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Plant Physiology

The Effect of Microelements on the Activities of Peroxidase and Polyphenol Oxidase in Tomato Leaves and Fruit

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ABSTRACT. The influence of microelements (B, Zn, Cu, Co, Mn) on the peroxidase and polyphenol oxidase activity in tomato leaves and fruit was studied. Activity of the enzymes under study varies in different phases of plant vegetation. It can be explained by the fact that the tomato plant bears the technically, physiologically mature and crude fruit as well as buds and blossoms together. In both control and in the microelement group with the increase of the peroxidase activity the polyphenol oxidase activity decreases. Compared to the control group the polyphenol oxidase activity increased the most in the phase of vegetation of 4-5 leaves, after treatment with boron and then with cobalt. Boron and zinc have similar action on the enzyme activity. Under the action of boron the polyphenol oxidase activity especially increases in the phase of vegetation of 4-5 leaves and in the later phase decreases. The peroxidase activity changes accordingly. After presowing treatment of tomato seeds with zinc the peroxidase activity decreases in leaves (in their early phase of vegetation) and fruit. It reaches its maximum in leaves in the fruit bearing period. Under the influence of copper and cobalt the peroxidase activity in fruit increases. In leaves it increases in the late phase of vegetation. The manganum influence on the the activity of polyphenol oxidase is less in the early phase of vegetation than in the late phase. In tomatoes with the increase of polyphenol oxidase activity the peroxidase activity decreases. *© 2015 Bull. Georg. Natl. Acad. Sci.*

Key words: *peroxidase, polyphenol oxidase, microelements, microfertilizers, seed preprocessing, enzyme activity.*

Microelements are necessary for normal processes of metabolism in the living bodies. They are related to vitamins, hormones, enzymes, therefore, play a significant role in growth and development of the body [1].

The source of micro elements is the soil with the chemical elements of mother rock as well as the gases, meteorite showers, smoke, volcanoes. Microelements occur in the solid particles of the soil and in the surface colloids. Microelements content in soil is dynamic in vegetation periods, for example, manganum, copper and iron content is in greatest amount in spring, when there is great amount of moisture and the unaerobic processes are going on. In early spring these elements are less absorbable for plants. Microelements content in different types of soil is different, for example, zinc accumulates in meadow and reeded podzolic soils, while they are in least amount in dark podzolic soils; copper accumulates in irrigated and reeded soils; cobalt content does not differ according to the soil types. The content of manganum and molybdenum is reduced in the deeper layers of soil.

Microelements introduced to plants through fertilizers stimulate their growth and development, and increase the crop. However, microelements have different action on different species. Besides, one and the same microelement has different effect on one and the same plant in different phases of vegetation. Therefore, it is necessary to estimate the exact doze of microelements according to the soil type.

Microelements in plants should not exceed a certain control point, for example, Mn – 40.0, Mo -0. 20, Co-1.5, Zn -0.3, B -0.5 mg/kg. They can be delivered to plants in three ways: 1) by means of roots through introduction of microelements into soil; 2) by means of spraying the leaves with weak solution; 3) by means of presowing treatment of the seed in aqueous solution of salts of microelements. Presowing treatment is more effective for studying the effect of microelements, because in such a case the seeds can absorb maximum amount of microelements, the embryo begins development earlier and the microelements influence spreads on the new cells generated by the embryo division [2].

We studied the effect of microelements (B, Zn, Cu, Co, Mn) on the activity of the enzymes - peroxidase and polyphenol oxidase in tomato leaves and fruit. Peroxidase (EC 1.11.1.7) is one of the enzymes of the process respiration. It belongs to the group of oxidoreductase. Peroxidase contains heme (protoporphyrin IX). The enzyme causes oxidation of some organic compounds such as phenols, amines and some other heterocyclic compounds with hydrogen peroxide [3].

$$\text{Donor -}H_2O_2\text{=oxidized donor+}H_2O$$

Unlike the catalase the peroxidase does not cause oxidation of hydrogen peroxide and spirits. Under the action of catalase H_2O_2 is decomposed into molecular oxygen and water while the peroxidase can restore H_2O_2.

