BIOLOGIA PLANTARUM 43 (1): 35-39, 2000

Effects of some growth regulators on young iron deficient maize plants

V.R. NENOVA and I.G. STOYANOV

Institute of Plant Physiology "M. Popov", Bulgarian Academy of Sciences, Akademik G. Bonchev St., Bl. 21, Sofia 1113, Bulgaria

Abstract

Young maize plants, grown hydroponically, were supplied with 1/10 the optimal amount of iron (0.75 mg dm^{-3}). Foliar treatments with solutions, containing N^6-benzyladenine (BA), indole-3-acetic acid (IAA) or (2-chloroethyl)-trimethylammoniumchloride (CCC) were conducted after chlorosis had been well manifested. Changes in growth, chlorophyll content, rate of photosynthesis, catalase and peroxidase activities in leaves, and the contents of Fe, Cu, Zn, Mn, and P in leaves were recorded. Growth regulators improved (CCC, IAA) or aggravated (BA) the physiological state of chlorotic plants. Their effect might be explained by changes in Fe transport towards the leaves, by increased efficiency of Fe utilization, and by effects on plant metabolism not involving Fe.

Additional key words: benzyladenine, CCC, chlorophyll, chlorosis, indole-3-acetic acid, nutrient elements, phytohormones, *Zea mays*.

Introduction

Iron deficiency alters the content of endogenous phytohormones in plants (Stoyanov and Tha 1981, Bozova and Stoeva 1982). On the other hand, hormones regulate ion absorption and ion distribution in the whole plant; however, as far as Fe is concerned, limited and often contradictory information is available (Landsberg 1984). The effect of naphthyleneacetic acid on growth and chlorophyll content of field grown peas was analogous to the effect of sequestrene 138-Fe (Sahu *et al.* 1987). An IAA foliar spray tended to improve the chlorophyll content and the dry matter accumulation in field experiments with peanut, grown on calcareous soil (Singh and Sahu 1993). Chlorotic maize plants, grown hydroponically, became green when sprayed with 100 μM IAA (Mengel and Geurtzen 1988). N^6-benzyladenine increased the content of chlorophyll and protein in leaves of Fe deficient chlorotic wheat plants (Bozova and Stoeva 1982).

In the present study we examined some of the physiological and biochemical changes in young Fe-deficient maize plants after foliar sprays with IAA, BA and CCC.

Material and methods

Plants and growth conditions: Five greenhouse experiments were carried out from May to September with maize plants (*Zea mays* L., hybrid Knezha-650). Seven-day-old seedlings grown from seeds germinated in wet paper rolls were transferred to plastic 1 200 cm^3 boxes, two plants per box. The maize plants were grown hydroponically in Hoagland-Arnon nutrient solution I, replaced twice weekly. Micronutrients were supplied according to Hoagland's "A-Z" solution with the addition of $(NH_4)_6Mo_7O_{24}$. Iron was supplied as FeEDTA in concentration 7.5 mg dm^{-3} Fe for control plants and 0.75 mg dm^{-3} Fe for iron deficient plants. In the daytime

Received 22 December 1998, *accepted* 5 October 1999.

Abbreviations: BA - N^6-benzyladenine; CCC - (2-chloroethyl)trimethylammoniumchloride; IAA - indole-3-acetic acid; F_0 - initial fluorescence; F_v - variable fluorescence; F_m - maximum fluorescence; GDHP - guaiacol dehydrogenated product.

Acknowledgements: This research was financially supported by the National Science Fund of the Bulgarian Ministry of Education and Science, Grant- B-420. The authors are grateful to Mrs. Ana Trifonova for excellent technical assistance.

Fax: (+359) 2 739952, e-mail: stoyanov@obzor.bio21.bas.bg

the plants were grown under natural conditions, except when unfavourable climatic conditions took place (rain, strong wind, air temperature below 15 °C). During the night the plants were taken into the greenhouse.

