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Modification of cultivar-specific regeneration capacity of potato explants by phytohormones and by *Agrobacterium* oncogenes

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Abstract

To overcome low, genotype dependent regeneration capacity of some commercially important potato cultivars, two alternative treatments of stem primary explants were employed: modification of the hormonal composition of inductive MS medium and insertion of some *Agrobacterium* oncogenes. A replacement of 6-BAP with zeatin in the inductive MS medium stimulated bud/shoot formation in only two of three tested cultivars with naturally low regeneration capacity. GA_3 did not affect the bud initiation phase (*i.e.* regeneration capacity of cultivars), it only stimulated shoot development. The insertion of some bacterial oncogenes (in particular genes 4, *rol*B, C and 5) enhanced bud/shoot formation, especially in the case of low-regenerating cultivars.

Introduction

Low regeneration capacity of numerous plant genotypes represents a serious obstacle in the introduction of genetic engineering techniques to conventional breeding processes. Also for practical commercial use of potato (*S. tuberosum* L.) somaclones, transformed plants and/or somatic hybrids it is desirable to have simple, universal and genotype independent protocol to regenerate new plants from *in vitro* cultures. The genotype dependence of regeneration in potato tissue cultures has been frequently reported (Webb *et al.* 1983, Wheeler *et al.* 1985, Coleman *et al.* 1990, Opatrná *et al.* 1990) and interaction between the genotype (variety, cultivar) and the particular regeneration protocole demonstrated. In spite of announcements of the verification of some "genotype-independent" methods for the regeneration of potato

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Abbreviations: 6-BAP - 6-benzylaminopurine; Claphoran - sodium cefotaxime; IAA - indoleacetic acid; ILA - indolelactic acid; ipt - isopentenyltransferase

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plants from various primary explants and/or tissue cultures (De Block 1988, Hulme *et al.* 1992) the problem of poor regeneration capacity remains. Its further investigation provides not only practical results, but also a valuable information about the regulatory mechanisms in plant morphogenesis concerning mainly the role of phyto-hormones.

In a previous study, cultivar-specific regeneration capacity of stem internode sections (SIS) of a wide range of Czechoslovak potato cultivars was examined (Opatrná *et al.* 1990). According to their response to the regeneration media containing cytokinin 6-BAP and auxin IAA (the two-step method beeing used) the cultivars were distributed to three groups: cultivars with high, medium and low bud regeneration capacity. The cultivar-specific response was relatively stable under standard conditions. The low regeneration capacity was not enhanced by modifications of IAA/6-BAP ratio. However, the possibility that the application of other cytokinins (*e.g.* zeatin) could modify this response scale should not be omitted.

The actual level of the phytohormones in plant tissues, able to modulate their regeneration capacity can be also affected by the alternative way. The synthesis and metabolism of auxins and cytokinins can be encoded by oncogenes carried by transferred DNA (T-DNA) of *Agrobacterium* (for review see Zambryski 1988, Zambryski *et al.* 1989, Walden and Schell 1990, Meyerhofer *et al.* 1991). *A. tumefaciens* genes iaaM (gene 1) and iaaH (gene 2) encode the synthesis of auxin IAA (Schröder *et al.* 1984, Inze *et al.* 1984, Akiyoshi *et al.* 1984). Gene 5 encodes the synthesis of indole-3-lactate (ILA), an auxin analogue. ILA was found to autoregulate its own synthesis *(via* inhibition of auxin induction of gene 5 promotor) and compete with IAA for *in vitro* binding to purified cellular auxin-binding proteins. In this way it probably modulates a number of auxin responses in transformed plants (Körber *et al.* 1991). On the contrary, the synthesis of cytokinin zeatin is encoded by gene ipt Z = gene 4 (Medford *et al.* 1989).

More recently, the morphoregulatory role of some oncogenes of *A. rhizogenes*, in particular genes *rol*A,B,C,D has been systematically studied (Spena *et al.* 1987, Estruch *et al.* 1991a,b, Van Altvorst *et al.* 1992). Their common expression leads to an increased auxin sensitivity, which might be attributed either to an increased auxin binding in plasmalemma or to the increase of free auxin level through the lysis of its complexes. The *rol*B gene alone codes for β-glucosidase, which probably hydrolyses indole-β-glucosides (Estruch *et al.* 1991a). On the contrary, gene *rol*C codes for a cytokinin-β-glucosidase which is able to hydrolyse at least two different N-glycosides (Estruch *et al.* 1991b). The mechanism of action of some other T-DNA genes able to modify the sensitivity of transformed cells to phytohormones (as gene 6b - Hookyaas *et al.* 1988, Tinland *et al.* 1989) remains unkonown.

