ACTA AGROBOTANICA
Vol. XXIX, (1): 99-105, 1976

The activity of ascorbic acid and catechol oxidase, the rate of photosynthesis and respiration as related to plant organs, stage of development and copper supply

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(Received: July 19, 1975)

Abstract

Some experiments were performed to investigate the physiological role of copper in oat and sunflower and to recognize some effects of copper deficiency. Oat and sunflower plants were grown in pots on a peat soil under copper deficiency conditions (−Cu) or with the optimal copper supply (+Cu). In plants the following measurements were carried out: 1) the activity of ascorbic acid oxidase (AAO) and of catechol oxidase (PPO) in different plant organs and at different stages of plant development, 2) the activity and the rate of photosynthesis, 3) the activity of RuDP-carboxylase, 4) the intensity of plant respiration. The activity of AAO and of PPO, and also the rate and the activity of photosynthesis were significantly lower under conditions of copper deficiency. The activity of both discussed oxidases depended on: 1) the plant species, 2) plant organs, 3) stage of plant development. Copper deficiency caused decrease of the respiration intensity of sunflower leaves but it increased to some extent the respiration of oat tops. Obtained results are consistent with the earlier suggestion of the authors that the PPO activity in sunflower leaves could be a sensitive indicator of copper supply of the plants. farther experiments are in progress.

OBJECT OF INVESTIGATION

The main purpose of our experiments was to investigate: 1) are there any differences in the activity of the two copper-proteid oxidases (i.e. ascorbic acid oxidase and catechol oxidase) in different organs of plants and also in different stages of development of the plant, 2) is there any relationship between the activity of these enzymes, photosynthesis and respiration intensity in copper deficient or copper sufficient plants. These

* The abstract of this paper has been published in Abstracts of Papers Presented at the XII International Botanical Congress, Leningrad, July 3-10, 1975, s. 313.

problems are very important to recognize the role of copper-proteid oxidases and the biochemical functions of copper in plants; this subject is still discussed in the literature (Samorodova-Bianki 1971; Shkolnik 1974; Trojanowski 1963, 1964).

METHODS

Experiments have been started since 1973. The experimental plants: oat (*Avena sativa L.* cv. 'Przebój II') and sunflower (*Helianthus annuus L.* cv. 'Wiernik') were grown in pots under greenhouse conditions on a copper deficient peat soil. Plants were supplied properly with a mineral nutrient solution without or with copper (−Cu — control, +Cu — the optimal dose of $CuSO_4$). The same plants were used in earlier experiments of the authors (Ruszkowska, Łyszcz 1975a, b; Ruszkowska et al. 1975) and they were recognized as the plants very sensitive to copper deficiency and suitable for copper-proteid oxidases assay.

The ascorbic acid oxidase (AAO) activity was determined in homogenates of plant tissues by means of the titration method according to Ostrovskaya (1961). The activity of the enzyme was expressed as micromoles of ascorbic acid oxidized by 1 g of fresh weight of plants during 1 minute, at pH 6,1 and temp. 20-22°C.

The catechol oxidase (PPO) activity was determined in homogenates of plant tissues using the colorimetric method of Smith and Stotz (1949). Results were expressed as micromols of 2,6-di-chloroindophenol (of Eastman firm) oxidized by catechol oxidase contained in 1 g of fresh plant tissues during 1 minute at pH 7,5 and temp. 20-22°C.

The activity and the rate of photosynthesis were determined by using ^{14}C procedure. Results were expressed as a radioactivity of 1 g of dry matter of plant material.

RuDP-carboxylase activity was determined by means of ^{14}C procedure, according to the Björkman's method in modification of Bowes and Ogren (after Wojcieska 1973). Results were expressed as a radioactivity of 1 g of fresh weight.

Respiration of plant organs was determined by means of Infralyt apparatus (Junkalor — Dessau) operating according to the principle of absorption of infra-red rays by CO_2.

RESULTS

1. The activity of ascorbic acid (AAO) and catechol (PPO) oxidases in plants was in all cases significantly lower under conditions of copper deficiency than under optimal levels of this trace element (Table 1-2). These results confirmed the earlier findings of authors and other workers.