Foliar treatments with aqueous solutions of growth regulators, containing 0.05 % Tween 80 as surfactant, were conducted twice, the first application when chlorosis of young leaves was well manifested (20 to 22-d-old seedlings) and the second - 6 d later. The following substances were used: 10 and 20 mg dm^{-3} N^6 benzyl-adenine (*Fluka*, Buchs, Switzerland), 50 and 100 mg dm^{-3} 3-indolylacetic acid (*Fluka*), 2 and 4 g dm^{-3} (2-chloroethyl) - trimethylammoniumchloride (*Cyanamid Inc.*, New Jersey, USA). Control plants were sprayed with distilled water containing Tween. The analyses were made in four replications 7 - 10 d after the second treatment and the youngest fully developed leaves were used.

Methods: The contents of chlorophylls were measured after acetone extraction according to Arnon (1949). The photosynthetic rate was measured on intact plants with a portable system *Li-6000* (*Li-Cor*, Lincoln, USA). Fluorescence was measured using a pulse modulation chlorophyll fluorometer (*PAM 101*, *H. Walz*, Effeltrich, Germany) as described by Schreiber *et al.* (1986) in leaf discs with 10 mm diameter.

Enzyme activities were measured in crude extracts, prepared as described earlier (Nenova and Stoyanov 1995). Catalase (EC 1.11.1.6) activity was determined according to Aebi (1983) and peroxidase (EC 1.11.1.7) by the guaiacol test (Chance and Maehly 1955). Total (on fresh biomass basis) and specific (on protein basis) activities were calculated. Protein content in enzyme extracts was determined by using precipitation with amido black 10 B followed by measuring the absorbance in the supernatant (Buzun *et al.* 1982).

For determination of the content of nutrient elements, all leaves of the plants were oven dried at 60 °C. The concentrations of Fe, Zn, Mn and Cu were measured with the use of Atomic Absorption Spectrophotometer (*AAS 3*, *Carl Zeiss*, Jena, Germany) after dry digestion at about 500 - 550 °C. Phosphorus was measured spectrophotometrically according to Kojuharov (1960). A definite volume from the solution was put in a 100 cm^3 measuring flask and was neutralised. Five cm^3 1.2 % solution of $(NH_4)Mo_7O_{24}.4\ H_2O$ and 5 cm^3 of 0.12 % solution of $(NH_2)_2.H_2SO_4$ (both dissolved in 12 % H_2SO_4) were added and the flask was filled with distilled water up to 9/10 of its volume. One cm^3 0.5% solution of $SnCl_2$, prepared in 3 % HCl was added and 90 min after the solution was brought to its final volume the absorption at 720 nm was read.

Results and discussion

Maize plants, supplied with 0.75 mg dm^{-3} Fe accumulated 50 % less biomass as compared to the control plants (Table 1). The chlorophyll content and the photosynthetic rate per leaf area decreased (Table 2). As shown in our previous work iron deficiency resulted in significant changes in biomass accumulation and distribution between vegetative organs of young maize plants. There were changes in the pigment content and their proportions, changes in photosynthetic rate, and in carbon metabolism of leaves (Nenova and Stoyanov 1993). Since Fe is involved in chlorophyll biosynthesis (Pushnik *et al.* 1984) and destruction (Almela *et al.* 1983), and is important for the maintenance of the whole photosynthetic apparatus as well, Fe-deficiency has been found to have significant effects on the structure and physiology of maize chloroplasts (Stocking 1975). The lower F_v/F_o and F_v/F_m ratios (Table 2) might be explained by the decreased functional activity of photosystem II (Georgieva and Yordanov 1993).

The activities of both Fe-containing enzymes studied decreased in the leaves of chlorotic plants, catalase being more susceptible to Fe deficiency (Table 3). Since the protein content in the enzyme extracts also decreased, changes in specific activities were less pronounced than those for total activities. Metalloenzymes may be functional indices of the micronutrient supply in plants. In contrast to leaf analysis, this approach can distinguish between biologically functional metal and its inactive fraction (Del Rio 1983). Peroxidase, and especially

Table 1. Effects of growth regulators on growth of Fe-deficient maize plants. Values in one column followed by the same letter are not significantly different at $P = 0.05$ according to the Student's *t*-test.

