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The Effect of Potassium-Deficiency on Diamine Oxidase Activity in Pea

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Abstract. Pea plants grown in nutrient solution in which K^+ ions were equimolarly replaced with Na^+, NH_4^+ or Rb^+ did not show morphological symptoms of potassium-deficiency. The activity of diamine oxidase in these plants was higher than in controls. Similarly higher diamine oxidase activity was found in plants grown in a complete nutrient solution supplemented with putrescine.

Plants grown under potassium-deficient nutrient conditions contain increased level of putrescine (SMITH 1968, 1970a, 1971, SMITH and RICHARDS 1962, RICHARDS and COLEMAN 1952, COLEMAN and RICHARDS 1956, HACKET *et al.* 1965, MURTY *et al.* 1971).

The amount of accumulated putrescine is approximately equivalent to the difference in potassium content between the normal and potassium-deficient plants (SMITH 1970a). Potassium-deficit can be compensated by this organic cation only to one third without a visible damage of plants (MURTY *et al.* 1971). The increase in putrescine content has been explained by a disorder in nitrogen metabolism and in cation-anion equilibrium resulting in an increased acidity, which enhances formation of enzymes catalyzing synthesis of basic amines (COLEMAN and RICHARDS 1956, SMITH 1971). These amines are functional in adjustment of intracellular pH. According to SMITH (1973) the accumulation of putrescine is also affected by calcium, phosphorus, sulphur, magnesium and manganese nutrition. Excessive accumulation of putrescine during potassium-deficiency causes necrosis on leaves. This harmful effect of putrescine was proved by RICHARDS and COLEMAN (1952) and COLEMAN and RICHARDS (1956) by its addition to the nutrient solution containing optimum concentration of potassium.

Putrescine is oxidatively deaminated by diamine oxidase which has been detected in pea, soybean, red clover, lupine and lavender plants (KENTEN and MANN 1952, SUZUKI 1973). The changes in activity of diamine oxidase

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in pea plants grown under potassium-deficient nutrient conditions are investigated in this communication.

Material and Methods

Pea plants (*Pisum sativum* L. cv. 'Liliput') were grown in a stationary water culture without aeration. Acidity of nutrient solution was adjusted to pH of the control solution (pH 4.6) with 0.1 N NH_4OH and NaOH, respectively. Growth tanks were placed either in a greenhouse, where plants were grown under natural illumination (May) at 22 ± 5 °C (first experiment), or in a dark room with constant temperature 25 °C (second experiment). These conditions were chosen with respect to the expected significant effect of putrescine on diamine oxidase synthesis. The activity of this enzyme is increased up to three times when plants are grown in darkness (MACHOLÁN and MINÁŘ 1974).

The fresh weight and diamine oxidase activity (MACHOLÁN and MINÁŘ 1974) were estimated separately for shoots and roots of collected plants. The enzyme activity has been expressed in international units (U), *i.e.* in μmol of oxidized putrescine after 1 min of incubation at 25 °C.

Effect of Potassium Deficiency

Ten four days old pea seedlings were planted into each growth tank containing 4 l of Richter's nutrient solution, in which potassium salts (KH_2PO_4, KNO_3) were equimolarly replaced with sodium, ammonium or rubidium salts. Control plants were grown in Richter's solution with potassium salts.

Effect of Putrescine

Pea seedlings were planted similarly as in the first experiment. The Richter's solution was supplemented with putrescine dihydrochloride to the final concentration 10^{-3} M, 10^{-4} M, 10^{-5} M and 10^{-6} M, respectively.

Results

Effect of Potassium Deficiency

The potassium deficiency least affected the plants grown in nutrient solution containing sodium instead of potassium. On the other hand the employed concentration of rubidium had a toxic effect on plants at the end of the experiment (Fig. 1).