2. The activity of both discussed oxidases depended on the species of the plant. In oat plants the middle levels of AAO activity there were found (Table 1) but hardly any traces of PPO activity were observed (data not presented). It should be noticed that the PPO activity in oat plants was determined in discussed experiments at the stage of tillering, shooting, and after earing. At the same stages a marked activity of PPO was found in cereals by other authors (after Samorodova-Bianki 1971). This problem needs further experiments. On the other hand rather high PPO activity was stated in sunflower plants; besides the AAO activity was there several times higher than in oat plants (Table 2).

3. The activity of discussed oxidases depended on plant organs and on stage of the plant development.

Oat

A middle values of AAO activity was stated in oat leaves at the tillering stage; their activity decreased at the stage of shooting and earing

Table 1

The influence of copper on the ascorbic acid oxidase (AAO) activity in oat plants in $\mu mol \cdot min^{-1} \cdot g^{-1} \cdot 10^{-2}$ of fresh weight

Stage of development and plant organ	AAO activity	
	−Cu	+Cu
1. Tillering		
leaves	4.1	17.3
roots	14.6	21.2
2. Shooting		
leaves	0	0.4
culms	2.4	21.7
roots	10.3	20.9
3. After earing		
upper leaves	2.2	4.2
lower leaves	2.4	18.5
culms — upper part	2.8	3.5
culms — lower part	4.5	8.2
grain	— *	19.9
roots	7.1	20.7
4. Before ripening		
upper leaves	4.3	55.4
lower leaves	2.0	2.7
culms — upper part	1.2	1.3
culms — lower part	4.4	21.2
grain	— *	5.7
roots	9.8	27.7

* — no grain.

(Table 1). Later on the AAO activity increased rapidly in older leaves getting yellow (Table 1 — upper leaves, the stage before ripening). Even in half dead leaves it was stated some activity of this enzyme (Table 1 — lower leaves, the stage before ripening). The AAO activity was rather high in lower part of culms and in immature grains; during the ripening of grains it decreased. It should be noticed that in oat roots the AAO activity was high during the all examined stages of vegetation.

Sunflower

In the tops of sunflower the PPO activity increased with the development of the plants and it was high in all metabolically active centres (i.e. in young leaves, in upper parts of stems, in buds, and in immature seeds). In the roots the PPO activity was rather low during the all time of vegetation (Table 2). The AAO activity in sunflower tops was higher than in roots and it increased with the time of plant growth (Table 2).

4. A close relationship was found between the copper nutrition and photosynthesis (Table 3; data present measurements carried out on the youngest fully expanded leaves at different stages of plant development).

The rate of photosynthesis as well as the activity of RuDP-carboxy-

Table 2

The influence of copper on the catechol oxidase (PPO) and on the ascorbic acid oxidase (AAO) activity in sunflower plants

Stage of development and plant organ	PPO activity in $\mu mol \cdot min^{-1} \cdot g^{-1}$ of fresh weight		AAO activity in $\mu mol \cdot min^{-1} \cdot g^{-1} \cdot 10^{-2}$ of fresh weight	
	−Cu	+Cu	−Cu	+Cu
1. Stage of 6-8 leaves				
leaves	11.3	17.8	31	89
stems	3.2	20.6	10	80
roots	3.6	4.4	27	46
2. Before flowering				
leaves	6.2	24.0	61	139
stems	3.0	13.6	76	166
roots	3.4	6.5	33	54
3. Flowering				
upper leaves	2.0	45.2	90	262
lower leaves	2.8	16.3	58	136
stems — upper part	2.6	17.1	77	290
stems — lower part	0.9	8.7	18	129
buds	3.2	47.6	154	303
flowers	3.2	28.9	76	296
seeds	2.2	46.4	127	332
roots	1.4	6.6	35	111

lase were much higher in the oat plant leaves sufficiently supplied with copper in comparison to the copper deficient ones (Table 3).