Fe conc. [mg dm^{-3}]	Growth regulators [mg dm^{-3}]	Fresh biomass [g $plant^{-1}$]	Dry biomass [g $plant^{-1}$]
7.50	H_2O	46.48 a	4.21 a
0.75	H_2O	25.54 b	2.03 cd
0.75	IAA (50)	28.54 bc	2.20 bcd
0.75	IAA (100)	31.40 c	2.55 b
0.75	BA (10)	25.14 b	1.83 de
0.75	BA (20)	20.41 d	1.66 ef
0.75	CCC (2000)	29.86 c	2.58 b
0.75	CCC (4000)	24.08 bd	2.02 cf

Table 2. Effects of growth regulators on chlorophyll *a+b* content, photosynthetic rate, and characteristics of chlorophyll fluorescence (F_v/F_0 and F_v/F_m ratios) of Fe-deficient maize plants. Values in one column followed by the same letter are not significantly different at $P = 0.05$.

Fe [mg dm^{-3}]	Growth regulators [mg dm^{-3}]	Chlorophyll [mg g^{-1}(f.m.)]	Photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]	F_v/F_0	F_v/F_m
7.50	H_2O	1.8977 a	31.89 b	4.257 a	0.810 a
0.75	H_2O	0.8158 d	13.22 c	2.689 c	0.716 c
0.75	IAA (50)	0.6827 e	34.58 ab	3.517 b	0.779 b
0.75	IAA (100)	0.9561 c	41.84 a	3.119 bc	0.757 bc
0.75	BA (10)	0.3931 f	21.08 bc	2.943 c	0.745 c
0.75	BA (20)	0.4094 f	29.11 bc	3.061 c	0.753 c
0.75	CCC (2000)	1.1992 b	31.70 ab	2.966 c	0.748 c
0.75	CCC (4000)	1.2261 b	25.36 bc	3.300 bc	0.767 bc

catalase activities, as well as total chlorophyll content, are shown to reflect to a certain degree the Fe nutritional status of plants (Nenova and Stoyanov 1995). On the other hand the activities of enzymes (especially the peroxidase activity) are shown to be susceptible to phytohormones, thus making difficult their application as indices for Fe nutritional status in our experiments.

The relatively high Fe concentration in leaves (decreased by not more than 15 - 20 %) accompanied by increased concentrations of P, Cu, Zn and Mn suggested that the observed functional disorders might be due to a greater extent to the inactivation of iron than to its

Table 3. Effects of growth regulators on activities of catalase and peroxidase in leaves of Fe-deficient maize plants. Values in one column followed by the same letter are not significantly different at $P = 0.05$. GDHP - guaiacol dehydrogenated product.

Fe [mg dm^{-3}]	Growth regulators [mg dm^{-3}]	Catalase [nmol(H_2O_2) g^{-1}(f.m.) s^{-1}]	[nmol(H_2O_2) mg^{-1}(protein) s^{-1}]	Peroxidase [nmol(GDHP) g^{-1}(f.m.) s^{-1}]	[nmol(GDHP) mg^{-1}(protein) s^{-1}]
7.50	H_2O	9.53 a	0.86 a	270.25 a	24.37 ab
0.75	H_2O	4.77 d	0.66 b	161.57 e	22.42 abc
0.75	IAA (50)	5.40 c	0.65 b	186.53 d	22.52 bd
0.75	IAA (100)	6.03 b	0.62 b	208.27 c	21.20 cd
0.75	BA (10)	3.07 e	0.36 cf	138.07 f	16.28 e
0.75	BA (20)	2.55 e	0.34 ef	96.93 g	12.82 f
0.75	CCC (2000)	5.40 bcd	0.54 b	255.57 a	25.53 a
0.75	CCC (4000)	3.50 e	0.39 ce	226.18 b	25.45 a

shortage (Table 4). Only a portion of Fe in plants (designated as "active Fe") is supposed to participate in or to be available for metabolic reactions or for incorporation into molecular structures (Pierson and Clark 1984). The imbalance between nutrient elements might have an influence on Fe transport to the sites of action and on Fe function, especially when competition between elements takes place (Robson and Pitman 1983). Therefore, treatments, that increase Fe uptake and transport and/or that increase Fe mobility and efficiency might improve the Fe nutritional status of plants at a given external Fe supply.

