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THE EFFECT OF MICROELEMENTS ON PEROXIDASE AND POLYPHENOLOXIDASE ACTIVITY IN PEPPER LEAVES AND FRUITS

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The paper deals with the impact of micro elements (B, Zn, Cu, Co, Mn) on the activity of ferments – peroxidase and polyphenoloxidase in th eleaves and fruit of pepper (Capsicum annum). Enzyme activity varies according to different vegetative phases of a research plant. Both in the control and microelement variants during higher activity of peroxidase, polyphenoloxidase activity is rather low. Due to boron activity polyphenoloxidase activity particularly increases when 4-5 leaves appear, in later phases it decreases. While treating seeds with zinc peroxidase activity decreases in pepper leaves in the early phase of vegetation and fruits, and even reaches a maximum when fruits start to appear. Copper and cobalt increase peroxidase activity in the leaves and fruits in the late phase. Manganese effect on polyphenoloxidase activity in the early phase of vegetation is lower than in the later phase. In pepper fruits polyphenoloxidase activity increases, while peroxidase activity is reduced.

INTRODUCTION

Micronutrients are essential for normal metabolism in living organisms, they are connected with vitamins, hormones, enzymes, and therefore play an important role in the growth and development of organisms [1].

The source of micronutrients is the chemical elements of mother-rock in soil, as well as gases, meteoric precipitation, smoke, volcanos.Microelements are found in the hard phase particles and surface colloids.The content of microelements in soil in the vegetation period is dynamic, e.g. manganese, copper, and iron content is high in spring, when humidity is high and anaerobic processes are under way. Insummer the available form of these elements for plants reduces. In different types of soils the content of microelements is different, e.g. zinc accumulates in meadow and podzolic gley soils, while in dark gley soils it is in less quantities, copper accumulates in irrigated and gley soils, cobalt is not characterized by the differentiation according to soil types. Manganese and molybdenum content in the deeper layers of the soil decreases.

Provision of plants with micronutrients in the form of microfertilizers improves its growth, increases productivity. However, microelements differently influence different species (varieties) of plants, moreover, the same microelement different influences the same plant in a different phase of vegetation, so it is necessary to determine the exact dose according to soil types.

Plants can be provided with microelements when their amount does not exceed a certain level, for example, Mn - 40,0 Mo -0, 20, Co-1,5 Zn -0,3, B -0,5 mg / kg.

Microlements may be provided in three ways: 1) through root system by application of fertilizers in the soil, 2) by spraying weak solution on leaves, 3) pre-sowing treatment of seeds with microelements salt solution. Pre-sowing treatment of seeds is more effective to study the influence of microelements, as in this case, the seeds best absorb all the minerals, fetal growth starts early, metabolism becomes more active, microelements influence the new cells formedafter the division of embryonic cells [2].

We studied the effect of microelements (B, Zn, Cu, Co, Mn) on the activity of ferments - peroxidase and polyphenoloxidase on pepper leaves and fruits. Peroxidase (f.c.1.11.1.7) is one of the respiratory enzymes, it belongs to a group oxidoreductase. Peroxidase includes iron-porphyrine IX []. The enzyme causes the oxidation of some of organic compounds: phenols, amines, heterocyclic compounds, etc.by hydrogen peroxide (3).

Donor + H_2O_2 = oxidized donor+ H_2O

Unlike catalase peroxidase does not oxidize alcohol and hydrogen peroxide, under catalase activity H_2O_2 dissolves into molecular oxygen and water, while the peroxidase restores H_2O_2.

Peroxidase in interaction with H_2O_2 creates intermediate compounds which have different spectral properties. Weknow 4 types of such a complex. According to Chans the process is as follows:

Peroxidase Fe3 ++ H_2O_2 - complex I

Complex I + AH_2- Complex II + AH

Complex II + AH_2 + - peroxidase F3 ++ AH

The complex is formed immediately after adding H_2O_2, is unstable compound, colour is green, it is quickly transformed into a complex II. The complex is formed by the excess quantity of H_2O_2- and has a red color, these compounds tend to have catalytic activity, their formation causes a delay in the action of peroxidation [3,4].

Today we know 6 types of peroxidase :

1. Classic peroxidase (f.c. 1.11.1.7.)
2. NAD Peroxidase (f.c.1.11.1.1)
3. NADP peroxidase (f.c.1.11.1.2)
4. fatty acids peroxidase (f.c. 1.11.1.3)
5. glutationperoxidase (f.c. 1.11.1.9)
6. citochromeperoxidase (f.c.1.11.1.5)

Peroxidase protects cells from the harmful effects of H_2O_2, is actively involved in the metabolism of a cell. It is localized mainly in mitochondrias and chloroplasts, some scholars acknowledge the presence of peroxidase in ribosomes and core. Peroxidase regulates plant growth process, because it is part of the indloxidaseenzyme system [3-5].

