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THE ROLE OF COBALT IN FORAGE LEGUMES

Dalibor Tomić[1], Vladeta Stevović[1], Milomirka Madić[1], Miloš Marjanović[1], Nenad Pavlović[1], Đorđe Lazarević[2], Mirjana Petrović[2], Vladimir Zornić[2], Jasmina Knežević[3]

Abstract: The growth and metabolism of plants, especially on acidic soils, largely depend on the concentration of cobalt (Co) in the soil, i.e. the rhizosphere. An optimal supply of cobalt is essential for N_2 fixation of Rhizobium bacteria that are in symbiotic relationships with leguminous plants, influencing their better growth and supplying them with nitrogen. When there is a lack of Co in the plant, the organic production of legumes falls. Indirectly or directly, Co also affects other metabolic processes in plants. The aim of the work was to analyze the importance of optimal provision of forage legumes with cobalt for obtaining high and quality yields of forage and seeds.

Keywords: cobalt, forage legumes, mineral nutrition

Introduction

Cobalt is not classified in the group of essential elements for plants, but it is in the group of useful elements (Bakkaus et al., 2005). The normal concentration of cobalt in the dry matter of plants is low and ranges from 0.1-10 μg g^{-1}. The distribution of cobalt in plants depends on the plant species, and it is generally highest in the leaf. The concentration of cobalt in the leaf of forage plants varies from 0.6-3.5 ppm. It is most abundant in legumes (Palit et al., 1994), for which it is particularly important for the process of nitrogen fixation. The distribution of cobalt in plant organs also depends on the stage of plant development. In the early phase of plant development, large amounts of cobalt are absorbed in leaves and stems (Kenesarina, 1972), while before flowering and until maturation, the largest amounts are found in nodules. Plant organs contain cobalt in the following ascending order: roots, leaves, seeds, stems.

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (dalibort@kg.ac.rs).

[2] Institute for Forage Crops, Globoder bb., 37251, Serbia.

[3] University of Priština, Faculty of Agriculture, Kopaonička, 38219, Lešak, Serbia.

According to Palit et al. (1994) the growth and metabolism of plants, especially on acidic soils, depend to a large extent on the concentration of cobalt in the soil, i.e. the rhizosphere.

Cobalt affects plant metabolism and growth and is an essential component of many enzymes and coenzymes. According to Kobayashi and Shimizu (1999), cobalt is used in a limited number of enzymes, unlike iron and zinc. However, there are several enzymes that are activated by cobalt ions: E. coli acetylornithinase, Methanobacterium thermoautotrophicum cyclic 2,3-diphosphoglycerate hydrolase, α-d-mannosidase. Cobalt was not found in enzymes that enter the respiratory chain.

Cobalt is also important for normal leaf development, inhibition of ethylene biosynthesis and stimulation of alkaloid biosynthesis (Farooq et al., 2012). It is a component of vitamin B_{12} and cobamide coenzyme and thus helps in the process of fixing molecular nitrogen in the root nodules of legumes (Tomić, 2017). The positive effects of cobalt are reflected in slowing down the aging of leaves, increasing resistance to drought, regulating the accumulation of alkaloids in medicinal plants, inhibiting ethylene biosynthesis (Palit et al., 1994). Indirectly or directly, cobalt affects the metabolic processes of plants, such as: lipid metabolism, auxin turnover, photochemical activity of chloroplasts, etc. (Petrović and Kastori, 1992).

The aim of the work was to analyze the importance of optimal provision of forage legumes with cobalt for obtaining high and quality yields of forage and seeds.

Cobalt in soil

The total concentration of Co in the soil is 1-40 mg kg^{-1}. It includes Co associated with insoluble minerals or within stable crystal structures, which is not available to plants. According to Collins and Kinsella (2011), pedogenetic factors including soil pH value, concentration of total, extractable, isotopic and water-soluble cobalt can be found in specific correlations with cobalt content in plants. Therefore, understanding the factors that influence cobalt uptake is necessary for continuous plant production as well as for the possibility of remediation of contaminated systems. Kukier et al. (2004) found a significant positive correlation between soil pH and plant cobalt content. Contrary to this, a large number of studies indicate a negative correlation between soil pH and cobalt uptake by plants (Kukier et al., 2004; Faucon et al., 2009). This means that, in general, a universal relationship between soil pH and cobalt uptake cannot be concluded, but that it depends on many factors, most of all on the type and properties of the soil. Lee et al. (2004) indicate the existence of a significant negative correlation between soil iron content and cobalt uptake. A higher concentration of manganese can negatively affect cobalt uptake (Faucon et al., 2009). According to McKenzie (1972), high amounts of manganese and humus in the soil inhibit cobalt uptake. Most of the cobalt in the soil is fixed in this way and is therefore unavailable to plants. Collins and Kinsela (2010) indicate the importance of the influence of soil solution composition on cobalt uptake. Numerous studies indicate that with an increase in the concentration of easily soluble cobalt in water, its absorption from the soil also increases (Li et al., 2009).