Interaction of the peroxidase with H_2O_2 forms intermediate compounds which have different spectral properties. Today there are known 4 types of such compounds. According to Chance the process is going on as follows:

peroxidase Fe^{3+}+ H_2O_2 – complex I
complex I + AH_2 – complex II+AH
complex II+AH_2+ – peroxidase F^{3+}+AH

Complex I is formed as soon as H_2O_2 is added. It is an unstable compound with green color which can be easily transformed into complex II. Complex II is formed from the excess amount of H_2O_2 and has red color. These compounds are not characterized by catalytic action. Their formation hinders the peroxidase action [3,4].

Today there are known 6 types of peroxidase:

1. Classical peroxidase (EC 1.11.1.7.)
2. NADH peroxidase (EC 1.11.1.1)
3. NADH peroxidase (EC 1.11.1.2)
4. Fatty-acid peroxidase (EC 1.11.1.3)
5. Glutathione peroxidase (EC. 1.11.1.9)
6. Cytochrome peroxidase (EC 1.11.1.5)

Peroxidase protects the cell from harmful action of H_2O_2 and actively participates in cell metabolism. It is mainly localized in mitochondria and chloroplast. Some researchers admit existence of peroxidase in ribosomes and nucleus. Peroxidase regulates the process of plant growth and development, because it is the part of the indoxylic enzyme system [3-5].

Polyphenol oxidase (EC 1.10.3.1.) contains copper, which has an influence on different o-diphenols. The enzyme activates molecular oxygen and oxidizes dioxyphenol (sometimes even the monophenols) [1]. Diphenoloxidases catalyze two strong reactions of different mechanisms: 1) o-dioxyphenol oxidation into o-chinon (i.e. "catechinoloxidase activity") and 2) monooxyphenol hydroxilization forming o-dioxyphenol (cresol action). The enzyme is widely spread in plants. It is the result of the polyphenol oxidase action when fruit and vegetable change color on air as soon as they are cut. The enzyme is easily

Table 1. Influence of microelements on the activity of peroxidase in tomato leaves and fruit (%)

Versions	Phase of 4-5 leaves appearing	Phase of 6-7 leaves appearing	Phase of blossoming	Phase of fruit appearing	Phase of mature fruit	Phase of technical ripening of fruit
Control	4.6±0.01	12.2±0.01	12.2±0.01	8.2±0.01	10.0±0.01	2.0±0.01
H_3BO_3	4.0±0.01	10.6±0.01	10.6±0.01	34.0±0.01	6.0±0.01	8.2±0.01
$ZnSO_4$	4.4±0.01	9.4±0.01	9.4±0.01	22.6±0.01	8.2±0.01	15.0±0.01
$CuSO_4$	1.0±0.01	6.8±0.01	6.8±0.01	15.0±0.01	10.0±0.01	6.8±0.01
$CoCL_2$	3.0±0.01	6.2±0.01	6.2±0.01	12.4±0.01	2.5±0.01	13.0±0.01
$KMnO_4$	14.8±0.01	11.4±0.01	11.4±0.01	16.8±0.01	4.8±0.01	10.0±0.01

solved in water. Therefore, it is not easy to define its localization in the cell. Supposedly, they occur in mitochondria and plastids, though some scientists have different view point, therefore, the question of its localization requires investigation and specification [6]. Microelements influence the enzyme activity [7].

Materials and Methods

The object of study was tomato species "market surprise" (Lycopersicum esculentum Mill), which is widespread in our conditions. Before sowing the tomato seeds were treated in 0.02%-solutions of $ZnSO_4$, $CuSO_4$, $CoCl_2$, $KMnO_4$, H_3BO_3. The control seeds were processed in distilled water. After drying the seeds were sown on an experimental plot. Its biochemical analysis on the content of microelements was preliminarily carried out. The material (the leaves of the middle layer) for analysis was taken at 12 o'clock of the day.

The activity of the enzyme peroxidase was defined in the acetone preparation obtained from the raw material.

