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In vitro Induction of Axillary Potato Microtubers and Improvement of Their Quality

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Abstract. Germinated tubers of selected cultivars Kera, Resy, Nicola and Oreb were made healthier by heat treatment. They were derived from germ explants on MS media with the addition of BAP 1 mg l^{-1} and IAA 0.2 mg l^{-1}. After sufficient multiplication of stems, optimum conditions of the photoperiod were followed for the induction of axillary microtubers on stem segments in media with BAP 10 mg l^{-1} and 8% sucrose. The ability of tuberization is different: the early cvs. Kera and Resy induce earlier tubers at a long photoperiod and late cvs. Nicola and Oreb tuber rather at a short photoperiod. In suitable photoperiods the inhibitory substances accelerate the induction of axillary tubers and limit the formation of adventitious roots. Synthetic inhibitors applied in induction media increase the number of the tubers.

The quality of tubers was affected by the addition of a mixture of amino acids: aspartic, glutamic, lysine and proline in concentrations of 12.5, 25 and 50 mg l^{-1} into the induction media. In the course of cultivation the types which were growing well, formed tubers and increased the volume were selected. The representation of amino acids in the tubers was not significantly affected, there was only an increase in proline. The higher content of amino acids was reflected in the increase of proteins in the tubers. The selected clones are further multiplied.

The explant techniques find steadily increasing application in the growth programmes. They allow a quick multiplication of selected individuals, preserve good qualities of the mother plant and contribute to the elimination of the present cultivars from virus and fungus pathogens. By means of suitable induction of stem segments of potatoes it is possible to obtain axillary tubers in a short time and to affect their composition. By refining the conditions of tuberization both the morphogenesis and the quality of tubers can be regulated. BURI (1971) reports besides nucleotides also some amino acids, above all aspartic and glutamic acids, which improve the gustatory properties of tubers. Proline tastes sweet, alanine and lysine are evaluated as neutral. Possibilities are being sought of increasing the content of amino acids for improving the tuber quality.

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The objective of the present study was to multiply the healthy material specifying the conditions for the induction of tuberization of early and late cultivars, including the specification of the share of inhibitory substances. The application of amino acids in the media was to affect the composition of reserve substances in the tubers and to contribute to the improvement of tubers.

MATERIAL AND METHODS

Explants of early cvs. Kera and Resy and late cvs. Nicola and Oreb were derived from the germs of potatoes after heat treatment. The tubers were exposed for 15 days to temperatures of 39 °C during the day and 25 °C at night according to SOLDAÑA and DAWSON (1982), and from the surviving germs axillary shoots for stem formation were removed. Part of the cultures of Kera and Oreb were complemented from the collection of the Research Institute of Potato Improvement in Havlíčkův Brod.

Increase in the multiplication coefficient was stimulated by the addition of BAP 1 mg l^{-1} and 0.2 mg l^{-1} into MURASHIGE and SKOOG medium (1962) (MS). Photoperiod was 12, 14 and 16 h under the total illuminance of 3000 lx from fluorescent tubes and bulbs. The temperature of cultivation was 21 ± 2 °C. Stimulations of axillary bud regeneration are shown in Table 1.

The induction of axillary tubers on stem segments 3 cm long was carried out on MS media with BAP 10 mg l^{-1} and 8% sucrose according to PALMER and SMITH (1969) during a long or short photoperiod. The nodal segments usually contained 3 leaves with axillary buds, some of which changed into microtubers. The effect of different photoperiods on forming tubers in early and late cultivars after 15 and 30 days of cultivation is shown in Table 2.

