Phosphorus compounds in callus tissue of *Nicotiana tabacum* L. treated with morphactins IT 3233 and IT 3456

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Abstract

The morphactins under examination blocked the growth of tobacco callus tissues at as low concentrations as 1 mg/dm³ (IT 3456) and 10 mg/dm³ (IT 3233). The growth inhibition was accompanied by an increase in dry matter content.

An increment in total P level induced by morphactins was the result of inorganic P accumulation. It could be supposed that the compounds tested influence the permeability of plasma membranes.

No direct relationship has been found between the action of morphactins on growth and the level of phosphorus compounds in tissues.

It seems that both the morphactins have a little different mode of action.

INTRODUCTION

Morphactins are known since 1964 as a group of growth regulators containing biologically active derivatives of 9-hydroxyflourene-(9)-carboxylic acid. These compounds induce among others things: callus formation, growth inhibition, disturbances of normal geotropic response and they also abolish the apical dominance in shoots. In consideration of these effects morphactins found practical application for instance in plant propagation and thick plant cover providing. The results of investigations on morphactins were presented by Schneider (1970). Only very few data were concerned with the influence of morphactins on biochemical processes.

Our previous study (Chirek — 1974, pl. I, II) dealing with the effect of IT 3233 on the growth and metabolism of tobacco tissues grown in vitro has shown that callus tissue is particularly sensitive to this regulator. It responded by growth inhibition and some changes in nitrogen metabolism, and by decreased protein synthesis.

The object of the present research was to examine the effect of morphactins on phosphorus compounds content. The evidence concerning the

influence of morphactins on phosphorus metabolism is very scanty and fragmentary. K o n o p s k a et al. (1970) found an increase in orthophosphates level with a simultaneous decrease of organic P in the roots of pea seedlings treated with morphactin. B o r n m a n (1976) citing the results of investigation concerning the effects of morphactins on the activity of enzymes connected with phosphorus metabolism found these effects to be opposed. Thus the physiological role of morphactins in that connection is still unknown.

In this study a comparison of effectiveness of both morphactins IT 3233 and IT 3456 representing 2 series of fluorene derivatives — flurenols and chlorflurenols — was also taken into consideration.

MATERIAL AND METHODS

Callus tissue of *Nicotiana tabacum* L. var. White Burley isolated in 1962 (Rennert, 1965) was grown on a solid medium of Murashige and Skoog (1962) modified by Linsmaier and Skoog (1965). The medium was supplemented with growth regulators: kinetin — 250 $\mu\text{g}/\text{dm}^3$, IAA — 2 mg/dm^3 and morphactin in concentrations: 1, 10, 40 mg/dm^3 in the case of IT 3233 preparation (n-butyl 9-hydroxyfluorene-(9)-carboxylate) and 0.1, 1, 10, 20 mg/dm^3 in that of IT 3456 (methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate). Morphactins were obtained from the E. Merck AG laboratories.

pH of the media was adjusted to 5.5. For each experimental series a control without morphactins added was performed.

The tissues were grown for 5 weeks in an incubator at $26^\circ \pm 1^\circ \text{C}$ under continuous fluorescent light. Then the tissue colonies were weighed, dry matter, total phosphorus and P-fractions were determined. Fractionation of P compounds was done according to the method of Ogur and Rosen modified by Holden (1952). 5 g samples of fresh tissue were used.

Phosphorus was determined by the method of Kuttner and Lichtenstein modified by Holden and Pirie (1955).

The experiments were repeated 4-6 times. The standard deviation of the mean and the significance of differences were calculated by Student's test.

RESULTS AND DISCUSSION

Growth. An average growth of tissue colonies expressed as fresh weight of colonies after 5 weeks of culture and dry matter contents in them are presented in tables 1A and 1B. Both morphactins slacken the rate of growth of the tobacco callus tissue, but IT 3456 acts at 10 times lower concentration than does IT 3233. A higher activity of the IT 3456 preparation has been reported by Schneider (1964).

Table 1

Fresh weight and dry matter of *Nicotiana tabacum* L. callus tissues cultured (for 5 weeks) on medium with morphactin

A		IT 3233			
Fresh and dry matter	Concentration M, mg/dm ³				
	0 control	1	10	40	
fresh weight of colony (mg)	1701±196	1751±235	1356±226	**672±84	
dry matter %	6.0±0.18	6.29±0.37	6.57±0.22	*7.58±0.47	
B		IT 3456			
Fresh and dry matter	Concentration M, mg/dm ³				
	0 control	0.1	1	10	20
fresh weight of colony (mg)	1509±51	1512±119	**1248±24	**880±50	**680±53
dry matter (%)	6.04±0.17	6.24±0.18	6.78±0.28	*7.66±0.49	**7.96±0.16

n=5 for A, n=6 for B

n — number of replicates

* — difference significant at $\alpha = 0.05$

** — difference significant at $\alpha = 0.01$

No distinct effect on the growth of these tissues has been observed in the case of IT 3456 at 0.1 mg/dm³ concentration and of IT 3233 at 1 mg/dm³.

