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BRIEF COMMUNICATION

Acid Phosphatase Activity in Maize Leaves as Related to their Evolution and Phosphorus Deficiency

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Abstract. The effect of phosphorus deficiency on the activity of acid phosphatase of the first, second and third leaves of maize plants was followed. The supernatant obtained by centrifuging the homogenate of plant tissue at $1500 \times g$ was further centrifuged at $18\,000 \times g$, the sediment marked as fraction II and the supernatant as fraction III. Acid phosphatase activity of fraction II of the first to third leaves was for the whole period of culture higher in plants grown in the nutrient solution without phosphate. In fraction III this relation was established in the first leaf, after 3 days of culture in the second leaf and after 5 days in the third leaf. In all leaves higher enzyme activity was unambiguously determined in fraction III when compared with fraction II. Higher acid phosphatase activity was established in those leaves which were younger in their development, particularly in the first days of culture. With the ageing of leaves the enzyme activity decreased.

In studying the effect of phosphorus deficiency in the nutrient solution on the activity of acid phosphatase of maize root, many times higher enzyme activity was proved in the homogenate of subcellular structures of plants cultured in the medium without phosphorus (KUMMEROVÁ 1983). The vertical analysis of the root confirmed the assumption that acid phosphatase activity in these plants is located above all in the cytosol fraction of the apical part of the root (KUMMEROVÁ 1986).

For judging the rate of phosphorus deficiency in the plant from the point of view of the integrity of the plant organism BESFORD (1978a), BESFORD and SYRED (1979) and — last but not least — BARRET-LENNARD *et al.* (1982) studied acid phosphatase activity also in the overground parts of plants.

The characteristics of the overground part of plants in this respect are, however, difficult to define owing to the redistribution and reutilization abilities of the phosphorus anion and the fact that it is irreplaceable particularly in the energetic metabolism. Thus it is known that phosphorus is translocated into sites with intense photosynthetic activity.

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TABLE 1

Acid phosphatase activity of the IIIrd fraction of the first, second and third leaves of maize cultured in nutrient solution with (+P) and without (−P) phosphate

Time of plant culture in nutrient solution [d]			Acid phosphatase activity [μmol P g^{-1} (fr. mass) h^{-1}]		
			leaf		
			1st	2nd	3rd
0		$\bar{x} \pm s_{\bar{x}}$	82.56 ± 3.86	103.22 ± 1.11	
		t		5.14^{++}	
2	+P	$\bar{x} \pm s_{\bar{x}}$	66.73 ± 0.15	110.17 ± 0.29	128.78 ± 5.06
		t		135.75^{++}	3.67^{+}
	−P	$\bar{x} \pm s_{\bar{x}}$	75.40 ± 2.11	97.16 ± 0.28	121.78 ± 1.81
		t		10.22^{++}	13.45^{++}
4	+P	$\bar{x} \pm s_{\bar{x}}$	54.78 ± 2.77	63.41 ± 0.14	108.68 ± 3.53
		t		3.11^{+}	12.82^{++}
	−P	$\bar{x} \pm s_{\bar{x}}$	68.65 ± 1.53	83.18 ± 0.70	97.18 ± 2.48
		t		8.64^{++}	5.44^{++}
6	+P	$\bar{x} \pm s_{\bar{x}}$	59.00 ± 0.58	59.32 ± 0.77	66.00 ± 0.99
		t		0.33	5.34^{++}
	−P	$\bar{x} \pm s_{\bar{x}}$	66.28 ± 0.70	70.86 ± 0.40	80.15 ± 7.18
		t		5.72^{++}	3.08^{+}
8	+P	$\bar{x} \pm s_{\bar{x}}$	56.27 ± 2.23	59.08 ± 1.67	64.50 ± 2.09
		t		1.01	2.02
	−P	$\bar{x} \pm s_{\bar{x}}$	58.84 ± 0.83	67.12 ± 1.70	78.54 ± 0.52
		t		4.38^{+}	6.45^{++}

No. of repetitions (experiments) (n) = 3;
Differences between the phosphatase activity of individual leaves $^{+}$ = significant (t = 2.78), $^{++}$ = highly significant (t = 4.60).