The diamine oxidase activity (U.g^{-1} fresh weight) in shoots was decreasing in all examined experimental variants. This decrease was very sharp at the beginning of the experiment and became gentle after 19 days of cultivation. During the whole experimental period the diamine oxidase activity was the lowest in the case of control plants. Higher activity was found in plants grown in nutrient solution with sodium and ammonium and the highest activity in plants cultured in rubidium-supplemented solution. Similar results were obtained for roots where the diamine oxidase activity (U.g^{-1} fresh weight) was also gradually decreasing during cultivation. However, a temporary increase in its activity was recorded in plants grown in solution with ammonium between the 19th and 32nd day of their cultivation. Simultaneously necrotic spots appeared on leaves of these plants, which did not occur on leaves of other variants. Starting from the 19th day of cultivation a gradual increase in diamine oxidase activity was recorded in roots of plants grown in rubidium-containing solution. The activity was increasing till the end of the experiment and it seems probable that this increase is caused by a gradual dying of plants in this variant (Fig. 2). The diamine oxidase activity expressed in U per one plant was lower in all experimental variants than in control plants. At the end of the experiment a bulky formation of nodules was observed on roots of plants grown in solution with sodium salts.

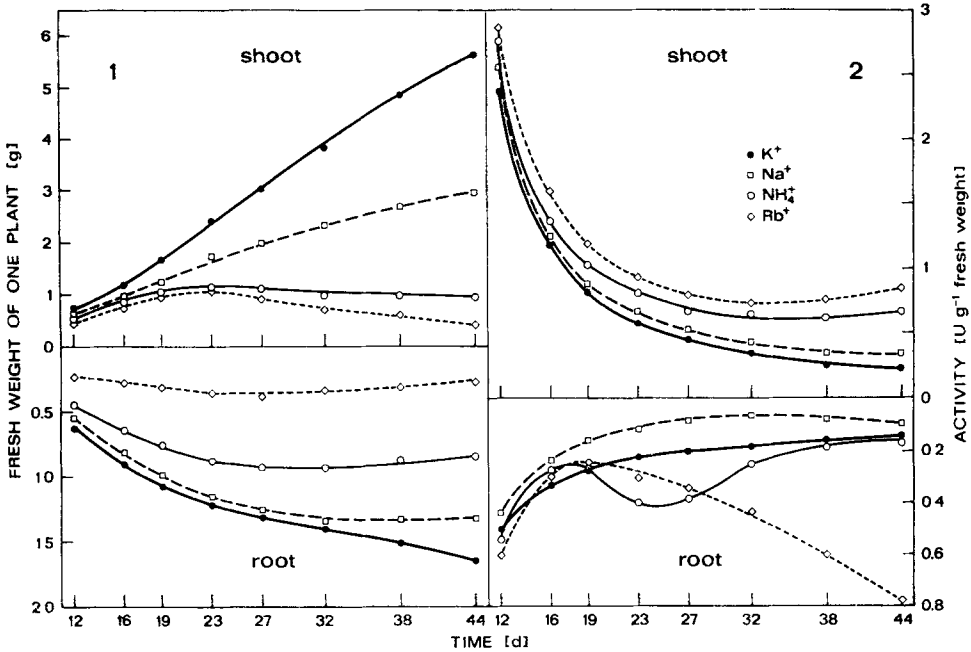

Fig. 1. Growth of pea seedlings in Richter's nutrient solution (K^+) and in solution in which potassium salts were replaced with sodium (Na^+), ammonium (NH_4^+) and rubidium (Rb^+) salts, respectively.

Fig. 2. Diamine oxidase activity in pea seedlings grown in Richter's nutrient solution (K^+) and in solutions in which potassium salts were replaced with sodium (Na^+), ammonium (NH_4^+) and rubidium (Rb^+), respectively.

Effect of Putrescine

The extent of unfavourable effect of putrescine on fresh weight of plants is directly dependent on its concentration in nutrient solution (Fig. 3). Similarly to the first experiment, the diamine oxidase activity ($U.g^{-1}$ fresh weight) was decreasing with the increasing age of plants. Its activity in shoots of six days old plants (1st analysis) was the highest in the case of control plants. Two days later, however, the enzyme activity in plants of all experimental variants was already higher than in control and this difference remained till the end of the experiment. The differences in diamine oxidase activity between control and putrescine-treated plants were increasing with increasing putrescine concentration from 10^{-6} M to 10^{-3} M. When compared with the control, the highest tested putrescine concentration decreased diamine oxidase activity by 20 % in 8 days old plants, but after 19 days of cultivation its activity was by 50 % higher than that of the control. The enzyme activity in shoots expressed in U per one plant is, in general, higher in all experimental variants than in control plants during the whole period of cultivation. Maximum activities were again recorded in plants grown in solutions with the highest putrescine concentration. Higher activity of diamine oxidase ($U.g^{-1}$ fresh weight) than in control was also found in roots of plants from variants with 10^{-5} M and 10^{-6} M putrescine. This increased activity was,

