Short Communications

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Enhanced Activity of Pyruvic Kinase in the Potassium-Deficient Wheat Leaves

Sir:

Potassium is one of the three essential elements for the plant growth. Though considerable attention has been paid to elucidate its role in the plant life, the crucial nature has still remained obscure. Several enzymes have been shown to require potassium ion for their catalytic action, among which the stimulation of the pyruvic kinase activity by K^+ , NH_4^+ , and Rb^+ has been the best known example.¹⁾ During the course of our study on the analysis of enzymic patterns in the potassium-deficient wheat leaves, we have found that the activity of pyruvic kinase was markedly enhanced. The present report deals with this observation. Our finding was in a sharp contrast to the previously reported one of Evans which had shown the decline of the pyruvic kinase activity in the potassium-deficient pea plants.²⁾

Wheat plants (variety of Norin No. 61) were grown in an acid-washed sea sand bed supplemented with the growth media of Evans with or without potassium. Composition of the standard nutrient medium was as follows (in mmoles per liter): $Ca(H_2PO_4)_2 \cdot H_2O$, 0.5; $CaCl_2 \cdot 2H_2O$, 0.25; $Ca(NO_3)_2 \cdot 4H_2O$, 3.0; $MgSO_4 \cdot 7H_2O$, 1.0; K_2SO_4 , 2.4; and Fe-citrate, 0.018.

At the growing stage of eleventh day, 5 g of the first leaf of the plants was macerated with 15 ml of 0.05 M Tris buffer (pH 7.5), and resulting suspension was spun at $10,000 \times g$ for 10 minutes. Supernatant fluid was applied to a column of Sephadex G 25, and the

eluate was used as an enzyme preparation. All the experiments were carried out at 0 to 4°C, and enzyme activity was measured within 30 minutes after preparation of the enzyme. Composition of the standard reaction mixture was (in μ moles): Tris buffer (pH 7.4), 50; tricyclohexylammonium salt of phenolphosphopyruvic acid, 1.5; sodium salt of ADP, 2.5; $MgSO_4$, 10; and KCl, 50 in a total volume of 1.0 ml. To inhibit phosphatase which was present in the crude enzyme preparation, 1.25 μ moles of Tris-molybdate, sufficient to suppress the breakdown of phosphate ester, was added.

After incubation for 10 minutes at 37°C, the reaction was stopped by adding 1.0 ml of 0.125% 2,4-dinitrophenylhydrazine in 1 N HCl and the formation of pyruvate was assayed after the method of Kachmar and Boyer.³⁾ A reaction mixture without ADP addition was served as a negative control in the enzyme assay in each system. It was confirmed that the enzyme activity linearly increased for at least 15 minutes and was proportional to the enzyme concentration over the range employed.

The pattern of the pyruvic kinase activity of both potassium-supplemented and -deficient plants, expressed as the specific activities, as a function of growing days of plants, is diagrammatically presented in Fig. 1. It is clearly shown that a significant increase in enzyme activity occurred in the potassium-deficient wheat leaves after 10 days of germination. In spite of the appearance of

1) G. Miller and H. J. Evans, *Plant Physiol.*, **32**, 346 (1957).
2) H. J. Evans, *ibid.*, **38**, 397 (1963).

3) J. F. Kachmar and P. D. Boyer, *J. Biol. Chem.*, **200**, 1 (1953).

FIG. 1.

TABLE I. EFFECT OF K⁺ ADDITION ON THE PYRUVIC KINASE ACTIVITY IN THE K-DEFICIENT WHEAT LEAVES

Days	Plants					
	K(+)		K(-)		K(-)→K(+)*	
	a)	b)	a)	b)	a)	b)
10	0.054	32.4	0.102	4.6	—	—
13	0.055	53.0	0.202	3.7	0.065	35.4

* K(9.6 mmoles per liter) was added to the K-deficient plants at the growing stage of 10th day, and both enzyme assay and K content determination were made at 3 days afterwards (13th day).

a) Pyruvic kinase activity (μmoles of pyruvate formation in 10 minutes per mg protein).

b) K content (mg K per g dry weight).

prominent potassium-deficiency symptom in wheat leaves, the difference of the growth rate between potassium-supplemented and

-deficient leaves was not remarkable during the whole growth period.

The effect of potassium addition to the mineral-deficient plants after a certain period was tested, and the specific enzyme activity of leaf extract at the 3 days later was found to be much lower than that in the plants before K⁺ supplementation as shown in Table I.

It is interesting to note that a partial restoration of the mineral deficiency was apparently reflected in the decrease in activity of potassium-dependent enzyme, and our results may suggest the regulation of pyruvic kinase by the potassium content in wheat leaves.

It is noteworthy that Evans had grown plant for more than one month in the condition of potassium-deficiency.²⁾ Hence, discrepancies of the observation of Evans' and ours might most probably be attributable to the difference of experimental conditions employed. Details of this investigation will be presented in a forthcoming paper.

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