf

Lindsay WL (1979). Chemical Equilibria in Soils, Wiley-Interscience, New York

Liu Z (1991). Agricultural chemistry of microelement. Agriculture Press, China, pp 250-252.

Liu P, Y, YA (2000). Effect of molybdenum and boron pollution on quality of soybean. Chin. J. Appl. Environ. Biol. 9:594-597.

Liu P (2001). The research development of molybdenum and boron nutrition in soybean. China Agric . Sci. Bulletin. 17:41-44.

Liu P (2002) Effects of the stress of molybdenum on plants and the interaction between molybdenum and other element. Agri-environmental Protection. 21:276- 278.

Lopez RS, Anali ER, Rao MA, Maria De La Luz Mora, Alvaer M, Gianfreda L (2007). Manganese and molybdenum affect acid phosphatases from potatoes. Acta Agr. Scand. B-S P. 57:65-73.

Lucrecia M, Ramos G, Boddey RM (1987). Yield and nodulation of *Phaseolus Vulgaris* and the competitivity of an introduced *Rhizobium* strain: Effects of lime, mulch and repeated cropping. Soil Biol. Biochem. 19:171-177.

Lunze L, Kimani PM, Ngatoluwa R, Rabary B, Rachier GO, Ugen MM, Ruganza V, Awad elkarim EE (2007). Bean Improvement for Low Soil Fertility Adaptation in Eastern and Central: In: Advances in integrated soil fertility research in Sub-saharan Africa: challenges and opportunities (Eds Bationo E, Waswa B, Kihara J, Kimetu J), Dordrecht, The Netherlands: Springer pp. 325-332.

Makoi JHR, Ndakidemi PA (2008). Selected soil enzymes: examples of their potential roles in the ecosystem. Afr. J. Biotechnol. 7:181-191.

Makoi JHR, Ndakidemi PA (2007). Biological, ecological and agronomic significance of plant phenolic compounds in rhizosphere of the symbiotic legumes. Afr. J. Biotechnol. 6:1358-1368.

Mandal PS, Mandal LN (1998). Effect of molybdenum, phosphorus and lime application to acid soils on dry matter yield and molybdenum nutrition of lentil. J. Plant Nutr. 21:139-147.

Marschner H (1995). Mineral Nutrition of higher plants. Academic Press, San Diego.

Montanaro G, Dichio B, Xiloyannis C (2007). Response of photosynthetic machinery of field grown kiwifruit under mediteranean condititions during drought and re-watering. Photosynthetica. 45(4):533-540.

McCammon JA, Harvey SC (1987). Dynamics of Proteins and Nucleic Acids. Pp 234. Cambridge University Press, Cambridge.

Mcbride MB (2005). Molybdenum and copper uptake by forage grasses and legumes grown on a metal-contaminated sludge site. Commun Soil Sci. Plant Anal. 36:2489-2501.

Mendel RR, Hansch R (2002). Molybdoenzymes and molybdenum cofactor in plants. J. Exp. Bot. 53:1689-1698.

Mloza-Banda HR, Ferguson AE, Mkandawire ABC (2003). The common bean (*Phaseolus vulgaris* L.) genetic resources in Malawi: Implications for conservation, utilization and production. Bunda J. Agric. Envron. Sci. Tech. 1:1-13.

Min Y, Yue-Jin C, Hong-Dong X, Hui-Zhen W, Fang W, Xiu-Hua S (2005). Influences of Boron and Molybdenum on Active Oxygen Species in Turfgrass *Seashore Paspalum* under low temperature. Acta Agron. Sin. 31:755-759.

Mukoko OZ, Galwey NW and Allen DJ (1995). Developing cultivars of the common bean (*Phaseolus vulgaris* L.) for southern Africa: bean common mosaic virus resistance, consumer preferences and agronomic requirements. Field Crop Res. 40:165-177.

Munns DN (1978). Soil acidity and nodulation. In. Mineral Nutrition of legumes in tropical and subtropical soils. (Andrew CS and Kamprath AJ. Eds), pp 247-263. CSIRO, Melbourn, Australia.

