Potato Res. 23 (1980) 155–166

The effect of growth regulators on meristem tip development and in vitro multiplication of *Solanum tuberosum* L. plants

F. J. NOVÁK[1], J. ZADINA[2], V. HORÁČKOVÁ[2] and I. MAŠKOVÁ[1]

[1]Czechoslovak Academy of Sciences, Institute of Experimental Botany Prague, Sokolovská 6, 772 00 Olomouc, Czechoslovakia
[2]Research Institute for Potato Growing and Breeding, 580 01 Havlíčkův Brod, Czechoslovakia

Accepted for publication: 23 October 1979

Zusammenfassung, Résumé p. 164

Additional keywords: auxins, cytokinins and gibberellic acid, shoot and root development, multiple shoot formation, continual in vitro multiplication

Summary

We studied the effect of β-indoleacetic acid (IAA), α-naphthylacetic acid (NAA), kinetin (K), 6-benzylaminopurine (BAP) and gibberellic acid (GA_3) on development of isolated meristems of four potato cultivars (subsp. tuberosum). GA_3 had a stimulating effect on in vitro meristem growth. The differences among the cultivars are presented. K + IAA + GA_3 induced formation of multiple shoots. We describe a method of continual plant multiplication under in vitro conditions and propose the produce for maintenance, multiplication and virus eradication of potato germplasm and cultivars.

Introduction

Plant development from meristem tips under in vitro conditions has been described in many species, and in potato this method has been used to eradicate virus infection and to derive virusfree clones. The reviews of Petrů (1976) and Mellor & Stace-Smith (1977) present a recent survey of literature and culture techniques.

In vitro culture can be also applied for the maintenance and multiplication of germplasm and cultivars. These methods could be highly topical in vegetatively propagated species, especially in potato, offering an alternative to expensive traditional procedures used until now (Westcott et al., 1977).

The objective of our work was to study hormonal control of the development of isolated potato meristem tips in four cultivars of the present Czechoslovak assortment.

The aim of the trials was to work out the methods of multiplication and maintenance of potato plants under in vitro conditions for genetic and breeding purposes.

Materials and methods

Meristem tips were excised from sprouts 10–15 cm long of *Solanum tuberosum* L.

subsp. *tuberosum*, cv. Blanik, Cira, Nora and Radka. The tubers were planted out in a glasshouse at the end of March and resulting sprouts were grown under artifical illumination provided by fluorescent lamps of 5000 lx.

Apical parts of the sprouts were rinsed in 70 % ethanol and sterilized with 3 % calcium hypochlorite for 30 min. Afterwards, they were washed 5 times with sterile distilled water. The meristem tips, 300–500 μm long, with 2 leaf primordia, were isolated under a stereo-microscope and placed level on 5 ml of agar medium in test tubes with aluminium caps. The composition of the culture medium was as follows: macro- and microelements and FeEDTA according to Murashige & Skoog (1962), the organic component of the medium B-5 (Gamborg et al., 1968), 100 mg/litre inositol, 30 g/litre sucrose and 8 g/litre agar. The pH of the medium was adjusted to 5.7. Growth regulators – IAA, NAA, K, BAP and GA_3 – were added to the medium at various concentrations as given below. The medium was sterilized by autoclaving, except for the GA_3 medium which was sterilized by filtration.

The cultures were held at 26–28 °C and illuminated for 16 h daily under white fluorescent lamps giving 1200 lx. Growth and development of meristem tips were evaluated after 72 days of in vitro culture. Two sets each of twenty meristem tips (one per test tube) were cultured for each hormone treatment. The plants obtained were cloned under sterile conditions (see 'Results'). Plants were decapitated and placed horizontally on the surface of perlite saturated with Hoagland's solution (Gamborg & Wetter, 1975), containing 0.1 μM IAA. Flasks with perlite were put in a cold glasshouse at 15–18 °C under high-pressure sodium lamps (light intensity 8000 lx).

Rooted plants were transplanted directly into pasteurized garden soil.

Results

The developeent of isolated meristem tips was poor on the basal medium without growth regulators and the explants eventually died.

Effect of auxins

The addition of IAA or NAA, alone to the basal medium within the cofcentration range of 0.1–10.0 μM was insufficient to induce whole plant development from meristem tips (Fig. 1, Table 1).

