BIOLOGIA PLANTARUM (PRAHA)

9 (5) : 383—391, 1967

The Effect of Various Microelements on Changes in the Activity of Certain Enzymes in Hydroponically Cultivated Barley During the First Period of Growth

J. PAVEL and JIŘINA ŽÁKOVÁ

Department of Biochemistry, University of Agriculture, Brno*

Received January 26, 1967

Abstract. The paper deals with the effect of the application of certain ions (Zn^{2+}, $B_4O_7^{2-}$, Mn^{2+}, Cu^{2+}, MoO_4^{2-}, Co^{2+}) on the development of the activity displayed by amylase, urease, and glutamate-oxalacetate (GOT) in hydroponically cultivated barley during the first seven days of ontogenesis. The enzymes were studied separately in the overground and root parts of the plants. The following main conclusions can be drawn from the findings made:

1) The amylase activity of the control plants in connection with their development diminished slightly in the overground part, while it was relatively constant in the roots. Due to the effect of certain elements tested, this activity increased pronouncedly (significant differences were confirmed in variants containing MoO_4^{2-}, Zn^{2+}, and Co^{2+}).

2) The urease level in the overground part of the control plants remained low throughout the entire experimental period and was only little influenced by the individual microelements under study (with the exception of $B_4O_7^{2-}$). On the other hand, the urease activity in the root system increased markedly, the $B_4O_7^{2-}$ ions exhibiting a significantly stimulating effect.

3) The activity of glutamate-oxalacetate transaminase (GOT) in the overground part of the control plants increased only slightly in the course of the initial development, while in the root system it grew relatively more pronouncedly. In the overground part of the plants all the microelements administered displayed a significantly depressive effect on the development of this enzyme activity; in the roots, the inhibitory action was maintained only in the last experimental period in variants with Zn^{2+} ions.

The stimulating activity of microelements results primarily from their ability to form structural or functional components of enzymes. Seen from this aspect, it is bivalent cations that are significant. These cations fulfil the function of a free bond between enzyme and protein and are able to interact and fulfil biochemically identical functions (PEYVE, 1960).

SHKOLNIK (1958) reports that manganese, zinc, molybdenum, and copper activate oxireduction enzymes in plants. Zinc in cotton plants, in addition to polyphenoloxidase and peroxidase, increases the activity of lipase, protease, and invertase (KUSTOVA, 1964). The effect of copper on the action of protease, amylase, lipase, peroxidase and on the content of proteins, sugars, and fats was studied by OKUNCOV and RONZHINA (SHKOLNIK, 1959). AGARWALA,

* *Address*: Brno, Zemědělská 1.

SHARMA and KUMAR (1964) followed the effect of combined doses of manganese and copper on the activity of enzymes of the Fe-protein type in barley plants, RATNER and AKIMOCZKINA (1964) studied the interaction of the effect produced by molybdenum and certain hydrophilous vitamins on the activity of nitrate reductase in soya beans, etc.

Other studies concerned the influence of ions on the activity of urease and glutamate-oxalacetate transaminase (GOT). SUMNER (1951),f or instance, reported that urease was inactivated by Cu^{2+}, while DOBY, 1965, demonstrated that Co^{2+} was also responsible for this action. GOT wasr eported to be activated by iron compounds (SANWAL, ZINK and DIN, 1964).

Besides the changes in the systems of these enzymes, the action of microelements manifests itself also in other connections, e.g. by an increase of germinating energy, photosynthesis, respiratory intensity, elevated synthesis of chlorophyll, carotene and vitamins, improved glycide exchange and also by action on the synthesis of amino acids and proteins through intensive nitrogen assimilation (DOBROLUBSKY 1958; PEYVE 1960; BERZIN 1961).

The wide importance of microelements for plants, which is but relatively little elucidated as regards their action on the activity of amylase, urease, and GOT transaminase, i.e. enzymes linked with nitrogen-glycide metabolism of plants, is evident. The present paper represents a contribution to existing data.