TABLE 1

Stimulation of axillary bud regeneration on potato stem segments by means of growth regulators after 30 days of cultivation on MS media. Mean values are from 100 stem segments in each experimental series

Cultivars	Control			Growth regulators BAP + IAA		
	Stems		Roots	Stems		Roots
	No.	Length	Length	No.	Length	Length
Kera	4.5 ± 0.4	10.3 ± 1.8	7.2 ± 0.6	12.3 ± 1.9*	7.8 ± 1.2	0
Resy	6.8 ± 0.5	9.6 ± 1.3	9.2 ± 0.6	13.6 ± 1.4*	6.3 ± 1.9	0.8 ± 0.1
Nicola	4.3 ± 0.5	11.6 ± 2.3	6.9 ± 0.4	10.8 ± 1.0*	4.1 ± 0.9	0
Oreb	6.9 ± 0.7	12.3 ± 1.9	9.3 ± 0.8	14.2 ± 1.8*	8.6 ± 1.6	1.2 ± 0.1

* significant at $P = 0.05$.

TABLE 2

Induction of axillary microtubers on a long and a short photoperiod after 15 and 30 d of cultivation on Palmer and Smith media. Mean values are calculated from 100 stem segments in each experimental series

Cultivars	Photoperiod			
	Long		Short	
	% of tubers		% of tubers	
	15 d	30 d	15 d	30 d
Kera	36.2 ± 2.8**	50.3 ± 3.6**	12.6 ± 0.9	23.6 ± 1.7
Resy	28.7 ± 2.3**	42.8 ± 3.8**	11.8 ± 1.6	18.9 ± 2.3
Nicola	19.3 ± 2.0	28.7 ± 3.1	31.1 ± 2.9**	41.0 ± 3.7*
Oreb	12.7 ± 0.9	24.2 ± 2.6	39.4 ± 3.1**	58.9 ± 3.5**

** highly significant at $P = 0.01$
* significant at $P = 0.05$

The preliminary determination of endogenous substances diffusing from leaves into agar blocks on the 4th day of the photoperiodic induction was carried out by a biotest for cytokinins according to CONRAD (1974). Besides colour differences of β-cyanine also the length of hypocotyls and roots of *Amaranthus caudatus* was followed. Simultaneously for comparison agar blocks with BAP, GA_3, IAA and ABA at concentrations of 0.5 and 1.0 mg l^{-1} were used. The absorbance was measured at 542 nm.

In each experimental series, the endogenous substances diffused from 10 adult leaves of different induced potato stems into 10 × 10 × 3 mm agar blocks. After 4 h diffusion leaves were removed and into pits of agar block 10 pregerminated seeds of *Amaranthus* were slid. After 4 days the roots grew through and the length of roots and hypocotyls was measured. This biotest of growth of organs is based on the presence of stimulations and inhibitions according to FRANKLAND and WAREING (1961). Differences in the character of diffuse substances from potato leaves in long or short photoperiod are given in Table 3.

The effect of synthetic inhibitors on the formation of axillary tubers was evaluated after 15 days of cultivation at a 14-h photoperiod (Table 4). Conspicuous inhibitions appeared in the production of sessile or oval shaped microtubers with a different degree of dormance (Fig. 1 B, C).

In the experiments with higher concentrations of amino acids the explants were transferred every 30 days into new media. In the same time the types were selected that continued the formation of tubers and those that formed rather axillary shoots were eliminated from multiplication.

The representation of amino acids in tubers after a 6-month cultivation on media with the addition of 12.5, 25 and 50 mg l^{-1} of a mixture of aspartic and glutamic

TABLE 3

Differences in character of diffuse substances from basal potato leaves after the 4th day of photoperiod induction ascertained by bioassays and expressed in % of root and hypocotyl length and colour intensity in cotyledons of *Amaranthus caudatus*

Cultivars	Photoperiod					
	Long			Short		
	values of biotests related in % of controls					
	Roots	Hypocotyls	Cotyledons	Roots	Hypocotyls	Cotyledons
Kera	−28.8*	−34.4*	−12.1	+35.2	+43.1	+7.0
Resy	−21.2*	−36.2*	−18.6*	+29.6	+41.6	+6.9
Nicola	+22.9	+46.3	+9.3	−30.8*	−2.6*	−15.2*
Oreb	+37.7	+39.5	+5.8	−43.4*	−14.1*	−17.4
		Inhibitions			Stimulations	
		Stimulations			Inhibitions	

Differences ± 20 % are significant.

acids, proline and lysine were evaluated on automatic analyser of amino acids AAA 339 of Mikrotechna, Praha by the workers of the Research Institute of Fodder Plants at Troubsko near Brno. The differences in the content of proteins in the tubers (Table 5) were followed photocolorimetrically according to BRADFORD (1976) and refractometrically according to HOFF (1975).