however, preserved till the end of cultivation only in roots of plants which were grown in solution containing 10^{-5} M putrescine (Fig. 4). This dependence is even more pronounced when the enzyme activity is expressed in U per roots of one plant.

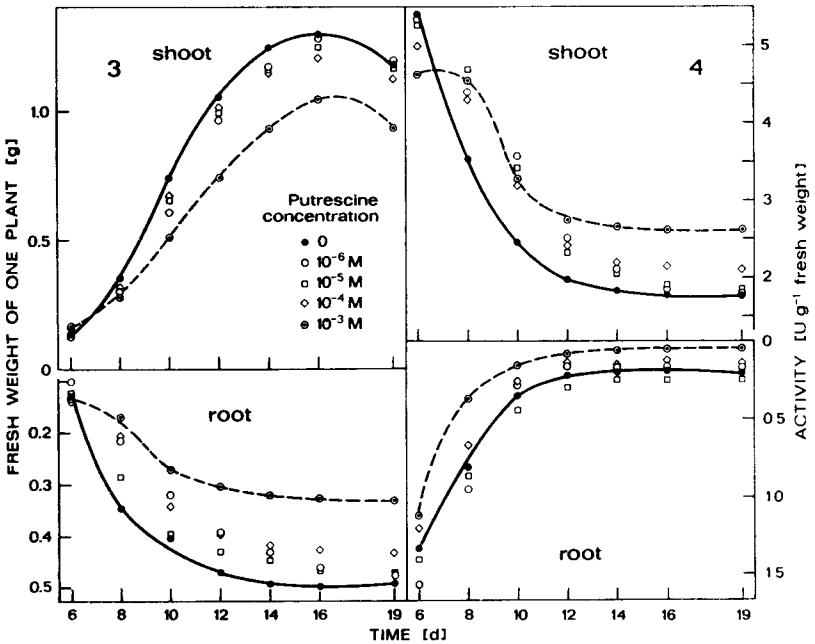

Fig. 3. Growth of pea seedlings in Richter's nutrient solution (control) and in solutions containing various concentrations of putrescine.

Fig. 4. Diamine oxidase activity in pea seedlings grown in Richter's nutrient solution (control) and in solutions containing various concentrations of putrescine.

Discussion

Putrescine accumulated by potassium-deficient plants causes their gradual dying (Hackett *et al.* 1965, Smith and Richards 1962, Smith 1968, 1970b, 1971). We expected and proved that this is not the case in plants furnished with the diamine oxidase. Simultaneously it has been found in several experiments (one of which is presented here) that in potassium-deficient pea plants the activity of this enzyme in shoot is increased. This made us again assume a possible induction of diamine oxidase by its substrate (Růžička and Minář 1974). The results of the second experiment which are presented above and of other not yet published experiments are consistent with this hypothesis. It has been found that the activity of diamine oxidase is increased even when the developed plant seedlings are transferred to the nutrient solution supplemented with putrescine. The activity of this enzyme was also increased after swelling of seeds in this diamine. As compared with the roots, the enzyme activity in shoots was increased only after application of higher putrescine concentrations to the root system. This difference can be understood on the basis of Macholán and Minář's (1974) experiments which showed that putrescine taken up by roots is gradually degraded when

transported to the shoots. The activity of diamine oxidase in roots is repressed even by very low putrescine concentrations. This sensitivity is probably caused by the lower diamine oxidase activity in roots as compared with shoots.