Material and Methods

The experiments were performed on a hydroponically cultivated barley (*Hordeum sativum* L.) cv. Slovenský Dunajský trh. During cultivation the procedure suggested by the Institute of Microbiology, Czechoslovak Academy of Sciences, Třeboň (ÚVTI, 1963) after previous administration of microelements was applied. The control seeds (120 g) were steeped only in distilled water, the experimental seeds in solutions of $MnSO_4$ (0·025%), $Na_2B_4O_7$ (0·025%), $ZnSO_4$ (0·05%), $(NH_4)_2MoO_4$ (0·01%), $CuSO_4$ (0·025%) and $CoSO_4$ (0·05%), i.e. in concentrations recommended by Abutalybov et al. (1964) for stimulating protein synthesis in wheat and cotton. All variants were steeped for a period of twenty hours. During 4-day germination in the dark, the seeds were moistened with an automatic sprinkler twice a day as needed. The germinated grains were illuminated for seven days running with fluorescent tubes and lamps (6.000 ergs. cm^{-2}. sec^{-1}) at 24 to 26° C and 70 to 80 percent relative humidity. The roots of the plants were irrigated every day for one hour with Knop's nutrient solution devoid of microelements. For the control plants, a complete Knop's solution containing microelements was used.

After the first, fourth and seventh day of illumination, samples were taken for analyses from 400 plants, whose overground and root parts were treated separately. During the entire experimental period the growth was fresh, deep green, and had reached a mean height of 15 cm when the last sample was taken. The extracts for estimation of the enzymatic activity were obtain-

ed by triturating the plant material with fine silica sand and adding a buffer solution. Thus we tried to eliminate the possible influence of heat when using another method of homogenization.

The activity of the enzymes studied was determined by the photometric technique. Amylase activity was estimated according to the method of HOŘEJŠÍ et al. (1964); its units correspond to the amount of enzyme which under the conditions of the method hydrolyzes 10 mg out of the 60 mg of starch added. The resultant values are presented, converted to 1 g of dry matter.

Transaminase activity was established according to REITMAN and FRANKEL (1957) and is expressed in μmol of pyruvate per hour per 1 g dry matter at 37° C.

Urease activity was determined according to HOFFMANN (1962), its units corresponding to mg of ammonia nitrogen split off from the substrate (urea) and also converted to the unit of dry matter (1 g).

The results obtained were treated by variation-statistical methods.

Results

The experimental values obtained by analysis of succesive samples of material from the overground and root parts of the hydroponically cultivated barley variants were graphically treated and the significance of the effect produced by the individual microelements was defined by the method of variance analysis (Table 1).

The dynamics of the changes in amylase activity in the course of the experimental stages (Fig. 1) in comparison with the controls, shows a stimulating effect of the bulk of the tested microelements in the concentrations used, yet significantly higher values were recorded in the overground part only when MoO_4^{2-} was applied after the fourth and the seventh day of illumination. In the roots, significant changes appeared, on the one hand, in the initial growth phase of the variants with Co^{2+}; on the other hand, after the fourth and the seventh day of illumination in the variants containing Zn^{2+} (Table 1).

The inhibitive effect of the Cu^{2+} concentration in the roots, conspicuous especially after seven days of illumination, was statistically insignificant.

If we summarize the influence of the individually tested microelements on amylase activity, we can see that the most pronounced stimulative effects, confirmed by the significant differences in the overground part with MoO_4^{2-}, in the roots with Zn^{2+} and Co^{2+}, occur, for the most part, in the period between the fourth and the seventh day of illumination, i.e. not until a certain more advanced development stage of the plants is reached.

