Technical Journal, 4 (1977) 10 - 18

FUNCTIONS OF COPPER IN PLANT METABOLISM AND COPPER DEFICIENCY IN SOILS

S. MUTLU

Ankara Nuclear Research and Training Center

ABSTRACT

This review provides the published works on the function of copper in the plant metabolism and on the copper deficiency. The effect of the interaction between copper and the other mineral nutrients in soils to copper uptake is given. The general deficiency symptom has been described and the diagnosis and correction of copper deficiency in soils and plant are discussed.

ÖZET

Bu yazı, bakırın bitki metabolizmasındaki fonksiyonlarını ve bakır noksanlığını içeren araştırmaları derlemektedir. Toprak bakırı ile toprağın diğer mineral maddeleri arasındaki ilişkinin bitkinin bakır alımına etkisi ve bakır noksanlığının toprakda ve bitkide tanısı ve giderilmesi tartışılmıştır.

INTRODUCTION

The continuous increase in world population leads to a demand for more agricultural land and an increase in food production, which encourages man to carry out research for better quality crops and higher yields.

The great importance of micro - nutrients as copper in the field has become well recognised in the past 50 years. Recognition followed the incorrect amendment of agricultural soils and the reclamation of new land, which became a necessary expedient to cope with the world's diminishing food supplies.

As far as we know, copper has three functions in plant breeding. Firstly, it acts as a catalyser in some metabolic reactions. Secondly, it effects the chemical properties of soil. Thirdly, it protects the plants against fungus, germ and algae sort of primitive life form.

This paper will be concerned with the recent works done on copper in the plant metabolism and on the copper deficiency in soils.

COPPER IN PLANT METABOLISM

Copper occurs throughout the tissues of plants but it is concentrated mostly in the green leaves and in the germ of seeds. Green [18] has reported that plant tissues on average contain from 3 - 40 ppm. copper depending on species, soil, amount of fertilizer used, and other factors. Copper accumulates in organic form in some plant organs such as pollen anthers and seeds of cereals [24] and chloroplasts [20, 47, 60]. Although the function of this accumulated copper is not completely known, a minor amount of copper as copper ions and as metallo - enzymes (copper protein enzyme) is known to act as a catalyst in some oxidation - reduction reactions. In metallo - enzymes, copper in mixed valencies (Cu^{+} and Cu^{++}) is attached to O^{-}, N^{-} and S^{-} containing ligands in prosthetic groups of proteins [26]. The catalytic porperties of the copper protein enzymes are remarkably higher and more specific than the copper-ions [44].

Many of the chemical reactions which take place in respiration, photosynthesis and nitrogen metabolism are regulated by copper enzymes and to a lesser extent by ionic copper. Excess or shortage of this element will effect the chemical reactions and hence alter the entire metabolism of the plant. The functions of copper in different parts of plant metabolism were discussed below.

1. Copper in plant respiration

The copper protein enzymes, which catalyze the reaction of molecular oxygen with electrons liberated from various respiratory substances and intermediates, are called «oxidases» or «terminal oxidases». The main copper - protein oxidases in plants include cytochrome oxidases and ascorbic acid oxidase, and these are unevenly distributed among plant species and within individual plants [45].

Cytochrome oxidase is located exclusively in mitochondria and mitochondrial fragments and is responsible for cell respiration [28]. It occurs in most animals, plants and some bacteria [12, 32]. Unfortunately the role of copper in cytochrome oxidase has not been studied in higher plants, but studied in some bacteria and animals [44].

Phenol oxidases (polyphenol oxidase, tyrosinase and laccase) occur in most plants [39, 33] and fungi [39] but occurrence of laccase in the animal kingdom has yet to be described. Polyphenol oxidase is responsible for the formation of brown, red and tan pigments, melanins [39]. The location of polyphenol oxidase in the chloroplasts of spinach beet has been reported by Arnon [2].

Ascorbic acid oxidase is present in the soluble and the mitochondrial fragments of the most plant tissues [38], and is also found within the cell wall fractions [8, 12. 32]. Although the most usual terminal oxidase is cytochrome oxidase, ascorbic acid oxidase (in apple fruit, swedes and barley) and polyphenol oxidase (in potato tubers and apples) are also thought to act as terminal oxidases in some cases [45, 59].