Chlorotic plants sprayed twice with growth regulators manifested some positive or negative changes in the indices studied as compared to untreated chlorotic plants, but they differed significantly from green control plants (Tables 1 - 4). Physiological responses to individual growth regulators varied among experiments, probably depending on climatic conditions. Such unstable effect of leaf sprays with growth regulators (Goatley and Smidt 1990) or with Fe-containing substances (Mortvedt 1986) applied to correct chlorosis was also reported by other authors. It might be explained by the secondary nature of the most of observed metabolic changes (Muromtzev *et al.* 1987). The chlorophyll content was found to be the most sensitive and stable index in our experiments.

The greatest increase in chlorophyll content in chlorotic plants (on the average by 20 - 30 %) was found after treatment with CCC (Table 2). There was no significant difference between both applied concentrations. In some of the experiments the photosynthetic rate also increased. The slight increase in

the total enzyme activities was due to the rise of protein concentration in the leaf extracts by about 20 % (Table 3). The specific peroxidase activity was nearly the same as in control plants. The specific catalase activity tended to decrease additionally, especially at the high concentration of CCC. At 4 mg dm^{-3} CCC tended to increase the Mn concentration in the leaves (Table 4). The rest of the nutrient elements did not change significantly at both applied concentrations. Reduction in the endogenous content of gibberellins is thought to be the main reason for the growth-regulating properties of the retardant (Grossmann 1992). As in chlorotic maize

Table 4. Effects of growth regulators on concentrations of P, Fe, Cu, Zn and Mn in leaves of Fe-deficient maize plants. Values in one column followed by the same letter are not significantly different at $P = 0.05$.

Fe [mg dm^{-3}]	Growth regulators [mg dm^{-3}]	P [mg g^{-1}(d.m.)]	Fe [μg g^{-1}(d.m.)]	Cu [μg g^{-1}(d.m.)]	Zn [μg g^{-1}(d.m.)]	Mn [μg g^{-1}(d.m.)]
7.50	H_2O	3.28 c	192.8 b	13.4 c	42.0 d	22.3 d
0.75	H_2O	3.58 b	154.0 def	14.8 ab	105.0 b	36.8 c
0.75	IAA (50)	3.28 c	188.8 bc	15.8 a	75.0 c	37.3 c
0.75	IAA (100)	3.66 b	206.5 a	15.7 ab	82.5 c	37.5 c
0.75	BA (10)	3.66 b	138.5 f	15.2 ab	117.5 b	41.5 b
0.75	BA (20)	4.15 a	164.8 cde	16.6 ab	142.5 a	61.0 a
0.75	CCC (2000)	3.66 b	166.8 d	15.8 ab	102.5 b	35.7 c
0.75	CCC (4000)	3.36 c	153.5 e	15.0 b	107.5 b	41.3 b

plants, the content of endogenous gibberellins had already been decreased by Fe deficiency (Stoyanov and Tha 1981), CCC did not affect plant height (data not shown). The low concentration of CCC improved the biomass accumulation of chlorotic plants. The rise in the dry biomass was greater than the rise in the fresh biomass, because of the decreased tissue water content. The high concentration of CCC did not change the dry biomass and tended to decrease the fresh biomass. The treated leaves of these plants had isolated necrotic spots.

The beneficial effect of 2 mg dm^{-3} CCC sprays on chlorotic plants might be explained partially by the acidic nature of the solution (pH = 3.7). Mengel *et al.* (1994) established highly significant negative correlation between leaf apoplast pH and chlorophyll content. Depressing leaf apoplast pH by simply spraying chlorotic leaves with an acid led to a regreening of the leaves (Mengel 1994). At the same time a significant and prolonged drop in the leaf pH could not be expected in our experiments as applied solutions were not buffered. CCC might improve the metabolism without involving Fe at all as the green colour of the foliage often is intensified after treatment with growth retardants (Grossmann 1992).