Nadiya EL, Sandeno JM, McGrath D, Dick RP (1997). Integrative Biological Indicators for detecting changes in soil quality. Am. J. Alternative Agric. 15:26- 36.

Nautiyal N, Chatterjee C (2004). Molybdenum stress- induced changes in growth and yield of Chickpea. J. Plant Nutr. 27:173-181.

Ndakidemi PA, Dakora FD (2003). Legume seed flavonoids and nitrogenous metabolites as signals and protectants in early seedling development. Funct. Plant Biol. 30:729-745.

Ndakidemi PA (2005). Nutritional characterization of the rhizosphere of symbiotic cowpea and maize plants in different cropping systems. Doctoral degree Thesis. Cape Peninsula University of Technology, Cape Town, South Africa. p. 150.

Ndakidemi PA (2006). Manipulating legume/cereal mixtures to optimize the above and below ground interactions in the traditional African cropping systems. Afr. J. Biotechnol. 5:2526-2533.

Neumann G, Romheld V (2001). The release of roots exudates as affected by the plant's physiological status in the rhizosphere. (Pinton R, Varanini Z, Nannipieri P. Eds), pp. 41-93. Marcel Dekker Inc., New York.

Parker MB, Harris HB (1977). Yield and leaf nitrogen of nodulating and nonnodulating soybeans as affected by nitrogen and molybdenum. Agron J. 69:551-554.

Peoples MB, Lilley DM, Burnett VF, Ridley AM, Garden DL (1995). Effects of surface application of lime and superphosphate to acid soils on growth and N2 fixation by subterranean clover in mixed pasture sward, Soil Biol. Biochem. 27:663-671.

Pienaar WJ, Bartel EE (1968). Molybdenum content of vegetables and soils from the Vredendal and Langkloof areas. J. Dent. Assoc. S. Afr. 23(8):242-4.

Purvis ER (1955). Minor Elements in Plant Nutrition. Review of role of molybdenum in soils and plants. J. Agric. Food Chem. 3:666-669.

Randall PJ (1969). Change in nitrate and nitrate reductase levels on restoration of molybdenum to molybdenum-deficient plants. Aust. J. Agr. Res. 20:635-642.

Rengel Z (1999). Physiological mechanisms underlying differential nutrient efficiency of crop genotypes. In Mineral nutrition of crops: mechanisms and implications. (Rengel Z Ed.), pp 227-265. The Haworth Press, New York, USA.

Rhodes ER, Kpaka M (1982). Effects of nitrogen, molybdenum and cultivar on cowpea growth and yield on an oxisol. Commun. Soil Sci. Plant Anal. 13:279-283.

Sattelmacher B, Gerendas J, Thoms K, Bruck H, Bagdady NH (1993). Interaction between root growth and mineral nutrition. Environ. Exp. Bot. 33:63-73.

Singh SP (1999). Common bean improvement in the twenty-first century. Kluwer Academic Publisher, 420 p.

Smith KS, Balistrieri LS, Smith SS, Severson RC (1997). Distribution and mobility of molybdenum in the terrestrial environment. In molybdenum in agriculture (Gupta UC Ed.), Cambridge: CUP.

Smithson JB, Edje OT, Giller KE (1993). Diagnosis and correction of soil nutrient problems of common bean (*Phaseolus vulgaris*) in the Usambara Mountains of Tanzania. J. Agr. Sci. 120:233-240.

Speir TW, Ross DJ (1978). Soil Phosphatase and Sulphatase. In Soil Enzymes; (Burns RG Ed.), pp. 197-250. Academic Press: New York.

Spencer D, Wood JG (1954). The role of molybdenum in nitrate

reduction in higher plants. Aust. J. Biol. Sci. 7:425-473.

Ssali S, Keya SO (1986). The effects of phosphorus and nitrogen fertiliser level on nodulation, growth and dinitrogen fixation of three bean cultivars. Tropical Agriculture. 63:105-109.