The mean activity of urease in the overground and root parts is illustrated in Fig. 2 according to the tested variants and experimental periods. While in the overground part no changes in the level of this enzyme appeared as significant after the application of the tested microelements (with the exception of $B_4O_7^{2-}$ after the first and the seventh day of illumination — Table 1), a sharp increase occurred in the roots from the first to the seventh

day, particularly evident in the control plants. The variants with Co^{2+}, MoO_4^{2-}, and Cu^{2+} ions displayed in the root system a diminished mean activity of the studied enzyme.

Also the Zn^{2+} and Mn^{2+} ions exhibited a similar effect. In all these cases the influence was not statistically significant.

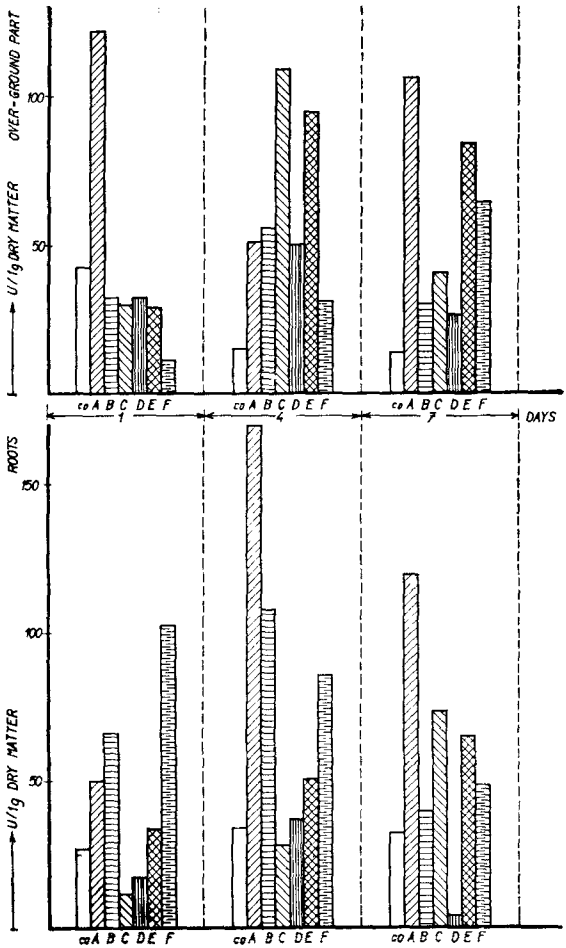

Fig. 1. Amylase activity in hydroponically cultivated barley plants (*Hordeum sativum*); 1, 4, 7 — days of illumination; co — control, A — Zn^{2+}, B — $B_3O_7^{2-}$, C — Mn^{2+}, D — Cu^{2+}, E — MoO_4^{2-}, F — Co^{2+}

Weighty changes in urease activity were also caused by the application of $B_4O_7^{2-}$. Of all the microelements, only boron (despite the decrease in the urease level on the seventh day of illumination) in the given concentration exhibited a significant stimulative effect in the root system in the course of the entire experimental period (Table 1). If we also take into consideration

the identical action of this microelement in the overground part, we can regard the influence of $B_4O_7^{2-}$ on urease activity as most significant.

The last enzyme to be studied was glutamate-oxalacetate (GOT) transaminase, the behaviour of which in the individual parts of the experimental plants is presented in mean values in Fig. 3. The changes in GOT activity in the overground parts of the control plants are relatively low during the first to the seventh day. In all variants with microelements, transaminase activity was lower than in the controls. It goes without saying that there exist differences in the degree of quantitative influence on the GOT level according to the individual microelements tested, but their effect is, for the most part, significantly repressive. An exception is the application of MoO_4^{2-}