Morphactin-induced inhibition of growth of tissues is accompanied by an increase in dry matter content up to 32% (see Figure 1).

Probably it is the result of the limitation of respiration processes in these tissues. The results of previous investigation concerning the effect of IT 3233 on the metabolism of tobacco tissues suggest such a conclusion (Chirek, 1974, p. II).

Dry matter accumulation induced by IT 3456 at 10 p.p.m. concentration was also observed by Lorenzen and Weisbrich (1969) in *Cardamine* seedlings and by Mehta et al. (1974) in *Cicer arietinum*.

There is evidence, however, of the possibility of dry matter decrease influenced by morphactins (Roversi, 1970).

In figure 1 the changes in fresh and dry matter of issue colonies influenced by morphactins are expressed in per cent as related to control (100%). Under the influence of IT 3456 fresh matter of a tissue colony decreases far more than dry matter. Therefore it can be concluded that

this regulator acts strongly on the cell enlargement connected with cell hydration.

Fig. 1. Changes in fresh and dry matter of tobacco callus tissue colonies treated with morphactin (M) (in % of control)

In the case of morphactin IT 3233 a decrease of fresh weight and that of dry matter are parallel suggesting that this compound inhibits the growth of colonies by way of reduction of both: the cell number and cell dimensions to a nearly equal degree.

Phosphorus. Phosphorus content (in mg/g of dry matter) in the tissues influenced by morphactin IT 3233 is given in table 2. At the highest concentrations (40 mg/dm³) of this preparation a significant increase in the total phosphorus content (by nearly 24%) was found. This increase is due mainly to inorganic P accumulation. RNA-P content increases a little. At lower IT 3233 concentrations no distinct changes in the level of phosphorus compounds were observed.

Morphactin IT 3456 induces, however, a significant increment in the level of total P at all the concentrations applied (Table 3). Most effective was IT 3456 at 1 and 10 mg/dm³ concentrations. P contents were higher by 43% and 30%, respectively than in the control.

Similarly as in the case of morphactin IT 3233 mainly the level of inorganic P increases. A slight increment in the content of organic P of fraction I and in that of phospholipid P were found.

DNA-P content (as related to dry matter) in tissues treated with the morphactins under examination has not been changed.

Figure 2 illustrates changes in inorganic P and total P in tissues treated with morphactins IT 3233 and IT 3456. Quite different relations of the changes, depending on the concentration of both preparations should be noticed.

Table 2

Phosphorus fractions in tobacco callus tissues treated with morphactin IT 3233 (in mg/g dry matter)

Conc.M mg/dm ³ fractions P		0 control	1	10	40
acid-soluble-P extracted with 0.2 N HClO ₄	inorg.-P	2.04±0.15	1.91±0.18	2.21±0.06	*3.05±0.20
	org.-P	1.09±0.08	1.22±0.10	1.02±0.06	0.97±0.08
	total	3.13±0.19	3.13±0.12	3.23±0.06	*4.02±0.26
lipid-P ethanol- ether 3:1		0.52±0.05	0.48±0.02	0.53±0.03	0.55±0.04
RNA-P 1 N HClO ₄ room temp.		0.72±0.01	*0.65±0.02	0.75±0.06	0.91±0.14
DNA-P 1 N HClO ₄ 37°C		0.26±0.02	0.22±0.01	0.24±0.03	0.26±0.04
sum fractions		4.63±0.20	4.49±0.10	4.75±0.09	*5.74±0.36
total-P		4.71±0.17	4.73±0.14	4.91±0.11	*5.65±0.27

n=4

Notations as in table 1

Table 3

Phosphorus fractions in tobacco callus tissues treated with morphactin IT 3456
(in mg/g dry matter)

Conc. M mg/dm ³ fractions P		0 control	0.1	1	10	20
Acid-soluble-P	inorg.-P	2.54±0.09	*3.23±0.22	**4.15±0.17	**3.53±0.15	2.89±0.19
	org.-P	0.94±0.04	0.93±0.13	1.03±0.05	*1.15±0.07	1.10±0.15
	total	3.48±0.13	*4.16±0.21	**5.18±0.20	**4.74±0.17	*3.99±0.12
lipid-P		0.48±0.02	0.57±0.06	*0.57±0.03	0.56±0.06	0.61±0.06
RNA-P		0.82±0.03	0.82±0.07	0.95±0.09	0.88±0.07	0.86±0.07
DNA-P		0.29±0.05	0.28±0.03	0.29±0.03	0.29±0.03	0.29±0.03
sum fractions		5.07±0.13	*5.84±0.22	**6.98±0.20	**6.47±0.09	*5.76±0.12
total-P		5.05±0.17	**5.95±0.18	**7.22±0.12	**6.61±0.10	*5.81±0.15

n=5 or 4

Notations as in table 1.

P content in the tissues depends on several factors such as medium composition, age of tissue and so on.

Fig. 2. Changes in inorganic-P and total-P content in tobacco callus tissues treated with morphactins: IT 3233 and IT 3456

For example Rennert (1968) reports a lower P content in the same tobacco callus tissues cultured in White medium. Bee bread added to this medium increased the P content. Baranowska et al. (1972) determined phosphorus content in the same tissues cultured in a Murashige-Skoog medium, considering also the age of tissues. Tissues 4 weeks old showed the highest P content, 6 week-old ones, however, contained 50% less P. Our results of determinations (performed by a somewhat different method) are approximate to those obtained by the above mentioned authors for 4 weeks-old tissues.