The acetone preparation of 20 mg was placed in the test-tube and was added by 3.0 ml. m/15 phosphoric buffer (whose PH is equal to 6.0) 10 mg pyrogallol (solved in 1.0 ml water) and 1 ml 1.0 ml 1% 1.0% - H_2O_2. Reaction proceeded for 10 minutes. After that the solution was added by 5.0 ml ethyl alcohol for activation of the enzyme and then it was filtered. In the filtrate the optical density was defined by means of photo-electrometric colorimeter At 360 nm. The possible amount of the enzyme polyphenol oxidase was deducted from the received number. For that the test-tube was placed according to the above described general scheme of reaction in conditions of introduction of 1.0 ml water instead of 1% hydrogen oxide [1].

Results and Analysis

The results of the analysis are given in Tables 1 and 2.

In the phase of appearing 4-5 leaves the polyphenol oxidase activity in tomato leaves is greater than that of the peroxidase. In the next phase of vegetation of 6-7 leaves and in the phase of blooming the peroxidase activity increases exceeding the polyphenol oxidase activity. In the phase of fruit bearing the polyphenol oxidase activity increases again and in the phase of technical ripening of fruit the activity of both enzymes decreases that is related to the age of the plant. In the phase of vegtation of 4-5 leaves the greatest increase of the polyphenol oxidase activity is observed first in the plants treated with copper and then in those treated with cobalt compared to the control version. The action of boron (17.6) and zinc (17.4) equally increases the enzyme activity.

In case of boron the polyphenol oxidase activity is greater than that of the peroxidase. The peroxidase activity is observed to be decreased and the polyphenol oxidase activity increased in comparison with the control version. And in the phase of fruit bearing, on the contrary, the polyphenol oxidase activity is less decreased (12.5%) and the

Table 2. Influence of microelements on the activity of polyphenoloxidase in tomato leaves and fruit (%)

Versions	Phase of 4-5 leaves appearing	Phase of 6-7 leaves appearing	Phase of blossoming	Phase of fruit appearing	Phase of mature fruit	Phase of technical ripening of fruit
Control	12.4±0.01	4.2±0.01	4.0±0.01	16.0±0.01	0±0.01	1.9±0.01
H_3BO_3	17.6±0.01	2.8±0.01	9.6±0.01	14.0±0.01	0.4±0.01	1.6±0.01
$ZnSO_4$	17.4±0.01	8.0±0.01	8.8±0.01	10.4±0.01	4.0±0.01	1.0±0.01
$CuSO_4$	20.4±0.01	26.8±0.01	13.0±0.01	10.0±0.01	6.0±0.01	8.2±0.01
$CoCL_2$	18.4±0.01	32.0±0.01	0±0.01	0.8±0.01	2.0±0.01	15.0±0.01
$KMnO_4$	13.4±0.01	1.6±0.01	14.0±0.01	8.40±0.01	5.0±0.01	2.4±0.01

peroxidase activity is 4-times increased. In the phase of technical ripening of fruit the polyphenol oxidase activity is greater than that of the peroxidase. In the late phases of vegetation the peroxidase activity increases more than that of the polyphenol oxidase in the zinc version. As it was in case of the boron action, in those phases the peroxidase activity is greater than that of the polyphenol oxidase (the latter decreases in comparison with the control group).

Copper treatment greatly increases the activity of polyphenol oxidase. Also, copper has positive action on the peroxidase activity in the late phases of vegetation. With this respect the plants treated with copper significantly differ from the control group.

Cobalt increases the polyphenol oxidase activity in the phase of vegetation of 6-7 leaves, while the activity of the enzyme is zero in the phase of blooming. In cobalt version the activity of polyphenol oxidase is greatest among the other versions.

Manganum effect on the activity of the enzymes under study is revealed in the later phases. The peroxidase activity is more increased.

In the immature fruit of tomato the polyphenol oxidase activity is less than that of the peroxidase. In the boron version the polyphenol oxidase activity is decreased (0.4), and the peroxidase activity is also reduced. The zinc and copper treatment increases polyphenol oxidase activity in tomatoes. Unlike the other microelements in manganum version the activity of both enzymes is similar.