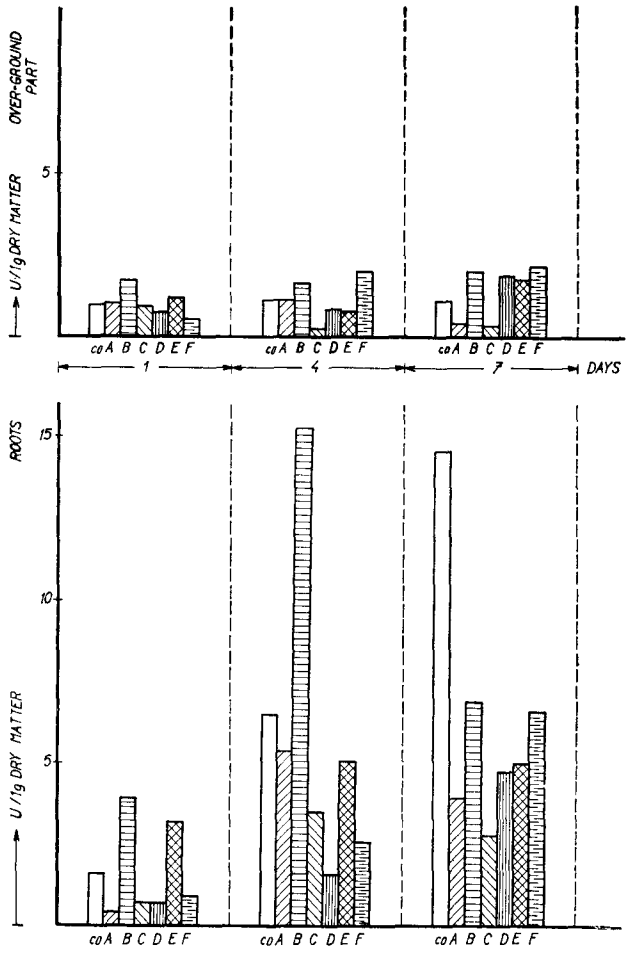

Fig. 2. Urease activity in hydroponically cultivated barley plants (*Hordeum sativum*); 1, 4, 7 — days of illumination; co — control, A — Zn^{2+}, B — $B_4O_7^{2-}$, C — Mn^{2+}, D — Cu^{2+}, E — MoO_4^{2}, F — Co^{2+}.

for the first and the fourth day of illumination, when the recorded diminution lies within the limits of experimental error; therefore the values found in the individual cases can be considered identical with the activities displayed by the control plants.

Transaminase activity in the roots exhibits the same tendency of change as urease. The level of this enzyme rises here with a delay in the time of growth, the highest values being registered again in the control plants after seven days of illumination. The values of the activity of this enzyme upon the application of MoO_4^{2-} and Co^{2+} after the first day of illumination, even though they are rather higher as comppared with the controls, show no significant difference. The only significant influence (diminution) was confirmed in the Zn^{2+} — treated variants after seven days of illumination.

