Acta Botanica Academiae Scientiarum Hungaricae Tomus 12, pp. 89—94 (1966)

CHANGES OF ARGINASE ACTIVITY AND THE DEVELOPMENT OF ADVENTITIOUS ROOTS IN LUPINUS ALBUS

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(Received April 16, 1965)

Introduction

According to recent investigations the activity of genes governing the organic processes in the cell is highly influenced by the histones being in close association with the nuclear deoxyribonucleic acid (DNA) [2]. In the histone-DNA complex the DNA molecule is inactive and regains its function ability only after the disappearing of histones from the complex.

The above outlined role of histones is also proved by their behaviour in plant cells [1]. Accordingly, in plant organisms out of all DNA molecules only those being free are active. New organs, e.g. flowers or adventitious roots may, therefore, only appear, if previously certain DNA molecules liberate and histone molecules decompose. As arginine is an essential part of the histones, their supposed changes in connection with the development of adventitious roots were examined through arginine metabolism.

Material and methods

Measurements were performed in a plant growth chamber at 25°C using rooted and 4 to 5-day-old derooted seedlings of *Lupinus albus* grown in Pfeffer medium. For the establishment of arginase activity in all cases a hypocotyl extract was made from 2 g hypocotyls with 0.5 ml of a phosphate buffer of pH 7.4. The optimum pH-value of *Lupinus albus* hypocotyls ranged from 8.0 to 8.4, and was fixed with 0.3 per cent arginine. The reaction mixture was incubated at 25°C in darkness for 16 hours.

For the assessment of the activity two methods were used.

I. By the modified procedure of WEIL and RUSSEL [9] the amount of CO_2 released from the urea decomposed by the endogenous urease was established. Under the influence of arginase and in a solution of suitably fixed pH value the arginine hydrolyzes into ornithine and urea. This optimum pH value was for *Lupinus albus* hypocotyls 8.4 instead of 9.5 according to WEIL and RUSSEL. The developed urea was decomposed into CO_2 and NH_3 by the endogenous urease. For the manometric measurement of CO_2 0.25 ml of incubated solution were used from which the CO_2 was freed by 1 ml citrate buffer of 5.0 pH. To decrease the error, to the 0.25 ml sample 0.25 ml of distilled water were added. Each measurement took 15 minutes.

II. Using paper chromatography the activity was established with the Sakaguchi reagent on the strength of the decomposed arginine. From the extract incubated with arginine a solution quantity of 20 μl was placed on the filter paper (SS 2043-B), run for 16 hours in a solution of pyridine, acetic acid and water (50 : 35 : 15) and developed with the Sakaguchi reagent after drying. The amount of arginine was assessed with a Zeiss Eri extinction registrator.

Results

The trend of arginase activity in rooted and 4 to 5-day-old derooted seedlings of *Lupinus albus* is shown in Fig. 1. The arginase activity of young *Lupinus albus* seedlings put into the medium gradually decreased with advancing age (Fig. 1*a*).

The arginase activity in the hypocotyl of 4 to 5-day-old derooted

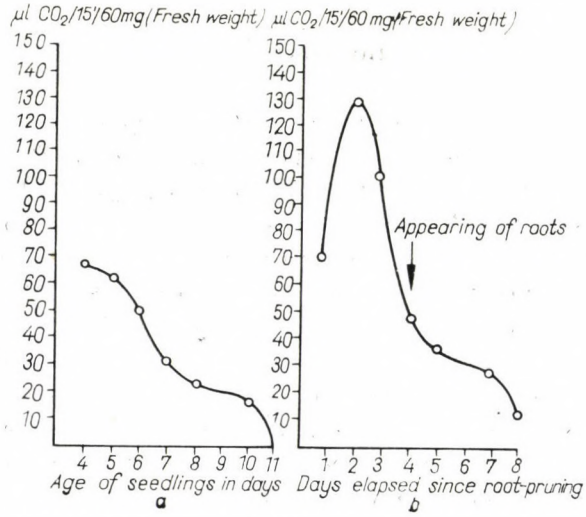

Fig. 1. Changes of arginase activity in hypocotyls of rooted and derooted *Lupinus albus* seedlings according to age

a = Rooted controls
b = Root-pruned at the age of 4 days

seedlings increased considerably in 1 to 2 days after derooting and diminished progressively while new roots appeared and developed (Fig. 1*b*).

Measurement results on arginase activity obtained by paper chromatography are summarized in Table I and reveal that — similarly to data by the WARBURG method — the arginase activity of derooted seedlings was about double that in rooted seedlings of identical age.

Comparing the data of Table I and Fig. 1 it turns out that the change of arginase activity was followed by that of urease activity in a day and that urease remained active even for 4 days after arginase activity had ceased. From this it may be concluded that urea comes into being not only from arginine but also in an other way. Really, urea may develop from purines also [5].

The temporal difference in the activity change of examined enzymes is in conformity with the fact that — if an inductive enzyme synthesis is

supposed — the activity of urease increases under the influence of the urea decomposed from arginine, but a certain time is required for the developing of urea from arginine necessary for arginase induction.