Plant amine oxidase is also a copper enzyme found in peas and some other plants [23, 37]. It is less widespread than the other plant oxidases.

2. Copper in photosynthesis

Plastocyanin, a nonautoxidizable copper protein complex, influences the formation of strong reducing agents in the electron transport chain between the two photosystem (System II and System I) of photosynthesis [4, 29, 31]. It is ubiquitous among various plants and algae. Most of the copper in chloroplasts is contained in plastocyanin, which contains copper to an extent up to 1.1 % of its weight [5, 30, 54].

Although chlorophyll itself contains no copper, an increase in the chlorophyll content of some plants with copper application has been reported, and it was suggested that copper has a protective effect against chlorophyll destruction [16]. More recently in pot trials with wheat, Agrawal et al. [1] showed that application of 12.5 kg/ha $CuSO_4$ as a basal soil application 7 days before sowing, increased the chlorophyll content by 73 %, compared with the control given no copper. On the other hand 25 kg/ha $CuSO_4$ as a basal application or foliar spray gave lower chlorophyll content than the control. Copper deficiency has been directly associated with a reduced rate of apparent photosynthesis of young tung trees [35] and with reduced net photosynthesis of flag leaves of Kloka wheat [49].

3. Copper in nitrogen metabolism

Nitrate, nitrite and hyponitrite reductases are key enzymes in the nitrogen metabolism of plants. Copper is required for the prosthetic group of nitrite and hyponitrite reductase [48].

Copper deficiency in the fungus **Neurospore crassa** wild type 146 causes an appreciable increase in the nitrate reductase activity [48]. A high accumulation of nitrates and amino acids in copper deficient tomatoes [53] and Thatcher wheat [7] has ben reported. Rasheed and Seeley [56] found that an increase in protein content, resulting from high application of nitrogen, inactivated copper in plants and might induce or accentuate copper deficiency [27].

4. Copper in carbohydrate and fatty acid metabolism

In carbohydrate and fatty acid metabolism, copper does not actually become associated with any enzyme, but somehow the end-products are altered, depending on the amount of copper in the plant.

The addition of copper to wheat (two weeks old) reduces amylase and lipase activity, as well as the sugar and fat content, but increases the synthesis of pentosans relative to the copper deficient tissue [50]. 46 day old copper sufficient wheat plants show a marked decrease in organic acid and simultaneously a large increase in sugar content, where as copper deficient plants do not show these changes [7].

COPPER DEFICIENCY

Copper makes up only 0.0001 % of the earth's crust. The amount of copper in soils varies; a normal agricultural soil is estimated to contain from 1 to over 50 ppm. The copper found in soils can be divided into three forms: water-soluble, exchangeable and fixed. Water soluble and exchangeable copper will determine the available soil-copper. The factors generally recognised as directly infuencing the available copper content of soil are; pH, humus content, the nature of previous crop, the porportion of sand to clay, the presence of other chemical elements with which the copper could interact. It is possible for several of these factors to be active at the same time. The copper deficiency frequently occurs in the soils of high organic content, particularly in the newly cultivated peat and in the alkaline peat soils which have appreciable amounts of ferrous iron. In the newly reclaimed deep light peats,in which copper levels are very low, copper deficiency is increased by drainage and by lowering the water table. The sandy and open gravel soils also lack sufficient amount of available copper, and the shortage of rainfall will decrease the sufficient amount of available quantities in these soils.

All plants vary to some extent in their mineral requirement in another way in their sensitivity to the mineral nutrients. Copper deficiency has been more severe in its effects on cereals and citrus. Also the severe copper deficiency has been found in onions, cocsfoot (Dactylis glomerata) grown for seed, and couch grass (Agropyron repens). On the fen peats, sugar beet, and potatoes have benefitted occasionally; celery normally receives copper as a protective fungicide. The interaction between copper and other mineral nutrients is influenced by a host of factors, such as temperature, rainfall, drainage,soil pH, etc., but the most important determining influences are the nature of the plant and the other mineral nutrients.