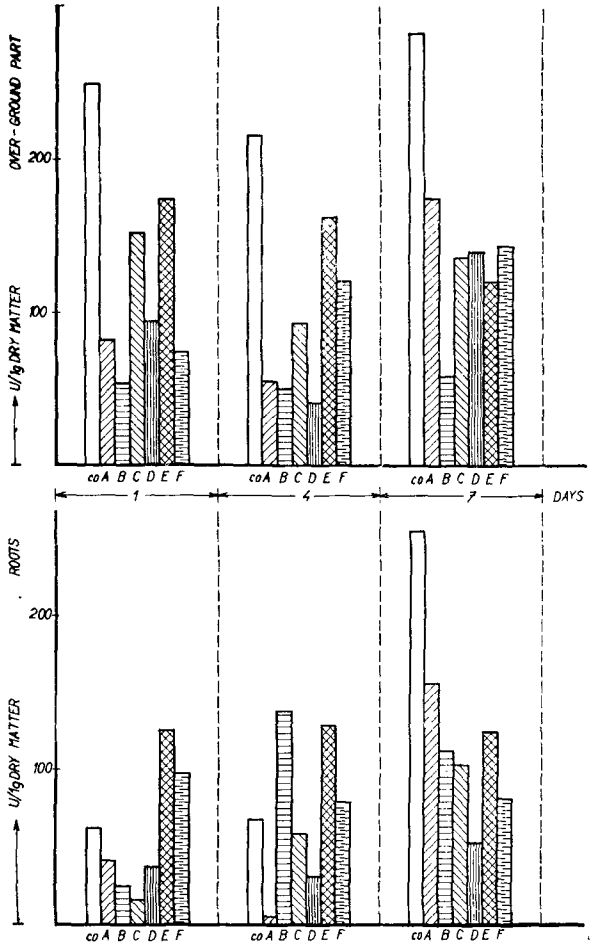

Fig. 3. Glutamate-oxalacetate transaminase activity in hydroponically cultivated barley plants (*Hordeum sativum*); 1, 4, 7 — days of illumination; co — control, A — Zn^{2+}, B — $B_4O_7^{2-}$, C — Mn^{2+}, D — Cu^{2+}, E — MoO_4^{2-}, F — Co^{2+}

Table 1a

Significance of influence of microelements on the activity of enzymes in the overground part of barley *(Hordeum sativum)* defined by variance analysis

Micro-elements	Amylase			Urease			GOT		
	Day of illumination								
	1	4	7	1	4	7	1	4	7
Zn^{2+}	−	−	−	−	−	−	+	+	+
$B_4O_7^{2-}$	−	−	−	+	−	+	+	+	+
Mn^{2+}	−	−	−	−	−	−	+	+	+
Cu^{2+}	−	−	−	−	−	−	+	+	+
MoO_4^{2-}	−	+	+	−	−	−	−	−	+
Co^{2+}	−	−	−	−	−	−	+	+	+

Table 1b

Significance of influence of microelements on the activity of enzymes in the roots of barley *(Hordeum sativum)* defined by variance analysis

Micro-elements	Amylase			Urease			GOT		
	Day of illumination								
	1	4	7	1	4	7	1	4	7
Zn^{2+}	−	+	+	−	−	−	−	−	+
$B_4O_7^{2-}$	−	−	−	+	+	+	−	−	−
Mn^{2+}	−	−	−	−	−	−	−	−	−
Cu^{2+}	−	−	−	−	−	−	−	−	−
MoO_4^{2-}	−	−	−	−	−	−	−	−	−
Co^{2+}	+	−	−	−	−	−	−	−	−

The conclusions can still be supplemented by an analysis of the coincidence of the tendencies mentioned with the findings of the authors cited in the introductory part of this paper. As SUMNER (1951) demonstrated, the depressive effect of Cu^{2+} ions on the intensity of urease activity manifested itself in the experiments markedly only in the roots. The same effect is attributed, besides Cu^{2+} ions, by DOBY (1965) to Co^{2+} ions as well. It was found that in our case the effect of this microelement manifested itself repressively again only in the roots. In the overground parts of the experimental plants, urease activity after the application of the ions mentioned in the more advanced development stage was even higher than in the controls.

On the basis of the results achieved it would be possible to widen, for example, the conclusions arrived at by KUSTOVA (1964) about the stimulating effect of zinc on the activity of certain enzymes and about the demonstrated effect of this element on stimulating the activity of amylase and/or to draw further analogies, etc. On the whole it can still be added that our conclusions are typical, above all, for the chosen experimental conditions, i.e. for the hydroponic method of cultivation with a given concentration of the tested microelements and for a given development stage of the plants under study.

References

Agarwala, S. C., Sharma, C. P., Kumar, A.: Interrelation of iron and manganese supply in growth, chlorophyll and iron porphyrin enzymes in barley plants. — Plant. Physiol. **39** : : 603—609, 1964.

Doby, G.: Plant Biochemistry. Budapest, 426—428, 1965.

Hoffmann, E.: in: Bergmeyer, H. U. (ed.): Methoden der enzymatischen Analyse. Pp. 913—916, Weinheim, 1962.