A technique of the insertion of some *Agrobacterium* genes into the potato genome has been used to investigate their effect on a phenotype of the transformed plants, including the ability of flowering, shoot development, tuberization (Ooms and Lenton 1985, Ooms *et al.* 1985, 1987, Hänish ten Cate *et al.* 1988, Schmülling *et al.* 1988, Tavazza *et al.* 1988, Visser *et al.* 1989, Ondřej *et al.* 1989, 1990, Higgins *et al.* 1992).

These results initiated our effort to examine the effect of the above genes on regeneration capacity of our model cultivar scale. We decided to study the response of stem internode sections (SIS) of usually well, moderately and poorly regenerating cultivars to either these "hormonal" genes or to the modified hormonal composition of the regeneration media. In this case, the effect of zeatin as a substitute for 6-BAP and gibberellin as another type of plant growth regulator was tested.

Material and methods

Plant material, regeneration protocols: The following potato (*Solanum tuberosum* L.) cultivars were used: *a*) with high regeneration capacity - local cvs. Kamýk and Lada, together with the Dutch cv. Desirée as frequently used model genotype; *b*) with medial bud regeneration capacity - local cv. Lukava; *c*) with very low to negligible regeneration capacity local cvs. Blaník, Karla, Karin and Ostara.

The primary stem internode explants (*ca.* 5 - 7mm) were taken from regularly *in vitro* cloned plantlets at 6 - 7 weeks after subculturing and inoculated on agar medium (0.7 %), containing MS salts and vitamins, supplemented with 30 g l^{-1} saccharose. The two-step regeneration method was employed (Opatrný and Müllerová 1986), consisting of 4-week induction phase on MS1 medium supplemented with 2.1 mg l^{-1} of 6-BAP, 40 mg l^{-1} of adenine and 0.1 mg l^{-1} of IAA, after which the explants were transferred to medium MS-2, containing only 0.1 mg l^{-1} IAA. On this medium the already initiated buds developed further. They can be excised some weeks later and rooted to develop into complete plants.

Two alternative induction agar media of modified hormonal composition were used: *a*) PB1 containing 2 mg l^{-1} zeatin, 40 mg l^{-1} adenine, 0.02 mg l^{-1} NAA and 0.01 mg l^{-1} GA_3, and *b*) PB2 containing 1 mg l^{-1} BAP, 40 mg l^{-1} adenine, 1 mg l^{-1} IAA and 10 mg l^{-1} GA_3. Cultures were grown in growth chamber conditions under a 12 h photoperiod, with an irradiance of 220 μmol m^{-2} s^{-1} and day/night temperature 22/18 °C.

At about 50 stem explants per each cultivar were used in all cultivar/protocol experiments, in three replicates at least, with a high reproducibility of the response.

Bacterial strains and plasmids: Various *Agrobacterium tumefaciens* strains with different oncogene carrying T-DNA constructs were applied:
-C58C1 (pGVL 2260; pGV 941) carrying NPT II gene driven by Nos promoter (Deblaere *et al.* 1985)
-LBA 4466 (Hooykaas *et al.*1988) with 6b gene under its own promoter
-GV3101 (pMP90RK; pPCV.002CaMVBT) carrying *Agrobacterium rhizogenes* gene *rol*B driven by CaMV 35S promoter and with CaMV 35S terminator
-GV3103 (pMP90RK; pPCV.002CaMVC) carrying *Agrobacterium rhizogenes* gene *rol*C driven by CaMV 35S promoter
-GV3103 (pMP9ORK; pPCV.702g5) carrying *Agrobacterium tumefaciens* gene 5 under 35S CaMV promoter (Spena *et al.* 1987)

-LBA4404 (pLA4404; pCB 1341) containing *Agrobacterium tumefaciens* genes 1,2,4,5 driven by their own promoters
-LBA4404 (pLA4404; pCB1347) carrying Ti-plasmid gene 4 under its own promoter
-LBA4404 (pLA4404; pCB1363) carrying Ti plasmid gene 4 and 1 under their own promoters (Ondřej *et al.* 1989)
All of the binary vectors include NPTII gene with plant transcription/translation signals.

