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EFFECTS OF KINETIN AND 4PU-30 ON THE GROWTH AND THE CONTENT OF POLYPHENOLS IN TOBACCO CALLUS TISSUE

Yordanka Angelova, Snejana Petkova, Ekaterina Zozikova, Emilia Kotseva, Lubomir Iliev*

Acad. M. Popov Institute of Plant Physiology, Acad. G. Bonchev Str. , Bl. 21, 1113 Sofia, Bulgaria

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Summary. The effect of phenylurea cytokinin 4PU-30 [N_1-(2-chloro-4-pyridyl)-N_2-phenylurea] on the growth and the amount of total phenols in callus culture of *Nicotiana tabacum* L. var. Wisconsin 38 was studied. Callus grown on nutrition medium with kinetin was used as the control. The results showed that 4PU-30 induced greater accumulation of fresh and dry biomass than kinetin. The intensive callus growth was accompanied with accumulation of total phenols but there were no differences between the two variants. When results for total phenols were related to dry matter, higher content was established in callus with kinetin and there were no differences in contents of chlorogenic acid and rutin during the whole cultivation period. In the present work we found the opposite correlation between tobacco callus growth induced by different types of cytokinins and accumulation of total phenols. Additional investigations are needed to elucidate the specificity of their mode of action.

Key words: callus tissue, growth, kinetin, phenols, 4PU-30.

Abbreviations: 4PU-30 – N_1-(2-chloro-4-pyridyl)-N_2-phenylurea

Introduction

The plant cells cultivated *in vitro* synthesize phenolic compounds, however in some cases changes in the quality and quantity of the substances were registrated (Zagoskina

* Corresponding author, e-mail: *iliev@obzor.bio21.bas.bg*

et al., 1983). This is probably due to specificity of the tissue cultures as artificial biological systems in which the basic function of phenols is to interfere in cell proliferation (Nosov, 1994). The hormonal effectors of growth (including cytokinins) are important in induction of cell division and synthesis of secondary metabolites. Kinetin at optimal concentrations for the tobacco callus growth increased the chlorogenic acid amount (Filonova, 1985). Kinetin at inhibiting concentrations increased the synthesis of soluble phenolic compounds in the long-passaged tissue culture of *Camellia sinensis* (Zagoskina et al., 1983) and decreased it in tissue culture of *Cassia fistula* L. (Shah et al., 1976). It is well known that some phenylurea cytokinins are more active as compared to purine cytokinins as growth promoters in tissue cultures (Mok et al., 1987), however there is no enough available information about their influence on phenolic metabolism. Tereza et al. (1987) reported that 4PU-30 increased growth and suppressed alkaloid synthesis in tobacco callus culture.

The aim of the present investigation was to compare the effects of kinetin and the phenylurea cytokinin 4PU-30 on the amount of total phenols in growing tobacco callus tissue.

Materials and Methods

Callus culture of *Nicotiana tabacum* L. var. Wisconsin 38 was used. Callus was grown in 100 ml Erlenmayer flask on modified nutrition medium of Murashige and Skoog supplemented with 2.0 mg/l IAA and 0.2 mg/l kinetin (control) or 0.025 mg/l 4PU-30. Callus was cultivated at 26 °C and 70% humidity in continuous darkness and was maintained by subculturing every 4 weeks. Samples for analyses were taken on the 6th, 10th, 15th, 21th and 30th day of cultivation. Callus was dried at 105 °C until constant weight was reached.

Total phenols were estimated by the Folin method of Swein et al. (1959). Ethanol extract from dried callus was used in the reaction medium. Optical density was measured at 730 nm. Results were expressed as an equivalent to chlorogenic acid using a standard curve.

Chlorogenic acid and rutin amounts were determined after Hausermann et al. (1962) with flavon reagent (2-aminoethyl diphenylborinate). Colour development was measured at 381 nm for chlorogenic acid and at 448 nm for rutin. Standard curves with the same phenols were used for calculations.

The results were statistically analysed using Fisher's criteria. Data for fresh and dry weights are means of four experiments in six flasks each. For the other determinations data are means of three experiments in three replications each.