In chlorotic plants sprayed with IAA (50 or 100 mg dm^{-3}) the biomass increased at average by 20 % (Table 1). The chlorophyll content and the photosynthetic rate also rose in some of the experiments. The functional activity of photosystem II tended to be partially restored as seen by the increased ratios F_v/F_0 and F_m/F_0 (Table 2). The protein content in the leaf extracts increased by 10 -35 % depending on the experiment (data not shown). The higher concentration (100 mg dm^{-3}) seemed to be more effective, although it tended to decrease additionally the specific catalase activity (Table 3).

The favourable effect of IAA might partially be due to the increased Fe transport towards the leaves as the Fe concentration in leaves rose in some of the experiments (Table 4). In these plants the Zn concentration dropped by 15 - 20 % on average. Thus greater "Fe- activity" in leaves might correspond to the increased ratio Fe/Zn. The increased solubility of Fe containing compounds in leaf apoplast and the greater Fe uptake into the symplasm might follow the pH drop of the leaf apoplast. On the one hand the direct acidic action of IAA can not be completely neglected, as the pH of a solution of 50 mg dm^{-3} IAA was 2.9. On the other hand, IAA might stimulate the proton pumps in the plasmalemma of leaf cells thus lowering the pH of the apoplast (Mengel and Geurtzen 1988).

Cytokinins are known to stimulate the structural and biochemical differentiation of chloroplasts, to delay chlorophyll destruction during senescence and to increase the resistance of plants towards various unfavourable agents (Muromtzev *et al.* 1987). Moreover, Fe deficiency and BA act in opposite ways on chlorophyllase activity (Drazkiewicz 1994). In our experiments, BA aggravated the chlorosis. The chlorophyll content dropped additionally by 15 - 50 %, the effect of the higher concentration of BA being more stable. The photosynthetic rate decreased only in the experiments with the greatest decrease of total chlorophyll. The parameters of chlorophyll fluorescence were close to the parameters in untreated chlorotic plants (Table 2). The activities of catalase and peroxidase decreased additionally, although the protein content in the tissue extracts did not change or even tended to increase (Table 3). The simultaneous decrease of the chlorophyll content and of the activities of both studied heme

enzymes might be regarded as sign for limited heme supply in leaves. The synthesis of porphyrin and heme depends on the availability of "active iron" in the tissues. An additional inactivation of Fe in BA-treated chlorotic plants might be assumed from the changes in the nutrient elements concentrations (Table 4). The Mn concentration increased and in some of the experiments the P and Zn concentrations were greater too. The plant biomass of plants, treated with BA, also tended to decrease (Table 1).

The obtained results show that sprays with growth regulators may improve (CCC, IAA) or aggravate (BA) the state of young Fe deficient maize plants. The effect of growth regulators might be explained by changes in Fe transport towards the leaves, by increased or decreased efficiency of Fe and by effects on plant metabolism not involving iron.

References

Aebi, H.: Catalase. - In: Bergmeyer, H. (ed.): Methods of Enzymatic Analysis. 3rd Ed. Vol. 3. Pp. 273-286. Verlag-Chemie, Deerfield Beach 1983.

Almela, L., Garcia, A., Madrid, R.: [Iron deficiency in maize plants. Effect on chlorophylase activity, chlorophylls, pheophytins and proteins in leaves.] - Agrochimica **27**: 123-131, 1983. [In Span.]

Arnon, D.: Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-15, 1949.

Bozova, L., Stoeva, N.: [The effect of 6-benzylaminepurine on chlorotic wheat plants.] - In: "V. Kolarov" Higher Institute of Agriculture Scientific Works XXVII. Book 2 Plant Physiology. Pp. 127-132. Plovdiv 1982. [In Bulg.]

Buzun, G., Dzemuhadse, K., Mileshko, L.: [Protein determination in plants with the aid of amidoschwarz.] - Fiziol. Rast. **29**: 198-205, 1982. [In Russ.]

Chance, B., Maehly, A.: Assay of catalases and peroxidases. - In: Colowick, S.P., Kaplan, N.O. (ed.): Methods in Enzymology. Vol. 2. Pp. 764-781. Academic Press, New York 1955.

Del Rio, L.: Metalloenzymes as biological markers for the appraisal of micronutrient imbalances in higher plants. - Life Chem. Rep. **2**: 1-34, 1983.