Effect of cytokinins

Meristem tips grown on the medium with 0.1 μM K gave rise to complete plants with well developed leaves and roots (Fig. 1). The individual cultivars differed from one another in the frequency of grown (Table 1). The resulting plants could be grown on perlite. Higher K levels (1–10 μM) resulted in teratological shoot development and inhibited root development. Callus tissue formed on the plant base and gradually became necrotic.

BAP fully inhibited root development. Shoots developed on the media with 0.1 μM BAP (Fig. 1), the frequency is shown in Table 1. After transplanting to medium with 0.1 μM IAA the plants rooted (Fig. 2). Higher BAP concentrations resulted in a teratological shoot development with callus formation on the explant base.

Fig. 1. Development of meristem tips on the media with various concentrations of growth regulators after 72 days of in vitro culture.

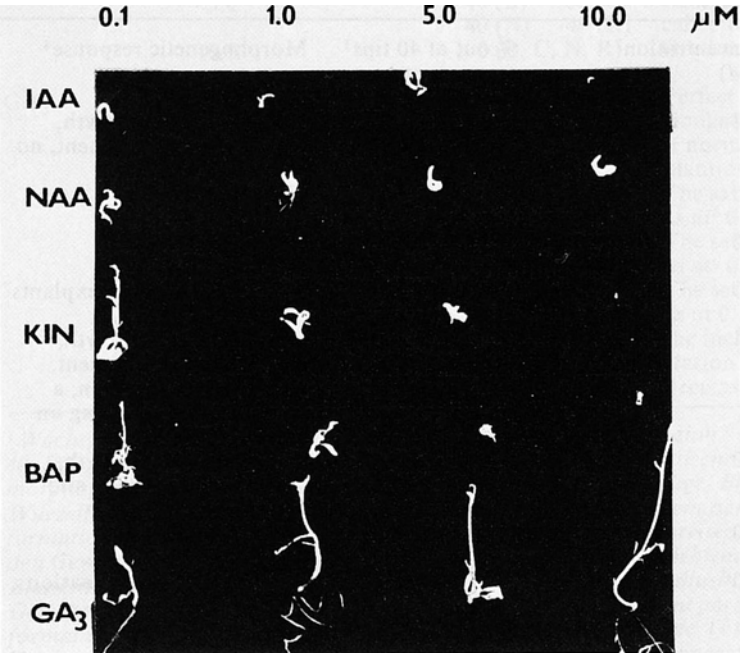


Abb. 1. Entwicklung von Meristemspitzen auf Nährböden mit verschiedenen Konzentrationen von Wachstumsregulatoren nach 72 Tagen bei in vitro-Kultur.
Fig. 1. Développement des méristèmes apicaux sur des milieux à différentes concentrations de régulateurs de croissance après 72 jours de culture in vitro.

Effect of gibberellin

GA_3 was the most effective hormone for both shoot and root development at all the tested concentrations (Fig. 1). Frequency of growth of the individual cultivars is given in Table 1. No callus was formed on explant bases. With increasing GA_3 concentrations leaves became narrow and elongated but normal growth resumed after transplanting into perlite.

Auxin-cytokinin interactions

Interactions between auxins and cytokinins in various combinations (a total of 64 variants) were tested on meristem tip cultures from cv. Cira only. IAA together with K stimulated shoot development within the whole range of IAA concentrations and at K levels 0.1, 1.0 and 5.0 μM. High K doses (10 μM) had an inhibitory effect in combination with all IAA concentrations applied. One meristem tip gave rise to 2–3 plants on the media with 1 and 5 μM K and 10 μM IAA. On the other hand, the combination of K and NAA within the tested range of concentrations appeared completely unsuitable for the growth of meristem tips of cv. Cira.

Table 1. Effect of growth regulators on growth and morphogenesis of meristem tips evaluted after 72 days of in vitro culture. The cultivars are marked as follows: (B) = Blanik, (C) = Cira, (N) = Nora, (R) = Radka.