RESULTS

Regeneration abilities of axillary buds on stem segments of both early cvs. Kera and Resy and late cvs. Nicola and Oreb are good, and can still be stimulated by application of 1 mg l^{-1} BAP and 0.2 mg l^{-1} IAA. Even if all cultivars preserve their morphological peculiarities, they react significantly to growth substances by a higher production of the axillary stems and limiting of roots. The lateral stems are shorter in experimental series. Growth rates of 100 stems are shown in Table 1 and regeneration capacity of axillary buds on 4 stem segments is given in Fig. 1A.

The induction of axillary tubers in long and short photoperiods after 15 and 30 days of cultivation on PALMER and SMITH (1969) media is given in Table 2. Cultivars Kera and Resy formed the microtubers earlier at a long 16 h photoperiod. In late cvs. Nicola and Oreb tubers were formed rather at the 12 h photoperiod. The values in Table 2 are the means of tuber induction of 100 nodal segments from 3 repetitions.

Suitable induction conditions were reflected by the formation of sessile axillary tubers of round shape. Less favourable conditions induced the formation of tubers on stolons and the buds on elongated tubers sometimes grew through (Fig. 1B).

TABLE 4

The effect of synthetic regulators on the induction of axillary tubers at a 14-h photoperiod after 15 d of cultivation. The mean values in % are calculated from 60 stem segments in each experimental series

Cultivars	Control	ABA 3 mg l^{-1}	CCC 100 mg l^{-1}	Coumarin 25 mg l^{-1}
Kera	23.1 ± 0.9	38.5 ± 1.3**	26.1 ± 0.8*	36.7 ± 1.3**
Resy	20.8 ± 0.7	34.1 ± 0.9**	30.6 ± 1.2**	39.0 ± 1.2**
Nicola	18.6 ± 0.8	24.7 ± 1.2**	28.3 ± 0.8**	28.9 ± 0.9**
Oreb	19.2 ± 0.8	39.7 ± 1.3**	30.6 ± 0.8**	32.3 ± 1.2**

** highly significant at $P = 0.01$
* significant at $P = 0.05$

The share of leaves in the induction of axillary bud on tuberization by the different length of photoperiod was complemented by following up the character of endogenous substances diffusing from leaves on the 4th day of the photoperiodic induction of stem segments. After a 4 h diffusion pregerminated seeds of *Amaranthus* were set into agar blocks, and after 4 days in the darkness the length of roots, hypocotyls and the intensity of β-cyanine coloration were measured. The different effects of endogenous substances in leaves from a long and a short photoperiod are given in Table 3.

In the leaves of early cvs. Kera and Resy induced by a long photoperiod the inhibitions prevailed, which also hampered the lengthening of roots and hypocotyls as well as the synthesis of β-cyanine. In the leaves of early cvs. induced by a short photoperiod the stimulations prevailed, since both the length of roots and hypocotyls and the intensity of violet-red colouration were greater.

An opposite reaction was that exhibited by the leaves of the late cvs. Nicola and Oreb. Under conditions of the long photoperiod the stimulatory character of diffuse substances prevailed, whereas at the short photoperiod the leaves showed inhibitory effects on the growth processes and on the synthesis of the colour substances. The

TABLE 5

Protein content [mg g^{-1}(fresh mass)] in tubers of cv. Nicola on PALMER and SMITH (1969) media with higher concentrations of amino acids. Averages from 3 repetitions

Method	Control	Amino acid conc. [mg l^{-1}(media)]		
		12.5	25	50
Bradford	1.05 ± 0.3	1.43 ± 0.4	1.52 ± 0.5	1.78 ± 0.5
Hoff	1.16 ± 0.5	1.38 ± 0.4	1.45 ± 0.5	2.18 ± 0.4

results indicate that the leaves of early and late cultivars have preserved the ability of different reactions to the photoperiod.