Transformation procedure: *Agrobacterium* suspensions were used for transformation. Bacteria were cultivated over night at 28 °C in minA medium (2.1 g l^{-1} K_2HPO_4, 0.81 g l^{-1} KH_2PO_4, 0.2 g l^{-1} $(NH_4)_2SO_4$, 0.1 g l^{-1} sodium citrate, 0.2 g l^{-1} $MgSO_4.7H_2O$, 2 g l^{-1} glucose, pH = 5.6). Then they were washed in 0.01 mol l^{-1} $MgSO_4$ and diluted using liquid MS hormone-free medium to a final density 5×10^6 cells per ml. A standard transformation technique was used (Ovesná 1991). Fresh SIS were pre-cultured for 24 h on MS1 agar medium, then incubated for 30 min in bacterial suspension and thereafter cocultivated for 48 h with *Agrobacterium* on MS1 agar medium.

After that, the explants were transferred onto the fresh MS1 medium, supplemented with 500 mg l^{-1} Claforan and 50 mg l^{-1} kanamycin-sulphate to kill bacteria and to select potential kanamycin resistant transformants.

T-DNA insertion into plant DNA of potentially transformed shoots was proved using Southern hybridization (Sambrook *et al.* 1989).

Results and discussion

Cultivar-specific regeneration capacity of non-transformed SIS and the effect of growth regulators: Changes in growth regulator contents of culture media enhanced to a certain extent the regeneration capacity of the explants of some cultivars, including that of usually poorly regenerating. Both the frequency of bud/shoot formating explants and the total number of regenerated buds+shoots per explant were increased (Table 1 a,b).

Supposing a high physiological uniformity of the model system (stem explants taken from the plantlets of the same "age", precultured under the same conditions of *in vitro* culture) substantial differences in the susceptibility of various cultivars to phytohormones were again proved. The original response scale (Opatrná *et al.* 1990) was only partially modified by the changed hormonal composition of the media.

An enhancing effect of GA_3 (medium PB2) on the development of bud primordia was observed only in usually well regenerating cvs. Kamýk and Desirée. No direct stimulatory effect of GA_3 on the bud initiation phase was detected even by anatomical analysis (Burdová, unpublished results).

The application of zeatin instead of BAP resulted in a certain improvement of the regeneration capacity of cvs. Kamýk (well regenerating) and Lukava (moderately regenerating). In case of low regenerating cultivars, the bud initiation was significantly stimulated by zeatin in cvs. Karin and Karla, but not in cv. Ostara.

These results indicate the existence of different mechanisms underlying phenomenon of "low regeneration capacity" of different genotypes.

Table 1. Cultivar-specific bud/shoot regeneration capacity of stem explants on various inductive media evaluated on 65th day of cultivation (*a*), 35th and 65th day of cultivation (*b*).

a

Frequency [%] of explants regenerating buds or shoots	Desirée	Kamýk	Lukava	Karla	Karin	Ostara
MS1/MS2	68	97	63	30	0	0
PB1	100	100	88	90	95	68
PB2	73	73	25	23	10	33

b

Total number of buds+shoots per explant	Desirée		Kamýk		Lukava		Karla		Karin		Ostara	
	35	65	35	65	35	65	35	65	35	65	35	65
MS1/MS2	0.4	1.6	1.7	5.0	1.7	3.4	0	0.3	0	0	0	0
PB1	1.1	7.1	2.0	4.3	3.3	4.6	0.4	6.4	1.3	2.0	0	1.4
PB2	7.0	9.0	7.0	8.8	1.0	1.7	0	1.1	0	0.6	0	0.3

The effect of oncogene insertion: Transformation of explants by *ipt* gene 4 stimulated regeneration of buds and increased frequency of regenerated shoots per explant in almost all the cultivars treated. Shoot formation and subsequent regeneration of complete plantlets was achieved even in a case of poorly regenerated cv. Ostara (Table 2). Morphology of the transformants was markedly altered in most cases. Pronounced shoot branching resulted probably from the suppression of shoot apical dominance by increased endogenous cytokinin level, atypical pale leaves appeared. Rooting of such shoots was slow and difficult (data not given).

Transformation by constructs carrying gene 4 in combination with genes 1, 2 and 5 resulted mainly in crown gall formation.