Growth regulators[1]	Concentration[2] (μM)	% out of 40 tips[3]	Morphogenetic response[4]
IAA	0.1	40 (B) 50 (C) 40 (N) 60 (R)	Imperfect shoot growth, partial leaf development, no root formation[5]
	1.0	80 (B) 70 (C) 80 (N) 80 (R)	The same as at[6] 0.1 μM
	5.0	40 (B) 10 (C) 50 (N) 80 (R)	The same as at[6] 0.1 μM
	10.0	0 (B, C, N, R)	Gradual necrosis of explants[7]
NAA	0.1	100 (B) 50 (C) 40 (N) 70 (R)	Imperfect shoot growth, partial leaf development, sign of root formation, a small callus developing on explant base[8]
	1.0	80 (B) 30 (C) 30 (N) 80 (R)	Imperfect shoot growth, more intensive callus and root formation[9]
	5.0	60 (B) 30 (C) 0 (N) 60 (R)	The same as at[6] 1.0 μM
	10.0	40 (B) 20 (C) 10 (N) 20 (R)	Prevailing callus formation without any sign of organized growth[10]
K	0.1	80 (B) 60 (C) 60 (N) 90 (R)	Development of plants with perfect leaves and roots, no callus formation, some plants are transplantable into perlite[11]
	1.0	60 (B) 30 (C) 80 (N) 80 (R)	Imperfect shoot growth, teratological leaf development, suppressed root formation, a gradually necrotizing callus is formed on the explant base[12]
	5.0	20 (B) 20 (C) 20 (N) 0 (R)	Prevailing teratological shoot development, callus is formed on the explant base[13]
	10.0	0 (B, C, N, R)	Gradual necrosis of explants
BAP	0.1	60 (B) 70 (C) 100 (N) 60 (R)	Formation of plants with small leaves, exceptional multiple shoot formation, rooting takes place after transfer to the medium with 0.1 μM IAA, possibility of plant transplantation to perlite[14]
	1.0	100 (B) 100 (C) 100 (N) 90 (R)	Growth of teratological character, callus formation on explant base, suppressed root formation[15]

Table 1 (continued).

	5.0	40 (B)	30 (C)	As at 1.0 μM, more intensive
		40 (N)	40 (R)	callus formation[16]
	10.0	0 (B, C, N, R)		Gradual necrosis of explants[7]
GA_3	0.1	100 (B)	100 (C)	Perfect plant development,
		100 (N)	100 (R)	elongated character of plants is normalized after transplantion into perlite[17]
	1.0	80 (B)	80 (C)	The same
		90 (N)	100 (R)	as at[6] 0.1 μM
	5.0	100 (B)	80 (C)	The same
		100 (N)	90 (R)	as at[6] 0.1 μM
	10.0	60 (B)	40 (C)	The same
		100 (N)	30 (R)	as at 0.1 μM, though with the increasing GA_3, concentration leaf blade area is decreasing[18]