Drazkiewicz, M.: Chlorophyllase: occurrence, functions, mechanism of action, effects of external and internal factors. - Photosynthetica **30**: 321-331, 1994.

Georgieva, K., Yordanov, I.: Temperature dependence of chlorophyll fluorescence parameters of pea seedlings. - J. Plant Physiol. **142**: 151-155, 1993.

Goatley, J., Smidt, R.: Seedling Kentucky Bluegrass growth responses to chelate iron and biostimulator materials. - Agron. J. **82**: 901-905, 1990.

Grossmann, K.: Plant growth retardants: their mode of action and benefit physiological research. - In: Karssen, C.M., van Loon, L.C., Vreugdenhil, D. (ed.): Progress in Plant Growth Regulation. Pp. 788-797. Kluwer Academic Publishers, Dordrecht 1992.

Kojuharov, M.: [Colorimetric determination of phosphoric acid as molybdenum blue with hydrazine sulfate and $SnCl_2$ as combined reducing agent.] - In: Girginov, G., Kirev, P., Levenson, E., Radutchev, S., Todorov, T., Christov, A., Christov, M. (ed.): Scientific Works of Higher Agricultural Institute "G. Dimitrov". Vol. 8. Pp. 26-49. Zemisdat, Sofia 1960. [In Bulg.]

Landsberg, E.: Regulation of iron- stress- response by whole-plant activity. - J. Plant Nutr. **7**: 609-622, 1984.

Mengel, K.: Iron availability in plant tissues- iron chlorosis on calcareous soils. - Plant Soil **165**: 275-283, 1994.

Mengel, K., Geurtzen, G.: Relationship between iron chlorosis and alkalinity in *Zea mays*. - Physiol. Plant. **72**: 460-465, 1988.

Mortvedt, J.: Iron sources and management practices for correcting iron chlorosis problems. - J. Plant Nutr. **9**: 961-974, 1986.

Muromtzev, G., Tskanicov, D., Kulaeva, O., Gamburg, K.: Osnovy Khimicheskoï Regulyatsii Rosta i Produktivnosti Rasteniï. [Bases of Chemical Regulation of Growth and Productivity of Plants.] - Agropromizdat, Moskva 1987. [In Russ.]

Nenova, V., Stoyanov, I.: Physiological and biochemical changes in young maize plants under iron deficiency: 1. Growth and photosynthesis. - J. Plant Nutr. **16**: 835-849, 1993.

Nenova, V., Stoyanov, I.: Physiological and biochemical changes in young maize plants under iron deficiency: 2. Catalase, peroxidase, and nitrate reductase activities in leaves. - J. Plant Nutr. **18**: 2081-2091, 1995.

Pierson, E., Clark, R.: Ferrous iron determination in plant tissue. - J. Plant Nutr. **7**: 107-116, 1984.

Pushnik, J., Miller, G.: Iron regulation of chloroplast photosynthetic function: Mediation of PSI development. - J. Plant Nutr. **12**: 407-421, 1989.

Robson, A., Pitman, M.: Interactions between nutrients in higher plants - In: Läuchli, A., Bieleski, R.L. (ed.): Inorganic Plant Nutrition. Pp. 147-180. Springer-Verlag, Berlin 1983.

Sahu, M., Sharma, D., Jain, G., Singh, H.: Effect of growth substances, sequestrene 138-Fe and sulphuric acid on iron chlorosis of garden peas (*Pisum sativum* L.). - HortScience **62**: 391-394, 1987.

Schreiber, U., Schliwa, U., Bilger, W.: Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. - Photosynth. Res. **10**: 51-62, 1986.

Singh, D., Sahu, M.: Effects of phosphate carriers, iron, and indolylacetic acid on iron nutrition and productivity of peanut on a calcareous soil. - J. Plant Nutr. **16**: 1847-1855, 1993.

Stocking, C.: Iron deficiency and the structure and physiology of maize chloroplasts. - Plant Physiol. **55**: 626-631, 1975.

Stoyanov, S., Tha, H. Z.: [The relation between growth, gibberellin-like substances and abscissic acid in maize depending on various iron concentrations in the nutrient medium.] - Fiziol. Rast. (Sofia) **7** (2): 70-76, 1981. [In Bulg.]