Results and Discussion

Tobacco callus grown on nutrition medium with 4PU-30 accumulated more fresh and dry biomass than those with kinetin (Fig. 1). The differences were manifested on the

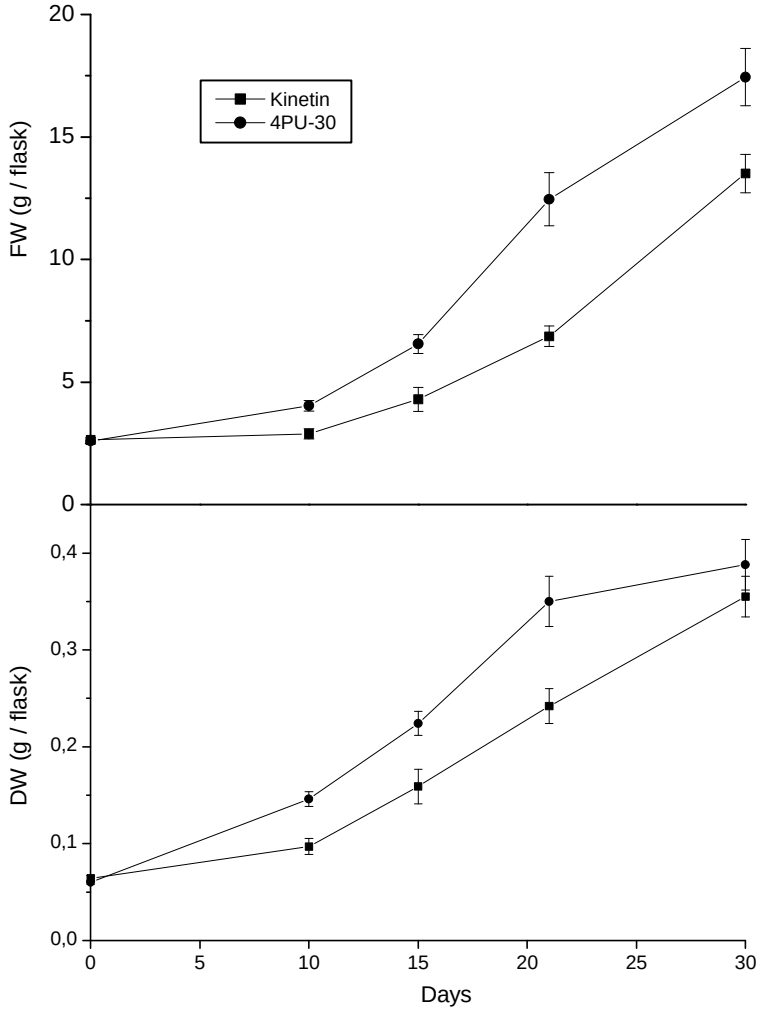

Fig. 1. Effects of kinetin and 4PU-30 on tobacco callus growth.

10th day of cultivation, but were best expressed on the 21th day, when 4PU-30 exceeded kinetin action with 81% and 45% respectively. The effect of 4PU-30 was demonstrated at a concentration eight times lower compared to kinetin. The highest physiological activity of 4PU-30 was established by Takahashi et al. (1978) in tobacco callus bioassay where in ten times lower concentration it promoted vigorous growth and

the fresh yield of callus was similar to the yield induced by N^6-benzyladenine. The biochemical action of the phenylurea cytokinins in the callus growth is not still understood and several theories have been proposed (Reynolds, 1986).

The changes in the amount of total phenols during the cultivation period were shown in Fig. 2. The results showed that tobacco pith callus kept its ability to synthesize phenolic compounds in quantities similar to those in tobacco stems (Petkova et al., 1994). An initial fast accumulation of phenols which stopped at the end of a lag-