Only callus tissue formation was observed after the insertion of gene 6b in usually well regenerating cv. Kamýk and moderately regenerating cv. Lukava. Gene 6b is supposed to modulate cytokinin activity in transformed tissues (Tinland *et al.* 1989) but our observations are difficult to interpret only in consistence with this hypothesis.

Very interesting results were obtained with the construct containing alone gene 5. As mentioned above, this gene codes for the ILA synthesis. ILA is produced by several soil bacteria, including *Agrobacterium* (Rovenská *et al.* 1988), as well as by plants but its physiological role has been unclear. According to Körber *et al.* (1991) transgenic tobacco calli expressing gene 5 regenerated shoots earlier than control wild type calli on appropriate culture media. When tobacco seeds from both the wild type and the gene 5 transformed plants were germinated on agar media containing high amount of NAA in combination with some cytokinins, only wild type seedlings were retarded. In contrast, on the hypocotyl of seedlings expressing gene 5 calli

appeared and developed in teratoma-like tissues with multiple shoots. ILA addition to another regeneration inducing media enhanced shoot formation by wild type calli. Similarly in our experiments the insertion of gene 5 into cvs. Kamýk, Karin and Ostara supported earlier onset of bud initiation and (in particular in cvs. Karin and Ostara) stimulated total bud formation (Table 2). Healthy looking, well rooting regenerants were obtained from these experiments.

Table 2. The morphogenetic effect of the transformation of stem explants by various *Agrobacterium* oncogenes.

Cultivar	Explant reaction	CO-1 0	CO-2 (+NPT) Km	Ti 1+2+4+5 Km	Ti 1+4 Km	Ti 4 Km	Ti 5 Km	Ti 6b Km	Ri *rol*B Km	Ri *rol*C Km
Kamýk	CG [%]	0	0	82	0	0	-	-	-	-
	SH [%]	85	78	0	71	74	-	-	-	-
	SH No	6.2	5.8	0	5.5	8.2	-	-	-	-
Lada	CG [%]	0	0	79	0	0	0	68	0	0
	SH [%]	89	82	0	62	79	84	0	86	87
	SH No	5.8	5.2	0	10.2	13.3	5.7	0	5.1	8.3
Lukava	CG [%]	0	0	-	-	0	-	52	-	-
	SH [%]	49	9	-	-	62	-	0	-	-
	SH No	4.1	2.5	-	-	7.2	-	-	-	-
Karla	CG [%]	0	0	93	-	0	-	-	-	-
	SH [%]	8	0	0	-	13	-	-	-	-
	SH No	1.8	0	0	-	2.0	-	-	-	-
Karin	CG [%]	0	0	91	-	0	0	-	0	0
	SH [%]	0	0	0	-	9	86	-	12	10
	SH No	0	0	0	-	2.3	6.8	-	2.2	3.4
Blaník	CG [%]	0	0	87	-	0	-	-	-	-
	SH [%]	0	0	0	-	16	-	-	-	-
	SH No	2.2	0	0	-	2.5	-	-	-	-
Ostara	CG [%]	0	0	91	-	0	0	-	0	0
	SH [%]	0	0	0	-	9	12	-	10	16
	SH No	0	0	0	-	3.1	6.7	-	5.3	5.7

CO-1 - untreated control cultivated on kanamycine-free medium; CO-2 - explants transformed with NPTII gene only; 1, 2, 4, 5, 6^b, *rol*B, *rol*C *Agrobacterium* oncogenes; Ti, Ri - *Agrobacterium* Ti or Ri plasmid; Km - regenerative medium supplemented with kanamycine (50 mg l^{-1}); CG - frequency of explants producing crown-galls; SH - frequency of explants regenerating shoots; SH - number of shoots produced per one explant. 50 explants were used in each variant and their response evaluated on 30^{th} day of cultivation.

Different responses of plants, germinated seeds and callus tissue to ILA content either in culture media or in transgenic plants may indicate that the auxin (IAA) antagonistic effect of ILA differs by the type and developmental stage of target plant tissue (Körber *et al.* 1991). From this point of view the insertion of gene 5 could be a method of chance for our aim to modify "hormonal response" of primary explants to

obtain regenerants but to minimalize the onset of unevitable physiological malformations among these somaclones.