In the leaves of young sunflower plants (8 leaves stage) in which symptoms of copper deficiency were hardly visible, no significant differences in the rate of photosynthesis and the activity of RuDP-carboxylase were found between −Cu and +Cu plants. At flowering, copper deficiency of leaves caused a decrease of the rate of photosynthesis but it increased the activity of RuDP-carboxylase. This phenomenon is difficult to explain and needs further investigations.

Table 3

Effects of Cu on the rate of photosynthesis and activity of the RuDP-carboxylase

Plant species and stage of development	Photosynthesis 10^3 cpm/g fr. wt.		RuDP -carboxylase activity 10^3 cpm/g fr. wt.	
	−Cu	+Cu	−Cu	+Cu
Oat				
shooting	1892.0	2902.9	22.5	43.3
earing	1906.0	2518.0	22.5	35.9
Sunflower				
8 leaves stage	1122.0	1172.0	38.1	38.3
flowering	762.9	1811.1	42.2	31.2

There was a positive effect of copper on the photosynthesis activity, especially in oat plants (Table 4). Response of the plants to the copper depended on the stage of development of the whole plants and especially of the leaves. The decrease in photosynthesis rate as a result of Cu deficiency was largest in the older plants and in the leaves developed later, when the symptoms of copper deficiency became clearly visible.

5. There were rather few results of our experiments on respiration

Table 4

Effect of copper on the activity of photosynthesis (10^3 cpm per plant)

Plant	Exposure I		Exposure II		Exposure III		Exposure IV	
	−Cu	+Cu	−Cu	+Cu	−Cu	+Cu	−Cu	+Cu
Oat	376	523	965	2669	1264	4441	378	1462
Sunflower	1027	1290	1967	3692	7272	11612	6237	8774

Explanations:

Exp.	Date	Oat	Sunflower
I	May 29	tillering	8 leaves stage
II	June 14	shooting	buds stage
III	July 3	earing	flowering
IV	July 16	grain filling	seeds filling

intensity of the examined plants. The results obtained indicated that copper deficiency caused the decrease of the respiration intensity of sunflower leaves before flowering: the intensity of respiration of −Cu leaves was then 2.9 and of +Cu leaves it amounted 4.39 (data expressed in mg of $CO_2 \cdot h^{-1} \cdot g^{-1}$ of dry matter). On the other hand under the copper deficiency conditions the respiration of oat tops at shooting stage was even higher in comparison with the plants sufficiently supplied with copper and it amounted 5.61 and 4.50, respectively. This difference of results could be explained as follows: in sunflower plants the AAO activity was much higher than in oat plants (compare Table 1 with Table 2). As the ascorbic acid oxidase is probably involved in the process of plant respiration thus the low level of this enzyme activity under conditions of copper deficiency in sunflower could be a sufficient cause of the decrease of the respiration of this plant. Some further experiments on that problem are needed.

6. The results obtained are consistent with the earlier suggestion of the authors that the PPO activity in sunflower leaves could be a sensitive indicator of copper supply of plants.

Further experiments are in progress. A more detailed discussion on studied problem will be presented in the next papers.

REFERENCES

Ostrovskaya L. K., 1961. Fiziologicheskaya rol miedi i osnovy primienieniya miednykh udobrenii. Izd. Ukrainskoy A.S.H.N., Kijev.

Ruszkowska M., Łyszcz S., 1975a. Wpływ dawek NPK i Cu na plony oraz na pobieranie miedzi i azotu przez rośliny w warunkach doświadczeń wazonowych. Pam. Puł. 62: 229–250.

Ruszkowska M., Łyszcz S., 1975b. Aktywność oksydaz miedzio-proteidowych jako wskaźnik zaopatrzenia roślin w miedź. Pam. Puł. 62: 251-263.

Ruszkowska M., Łyszcz S., Sykut S., 1975. The activity of catechol oxidase in sunflower leaves as an indicator of copper supply in plants. Polish J. Soil Sci. 8, 1: 67-74.

Samorodova-Bianki G. B., 1971. K biochimi miedproteidov v rastieniakh. Trudy po prikladnoi botanikie, genetikie i selekcii. 44, 1: 216-233.