[1] *Wachstumsregulatoren – Régulateurs de croissance;* [2] *Konzentration – Concentration;* [3] *% aus 40 Spitzen – % parmi 40 méristèmes apicaux;* [4] *Morphogenetische Reaktion – Réponse morphogénétique;* [5] *Mangelhaftes Triebwachstum, unvollständige Blattentwicklung, keine Wurzelbildung – Croissance des pousses imparfaite, développement partiel des feuilles, pas de formation de racines;* [6] *Dasselbe wie bei – Comme avec;* [7] *Fortschreitende Nekrosebildung auf den Geweben – Nécrose graduelle des explants;* [8] *Mangelhaftes Triebwachstum, unvollständige Blattentwicklung, Anzeichen von Wurzelbildung, eine kleine Kallusbildung an der Basis der Gewebekultur – Croissance des pousses imparfaite, développement partiel des feuilles, signe de formation des racines, un petit cal se développe à la base de l'explant;* [9] *Mangelhaftes Triebwachstum, intensivere Kallus- und Wurzelbildung – Croissance des pousses imparfaite, cal plus important et formation des racines;* [10] *Vorwiegend Kallusbildung ohne irgend ein Anzeichen von organisiertem Wachstum – Formation d'un cal sans signe de croissance organisée;* [11] *Entwicklung von Pflanzen mit vollkommenen Blättern und Wurzeln, keine Kallusbildung. Einige Pflanzen können in Perlit versetzt werden – Développement des plantes avec feuilles et racines parfaites, pas de formation de cal. Quelques plantes sont transplantables dans la perlite;* [12] *Mangelhaftes Triebwachstum, missgebildete Blattentwicklung, unterdrückte Wurzelbildung, ein allmählich nekrotisierter Kallus an der Basis der Gewebekultur wird gebildet – Croissance imparfaite des pousses, développement foliaire tératologique, pas de formation de racines, un cal se nécrosant peu à peu s'est formé à la base de l'explant;* [13] *Vorwiegend missgebildete Triebentwicklung, an der Basis der Gewebekultur wurde ein Kallus gebildet – Développement des pousses plutôt tératologique, un cal se forme à la base de l'explant;* [14] *Bildung von Pflanzen mit kleinen Blättern, aussergewöhnlich vielfache Triebbildung, Bewurzelung findet nach Uebersiedlung auf den Nährboden mit 0,1 μM IAA statt, Möglichkeit der Versetzung der Pflanzen in Perlit – Formation de plantes avec de petites feuilles, formation de pousses multiples exceptionnelle, la rhizogénèse a lieu après transfert sur le milieu contenant 0,1 μM de IAA, transplantation possible dans la perlite;* [15] *Wachstum mit missgebildetem Charakter, Kallusbildung an Pflanzengeweben, unterdrückte Wurzelbildung – Croissance à caractère tératologique, formation d'un cal à la base de l'explant, pas de formation de racines;* [16] *Wie bei 1,0 μM, stärkere Kallusbildung – Comme à 1,0 μM, formation plus intensive d'un cal;* [17] *Vollkommene Pflanzenentwicklung, die längliche Art der Pflanzen wird nach Versetzung in Perlit normalisiert – Développement parfait des plantes, le caractère allongé des plantes se normalise après transplantation dans la perlite;* [18] *Dasselbe wie bei 0,1 μM, obwohl mit zunehmender GA_3-Konzentration die Blattfläche abnahm – Comme à 0,1 μM, bien que avec les concentrations croissantes de GA_3 la surface des folioles diminue.*

Tabelle 1. Einfluss von Wachstumsregulatoren auf Wachstum und Morphogenese von Meristemspitzen, beurteilt nach 72 Tagen bei in vitro-Kultur. Die Sorten sind wie folgt bezeichnet: (B) = Blanik, (C) = Cira, (N) = Nora, (R) = Radka.

Tableau 1. Effet des régulateurs de croissance sur la croissance et la morphogénèse des méristèmes apicaux après 72 jours de culture in vitro. Les variétés sont notées ainsi: (B) = Blanik, (C) = Cira, (N) = Nora, (R) = Radka.

Fig. 2. Rooting of the plants on the medium with 0.1 μM IAA.

Abb. 2. Bewurzelung von Pflanzen auf dem Nährboden mit 0,1 μM IAA.
Fig. 2. Enracinement des plantes sur le milieu à 0,1 μM IAA.

A combination of BAP + IAA was insufficient to induce shoot or root growth under in vitro conditions. BAP + NAA stimulated the development of plants with a vigorous root system but only at the lowest concentrations of both growth regulators (0.1 μM). All other BAP + NAA combinations induced a bulky callus, which was yellowish and friable, showing no shoot or root regeneration. No shoots were formed even if the callus was transferred to the organ inducing medium described by Lam (1975).

Fig. 3. Multiple shoot formation on the medium with K (0.1 μM), IAA (0.1 μM) and GA_3 (5 μM) after 72 days of in vitro culture.

Abb. 3. Mehrfache Triebbildung auf dem Medium mit K (0,1 μM), IAA (0,1 μM) und GA_3 (5 μM) nach 72 Tagen bei in vitro-Kultur.
Fig. 3. Formation de pousses multiples sur le milieu contenant K (0,1 μM), IAA (0,1 μM), et GA_3 (5 μM) après 72 jours de culture in vitro.

Interactions of IAA, K, BAP and GA_3

The effect of GA_3 was tested in the following combinations: K + GA_3, K + IAA + GA_3, BAP + GA_3, BAP + IAA + GA_3 all at one concentration (0.1 μM) on all of the four cultivars. The above-mentioned combinations appeared to be suitable for meristem tip growth in all cases, except for cvs. Blanik and Nora, the meristem tips of which did not grow on the medium with BAP + GA_3. Meristem tips excised from cv. Cira gave rise to multiple shoots on the media with BAP. Some of these plants formed small tubers at the base. Optimal growth and rapid development of the plants were observed on the medium with K + IAA + GA_3.