A considerable cultivar specifity of morphogenetic response was also typical for plants transformed by *A. rhizogenes* oncogenes *rol*B and *rol*C. Bud regeneration from explants of well regenerating cv. Kamýk transformed by gene *rol*B was slightly depressed (lower number of buds per explant as well as lower frequency of regenerating explants in comparison with control - see Table 2). Surprisingly positive effect of both these genes exhibited poorly regenerating cvs. Karin and Ostara. To interpret these results in accordance with the classic hypothesis of auxin/cytokinin ratio determining the type of regeneration (low auxin/high cytokinin activity stimulates bud formation - see Skoog and Miller 1957) various mechanisms can be taken into account. As Estruch *et al.* (1991a) proposed, the phenotypical alterations of tobacco transgenic plants expressing *rol*B gene could result from increased intracellular auxin activity caused by the release of active auxins from inactive beta-glucosides. Preliminary analysis of our potato cultivars indicated pronouncedly higher free cytokinin (zeatin, zeatin-riboside, 2iP) level in the stem tissue of untransformed plantlets of poorly regenerating cvs. Lada than in well regenerating cvs. Blaník, Karin, Ostara (Macháčková, unpublished results). Thus the insertion of auxin-activating gene *rol*B could increase the internal level of IAA in an optimal way in case of some cultivars and in a supraoptimal way in case of others. Once bud primordia being initiated, their further development can be again differentially modulated by the activity of *rol*B promotor. As Altamura *et al.* (1991) described, natural *rol*B promotor is developmentally regulated and is active in the initial cells of all types of meristems. But in our constructs stronger CaMV promotor was used which probably nivelised gene *rol*B tissue specific expression.

The effect of gene *rol*C on the bud formation capacity of the explants was only partially similar to that of above mentioned Ti gene 4, although both these genes are supposed to increase free cytokinin level in transformed plant cells (see below). In well regenerating cv. Lada Ti gene 4 induced regeneration of numerous shoots exhibiting very short internodes and developmental blocks. On the contrary *rolC* transformation resulted in lower stimulation of shooting, but regenerated shoots were able to develop into complete normal-looking plantlets. In poorly regenerating cvs. Karin and Ostara buds, shoots were obtained as well as complete *rol* C plantlets. Compared to gene 4 a higher regenerative effectiveness was reached (Table 2).

To interpret these findings the results of Estruch *et al.* (1991b) can be applied. They concluded that developmental, physiological and morphological alterations caused by *rol*C expression in transgenic tobacco plants are primarily due to a modification of the cytokinine balance. *Rol*C codes for β-glucosidase, which is able to hydrolyse cytokinin N-glucosides and in this way to increase the actual free cytokinin level in some cells and tissues. Plant tissues can be of somatic mosaic character for a cytokinin-synthesizing gene, which phenomenon can elucidate the variable response of the explants of one cultivar toward "antagonistic" effect of *rol*B and *rol*C, respectively. Some phenotype changes of *rol* transformants were observed, for instance a modified shape and colour of leaves (but not wrinkled ones) or the root

system size. It is consistent with previous findings of Schmülling *et al.* (1988) or Van Altvorst *et al.* (1991).

Further characterization of more the 70 transgenic clones in field tests is being done.

To summarize our results we conclude that both approaches, *i.e.* "exogenous" treatment of hormonal balance in primary explants *via* modification of growth regulators content in culture media and "endogenous" treatment *via* insertion of bacterial oncogenes resulted in the effect on genotype-dependent morphogenetic response. The examples of the improved bud regeneration ability can be interpreted in consistence with the Skoog-Miller model of the role of auxin/cytokinin ratio in *de novo* organogenesis, *i.e.* as a result of the shift of cytokinin activity on local level. However, no universal protocol for support of bud initiation and further development in all poorly regenerating cultivars has been found. Further study is required to elucidate supposingly different mechanisms underlying the phenomenon of low regenerative response of different genotypes.

References

Akiyoshi, D.E., Klee, H., Amasino, R.M., Nester, E.W., Gordon, M.P.: Cytokinin:auxin balance in crown gall tumors is regulated by specific loci in the T-DNA. - Proc. nat. Acad. Sci. USA **81**: 5994- 5998, 1984.

Altamura, M.M., Archilletti, T., Capone, I., Constantino, P.: Histological analysis of the expression of *Agrobacterium rhizogenes rol*B - GUS gene fusions in transgenic tobacco. - New Phytol. **118**: 69-78, 1991.

Van Altvorst, A.C., Bino, R.J., Van Dijk, A.J., Lamers, A.M.J., Lindhout, W.H., Van der Mark, F., Dons, J.J.M.: Effects of the introduction of *Agrobacterium rhizogenes rol* genes on tomato plant and flower development. - Plant Sci. **83**: 77-85, 1992.