Polyphenoloxidase (f.c .1.10.3.1.) contains copper, influences a variety of O-diphenols. The enzyme activates molecular oxygen and oxydizes dioxiphenol, (sometimes even monophenols) (1). Diphenol oxidase catalyzes the reaction of the two very different mechanisms: 1) oxidation of dioxiphenol into O-chynon (so-called "catecholoxidase activity") and 2) monooxiphenolhydroxylation forming dioxiphenol (crezol activity). The enzyme is widely distributed in plants, fruits and vegetables. Quick coloring after cutting a fruit and vegetables is the result of polyphenoloxidase activity on the air. The enzyme is easily soluble in water and therefore it is difficult to determine its localization in cells, it is supposed that it is found in mitochondrias and plastides, although some researchers have a different opinion, therefore, the localization of the enzyme has to be studied and specified [6]. Microelements affect the activity of enzymes [7].

OBJECTIVES AND METHODS

The research material was widespread pepper (Capsicum annum L.). Pepper seeds variety "megruli" were processed in 0, 02% solution of $ZnSO_4$, $CuSO_4$, CoCl2, $KMnO_4$, H_3BO_3 for 24 hours before sowing, the control seeds were treated with distilled water. After drying seeds were sown on an experimental plot, the biochemical analysis of which on microelements had been done in advance. Testing material samples were collected at noon, the observation was done on the middle tier leaves.

Peroxidase enzyme activity was determined in the acetone preparation received from raw materials.

20 mg acetone preparation was placed in a tube and 3.0 ml M / 15 phosphate buffer was added, the ph of which is equal to 6,0, besides 10 mg of pirogalol dissolved in 1.0 ml. water and 1.0 ml 1,0% H2O2 was also added. The reaction lasts for 10 minutes. In this lapse of time to inactivate the enzyme, 5.0 ml. Ethyl alcoholis added and is filtered. Fin the filtrate with the photoelectric colorimeter on 360 nm optical density is determined. Then the possible value of polyphenoloxidase activity was taken from the received figure, for this, a special tube is placed according to the above reaction scheme, and instead of 1.0 ml 1% hydrogen peroxide 1.0 ml water is carried in [1].

RESULTS AND ANALYSIS

The results are shown in Tables 1 and 2 .

At the stage of 4-5- leaf appearance polyphenoloxidase activity is higher than peroxidase, later when 6-7 leaves appear and blooming starts, peroxidase activity increases and exceeds polyphenoloxidase activity.During fruiting polyphenoloxidase activity grows again, during technical ripeness activity of both enzymes is reduced, which is related to the age of the plant.

In comparison with control variants, at the 4-5 leaf stage polyphenoloxidase activity increased most when treated with copper, then with cobalt. As for boron (20.6) and zinc (20.4), they almost uniformly increased the activity of the enzyme.

In boron variant polyphenoloxidase activity prevails peroxidase activity, peroxidase activity decreased in comparison with the control variants, while polyphenoloxidase activity increased. Duringfruiting polyphenoloxidase activity showed less decline (12.5%), while the peroxidase activity increased nearly 4 fold. During technical ripeness polyphenoloxidase activity prevails peroxidase activity.

In the late phases of vegetation in zinc variants the peroxidase activity increases more than polyphenoloxidase activity, in the above phases, like in boron variant, peroxidase activity prevails polyphenoloxidase activity (in comparison with the control variants the activity of the latter decreases).

Copper processing particularly increases polyphenoloxidase activity, in the late phases of vegetation it has positive effects on peroxidase activity. Copper variant plants significantly differ from the control plants in this respect

Cobalt processing increases polyphenoloxidase activity at the 6-7-leaf stage, while during blooming the enzyme activity is close to zero. Peroxidase activity was higher in cobalt variant than in all the other variants.

The effectiveness of manganese on the research enzymes' activity revealed in later phases, this time peroxidase activity increases.

In immature pepper fruits polyphenoloxidase activity is less than peroxidase activity. In boron variant polyphenoloxidase activity is less (0.7), peroxidase activity is also reduced. Zinc and copper processing increased polyphenoloxidase activity in pepper fruits. Unlike other variants, in the manganese variant the activity of both enzymes is approximately the same.