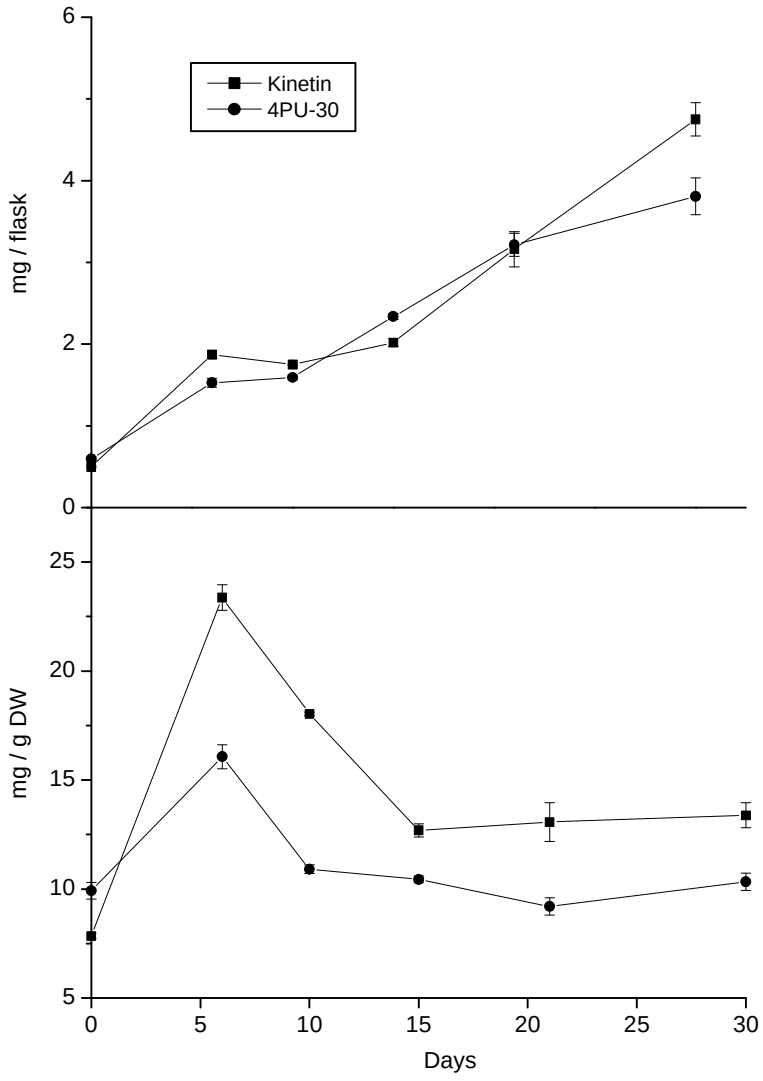

Fig. 2. Effects of kinetin and 4PU-30 on the amount of total phenols (in equivalent to chlorogenic acid) in tobacco callus tissue.

period was established. It may be due to the injure of callus and its adaptation to the new nutrition medium. The stage of intensive callus growth was accompanied with the accumulation of total phenols. Similar results were obtained by other authors, too (Koretzkaya et al., 1975; Shah et al., 1976). There were no significant differences between total phenol amounts in calluses grown with kinetin and 4PU-30. Differences were observed when phenol amounts were related to the dry matter. The initial fast accumulation of total phenols in both variants was followed by a sharp decrease and no changes till the end of the cultivation period were observed. Similar curves for phenol accumulation and PAL-activity in the beginning of the cultivation period were established with other callus and cell cultures (Ibrahim et al., 1976; Shah et al., 1976). Higher amount of phenols was established under the influence of kinetin. Similarly higher accumulation of alkaloids was obtained in tobacco callus grown on medium with kinetin compared with 4PU-30 (Tereza et al., 1987).

There were no significant changes in the amounts of chlorogenic acid and rutin in the control callus during the whole cultivation period (Table 1 and 2). In 4PU-30 dependent callus the amounts of chlorogenic acid were higher in the beginning of cultivation and reached the control levels at the end. By contrast, the amounts of rutin slowly increased troughout the cultivation period. Kinetin used at lower concentrations

Table 1. Effects of kinetin and 4PU-30 on the amounts of chlorogenic acid in tobacco callus.

Variants		Day							
		10		15		21		30	
		mg/g DW	%	mg/g DW	%	mg/g DW	%	mg/g DW	%
Kinetin		1.002	100	0.826	100	1.047	100	0.958	100
4PU-30		1.755	175	0.993	120	1.052	100	0.896	93
LSD	5%	0.911		N. S.		N. S.		N. S.	
	1%	1.320							

Table 2. Effects of kinetin and 4PU-30 on the amounts rutin in tobacco callus.

Variants		Day							
		10		15		21		30	
		mg/g DW	%	mg/g DW	%	mg/g DW	%	mg/g DW	%
Kinetin		0.366	100	0.345	100	0.328	100	0.373	100
4PU-30		0.250	68	0.228	66	0.411	125	0.436	116
LSD	5%	N. S.		N. S.		0.108		N. S.	
	1%					0.158			

formed fast division tobacco callus cells. When the concentration was higher, compact callus with differentiation of conducting elements was formed and higher amount of chlorogenic acid in it was established (Filanova, 1985). In our experiments kinetin and 4PU-30 caused only division of callus cells and this may explain the low level of chlorogenic acid.

We found the opposite correlation between tobacco callus growth induced by different types of cytokinins and the accumulation of total phenols. Similar correlation between growth and phenols was demonstrated at different concentrations of kinetin (Zagoskina et al., 1983), auxins (Ibrahim et al., 1976; Bagratishvili et al., 1980) and light (Koretzkaya et al., 1975). Moreover, there were cytokin-independent calluses in growth, but not in phenol accumulation (Bagratishvili et al., 1980). In this conection it is difficult to speculate about the specific cytokinin action. On the other hand, several lines of evidence indicate that kinetin can regulate callus growth by influencing some enzymes interfiering with secondary metabolism (Zagoskina et al., 1983, Filonova, 1985). There are no similar data on the 4PU-30 effect and further investigations are needed to elucidate its mode of action.

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