Smith F. G., Stotz E., 1949. A colorimetric method for the determination of phenol oxidase in plant material. J. Biol. Chem. 179 (2): 865-880.

Shkolnik M. J., 1974. Mikroelemienty v zhizni rastenii. Izd. „Nauka", Leningrad.

Trojanowski J., 1963. Rola biologiczna oksydazy katecholowej u roślin. Post. Bioch. 9: 187-200.

Trojanowski J., 1964. Roślinne oksydazy miedzioproteidowe. Post. Bioch. 10: 93-112.

Wojcieska U., 1973. Współzależność pomiędzy plonem, intensywnością fotosyntezy i aktywnością enzymów biorących udział w wiązaniu węgla. Post. Nauk roln. 1: 45-54.

Aktywność oksydazy askorbinianowej i oksydazy katecholowej w różnych organach roślin oraz intensywność fotosyntezy i oddychania w zależności od fazy rozwoju i zaopatrzenia roślin w miedź

Streszczenie

Badania miały na celu lepsze poznanie fizjologicznej roli miedzi w roślinach oraz skutków jej niedoboru. W ramach tych badań przeprowadzono doświadczenia wazonowe z owsem i słonecznikiem na torfie niskim w warunkach niedoboru miedzi (−Cu) lub przy optymalnym zaopatrzeniu roślin w miedź (+Cu). W roślinach oznaczano: 1) aktywność dwóch oksydaz miedzio-proteidowych (oksydazy askorbinianowej i oksydazy katecholowej) w różnych organach i w różnych fazach rozwojowych badanych roślin, 2) aktywność i intensywność fotosyntezy oraz aktywność karboksylazy RuDP jako wskaźnika intensywności fotosyntezy, 3) intensywność oddychania ciemniowego.

W warunkach niedoboru miedzi aktywność oksydazy askorbinianowej (AAO) i oksydazy katecholowej (PPO) była znacznie niższa w porównaniu z optymalnym poziomem Cu w roślinie (tabele 1, 2). W owsie stwierdzono średni poziom aktywności AAO i zaledwie ślady aktywności PPO, natomiast w słoneczniku wysoki poziom aktywności PPO i AAO. Aktywność obu badanych oksydaz zależała od organu rośliny oraz fazy jej rozwoju. W liściach owsa stwierdzono średni poziom aktywności AAO w okresie krzewienia, niski poziom — w czasie strzelania w źdźbło i wysuwania wiech, a bardzo wysoki poziom aktywności — w starych, żółknących liściach. Ponadto dość wysoki poziom aktywności AAO wykazywały zielone źdźbła i niedojrzałe ziarna owsa oraz — przez cały czas wegetacji — korzenie. W częściach nadziemnych słonecznika aktywność PPO wzrastała wraz z wiekiem rośliny i była wysoka we wszystkich metabolicznie aktywnych centrach, tj. młodych liściach, górnej części łodyg, pąkach i niedojrzałych nasionach. W korzeniach słonecznika aktywność PPO była niska w ciągu całej wegetacji. Aktywność AAO w roślinach słonecznika wzrastała wraz z wiekiem rośliny.

Stwierdzono współzależność między poziomem zaopatrzenia roślin w miedź a fotosyntezą. Mianowicie, intensywność oraz aktywność fotosyntezy, a także częściowo aktywność karboksylazy RuDP (w przypadku liści owsa) były dużo wyższe w roślinach dostatecznie zaopatrzonych w miedź niż w roślinach niedoborowych (tab. 2 i 3). Niedobór miedzi wpływał na osłabienie intensywności oddychania liści słonecznika, natomiast powodował pewien wzrost w intensywności oddychania części nadziemnych owsa. Wynik ten autorzy próbują tłumaczyć różną aktywnością AAO w badanych roślinach. Uzyskane wyniki są potwierdzeniem wniosków z poprzednich badań autorów (1975), że aktywność oksydazy katecholowej w liściach słonecznika może być czułym wskaźnikiem diagnostycznym przy wykrywaniu niedoboru miedzi w roślinach.