Hořejší, J. et al.: Zák ady chemického vyšetřování v lékařství. [The Fundamentals of Chemical Investigation in Medicine] — VIth edition, Praha 1964.

Reitman, S. and Frankel, S.: A colorimetric method for the determination of serum glutamic--oxalacetic and glutamic-pyruvic transaminases. — Amer. J. Chem. Path. **28** : 56, 1957.

Sanwal, B. D., Zink, M. W. and Din, G.: in Peach, K., Tracey, M. V. (ed.): Moderne Methoden der Pflanzenanalyse. 361—391, Springer-Verlag, Berlin-Göttingen-Heidelberg, 1964.

Sumner, J. B.; in: Sumner, J. B. and Myrbäck, (ed.): The Enzymes. Pp. 873—892, New York, 1951.

Ústav vědeckotechnických informací MZLVH: Současný stav hydroponického pěstování zeleniny a píce. [Institute of Scientifico-Technical Information, Ministry of Agriculture, Forestry, and Water management: The Present State of Hydroponic Cultivation of Vegetables and Forage Plants.] 22—30, Praha, 1963; 30—54, Praha, 1964.

Абуталыбов, М. Г., Алиев, Д. А.: Влияние микроэлементов на отток сахаров из листьев и передвижение этих веществ в растительном организме. — Сб. Роль минеральных элементов в обмене веществ и продуктивности растений. 121—125, Москва, 1964. [Abutalybov, M. G., Alyev, D. A.: The influence of microelements on the abstraction of sugars from the leaves and the behaveour of these substances in the plant organism]

Берзинь, А. Я.: Влияние микроэлементов на содержание витаминов Б-группы в растениях. Сб.: Микроэлементы и урожай, 297—304, Рига, 1961. [Berzin, A. J.: The influence of microelements on the B-vitamin content in plants.]

Добролюбский, О. К.: Предпосевная обработка семян и опрыскивание посевов растворами микроэлементов. — Земледелие, 2 : 65—69, 1959. [Dobrolubsky, O. K.: Presowing prepara ion of seeds and spraying of seeds with solutions containing microelements.]

Кустова, А. Х.: Влияние цинка на биохимические процессы тонковолокнистого хлопчатника. — Изв. АН Туркм. ССР, сер. биол. 3 : 3—10, 1964. [Kustova, A.: The effect of zinc on bi chemical processes in thin-fibrous cotton plants]

Окунцов, М. М., Ронькжина, О. А. в: Школьник, М. Я. (изд): Применение микроэлементов в сельском хозяйстве и медицине. 26—32, Рига, 1959. [Okuncov, M. M., Ronzhina, O. J.: The application of microe ements in agriculture and medicine]

Пейве, Я. В.: Микроэлементы и ферменты 8—15, Рига, 1960. [Peyve, J. B.: Microelements and Enzymes]

Ратнер, Е. И., Акимочкина, Т. А.: Сопряженное действие молибдена и некоторых витаминов группы «Б» на продуктивность симбиотической азотфиксации в сои. — Агрохимия 4 : 59—68, 1964. [Ratner. E. I., Akimoczkina, T. A.: Composite action of molybdenum and certain B-Vitamins on the produktivity of symbiotic fixation of nitrogen in soya beans]

Школьник, М. Я.: Влияние микроэлементов на синтез и передвижение углеводов. — Физиология растений, 5 : 393—399, 1958. [Shkolnik, M. J.: The effect of microelements on the synthesis and behaviour of sugars]

J. Pavel a Jiřina Žáková, katedra biochemie agronomické fakulty VŠZ v Brně, Zemědělská 1: **Účinek různých mikroprvků na změny aktivity některých enzymů u hydroponicky pěstovaného ječmene v prvním údobí růstu.** — Biol. Plant. **9** : 383—391, 1967.