Table I

The change of arginase activity in hypocotyls of rooted and derooted Lupinus albus seedlings according to age, on the basis of measuring the decomposed arginine with the methods of paper chromatography and densitometry

(Mean of 5 measurements)

Age of seedlings (days)	Days elapsed since derooting	Amount of exhausted arginine in percentage of that in boiled controls	
		in rooted seedlings	in derooted seedlings
5	1	35 ± 3.8*	70 ± 9.5
6	2	25 ± 3.1	55 ± 8.0
7	3	10 ± 0.3	13 ± 3.2
8	4	0	0

$$* \sigma = \sqrt{\frac{\Sigma' (x - \bar{x})^2}{N - 1}}$$

As adventitious roots appear in the lower part of hypocotyls (Fig. 2), the arginase activity was assessed both in the lower and upper section of hypocotyls. Measurement results on the supposed differentiation of arginase activity in hypocotyls are presented in Table II, which make evident that arginase activity is much more intensive in the rooting zone, *i.e.* the lower half of the hypocotyl, than in its upper part, where no adventitious roots develop.

Discussion

In the possible mechanism of rooting arginase activity may be characterized as follows: after root-pruning hypocotyl cells supposedly "post themselves up" — in some way or other — on the lack of roots. Subsequently in hypocotyl cells, exactly in genes governing the rooting processes, the decomposition of histone blocks commences and, on the other hand, the arginine originated from the latter activates the arginase.

However, beside the assumed influence of histones root organization and root development are also affected by many other factors. In rooting experiments with pea stalks and gooseberry cuttings it was observed that young

plants rooted much faster than older ones [4, 7]. It turned out that root-pruning was followed by increased activity of many enzymes and by higher respiration intensity [6].

The activity of glucose-6-P-dehydrogenase, other dehydrogenases, peroxidase and polyphenoloxidase increased in 1 to 5 days after derooting.

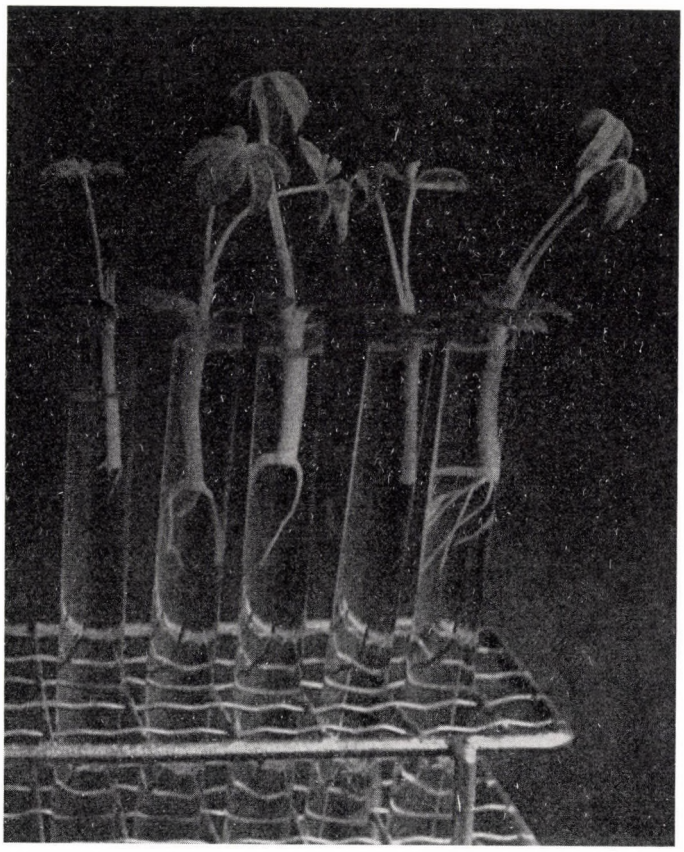

Fig. 2. Lupinus albus seedlings on the 8th day after derooting

This intensification was followed by a decomposition of proteins in the rooting organ and by a rise of the free amino acid content lasting until the roots appeared and turning into a decline afterwards. According to the aforesaid, arginine may be freed also in the course of protein decomposition taking place after derooting and may induce an increase of arginase activity. That in this process also the arginine freed from histones possibly participates was proved by observations on the differentiation of arginase activity (Table II) as well as by the data of SCHMIDT [8] and LIBBERT [7]. These authors

evidenced that in pea stalk the intensity of adventitious root formation decreased gradually upward on the stalk, similarly to the trend of arginase activity observed by the present authors. To a correlation of arginine and morphogenesis refer also the results obtained by DURANTON [3], according

Table II

Differentiation of arginase activity in the lower and upper part of hypocotyls of rooted and derooted Lupinus albus seedlings one day after pruning

(Mean of 5 measurements)

Methods applied	Upper half of hypocotyls	Lower half of hypocotyls
Paper chromatography: the amount of exhausted arginine in percentage of that in boiled controls	40 ± 7.0*	85 ± 10.8
Manometry: μl CO_2 obtained in 15 minutes from 60 mg of hypocotyls (fresh weight)	8 ± 3.0	104 ± 20.5

$$* \sigma = \sqrt{\frac{\Sigma' (x - \bar{x})^2}{N - 1}}$$

to whom free arginine increases during inflorescence induction and prior to rhizome flushing, but diminishes and disappears subsequently.

In continuation of the work reported here it will be attempted to corroborate the role of histones in rooting processes by direct histone measurements.

Summary

The arginase activity in hypocotyls of rooted and 4 to 5-day-old derooted *Lupinus albus* seedlings was measured manometrically and by paper chromatography. It showed an increase after derooting, but subsequently, with advancing age, a progressive drop. Arginase activity reached considerably higher values in the lower, rooting zone of the hypocotyls than in their upper rootless part. The correlations between arginase activity, rooting and histones are discussed.

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