1. Nitrogen

A heavy nitrogen fertilizer depresses copper concantration in the tops and the roots of some plants [9, 36] and induces or accentuates copper deficiency in cereals [11, 15, 27, 51] and in herbage [41, 42]. This may be caused by a change in the nitrogen metabolism [15] or by nitrogen-stimulated growth, which takes place to such extent that the absorbed copper nutrient is diluted to a deficient level [9].

2. Phosphate

Bingham and his co-workers [3] have demonstrated the influence of phosphatic fertilizer on trace elements in citrus. These workers have examined nineteen soils and find that, regardless of source, excess phosphate results in acute copper deficiency. Prosphate fertilizer increases the copper content of oats only in the presence of added copper and nitrogen [11].

3. Potassium

Potassium deficiency induced by copper sulphate amendment in tobacco plants grown on fine sandy loam has been reported [16].

4. Iron

Hewitt [22] found the excess copper led to chlorosis of the leaves of tomatoes and sugar beet similar to that produced by the shortage of iron, but oats and kale appeared not to be effected in this way. In chlorosis susceptible plants, copper and phosphate are much more effective in causing iron-chlorosis if applied together than if either element is applied alone, although phosphate has no effect upon the absorption of iron [6]. Copper induced iron-chlorosis is much worse during stem elongation in cereals [49], and it has been corrected by lowering the copper concentration in the culture solution during this period [43].

An opposite copper-iron antogonism was reported by Cheshire and his co-workers [11]. They found that the addition of iron to peat low in copper had no effect on the ccpper uptake by oats. However, they did find that copper application increased copper uptake to greater extent in the absence of iron than in its presence.

5. Manganese

In 1954, Forster reported the accumulation of excess manganese in the tops of crops grown in soils having an unusually high copper content. Habermann [19] later demonstrated the reversal of copper toxicity by manganese in several kinds of chloroplasts raactions (photoxidation of diketogulonic acid, Quinone-Hill reaction and Mehler reaction).

She suggested that manganese may provide a natural protective mechanism against a level of copper whieh might otherwise be toxic and that manganese ions influence chloroplast membrane permeability. In this respest, copper-manganese antogonism may be due to compotition for the sulphydryl groups present in the membrane for which copper has strong affinity.

6. Zinc

Zinc enhances the toxic effect of copper in flax grown in nutrient solution and the effect is further increased in the absence of boron [52]. This may be due to the intensive penetration of copper, or to boron exhaustion, which in turn promotes copper toxity in the presence of zinc. On the other hand, Chaudhry et. al.[9,10] have reported that zinc fertilizer depresses copper concentration in cereal crops grown on sandy soils, and in rice crops grown on rice growing areas in Pakistan by suppressing copper absorption.

7. Molybdenum

Copper-molybdenum antagonism in plants has been reported by Giordano [17]. In copper deficient areas, molybdenum toxicity has been found in the herbage of pastures on which sheep and cattle were affected by a «scouring disease» known as a teartness. This can be corrected by applying copper sulptate to the soil, or directly to the affected animals [16]. The application of copper fertilizer has been practised in parts of the South-west England, where this disease is known.

DİAGNOSİS OF COPPER DEFİCİENCY AND RECOMMENDED TREATMENTS

Copper deficiency appears to be simple deficiency of available copper in peat, sand and chalk solis. The soil analysis has only partial success in predicting this disorder since many factors (crop variety, weather, soil type and depth) influence the uptake of copper, but nevertheless it is useful for identifying soils containing small amounts of copper. Although there is some correlation between total soil copper and plant copper content, the diagnostic technique involving EDTA - extractable soil copper is found to be more satisfactory and so far, no better technique has been reported [27, 40, 51, 57]. In soil analyses, copper deficiency can occur when EDTA - extractable soil copper is below 2.5 ppm in peat and chalk soils [27, 51] and below 2.0 ppm in mineral soils [51]. The amount of available nutrients in the soil is sometimes referred to as labile pool. Recently copper-labile pool of deficient soils are determined sufficiently by isotophic exchange and spectrografic analysis [34].

So far, the severe, less severe, moderate and slight cases of copper deficiency have been observed in the plants grown in field.

In the severe cases, symptoms appear generally at the reproductive growth stage. Plants become pale-green with a chlorosis of tips and margins of the leaves; they lose turgidity, the foilage tends to flag and growth ceases. No flowers are produced and the roots are very poor, showing die-back and lack of regenaration. The tips of newly emerging leaves become chlorotic, wither and die without unrolling.