It seems also that the rate of tissue growth influences the P level. Tissues growing slower contain a little higher phosphorus content (see results for two control series in our experiments).

The morphactins under examination increase the level of phosphorus compounds as related to dry and to fresh matter of the tissues. The IT 3456 preparation is more effective than IT 3233. No simple relationship has been found between the action of these regulators on growth and phosphorus content. The greatest increase in total P took place at 1 mg/dm³ of IT 3456 where growth was inhibited only in 17%. In the case of IT 3233 however unabated an increase in P content was found at 40 mg/dm³ — where the growth inhibition reached 60%. It is characteristic and common for both morphactins that an increase in P content is due almost exclusively to an inorganic P increment. This proves at least in affected permeability and uptake of phosphorus stalls by the tissue cells or even perhaps an intensification of these processes induced by morphactins. Inorganic P

accumulation is probably due to the incomplete use of this element for the synthesis of organic compounds like ATP, nucleotides, nucleic acids. Per cent of organic P fractions in total P decreases in tissues treated with morphactins.

To obtain a more detailed picture of the mechanism of morphactin action on the metabolism of phosphorus compounds in tobacco callus tissues, the results of phosphorus determinations were related to 1 tissue colony, expressed in % of the control and shown in the diagrams (Fig. 3).

Fig. 3. Changes in P-fractions content in tobacco callus tissues treated with morphactins IT 3233 and IT 3456 (in % of control per one colony)

As seen, morphactin IT 3233 at higher concentrations (inhibiting growth) markedly decreases the level of all organic P fractions. A significant reduction of DNA-P content suggests a limitation of the cell proliferation in the colony. These results — similarly as previous ones, dealing with nitrogen metabolism (Chirek, 1974, p. II) — show that higher concentrations of morphactin IT 3233 slacken the metabolism of tobacco callus tissues, this resulting in growth inhibition.

Lower concentrations (0.1 and 1 mg/dm³) of morphactin IT 3456 increase inorganic P and lipid P contents in the tissue colonies, higher ones however reduce the contents of all P fractions including RNA and DNA, but to a lesser degree than morphactin IT 3233.

Salminen et al. (1972) did not find any distinct influence of IT 3456 (at 10 mg/dm³ conc.) on RNA synthesis in senescent sections of a bean endocarp.

It seems that these very few data do not allow to establish whether and how morphactins influence the metabolism of nucleic acids. As re-

gards the callus tissues under examination, further experiments should be concerned with the effects of morphactins on cell division in these tissues.

On the other hand there are some suggestions in the literature as to the possibility of an influence of morphactins on plasma membranes. This is mentioned by Bridges and Wilkins (1973) in connection with blocking of the auxin transport by morphactins.

Bornman (1976) found considerable changes in cellular membranes (such as endoplasmic reticulum, dictyosomes, thylakoids) of *Welwitschia callus* tissue showing a morphactin-induced growth inhibition. This author considers that the observed growth inhibition could be the result of inhibition of synthesis processes connected with cell wall formation and those in which membrane organelles take part.

Our data dealing with considerable changes in inorganic P (taken up from the medium) and an increase in lipid P (a component of plasma membranes) content in some cases also point to the influence of morphactins on plasma membranes.

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Związki fosforu w tkance kalusowej *Nicotiana tabacum* L. traktowanej morfaktynami IT 3233 oraz IT 3456

Streszczenie

Przedmiotem pracy było porównanie wpływu morfaktyny IT 3233 oraz IT 3456 na wzrost i zawartość związków fosforu w tkankach kalusowych tytoniu hodowanych „in vitro”.

Obie morfaktyny hamowały wzrost badanych tkanek; IT 3456 — już w stężeniu 1 mg/dm³, tj. 10 × niższym w porównaniu do IT 3233. Zahamowaniu wzrostu towarzyszyło podwyższenie zawartości suchej masy. Porównanie świeżej i suchej masy tkanek traktowanych morfaktynami sugeruje nieco inny mechanizm działania tych związków. IT 3456 działa silniej na wzrost objętościowy.

Zawartość fosforu całkowitego (na jednostkę suchej masy) wzrastała w tkankach traktowanych morfaktyną IT 3233 w stęż. 40 mg/dm³ oraz IT 3456 w stęż. 0,1—20 mg/dm³. Zwiększenie poziomu P-całkowitego było skutkiem zwiększenia P-nieorganicznego we wszystkich przypadkach. Tylko niewielkie zmiany wykazywały frakcje: P-organiczny — kwasorozpuszczalny, P-lipidowy i P-RNA.

Duże zmiany w zawartości P-nieorganicznego sugerują działanie morfaktyn na przepuszczalność membran plazmatycznych.

Nie stwierdzono prostej zależności pomiędzy działaniem morfaktyn na wzrost i poziom związków fosforu w tkankach.