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Cytokinin content in the potato plant cv. Bintje as affected by ratio of $Cl^- : SO_4^{--}$ nutrition

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Abstract

Cytokinin activity in the potato plant (*Solanum tuberosum* L.), estimated by the tobacco callus assay method, depends upon the ratio of $Cl^- : SO_4^{--}$ given to the plant (expressed as ppm) and upon the plant part or organ. The order of rising cytokinin activity for nutrient solutions tested, independet of the plant part or organ analysed, was as follows: 212 Cl : 127 S< 372 Cl : 40 S<0 Cl : 225 S. Similary, the plant parts showed the following order of rising cytokinin activity: roots, stems < leaf blades < tubers < leaf petioles, leafy tops.

INTRODUCTION

Exclusion of Cl^- from the nutrient solution inhibits growth of the potato plant (Borys 1967; Borys, Klejnowska 1972; Szczotka et al. 1973). This effect seems to be related to water stress conditions in such plants (Borys and Zielińska 1978).

The transpiration of potato plants is affected by both cytokinin (*cy*) and abscisic acid (ABA) supplied externally to potato leaves (Zielińska 1976). Leaf and root size and the level of *cy* activity showed an inverse relationship. The *cy* level in potato leaves dependend upon the $Cl^- : SO_4^{--}$ ratio in nutrient solution, in which the plants were grown (Borys and Jeske 1975).

Growth depends upon the water level in the plant. Several plant features related to water economy are profoundly affected by the $Cl^- : SO_4^{--}$ ratio, e.g., decreased Cl^- content in the nutrient solution resulted in a deficient water saturation, lower water potential, a higher rate of transpiration in the leaves, larger number of stomata and smaller root size (Borys and Klejnowska 1972; Borys and Jeske

1975; Borys and Zielińska 1978). Thus, it seemed useful to test the effect of the Cl^- : SO_4^{--} ratio on cytokinin activity, at the same growth stage in different parts of the cv. Bintje potato plant.

MATERIALS AND METHODS

Bud cuttings from potato tubers (*Solanum tuberosum* cv. Bintje) were kept until rooting in sand. The rooted cuttings were grown in aerated water cultures. The composition of the nutrient solutions is listed in Table 1. The nutrients kept constant were: K — 195, N — 105, P — 31, Na — 23, Ca — 100, Mg — 40, B — 0.1, Mn — 0.5, Fe — 1.0, Mo — 0.02, Cu — 0.01, Zn — 0.1 ppm.

Plants were grown in 3-liter glass-jars, each containing 2 plants. Each treatment was repeated four times. The treatments were arranged according to the randomised block design. The jars were kept at a constant temperature of 18°C (±1°C) and variable air temperature. The plants were grown until flowering with varying nutrients for the cytokinin test.

Table 1

Content of Cl^- and S (in ppm) in particular treatments

Treatment	Nutrient variables	
	Cl	S
A	372	40
C	212	127
F	0	225

At the time of full bloom, the plant parts were sampled and frozen with dry ice after recording the fresh weight. Ground frozen material was transferred into 70% boiling ethanol for 5 minutes, and left to cool for 24 hours. After this time the filtrate of ethanol was adjusted to pH 8.8 with NH_4OH, centrifuged three times, the supernatants combined, 20 cm^3 of saturated $(CH_3COO)_2$ Ba added and left for 24 hours at 9°C. The cool mixture was centrifuged, the supernatants collected and the residue washed with 200 cm^3 of ethanol, centrifuging each time. The combined supernatants were acidified with HCl to pH 2.5.

The acidic extract was run through the Dowex 50 W × 4 H^+ column. The column was washed in sequence with 500 cm^3 of 70% ethanol, 1500 cm^3 of water and eluated with 5n NH_4OH. The eluate, after congestion to 50 cm^3, was treated with NH_4OH to pH 8.0 and extracted three times with 100 cm^3 of butanol. The butanolic extracts were combined and evaporated to dryness. The residues were dissolved in water of pH 8.5.