It has been found that the potassium-deficient pea plants accumulate putrescine. This accumulation is probably related to the increased activity of arginine decarboxylase and N-carb-amylputrescine amidohydrolase (SMITH 1963, 1965).

Our experiments showed that these plants never die (with the exception of those cultivated in solution with rubidium) and that they grow up to flowering plants without symptoms of potassium deficiency. This especially proves true when potassium is replaced with sodium. It is very likely that a higher content of diamine oxidase maintains the putrescine in potassium-deficient pea plants on a reasonable low level which is not toxic. As was proved in our laboratory, barley and maize plants, in which the diamine oxidase was not detected (SMITH 1969, SMITH and STEVENS 1971), do accumulate putrescine when grown in potassium-deficient solution. Putrescine is accumulated to an amount that causes gradual dying of leaves starting from their apex.

WERLE *et al.* (1959) supposed that diamine oxidase has a certain physiological function in seed germination. Using specific inhibitors of this enzyme, diaminobutanone and diaminopentanone MACHOLÁN and MINÁŘ (1974) decreased its activity to the sensitivity limit of the used analytical method without affecting seed germination. The inhibitors or their degradation products affected later phases of growth. It is therefore necessary to look for the biological function of diamine oxidase not only in the early phases of growth and seedling nutrition but also in further developmental and growth processes. The present experiments showed that pea plants furnished with the diamine oxidase are not significantly affected by potassium deficiency.

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L. PAPRSKÁŘOVÁ a J. MINÁŘ (Brno): **Vliv deficience draslíku na aktivitu hrachové diaminoxidázy.** — Biol. Plant. **18** : 99—104, 1976.

Rostliny hrachu kultivované v živném roztoku, ve kterém byly ionty K^+ ekvimolárně nahrazeny ionty Na^+, NH_4^+ nebo Rb^+, nevykazovaly morfologické příznaky deficience draslíku. Tyto rostliny měly v nadzemní části vyšší aktivitu diaminoxidázy. Obdobně vykazovaly vyšší aktivitu diaminoxidázy rostliny kultivované v úplném živném roztoku, do kterého byl přidán putrescin.

BOOK REVIEW

HESS, D.: **Plant Physiology. Molecular, Biochemical, and Physiological Fundamentals of Metabolism and Development.** — Springer-Verlag Berlin—Heidelberg—New York 1975. 333 S. 36.30 DM.

The second edition of the book by Professor D. HESS (University of Hohenheim, Stuttgart, F.R.G.): Pflanzenphysiologie. Molekulare und biochemisch-physikalische Grundlagen von Stoffwechsel und Entwicklung. Uni-Taschenbücher Band 15, Verlag Eugen Ulmer, Stuttgart 1972 (cf. review in Biol. Plant. **15** : 302, 1973) has recently appeared in the translation of Dr. D. JARVIS at Springer-Verlag in the series "Springer Study Edition". The book deals with metabolism and development of higher plants based on molecular biology. Methodological details were mostly omitted for economy of space.

The metabolical part of the book (10 chapters) starting with the control of character formation by nucleic acids, continues with chapters on primary and secondary processes of photosynthesis and the chloroplast as the site of photosynthesis, on carbohydrates, biological oxidation and other components of plants and plant metabolism as fats, terpenoids, phenols, amino acids, alkaloids and porphyrins. In the second part of the book the developmental physiology of higher plants (9 chapters) is treated. It is for instance cell division, differential gene activity as principle of differentiation, regulation by internal and external factors, polarity and unequal divisions as fundamentals of differentiation, cell elongation, the formation of seeds and fruits, germination, the vascular system and its differentiation and function (including transport phenomena, transpiration), and flower formation (including vernalization and photoperiodism).

This Plant Physiology is modern, untraditional and didactically very well arranged. It presents a new, logically written review of the whole-plant physiology. The book is very well produced. It contains 248 instructive, mostly two-coloured figures, which profited much in this edition from being larger than in the German manual. A short bibliography (unusual is omitting the mutation sign "Umlaut" in German words), sources of illustrations and subject index are added.

The book is mainly intended to undergraduate students and biology teachers at secondary level as well as to research workers to provide them with digestic basic information from relative fields.

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