In the less severe cases, vegetation of plant can be normal but fruit formation is severaly affected.

In the moderate cases, the crops usually appears vigorous and healthy up till the harvest but most of the fruits fail to develop.

In the slight cases, no visual symptoms are observed except by an increase in yield with the use of copper fertilizer.

On the continuous appearance of copper deficiency, soil application of fine copper sulphate crystals at a recommended rate is advisable and the effect lasts at least 5 - 10 years. The apperanace of copper deficiency on a temporary basis on soils was found to be dependent to a large extent on weather conditions [58]. Previous works show that soil analyses are successful in diagnosing the severe copper deficiency in the field but are unsatisfactory for detecting temporary copper deficiency.

Recently, Mutlu [43] developed two methods to determine the temporary copper deficiency in cereal crops. These methods involves the determination of plant copper or an copper - enzyme which is active in that plant. In these determinations it is extremly important to ensure purity of chemicals, water and glass - ware and to use a sufficiently sensitive instrument. Also, it is important to take account of the ontogenetic changes in copper concentration in the plants and the variation in copper content that occurs between the different parts of the plant. However before these techniques it could be used routinely, it would be necessary to investigate their efficiency on the large number of different crops grown in the field.

CONCLUSION

The occurrence of copper deficiency has become more common all over the world. For obtaining the maximum and the good - quality crops, the future workers ought to develop the present diagnosing techniques for the different crops as well as to determine the copper requirement of each plant in the planted areas. Also, there is much to be done on the copper metabolism eg. what other reactions have been affected by

copper in the plant metabolism and why the plants store such a large quantity of copper and use very little from it, etc.

REFERENCES

[1] Agraval, H. P., Pandey, D. G., Indian J. Agric. Sci. 42 (1972) 230

[2] Arnon, D. I., Plant Physiol. 24 (1949) 1

[3] Dingham, F. T., Martin, J. P., Chastain, J. A., Soil Sci. 83 (1958) 24

[4] Bishop, N. I., Nature 204 (1964) 401

[5] Blumberg, W., Peisach, J., Biochem. et Biophys. Acta. 126 (1966) 269

[6] Borwn, J. C., Hollmes, R. S., Plant Physiol. 30 (1955) 457

[7] Borwn, J. C., Tiffin, L. O., Holness, R. S., Plant Plant Physiol. 33 (1958) 38

[8] Butt, V. S., Hallaway, M., Biochem. J. 69 (1958) 20

[9] Chaudhry, F. M., Loneragan, S. F., Aust. J. Agric. Res. 21 (1970) 865

[10] Chaudhry, F. M., Sharif, M., Latif, A., Qureshi, R. H., Plant and Soil 38 (1973) 573

[11] Cheshire, M. V., Dekok, P. C., Inkson, R. H. E., J. Sci. Fd. Agirc. 18 (1967) 156

[12] Clezy, P. S. Barret, J., Biochem J. 78 (1961)

[13] Dawson, C. R., Mallette, M. F., Advances in protein chem. 2 (1945) 179

[14] Felix, E. L., Phytopathology. 17 (1927) 49

[15] Gilbert, G. S., Plant Physiol. Lancaster. 26 (1951) 398

[16] Gilbert, F. A., Copper in nutrition. Adv. Agron. IV (1952) 147

[17] Giordano, P. M., Diss. Abstr. 25 (1964) 4337

[18] Green, H. H., Vet. Res., 50 (1938) 1185

[19] Habermann, H. M., Plant Physiol. 44 (1969) 331

[20] Hall, D. O., Whatley, F. R., The chloroplast. In «enzyme cytology» ed. D. Roodijn. Acad. Press (1967)

[21] Hammerich, P., The biochemistry of copper ed Peisach, J., Aisen, P., Blumberg W. E. (1966)

[22] Hewitt, E. J., J. Exp. Bot. 4 (1953) 59

[23] Hill, J. M., Mann, P. J. G., Rec. Asps. of Nit. Metab. Ist Long Ashton Symp. (1968) 149