On the basis of the above facts the relation of the enzyme activity to the development of leaves still remains a question. These problems have become the objective of our study of changes in the activity of acid phosphatase of subcellular structures of the individual maize leaves grown in media with sufficient and insufficient doses of phosphorus.

The experimental plant was maize (*Zea mays* L. cv. CE 170).

The composition of the culture media, the way of culture, acid phosphatase isolation from the plant material and the determination of the enzyme activity was described in detail elsewhere in this journal (KUMMEROVÁ 1986).

Acid phosphatase activity was determined separately in the individual leaves of the overground part (the first leaf — the oldest in development, the third — the youngest in development). The results given are average values from three experiments. They were evaluated statistically by means of the *t*-test.

The division of the overground part of the plants into individual leaves has enabled us, in contrast to conclusions by BESFORD (1978b, 1979) and KUMMEROVÁ (1983), to judge the relation of enzyme activity to the development of leaves.

TABLE 2

Acid phosphatase activity of the IInd fraction of the first, second and third leaves of maize cultured in nutrient solution with (+P) and without (−P) phosphate

Time of plant culture in nutrient solution [d]			Acid phosphatase activity [μmol P g^{-1} (fr. mass) h^{-1}]		
			leaf		
			1st	2nd	3rd
0		$\bar{x} \pm s_{\bar{x}}$	6.56 ± 0.87	8.93 ± 0.55	
		t	2.30		
2	+P	$\bar{x} \pm s_{\bar{x}}$	3.62 ± 0.17	5.20 ± 0.11	7.00 ± 0.84
		t	7.90++	2.14	
	−P	$\bar{x} \pm s_{\bar{x}}$	4.17 ± 0.13	6.33 ± 0.17	7.35 ± 0.78
		t	10.28++	1.29	
4	+P	$\bar{x} \pm s_{\bar{x}}$	3.21 ± 0.17	4.21 ± 0.13	5.91 ± 0.61
		t	4.76++	2.74	
	−P	$\bar{x} \pm s_{\bar{x}}$	3.85 ± 0.22	5.26 ± 0.13	6.00 ± 0.06
		t	5.64++	5.28++	
6	+P	$\bar{x} \pm s_{\bar{x}}$	2.14 ± 0.04	3.53 ± 0.22	5.21 ± 0.58
		t	6.31++	2.70	
	−P	$\bar{x} \pm s_{\bar{x}}$	3.14 ± 0.17	4.20 ± 0.07	6.81 ± 0.45
		t	5.88++	5.80++	
8	+P	$\bar{x} \pm s_{\bar{x}}$	2.50 ± 0.05	2.77 ± 0.02	6.04 ± 0.17
		t	5.40++	19.23++	
	−P	$\bar{x} \pm s_{\bar{x}}$	2.62 ± 0.06	3.37 ± 0.19	7.11 ± 0.12
		t	3.94	17.11++	

No. of repetitions (experiments) (n) = 3;
Differences between the phosphatase activity of individual leaves + = significant (t = 2.78), ++ = highly significant (t = 4.60).

The results obtained document the fact that the activity of acid phosphatase of the particular fraction (II) was higher for the whole time of culture in the leaves of plants grown in the nutrient solution without phosphorus (Table 3). The lowest values of enzymatic activities were found in the homogenate of the oldest, first leaf; the highest in the youngest, third leaf (Table 2).

Acid phosphatase activity was changing in the same leaf in the course of its ontogenetic development. The curves relating to the first and to the second leaves show objectively its drop in the course of the experiment. A partial growth of the enzymatic activity of the third leaf in the second part of the experiment can be interpreted as a symptom of the early phase of its development, from whose further course, after the appearance of further leaves, a drop can be assumed. The determined acid phosphatase activity of this fraction was roughly 10 times lower than the enzyme activity of the cytosol fraction (III).