Stafford H (1990). Flavonoid Metabolism. . pp. 201-216. Boca Ratan: CRC Press, Inc.

Staley TE, Brauer DK (2006). Survival of a genetically modified root-colonizing Pseudomonad and Rhizobium strain in an Acidic soil. Soil Sci. Soc. Am. J. 70:1906- 1913.

Sugiura Y, Tanaka H, Mino Y, Ishida T, Ota N, Inoue M, Nomoto K, Yoshioka H, Takemoto T (1981). Structure, properties and transport mechanism of Iron (III) complex of mugineic acid, a possible phytosiderophore. J. Am. Chem. 103:6979- 6982.

Tabatabai MA (1994). Soil enzymes. In: Weaver, RW, Augle S, Bottomly PJ, Bezdicek D, Smith S, Tabatabai A, Wollum A (eds.), Methods of soil analysis. Part 2. Microbial and biochemical properties, No. 5. Soil Science Society of America, Madison. pp.775-833.

Tanner PD (1978). Relations of sorption of molybdate and phosphate by clays and clay loams to soil pH and other chemical factors. Rhod. J. Agric. Res. 16:31-41.

Tanner PD (1982). The molybdenum requirements of maize in Zimbabwe. Zimbabwe Agric. J. 79:61-64.

Thibaud GR (2005). Molybdenum relationships in soils and plants. KwaZulu-Natal Department of Agriculture and Environmental Affairs, Cedara College, Private Bag X9059, Pietermaritzburg, 3200, South Africa. Available online: http://www.izasa.org/Documents/Zn_Fertilizer_Conf_06/Molybdenum%20relationships%20in%20soils%20and%20plants.pdf Available on line: 15/09/2009.

Togay Y, Togay N, Dogan Y (2008). Research on the effect of phosphorus and molybdenum applications on the yield and yield parameters in lentil (Lens culinaris Medic.). Afr. J. Biotechnol. 7:1256-1260.

Tu M (1992). Nutrition deficiency and fertilization of pasture in South China. Pratacultural Science 9:49-52.

Verhoeven JTA, Koerselman W, Meuleman AFM (1996). Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. Trends Ecol. Evol. 11(12):494-497.

Vieira RF, Cardoso EJBN, Vieira C, Cassini STA (1998). Foliar application of molybdenum in common beans. I. Nitrogenase and reductase activities in a soil of high fertility. J. Plant Nutr. 21:169-180.

Westermann DT (2005). Nutritional requirements of potatoes. Am. J. Potato Res. 82:301-307.

White PJ, Broadley MR (2003). Calcium in plants. Ann. Botany. 92:487-511.

Williams RJP, Frausto da Silva JJR (2002). The involvement of molybdenum in life. Biochem. Biophys. Res. Comm. pp. 293-299.

Wood M, Cooper JE, Holding AJ (1984). Soil acidity factors and nodulation of Trifolium Repens. Plant Soil. 78:367-379.

Wortmann CS, Kirkby RA, Aledu CA, Allen JD (1998). Atlas of common bean (Phaseolus vulgaris L.) Production in Africa, CIAT Cali, Colombia.

Wortmann CS, Lunze L, Ochwoh VA and Lynch JP (1995). Bean improvement for low fertility soils in Africa. African Crop Science J. 3(4):469-477.

XiaoJun Z, Jin Song Y, YongChao L, YunSheng L, XiaoYing Y (2004). Effects of exogenous calcium on photosynthesis and its related physiological characteristics of rice seedlings under salt stress. Sci. Agric. Sinica. 37:1497-1503.

Xue-Cheng S, Cheng-Xiao H, Qi-Lin T, Qiao-Qiao G (2006). Effects of molybdenum on photosynthetic characteristics in winter wheat under low temperature stress. Acta Agron. Sinica. 32:1418-1422.

Zhao J, Bai QY (2001). Alleviation of nitrate accumulation in vegetables by application of molybdenum. Agro-environmental Protection. 20:238-239.