Coleman, M., Waugh, R., Powell, W.: Genetical analysis of *in vitro* cell and tissue culture response in potato. - Plant Cell Tissue Organ Cult. **23**: 181-186, 1990.

Deblaere, R., Bytebier, B., De Greve, H., Deboeck, F., Schell, J., Van Montagu, M., Leemans, J.: Efficient octopine Ti-plasmid-derived vector for *Agrobacterium*-mediated gene transfer to plants. - Nucl. Acids Res. **13**: 4777-4788, 1985.

De Block, M.: Genotype-independent leaf disc transformation of potato (*Solanum tuberosum*) using *Agrobacterium tumefaciens*. - Theor. appl. Genet. **76**: 767-774, 1988.

Estruch, J.J., Chriqui, D., Grossmann, K., Schell, J., Spena, A.: The plant oncogene *rol*C is responsible for the release of cytokinins from glucoside conjugates. - EMBO J. **10**: 2889-2895, 1991a.

Estruch, J.J., Shell, J., Spena, A.: The protein encoded by the *rol*B plant oncogene hydrolyses indole glucosides. - EMBO J. **10**: 3125-3128, 1991b.

Hänish ten Cate, C.H., Ennik, E., Roest, S., Sree Ramulu, K., Dijkhuis, P., de Groot, B.: Regeneration and characterization of plants from potato root lines transformed by *Agrobacterium rhizogenes*. - Theor. appl. Genet. **75**: 452 - 459, 1988.

Higgins, E.S., Hulme, J.S., Shields, R.: Early events in transformation of potato by *Agrobacterium tumefaciens*. - Plant Sci. **82**: 109-118, 1992.

Hookyaas, J., Den Dulk-Ras, H., Schilperoot, R.A.: *Agrobacterium tumefaciens* T-DNA gene 6^b is an oncogene. - Plant. mol. Biol. **11**: 791-794, 1988.

Hulme, J.S., Higgins, E.S., Shields, R.: An efficient genotype - independent method for regeneration of potato plants from leaf tissue. - Plant Cell Tissue Organ Cult. **31**: 161-167, 1992.

Inze, D., Follin, A., Van Lijsebettens, H., Simeons, C., Genetello, C., Van Montagu, M., Schell, J.: Genetic analysis of the individual T-DNA genes of *Agrobacterium tumefaciens*: further evidence that two genes are involved in indole-3-acetic acid synthesis. - Mol. gen. Genet. **184**: 265 -274, 1984.

Körber, H., Strizhof, N., Staiger, D., Feldwisch, J., Olsson, O., Sandberg, G., Palme, K., Schell, J., Koncz, C.: T-DNA gene 5 of *Agrobacterium* modulates auxin response by autoregulated synthesis of a growth hormone antagonist in plant. - EMBO J. **10**: 3983-3991, 1991.

Mayerhofer, R., Koncz-Kalman, Z., Nawrath, C., Bakkeren, G., Crameri, A., Angelis, K., Redei, G. P., Hohn, B., Koncz, C.: T-DNA integration: a mode of illegitimate recombination in plants, - EMBO J. **10**: 697-704, 1991.

Medford, J.I., Horgan, R., El-Sawi, Z., Klee, H.J.: Alterations of endogenous cytokinins in transgenic plants using a chimaeric isopentenyl transferase gene. - Plant Cell **1**: 403-413, 1989.

Ondřej, M., Hrouda, M., Karavajko, N.N., Matoušek, J., Mikulovič, T. P., Pavingerová, D., Vlasák, J.: Transformation by *Agrobacterium* vectors and the study of functions of plant hormones. - In: Krekule, J., Seidlová, F. (ed.): Signals in Plant Development. Pp. 73-89. SPB Academic Publishers, The Hague 1989.

Ondřej, M., Macháčková, I., Čatský, J., Eder, J., Hrouda, M., Pospíšilová, J., Synková, H.: Potato transformation by T-DNA cytokinin synthesis gene. - Biol. Plant. 32: 401-406, 1990.

Ooms, G., Burrell, M.M., Karp, A., Bevan, M., Hille, J.: Genetic transformation in two potato cultivars with T-DNA from disarmed *Agrobacterium*. - Theor. appl. Genet. **73**: 744-750, 1987.