Conclusion

The activity of enzymes varies in different phases of vegetation of the plant under study. It can be explained by the fact that tomato plant bear technically and physiologically ripe fruit together with crude fruit and the buds and blossoms. In both versions (the controlled one and the one with microelements) with the increase of peroxidase activity the polyphenol oxidase activity decreases.

In comparison to the control versions in the phase of vegetation of 4-5 leaves the polyphenol oxidase activity is greatest under the action of copper, then comes cobalt, while boron and zinc action on the enzyme activity is similar. Under the action of boron the polyphenol oxidase activity is especially increased in the phase of appearing 4-5 leaves, while in later phases it is decreased and the peroxidase activity changes accordingly.

On presowing treatment of seeds with zinc the peroxidase activity in leaves (early phase of vegetation) and fruit decreases, while on vegetation of the fruit it reaches its maximum.

Under the action of copper and cobalt the peroxidase activity increases in leaves (late phase) and fruit.

Manganum has lesser effect on the activity of polyphenol oxidase in the earlier phase than in the later phase. In tomato fruit the activity of polyphenol oxidase increases and that of the peroxidase decreases.

მცენარეთა ფიზიოლოგია

მიკროელემენტების გავლენა პეროქსიდაზას და პოლიფენოლოქსიდაზას აქტიურობაზე პომიდვრის ფოთლებსა და ნაყოფში

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შესწავლილია მიკროელემენტების (B, Zn, Cu, Co, Mn) გავლენა ფერმენტების- პეროქსიდაზას და პოლიფენოლოქსიდაზას აქტიურობაზე პომიდვრის ფოთლებსა და ნაყოფებში. ფერმენტთა აქტიურობა საკვლევი მცენარის ვეგეტაციის სხვადასხვა ფაზაში ცვალებადობს. ეს მოვლენა შეიძლება იმითაც აიხსნას, რომ პომიდორში ერთი და იმავე დროს მცენარეზე არის ტექნიკური, ფიზიოლოგიური სიმწიფის და ახლადგამონასკვული ნაყოფი, ასევე კოკრები და ყვავილები. როგორც საკონტროლო ისე მიკროელემენტებიან ვარიანტებში პეროქსიდაზას მეტი მოქმედებისას პოლიფენოლოქსიდაზას ნაკლები აქტიურობა ვლინდება. საკონტროლო ვარიანტებთან შედარებით, 4-5 ფოთლის გამოღების ფაზაში პოლიფენოლოქსიდაზას აქტიურობა ყველაზე მეტად სპილენძით, შემდეგ კობალტით დამუშავებისას გაიზარდა, ბორის და თუთიის მოქმედება კი თითქმის ერთნაირად ზრდის ფერმენტის აქტიურობას. ბორის მოქმედებით პოლიფენოლოქსიდაზას აქტიურობა განსაკუთრებით იზრდება 4-5 ფოთლის გამოღებისას, გვიან ფაზაში კი მცირდება, შესაბამისად იცვლება პეროქსიდაზას აქტიურობა. თუთიით თესლების თესვისწინა დამუშავებისას პეროქსიდაზას აქტიურობა პომიდვრის ფოთლებსა (ვეგეტაციის ადრეულ ფაზაში) და ნაყოფში მცირდება, ნაყოფის გამოღებისას კი მაქსიმუმს აღწევს. სპილენძის და კობალტის გავლენით პეროქსიდაზას აქტიურობა დიდდება პომიდვრის ნაყოფში, ფოთლებში კი იზრდება გვიან ფაზაში. მანგანუმის მოქმედება პოლიფენოლოქსიდაზას აქტიურობაზე ვეგეტაციის ადრეულ ფაზაში უფრო ნაკლებია, ვიდრე გვიან ფაზაში. პომიდვრის ნაყოფში პოლიფენოლოქსიდაზას აქტიურობა იზრდება, პეროქსიდაზას აქტიურობა კი მცირდება.

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