[24] Hoeffner, W., Schammloefel, E., Voelker L., Planta 57 (1961) 608

[25] Honda, S. I., Plant Physiol 30 (1955 a) 174

[26] , Plant Physiol 32 (1955 b) 23

[27] Hooper, L. J., Davies, D. B., J. Sci. Fd. Agric 19 (1968) 733

[28] Karlson, P., Introduction to modern biochemistry. Acad Press (1965)

[29] Katoh, S., Nature 186 (1960) 533

[30] Katoh, S., Shiratory, I., Takamiya, A., J. Biochem. Tokya 51 (1962) 32

[31] Katoh, S., Pietro, S. A., Plastocyanin. In «the biochemistry of copper» ed. Peisach, J., Aisen, P., Blumberg, W. E. (1966)

[32] Lemberg, R., Clezy, P., Barret, J., Haematin enzymez. ed. Falk, J. E., Lemberg, R., Morton, R. K. Macmillan Company (Peroamon) New York (1962)

[33] Levine, W. G., Laccase. In «the biochemistry of copper» ed. Peisach, J., Aisen. P., Blumberg, W. E. (1966)

[34] Lopez, P. L., Graham, E. R., Soil Sci. 114 (1972) 295

[35] Loustalot, A. J. Burrows, F. W., Gilbert, S. O., Nason, A., Plant Physiol 20 (1945) 283

[36] Lucas, E. B., Proc. Soil Sci. Soc. Am. 10 (1945) 269

[37] Mann, P. J. G., Biochem, J. 79 (1961) 623

[38] Mapson, L. W., Annual Review of Plant Physiol 9 (1958) 581

[39] Mason, H. S., Polyphenoloxidase. In «the biochemistry of copper» ed. Peisach, J., Aison, P., Blumberg, w. E., (1966)

[40] Mitchell, R. L., Reith, J. w. S., Johnston, I. M., VI th Int. Congress Soil Sci. Paris. 2 nd. Symposium on plant analysis and fertilizer problems. I. R. H. O., Paris (1956) 249

[41] Mulder, E. G., V th. Internat. Grassl. Cong. Neth. (1949) 61

[42] Mulder, E. G., Pontificae Academiae Scientarum, Scripta Varia. 14 (1954) 215

[43] Mutlu, S., Investigation of copper deficieney in wheat and barley grown in culture solution. Univ. of Reading England. Ph. D. Thesis (1974)

[44] Mutuskin, A. A., Izv. Akad Nauk. SSSR Ser Biol. 5 (1970) 698

[45] Mc Elroy, W. D., Glass, B., In copper metabolism, John Hopkins Press Baltimore (1950)

[46] Mc Elroy, W. D. Nason, A., Borden's Rev. 17 (1956) 47

[47] Neish, A. C., Biochem, J. 33 (1939) 300

[48] Nicholas, D. J. D., J. Sci. Fd. Agric. 8 (1957) 15

[49] O'Connel, J. D , M. Sc. Report. Imperial Coll. Sci. Tech. (1969)

[50] Okunsov, M. M., Ron'zhia, O. A., Microelementy v Sel'sk. Khozi. Med. Riga. C. A. - 51 1956) 9807 f.

[51] Pizer, N. H., Caldwell, T. H., Burgess, G. R., Jones, J. L. O., J. Agric. Sci. 66 (1966) 303

[52] Porokhnevich, N. V., Soviet Plant Physiol. 17 (1970) 78

[53] Possingham, J. W., Australian J. Biol. Sci. 9 (1956) 539

[54] Pshenova, K. V., Mutuskin, A. A., Kolesnikov, L. A., Dokl. Akad. Nauk SSSR 170 (1966) 457

[55] Oureshi, R. H., Plant and Soil. 38 (1973) 573

[56] Rasheed, M. A., Seeley, R. C., Nature 212 (1966) 644

[57] Reith, J. W. S., J. Agric. Sci. 70 (1968) 39

[58] Soil Scientist Open Conference, N. A. A. S. M. A. F. F. ed. Evans c. (1965)

[59] Stiles, W., Trace elements in plants. Cambridge Univ. Press (1961)

[60] Whatley, F., Ordin, L., Arnon, D., Plant Physiol. 26 (1951) 414