When cobalt is added to the soil, geochemical reactions often result in its redistribution into the solid phase of the soil, so that there is no increase in the concentration of its easily soluble form (Wendling et al., 2009). This is also confirmed by Sherrell (1990), according to which the annual application of 0.09 mg kg^{-1} cobalt for nine years on loamy soil (pH 5.6) did not significantly increase the concentration of cobalt in white clover and alfalfa. Therefore, and considering the low mobility of cobalt in the plant (Austenfeld 1979) and its faster movement from the aerial part to the root compared to the opposite direction (Palit et al., 1994), foliar nutrition in relation to soil fertilization more effectively establishes its optimal status in plants. The same authors indicate that the movement of cobalt through the plant is faster in the descending path than in the opposite direction.

The role of cobalt in nitrogen fixation

Optimum provision of plants with cobalt contributes to enhanced nitrogen fixation in all Rhizobium species, and thus to the growth of legumes (Collins and Kinsela 2011). Cobalt is the central atom in the porphyrin structure of the coenzyme cobalamin and is essential for nodulation and development of bacteroids. Cobalt is a component of vitamin B_{12}, which is included in the composition of enzymes and coenzymes that participate in the process of nitrogen fixation in leguminous nodules (Mathur et al., 2006). Various studies indicate that vigor, nodule development and nitrogen content of legumes depend on the cobalt content of the soil. According to Vukadinović and Lončarić (1997), cobalt is a necessary element for symbiotic nitrogen-fixing microorganisms. When there is a lack of Co in the plant, the organic production of legumes falls. Das (2000) indicated that there are three specific cobalamin-dependent enzyme systems in rhizobium that can be attributed to the effect of cobalt on nodulation, ribonucleotide reductase and methylmalonyl coenzyme A mutase.

The application of cobalt in very small amounts to the aerial parts of plants or in the soil had a positive effect on symbiotic nitrogen fixation (Tomić et al., 2015a), yield components and seed yield in red clover (Tomić, 2017).

Powrie (1964) in alfalfa (*Medicago sativa* L.) and Ozanne et al. (1963) in subterranean clover (*Trifolium subterraneum* L.) determined that a good supply of soil Co influenced a significant increase in plant mass and an increase in the amount of fixed nitrogen.

Dilworth et al. (1979) state that the number of bacteroids and leghemoglobin content in nodules of blue lupine (*Lupinus angustifolius* L.) directly depends on the cobalamin content, and nitrogen fixation activity is dependent on both the cobalt status and the cobalamin content. The authors also conclude that lupine crown nodulation is significantly reduced under cobalt deficiency, most likely as a consequence of reduced nodule initiation and that normal nitrogenase activity cannot occur below the critical concentration of cobalt in nodules.

Pattanayak et al. (2000) state that the application of cobalt in a concentration of 250 mg kg^{-1} and 500 mg kg^{-1} of soil in cowpea (*Vigna sinensis* L.) influenced a significant increase in the number of nodules per plant, the number of effective nodules per plant, the mass of effective nodules per plants, accumulation of dry matter in plants, number of pods per plant and seed yield per hectare. The authors state that cobalt is an essential element for nodulation and fixation of atmospheric nitrogen by leguminous plants.

Adding cobalt to the soil during forage pea production, in the amount of 10-20 g ha^{-1} in the form of cobalt chloride, especially in combination with nitrogen fertilization, had an effect on increasing seed germination, leaf chlorophyll content, shoot length, root length, dry matter content matter in shoots and roots, nodules number per plant, flowers number per plant, pods number per plant, thousand seed weight, seed yield, nitrogen content in shoots and stem and cobalt content in nodules and shoots (Acbar et al., 2013).

Cobalt in the amount of 0.16 mg g^{-1} of soil influenced a significant increase in the number and mass of nodules, nitrogen concentration in nodules, leghemoglobin content, total biomass production, seed yield compared to untreated peanut plants (*Arachis hypogaea*) (Nadia et al., 2012).

According to the results of Jayakumar and Jallel (2009), foliar application of cobalt or its application over the soil had a positive effect on better nodulation, better growth, increase in plant dry matter yield and soybean seed yield.

Other roles of cobalt in forage legumes

Cobalt is important for the normal development of a number of physiological reactions in the process of photosynthesis (Lipskaya, 1972). In lower concentrations, cobalt has a positive effect on the Hill reaction with a simultaneous decrease in the amount of chlorophyll and an increase in the number of chloroplasts per unit of leaf area. The increase in photosynthesis activity can be associated with the redistribution of pigments (increase in the amount of chlorophyll b and decrease in the amount of chlorophyll a), or direct entry into the light and dark phases of Hill's reaction. The positive impact of the provision of Co to plants is manifested through increased chlorophyll content (Palit et al., 1994), greater thickness of the palisade tissue, increased number and size of chloroplasts (Lipskaya, 1972). Cobalt is an important element for the photosynthesis process in perennial legumes. The addition of cobalt to the nutrient solution at a concentration of 1 ppm in the form of $CoCl_2$ in soybeans led to an increase in the content of chlorophyll and vitamin B_{12} (Ahmed and Evans, 1960).