The share of inhibitory substances in tuberization under non-inductive conditions of the 14-h photoperiod is shown in Table 4. The addition of synthetic regulators: abscisic acid (ABA) 3 mg l^{-1}, chlorocholine chloride (CCC) 100 mg l^{-1} and coumarin 25 mg l^{-1} to the induction medium with BAP 10 mg l^{-1} and 8% sucrose increased the number of tubers. In the majority of cases tubers of round shape were formed, sessile or on short stolons (see Fig. 1 C lower row).

Another intention was to affect the quality of the reserve microtubers of the cv. Nicola by adding the mixture of amino acids: aspartic, glutamic and lysine into the nutrient medium in concentrations of 12.5, 25 and 50 mg l^{-1} in the induction medium with 10 mg l^{-1} and 8% sucrose. The application of higher concentrations of amino acids reduced the induction of tubers and supported the growth of lateral stems. The tubers were of elongated shape, growing through in their apical part (Table 1 C upper row). During transferring the explants with tubers types of round shape and yellow pith were selected (Fig. 1 D).

The analysis of amino acids in tubers cultured 6 months at different levels of amino acid mixture points to the ability of explants to metabolize three exogenously applied amino acids – aspartic, glutamic and lysine – whereas proline was accumulated in the tissues. The representation and content of endogenous amino acids in the tubers were not significantly affected. Data are not presented. There was only an expressive increase in proline content, especially in series with 25 mg l^{-1} where the quantity surpassed 83% and in series with 50 mg l^{-1} it was 102%.

The values of the colorimetrical determination of proteins (according to BRADFORD 1976) and the refractometric measurement (according to HOFF 1975) are listed in Table 5: the values are in mg g^{-1} fresh matter in the control and in experimental variants. Both methods indicate that the application of amino acids in the medium results in the increase in protein content of the tubers. The two methods may be used for the study of tuber proteins. Certain differences in *in vitro* tubers can be accounted for by the optimalization and utilization of potential abilities of the synthesis. Protein accumulation in selected tubers could be increased during long-time cultivation on PALMER and SMITH (1969) media with higher doses of amino acids.

DISCUSSION

The results indicate the role of growth regulators in the regeneration and morphogenesis of potato explants. To increase the multiplication coefficient, the stimulation of axillary meristems by cytokinins is necessary as well as the support of the formation of adventitious roots by auxin substances. The concentrations were chosen on the basis of data by ROEST and BOKELMANN (1976) and KOSTRICA *et al.* (1985). There are different data for the induction of axillary tubers. PALMER and

SMITH (1969) recomend a higher concentration of BAP and sucrose, KRAUS and MARSCHNER (1982) stress the importance of nitrogen and photoperiod for the tuberization, documenting the effects by changes in the ratio of the gibberellic and abscisic acids.

Tuberization appears as multiform phenomenon and some features suggest flowering. The phenomenon is photoperiodically dependent, the induction induces growth changes that are closely connected with the character of endogenous regulators. Many authors have tried to define these relations (EWING 1978, WAREING 1983). The induction of microtubers became a model for a more detailed study of these processes, making even the improvement of the tuber quality possible.

A different reaction of early and late cvs. to the photoperiod confirms the data of SLADKÝ (1985) on the effect of inhibitory substances in late cvs. in the short photoperiod. This is in agreement with the data by WAREING (1983), who also found that ABA from leaves supports the formation of axillary tubers of stem segments of *Solanum andigena* in the short photoperiod. Our results have extended these relationships by the discovery of inhibition in early cvs. in the long photoperiod.

The increase in amino acids concentration in the induction media stimulates the regeneration abilities of explants, verifying the possibilities of utilizing amino acids in the synthesis and storage of proteins in the reserve tissues of the tubers. The analyses have confirmed the metabolization of exogenously applied amino acids, with the exception of proline. It can be assumed that the exogenous applications stimulated the regeneration abilities of explants by means of a more active protein synthesis (KAMADA and HARADA 1984). Also RONCHI *et al.* 1984 report the stimulating effect on somatic embryogenesis of proline and serine, which enhance differentiation in regenerating cultures and offer an effective means to raise the frequency and yield of embryos.