Cytokinin activity was tested by Linsmaier and Skoog's (1965) method based on the growth of callus from tobacco pith tissue. The tissue was grown for 28 days and its fresh weight was recorded at the end of this period.

The significance of these results was evaluated by the F test and the LSD test at 0.5%.

RESULTS

The results of tests conducted for the presence of cytokininlike activity in potato plant parts, shown in Tables 2 and 3, indicate that the exclusion of Cl^- (except the salt impurities) from the nutrient solution resulted in a significant rise in *cy* activity (treatment F vs. A, C — Table 3). There is, however, no gradual rise in *cy* activity, corresponding to the drop in the supply of Cl^- and simultaneous rise of SO_4^{--} in the nutrient solution (Table 2, treatment A vs. C, e.g. leaf petioles or blades). On the contrary, a drop in *cy* activity is noted in the case of leafy tops and leaf petioles with a similar tendency noted in the blades and stems. The effect of nutrients is clearly visible in the case of leaf petioles but is not noticeable in the case of tubers or leafy tops (Table 2).

The *cy* level depends to a much higher degree upon the plant part than upon the $Cl^- : SO_4^{--}$ ratio. The content of *cy* in the analysed plant parts rises in the following order: roots = stems < leaf blades < tubers, leaf petioles = leafy tops.

Leaf petioles show a higher *cy* activity as compared to the blades, stems or roots, especially when one compares those activities in the treatment F with no Cl^-.

DISCUSSION

The size of plants, and their parts depends upon the balance of various growth regulators, including the *cy* level. Factors affecting this level can influence the size of plant organs. Among the nutritional factors affecting *cy* activity, up to the present time nitrogen and boron (Wagner and Michael 1971) and the $Cl^- : SO_4^{--}$ ratio (Borys and Jeske 1975) were studied. Nitrogen enhanced cytokinin synthesis in the roots of sunflower (Wagner and Michael 1971). Higher concentration of cytokinins was found in potato leaves given only SO_4^{--} as compared with the Cl^- and SO_4^{--} nutritional treatment (Borys and Jeske 1975).

The $Cl^- : SO_4^{--}$ ratio in the nutrient solution affects the size of most parts of the potato plant, larger dimensions being associated with an increased amount of Cl^- and decreased amount of SO_4^{--} (Harward et al. 1956; Borys 1967; Borys and Klejnowska 1972; Borys

Table 2

Cytokinin activity in potato organs cv. Bintje

Treatment *	Yield of potato organs as affected by the treatment fresh weight in g	Equivalent of μg kinetin in 100 g of fresh weight of the part tested
	Leafy top	
A	21.4	22.2
C	30.7	18.4
F	27.9	22.6
Mean	26.66	21.1
	Leaf blades	
A	64.2	10.3
C	71.4	9.3
F	50.1	13.2
Mean	61.9	10.9
	Leaf petioles	
A	24.1	22.2
C	30.9	15.3
F	18.9	31.3
Mean	24.63	22.9
	Stems	
A	93.1	5.2
C	116.3	4.6
F	76.2	7.1
Mean	95.2	5.6
	Tubers	
A	22.2	17.5
C	30.8	17.4
F	37.7	16.6
Mean	30.23	17.2
	Roots	
A	90.9	5.8
C	110.5	5.8
F	90.4	8.1
Mean	97.26	6.6

* See Table 1.

$LSD_{0.05}$ for organs 2.45, for nutrients 1.73, for interaction of organs × nutrients 4.24

Table 3

Cytokinin activity in potato plant organs as influenced by the $Cl^- : SO_4^{--}$ ratio in the nutrient solution *

Treatment	Equivalet of μg of kinetin in 100 g of fresh weight
A	**13.89**
C	**11.82**
F	**16.50**
LSD=0.05	**1.73**

* Means for all organs tested.

and Jeske 1975). This effect, observed also in the case of other species (Schmalfuss 1936; Seidler 1970), was related to the restriction of water uptake and facilited water loss in plants given sulphates (Schmalfuss 1936). Potato plants given no Cl^- and high SO_4^{--} (Tables 2, 3) showed higher *cy* activity as compared with plants supplied with Cl^- and SO_4^{--}.