Ooms, G., Karp, A., Burrell, M.M., Twell, D., Roberts, J.: Genetic modification of potato developement using Ri T-DNA. - Theor. appl. Genet. **70**: 440-446, 1985.

Ooms, G., Lenton, J.R.: T-DNA genes to study plant development: precocious tuberisation and enhanced cytokinins in *A. tumefaciens* transformed potato. - Plant mol. Biol. **5**: 205-212, 1985.

Opatrná, J., Opatrný, Z., Kostřica, P.: [Genotype determination of growth and differentiation process in potato stem cultures.] - In: Griga ,M. (ed.): Somaklonální Variabilita a Její Využití ve Šlechtění Bramboru. Pp.105-115. Oseva, Šumperk 1990. [In Czech.]

Opatrný, Z., Müllerová, E.: Anatomical analysis of the organogenesis in potato (*Solanum tuberosum* L.) stem internode section system. - Genet. Selekt. (Sofia) **5**: 455-458, 1986.

Ovesná, J.: [Sulfonylurea-based herbicide resistance gene cloning and transformation of some crops.] - Thesis. Res. Inst. Crop. Product., Prague 1991. [In Czech.]

Rovenská, J., Kutáček, M., Opatrný, Z., Eder, J.: *Agrobacterium* T-DNA encoded IAA biosynthesis. - In: Kutáček, M., Bandurski, R.S., Krekule, J. (ed.): Physiology and Biochemistry of Auxins in Plants. Pp. 409-414. Academia, Praha 1988.

Sambrook, J., Fritsch, E.F., Maniatis, T.: Molecular Cloning. A Laboratory Manual. - Cold Spring Harbor Laboratory, Cold Spring Harbor 1989.

Schmülling, T., Schell, J., Spena, A.: Single genes from *Agrobacterium rhizogenes* influence plant development. - EMBO J. 7: 2621-2629, 1988.

Schröder, G., Waffenschmidt, S., Weiler, E.W., Schröder, J.: The T-region of Ti plasmid codes for an enzyme synthesizing indole-3-acetic acid. - Eur. J. Biochem. **138**: 387-391, 1984.

Skoog, F., Miller, C.O.: Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. - Symp. Soc. exp. Biol. **11**: 118-130, 1957.

Spena, A., Schmülling, T., Koncz, C., Schell, J.: Independent and syneristic activity of *rol* A, B and C loci stimulating abnormal growth in plants. - EMBO J. **6**: 3891-3899, 1987.

Tavazza, R., Tavazza, M., Ordas, R.J., Ancora, G., Benvenuto, E.: Genetic transformation of potato (*Solanum tuberosum*) - an efficient method to obtain transgenic plants. - Plant Sci. **59**: 175-181, 1988.

Tinland, B., Huss, B., Paulus, F., Bonnard, G., Otten, L.: *Agrobacterium tumefaciens* 6b genes are strain-specific and affect the activity of auxin as well as cytokinin genes.- Mol. gen. Genet. **219**: 217-224, 1989.

Visser, R.G.F., Jacobsen, E., Hesseling-Mienders, A., Schans, M.J., Witholt, B., Feenstra, W.J.: Transformation of homozygous diploid potato with an *Agrobacterium tumefaciens* binary vectors

system by adventitious shoot regeneration on leaf and stem segments. - Plant mol. Biol. **12**: 329-337, 1989.

Walden, R., Schell, J.: Techniques in plant molecular biology - progress and problems. - Eur. J. Biochem. **192**: 563-576, 1990.

Webb, K.J., Osifo, E.O., Henshaw, G.G.: Shoot regeneration from leaflet disc of six cultivars of potato (*Solanum tuberosum* subsp. *tuberosum*). - Plant Sci. Lett. **30**: 1-8, 1983.

Wheeler, V.A., Evans, N.E., Foulger, D., Webb, K.J., Karp, A., Franklin, J., Bright, W.J.: Shoot formation from explant cultures of fourteen potato cultivars and studies of the cytology and morphology of regenerated plants. - Ann. Bot. **55**: 309-320, 1985.

Zambryski, P.: Basic processes underlying *Agrobacterium* mediated DNA transfer to plant cells.- Annu. Rev. Genet. **22**: 1-30, 1988.

Zambryski, P., Tempe, J., Schell, J.: Transfer and function of T-DNA genes from *Agrobacterium* Ti and Ri plasmids in plants. - Cell **56**: 193-201, 1989.