V práci je sledován účinek aplikace některých iontů (Zn^{2+}, $B_4O_7^{2-}$, Mn^{2+}, Cu^{2+}, MoO_4^{2-}, Co^{2+}) na rozvoj aktivity enzymů amylázy, ureázy a transminázy glutamátu-oxalacetátu (GOT) **u** hydroponicky pěstovaného ječmene po dobu prvních sedmi dnů ontogeneze.

Studované enzymy byly sledovány samostatně v nadzemní a v kořenové části rostlin. Z dosažených poznatků lze zdůraznit tyto hlavní závěry:

1. Aktivita amylázy kontrolních rostlin v souvislosti s jejich vývojem se v nadzemní části mírně snižovala, v kořenech byla poměrně stálá. Účinkem některých zkoušených iontů se její aktivita velmi pronikavě zvyšovala (průkazné rozdíly byly potvrzeny u variant s MoO_4^{2-}, Zn^{2+} a Co^{2+}).

2. Hladina ureázy v nadzemní části kontrolních rostlin se udržovala po celé pokusné období na nízké úrovni a byla jednotlivými testovanými ionty (s výjimkou $B_4O_7^{2-}$) pouze málo ovlivněna.

Naproti tomu v kořenovém systému se aktivita ureázy postupně silně zvyšovala, přičemž i zde ionty $B_4O_7^{2-}$ vykazovaly průkazný stimulační efekt.

3. Aktivita transaminázy kyseliny L-glutamové-oxaloctové (GOT) se v průběhu počátečního vývoje v nadzemní části kontrolních rostlin zyšovala pouze mírně, v kořenovém systému však její aktivita vzrůstala relativně mnohem pronikavěji. Účinek všech zkoušených mikroelementů v nadzemní části rostlin se projevil průkazně depresivně na rozvoj aktivity tohoto enzymu; v kořenech inhibiční působení zůstalo zachováno pouze v posledním pokusném období u variant s ionty Zn^{2+}.

Й. Павел, Йржина Жакова, Кафедра биохимии агрономического факультета Высшей сельскохозяйственной школы, Брно: **Воздействие разных микроэлементов на изменение активности некоторых энзимов у ячменя, выращиваемого с применением гидропонии, на первой фазе его роста.** — Biol. Plant. 9 : 383—391, 1967.

Приводятся результаты исследований воздействия применения некоторых ионов (Zn^{2+}, $B_4O_7^{2-}$, Mn^{2+}, Cu^{2+}, MoO_4^{2-}, Co^{2+}) на развитие активности энзимов амилазы, уреазы и трансаминазы глутамата-оксалацетата (GOT) у ячменя, выращиваемого с применением гидропонии в течение первых семи дней его онтогенеза. Изучаемые энзимы были исследованы отдельно в надземной части растения и в корнях. На основании полученных результатов можно сделать следующие выводы:

1) Активность амилазы у контрольных растений, в ходе их развития несколько понижалась в надземной части растения, в то время как в корнях она оставалась относительно постоянной. Воздействие некоторых ионов повышало активность амилазы. Значимые различия найдены для MoO_4^{2-}, Zn^{2+}, Co^{2+}.

2) Уреаза у контрольных растений в надземной части находилась в течение всего опыта на низком уровне и отдельные микроэлементы (кроме $B_4O_7^{2-}$) почти не оказали никакого действия, в корневой же системе, наоборот, активность уреазы постепенно сильно увеличивалась, причем и здесь ионы $B_4O_7^{2-}$ имели стимуляционный эффект.

3) Активность трансаминазы кислоты L-глутамовой-оксалуксусной (GOT) в ранних этапах развития растений повышалась в надземной части контрольных растений лишь умеренно, в корнях это повышение было гораздо более резким. Все микроэлементы действовали в надземной части подавляюще. В корнях ингибиция имела место только в конце развития у вариантов с Zn^{2+}.