The water potential in potato leaves decreased with a diminished amount of Cl^- and increased SO_4^{--} in the nutrient solution (Borys and Zielińska 1978), and this effect corresponded to a gradual decrease in the dimensions of potato leaves, as well as roots (Borys 1967; Borys and Klejnowska 1972; Borys and Jeske 1975). A high *cy* content in the leaves may decrease their resistance to evaporation of water, producing low leaf turgor not only because *cy* causes the opening of stomata, but also because of a larger number of stomata per unit surface area (Borys and Zielińska 1978) and smaller root (Borys and Klejnowska 1972; Borys and Jeske 1975). and the present determinations of *cy* activity for the respective organs.

The roots are the site of *cy* synthesis. Thus, one would expect, incrased *cy* activity with increased root size. In fact, the size of roots was inversaly related to the *cy* activity in the leaves, roots and especially the petioles (Borys and Jeske 1975). This is also evident, when we compare the respective growth data of Borys and Klejnwska (1972).

The potato plants growing without Cl^- with high SO_4^{--} in the nutrient solution developed more short roots containing a large number of intensively dividing cells. In such a case, the higher *cy* activity would be understandable. However, no exact measurements were taken of root morphology.

Cy plays a significant role as a mobilizing agent, participates in stomatal movement, affects water movement in the conducting tissues and the degree of leaf hydration (Mothes 1960; Livne and Vaadia 1972; Zielińska 1976).

The growing tissues which directly receive streams of various substances from the roots, usually contain larger amounts of *cy* (Michael and Seiler-Kelbitsch 1972).

This is also reflected in a high *cy* activity of the young, growing top parts of the plant and the growing potato tubers. The stems and roots were found to have a low *cy* content. Unexpectedly, however, high *cy* activity was found in the petioles, especially pronounced in leaf petioles subjected to the F treatment (no Cl^- and highest SO_4^{--} content). The blades were found to contain much less *cy* than the petioles.

Cy moves along the transpiration stream. The intensity of transpiration is highest when the concentration of Cl^- is 0 and that of SO_4^{--} is at the highest level (treatment F, Borys and Zielińska 1978). If there exists a direct relation between loss of water trough the blades and the movement of *cy* from the roots to the blades, then one would expect the highest *cy* activity in the blades and not in the petioles. No reasonable explanation can be offered for the rather high *cy* activity in the petioles as compared to the blades.

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Wpływ żywienia Cl^- i SO_4^{--} na poziom aktywności cytokininowej w roślinach ziemniaka cv. Bintje.

Streszczenie

Określono poziom aktywności cytokininowej przy pomocy testu kalusa tytoniu, w poszczególnych częściach rośliny ziemniaka, wyrosłych na trzech poziomach Cl^- i SO_4^{--}.

Wykazano, że aktywność cytokininowa wzrastała, w większości organów, w zależności od stosunków nawożenia Cl i S; 212 ppm Cl : 127 ppm S$<$372 ppm Cl : 40 ppm S$<$0 ppm Cl : 225 ppm S. W kombinacji 0 ppm Cl : 225 ppm S wykazano najniższą zawartość cytokinin tylko w bulwach.

Zróżnicowanie aktywności cytokininowej między częściami roślin było następujące: korzenie, pędy $<$ blaszki liściowe $<$ bulwy $<$ ogonki liściowe, wierzchołki ulistnione.

Zmiany w poziomach aktywności cytokininowej omówiono nawiązując do wcześniejszego stwierdzenia korzystnego oddziaływania Cl^- na rozmiary blaszek i ogonków liściowych, a także korzeni, oraz zmian w cechach rośliny, związanych z gospodarką wodną roślin ziemniaka.