Amino acids participated in the induction of the selective pressure which permitted the multiplying of types which continued forming the tubers and which also increased the protein content. Our results are similar to a recent work of WHEELER (1988). They suggest that trends both in visual rating of axillary tubers and accumulation of proteins following the harvest index could be an acceptable assay for evaluation of tuberization capacity in the potato.

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Fig. 1 at the end of the issue.

BOOK REVIEW

TULLIUS, T. D. (ed.): METAL-DNA CHEMISTRY. ACS Symposium Series 402. – American Chemical Society, Washington, DC 1989. 213 pp. US + Canada $ 49.95, Export $ 59.95.

The importance of metals for biological systems has been recognized for a long time, mainly in their association with proteins. The study of the chemistry of metals and nucleic acids has begun only recently. This publication is based mainly on lectures presented at the symposium "Transition Metal-Nucleic Acid Chemistry", held at the 195th National Meeting of the American Chemical Society, Toronto, 1988. The introductory article by T. D. Tullius reviews the use of metal complexes as tools for molecular biology. Metal complexes are especially well-suited to use as probes of unusual DNA structures and as replacements for enzymes that are used to manipulate DNA. The other 10 articles focus *e.g.*, on the following topics: chemical nuclease activity of phenanthrolinecopper, excited-state modalities, interaction of porphyrins and metalloporphyrins with nucleic acid, searching for metal-binding domains, inorganic reagents as probes, 31P NMR spectroscopic investigations, base selective DNA cleavage with a cyclometallated palladium complex *etc.* Of special interest is the article on the Fur regulon of *E. coli* and on the structures and stabilities of metal-nucleotide complexes.

This publication should prove helpful to researchers involved in all aspects of DNA chemistry.

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Polyamine Accumulation in Aged Wheat Seeds

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Abstract. The present work was conducted to evaluate the content of the main polyamines (Spm, Spd, Put) in a series of naturally aged durum wheat seeds as well as the activities of the enzymes ODC and ADC involved in their biosynthesis. In dry seeds the content of polyamines, especially that of Spd, rose during ageing till 6 years and then declined sharply. However, an increase of PA content upon imbibition was observed only with the youngest seeds, while a decrease was found in the older ones.

The activities of ODC and ADC differed in aged seeds, the ODC activity being constant and lower than the ADC in the course of seed ageing. The ADC increased till the early ageing and decreased then in the very old, ungerminating seeds. Imbibition increased both enzyme activities in the youngest seeds only, in the older ones rather a decrease and changed ADC/ODC ration was found.

The obtained results are discussed in relation to the participation of these enzymes in the biosynthesis of polyamines during seed ageing and in the course of plant senescence or stress.

Additional index words: Triticum durum: spermine ; spermidine ; putrescine ; arginine decarboxylase ; ornithine decarboxylase ; ageing.

Recent investigations indicate that polyamines (PA) may play an important regulatory role in a variety of growth and developmental plant processes such as seed germination and growth, response to stress, senescence (GALSTON 1983, SLOCUM *et al.* 1984). Data were reported that reveal that exogenous PA may retard the progressive senescence of oat leaf protoplasts (ALTMAN *et al.* 1977), cause the inhibition of RNase activity and solute leakage from tuber disc of *Solanum* by stabilizing membranes against leakage (ALTMAN 1982a), inhibit protease activity (KAUR-SAWHNEY *et al.* 1982a, BALESTRIERI *et al.* 1987) and prevent chlorophyll breakdown in detached, dark-incubated leaves of several plants (ALTMAN 1982b, CHENG and KAO 1983).

The diamine putrescine (Put) and, to a lesser extent, spermidine (Spd) accumulate in cereal leaf segments exposed (YOUNG and GALSTON 1983, FLORES and GALSTON 1982) to osmotic stress. This in parallel with an increase in the activity of

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