From the tabular representation (Tables 1 and 3) of the enzymatic activity of the cytosol fraction (III) it is evident that phosphorus deficiency in the culture medium and eventually also in the plant tissue was first reflected from the beginning of the culture in increased acid phosphatase activity in the first leaf, oldest from the point of view of development. The increase in enzymatic activity as an internal signal of phosphorus deficiency was

TABLE 3

Percentual change in acid phosphatase activity of the IIIrd and IInd fraction of the first, second and third leaves of maize due to phosphate deficiency in nutrient solution

Time of plant culture in nutrient solution [d]	Acid phosphatase activity* [%]		
	leaf		
	1st	2nd	3rd
		IIIrd fraction	
2	$+12.99^{+}$	-11.81^{++}	-5.44
4	$+25.31^{+}$	$+31.17^{++}$	-10.59
6	$+12.33^{++}$	$+19.45^{++}$	$+21.43^{+}$
8	$+4.56$	$+13.60^{+}$	$+21.76^{++}$
		IInd fraction	
2	$+15.19$	$+21.73^{++}$	$+5.00$
4	$+19.93$	$+24.93^{++}$	$+1.52$
6	$+46.72^{++}$	$+18.98^{+}$	$+30.71$
8	4.80	$+21.66^{+}$	$+17.71^{++}$

* The data are related to acid phosphatase activity of plants growing in nutrient solution with phosphate; $^{+}$ = significant difference, $^{++}$ = highly significant difference.

noticed at a time when no external symptoms of its deficiency were perceptible.

The data by CLARKSON and SCATTERGOOD (1982) concerning the drop in the inner concentration of phosphorus and an increase in phosphorus translocation into leaves of later development at the initial stage of phosphorus stress can be completed also by the characteristics of acid phosphatase. Changes in the values of this enzyme activity of the second leaf after 3 days of culture and of the third leaf after 5 days witness the release of this phosphate anion from phosphate esters under the conditions of phosphorus deficiency. It is probable that the released phosphorus is translocated into the site of intense need, *i. e.* from the first, oldest leaf into a leaf which is younger in development, or from the second leaf into the forming third one. This quantitative aspect of the redistribution and reutilization process completes histochemical studies of BIELESKI and JOHNSON (1972) in *Spirodela* and also those of BESFORD and SYRED (1979).

The study has proved that phosphorus deficiency is regularly accompanied by an increase in acid phosphatase activity and thus the activity of this enzyme in plants can be utilized for judging the rate of phosphorus deficiency. These results contribute to the establishment of the theoretical basis on which it would be possible to build up practical processes for confirming the suitability of sufficient fertilization of stands with phosphorus.

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BOOK REVIEW

GLOVER, D. M., (ed.): DNA CLONING. A PRACTICAL APPROACH, Vol. I and II. — IRL Press, Oxford — Washington DC 1985. 190 + 245 pp. US $ 45.00.

The new addition to the series "Practical Approach" of IRL is aimed to extend and complement the existing laboratory manuals for DNA cloning.

The topics covered in the first of the two volumes include the use of λ vectors that permit a direct selection of recombinants in library building and the use of λ gt10 and λgt11 vectors for cDNA cloning. A set of plasmid vectors that direct the synthesis of fusion proteins are covered as are the pEMBL plasmids. Two approaches to the *in vitro* mutagenesis of DNA carried in "single-stranded vectors" are presented. There is a detailed description of several methods to attain a high efficiency transformation of *E. coli* with nacked DNA. The final chapter looks at a set of vectors with a broader host range which allows the propagation of plasmid recombinants within Gram negative bacteria other than *E. coli*. In the second volume, alternative bacterial hosts are explored, including *Bacilli* and *Streptomycetes*. The remaining part of the volume focusses on eucaryotic systems with chapters describing the transformation system in yeast, *Drosophilla* and in plants. The field of animal host-vector systems is rapidly evolving and so there may be a call for a separate volume to cover this area. Meanwhile, the general procedures for efficient introduction of DNA into cultured cells as well as some of the transformation vectors that can be used are also described.

A balanced mixture of descriptions and laboratory protocols should prove usefulness of these books to students and teachers of molecular biology. The techniques that are described will find widespread application by research workers at universities and in industry.

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