Cobalt plays an important role in the respiration process in leguminous plants, as well as in the regulation of transpiration because it affects the work of stomatal cells (Rauser and Dumbroff, 1981). According to Zeid (2001), the presence of cobalt in the nutrient solution is very important for seed germination and achieving high plant yields.

Delwiche et al. (1960) applied 0.1 μmol L^{-1} Co over the soil with the addition of Rhizobium inoculum in alfalfa and determined that there was a significant increase in green mass yield in comparison to the control. Also independently applied cobalt ions or Rhizobium inoculation, had an effect on higher plant growth. The nodules that were on treatment with the application of cobalt had a higher capacity for nitrogen fixation than those on the control variant, which was determined by the application of isotopic nitrogen N^{15} in the substrate.

Reith and Burridge (1983) applied 0.5 kg ha^{-1} of cobalt in the form of cobalt-sulfate and found an increase in the content of cobalt in the forage of several types of clover, to a greater extent on peat than on mineral soils.

The foliar addition of cobalt had in general a positive effect on seed yield and yield components in all cultivars of red clover (Tomić et al., 2014). The higher yield on the variant with cobalt is the consequence of the significant increase in flower number per inflorescence, i.e. seed number per inflorescence. Foliar treatment with cobalt achieved a positive impact on forage yield of the cultivars of red clover (Tomić et al., 2015b). The hay yield results are consistent with the results of forage yield.

Toxic effect of cobalt in plants

The boundary between useful and toxic concentrations of cobalt in forage plants is narrow. A high concentration of cobalt in plants is undesirable and can adversely affect its physiological and biochemical functions (El-Sheejh et al., 2003). In interaction with other elements, cobalt forms complexes. The cytotoxic and phytotoxic activity of these complexes depends on their physical and chemical properties (Palit et al., 1994). Competitive absorption and mutual activation of related metals are factors on which the effect of cobalt in reactions depends. Toxic concentrations of cobalt inhibit active ion transport in higher plants. Cobalt participates in the formation of chlorophyll b, but causes damage to plastids and affects the change in the structure and number of chloroplasts per unit area in the leaf. Its harmful effect when it is in excess is reflected in inhibiting the activity of PS2 and reducing the export of photoassimilates in the dark phase of photosynthesis. In C_4 plants, cobalt interferes with carbon dioxide fixation by inhibiting the activity of enzymes involved in that process. Cobalt slows down the processes of cytokinesis and karyokinesis. Excess cobalt hinders the synthesis of RNA and DNA, most likely by modifying a large number of endonucleases and exonucleases.

The toxic effect of cobalt on the morphology of plants manifests itself in the form of chlorosis, leaf fall, pale leaf nervure and reduced plant growth (Palit et al., 1994).

Foliar application of cobalt affected a significant reduction of the chlorophyll content in the leaves of all cultivars of red clover compared to the control variant without application of cobalt (Tomić et al., 2015c). The reason for this is the harmful effect of cobalt applied in larger quantities.

Conclusion

The concentration of cobalt in soils is 1-40 mg kg^{-1}, and in the dry matter of plants 0.1-10 μg g^{-1}. When cobalt is added to the soil, geochemical reactions often result in its redistribution in the soil, so that there is no increase in the concentration of its easily soluble form. In general, a universal relationship between soil pH value and cobalt uptake cannot be concluded, but that it depends on many factors, most of all on the type and properties of the soil. A significant negative correlation exists between the content of iron and manganese and the concentration of easily accessible cobalt in the soil. Due to all of the above, given the low mobility of cobalt in the plant and its faster movement from the above-ground part to the root compared to the opposite direction, foliar nutrition in relation to soil fertilization more effectively establishes its optimal status in plants.

Cobalt is included in the structure of vitamin B_{12}, which is an integral part of enzymes and coenzymes, important for the process of nitrogen fixation in all species of Rhizobium. An optimal supply of cobalt has a positive effect on growth and development, and thus on the forage yield and seeds of a large number of forage legumes such as: red clover, alfalfa, cowpea, subterranean clover, lupine, peas, peanuts, soybeans. The positive impact is reflected in the increase of nodules number per plant, effective nodules number per plant, effective nodules mass per plant, the accumulation of dry matter in plants, greater plant height, root length, an increase in the pods number per plant, flowers number per plant, pods number per plants, thousand seeds weight, content of chlorophyll in the leaf and others. In addition to the above, the optimal presence of cobalt in forage legumes has a positive effect on the intensity of the photosynthesis process, seed germination and vigor, yield components and seed yield.

A high concentration of cobalt in leguminous plants is undesirable because it can adversely affect physiological and biochemical functions in the plant, and the border between useful and toxic concentrations of cobalt is very narrow.

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