CONCLUSION

Enzyme activity varies in different phases of plant vegetation. This can be explained by the fact that on the pepper plant there is technically, physiologically ripe and newly appeared fruits, buds and flowers at the same time. Both in control and microelement variants durin increased activity of peroxidase less activity of polyphenoloxidase occurs.

In comparison with control variants, at the 4-5 leaf stage polyphenoloxidase activity increased most when treated with copper, then with cobalt. As for boron and zinc , they almost uniformly increased the activity of the enzyme.

In boron variant polyphenoloxidase activity particularly increases at the 4-5 leaf stage, in later phases it decreases. Peroxidase activity changes correspondingly.

In the early phases of vegetationin zinc variants the peroxidase activity decreases in leaves and pepper fruits, while during fruiting it reaches maximum.

Copper and cobalt increase the peroxidase activity in leaves in the late phase and fruits.

The effect of manganeseon polyphenoloxidaseactivity in the early phase of vegetation is lower than in the late phase, polyphenoloxidaseactivity in pepper fruits increases, while peroxidase activity decreases.

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ВЛИЯНИЕ МИКРОЭЛЕМЕНТОВ НА АКТИВНОСТЬ ПЕРОКСИДАЗЫ И ПОЛИФЕНОЛОКСИДАЗЫ В ЛИСТЬЯХ И ПЛОДАХ ОСТРОГО ПЕРЦА

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Изучено влияние микроэлеменентов (B, Zn, Cu, Co, Mn) на активность пероксидазы и полифенолоксидазы в листьях и плодах острого перца. Активность ферментов в разных фазах вегетации меняется. При повышенной активности пероксидазы выявляется пониженная активность полифенолоксидазы. При сравнении с контрольным вариантом в фазе появления 4-5 листа увеличение активности полифенолоксидазы больше всех отмечается при обработки растении медью, а потом кобальтом. Бор и цинк на увеличение активности ферментов действуют аналогично. Бор увеличивает активность полифенолоксидазы, особенно при появлении 4-5 листа, а в поздней фазе вегетации уменьшает, при этом меняется активтость пероксидазы. При использовании Zn при обработке семян перед посевом активность пероксидазы в листьях и плодах перца в ранней фазе вегетации уменьшается, а при появлении плодов достигает максимума. Влиянием кобальта и меди активность пероксидазы в плодах и в листьях в поздней фазе увеличивается. В ранней фазе вегетации действие марганца на активность полифенолоксидазы меньше, чем в поздней фазе. В плодах перца активность полифенолоксидазы увеличивается, а активность пероксидази уменьшается.

Table 1. *The effect of microelements on the activity of polyphenoloxidase in pepper leaves and fruits, (%)*

Variants	4-5 –leaf stage	6-7-leaf stage	blooming	fruiting	Immature fruits	Stage of technical ripening
Control	15.4±0.01	7.2±0.01	7.0±0.01	18.0±0.01	0±0.01	3.9±0.01
H_3BO_3	20.6±0.01	5.8±0.01	12.6±0.01	17.0±0.01	0.4±0.01	3.6±0.01
$ZnSO_4$	20.4±0.01	11.0±0.01	11.8±0.01	13.4±0.01	7.0±0.01	3.0±0.01
$CuSO_4$	23.4±0.01	29.8±0.01	15.0±0.01	13.0±0.01	9.0±0.01	11.2±0.01
$CoCL_2$	21.4±0.01	35.0±0.01	0±0.01	0.8±0.01	5.0±0.01	18.0±0.01
$KMnO_4$	16.4±0.01	3.6±0.01	17.0±0.01	11.40±0.01	8.0±0.01	5.4±0.01

Table 2. *The effect of microelements on the activity of peroxidase in pepper leaves and fruits, (%)*

Variants	4-5 –leaf stage	6-7-leaf stage	blooming	fruiting	Immature fruits	Stage of technical ripening
Control	7.6±0.01	15.2±0.01	13,8±0.01	112±0.01	13.0±0.01	5.0±0.01
H_3BO_3	7.0±0.01	13.6±0.01	9.4±0.01	37.0±0.01	9.0±0.01	112±0.01
$ZnSO_4$	7.4±0.01	12.4±0.01	7.0±0.01	25.6±0.01	11.2±0.01	18.0±0.01
$CuSO_4$	30±0.01	9.8±0.01	11.8±0.01	18.0±0.01	13.0±0.01	98±0.01
$CoCL_2$	60±0.01	9.2±0.01	18.6±0.01	15.4±0.01	5.5±0.01	16.0±0.01
$KMnO_4$	17.8±0.01	14.4±0.01	11.8±0.01	18.8±0.01	7.8±0.01	130±0.01