The effect of different concentrations of GA_3 (0.1–10 μM) with K (0.1 μM) and IAA (0.1 μM) was tested. The meristem tips were excised from cv. Blanik, Cira and Radka. Complete plants developed at all GA_3 concentrations, but increasing GA_3 levels resulted in decreasing leaf area and longer internodes. Multiple shoot formation occurred on the media with 5 and 10 μM GA_3, especially in cv. Cira and Blanik (Fig. 3).

Plant propagation under sterile conditions

The plants obtained from the meristem tip culture could be continuously propagated under sterile conditions on a simple mineral medium with the addition of 0.1 μM IAA. After decapitation the plants were placed horizontally on perlite saturated with

Hoagland's solution, where axillary buds began developing after 14 days. Complete plant formation, including roots, occurred after 2 months of culture.

The propagation process could be continuously repeated, one plantlet of meristemic origin being able to give rise to 5–8 plants in two months. The plants obtained could serve either as a source of explants for further meristem or callus cultures, or for transplanting into soil. After transplanting into soil, the plants produced mature tubers within 6–8 months. Plants maintained continuously under in vitro conditions can thus be a source of virus-free seed stock. The above-mentioned method of isolation could be applied especially to cultivars which are highly susceptible to virus infection.

Discussion

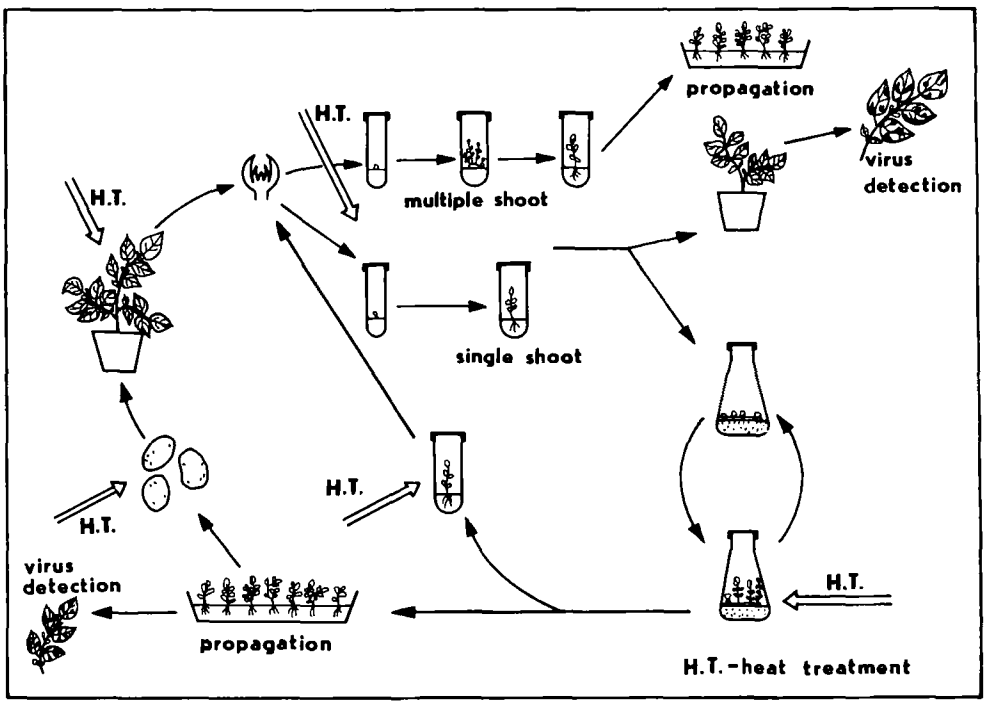
The present results show that perfect regeneration of potato plants from meristem tips can be obtained by appropriate growth regulators in the culture medium. IAA alone has only a slight effect on shoot and root organogenesis, but, in combination with K it exercises a stimulating effect on plant development. NAA at all the tested concentrations and in combination with cytokinins had a remarkable inhibitory effect on in vitro plant development in all the cultivars tested. This finding is in agreement with observation of Gregorini & Lorenzi (1974) and explains a low frequency of regenerants obtained by Morel & Martin (1955) in their initial work on the media with 0.001 mg/litre NAA. Chromova et al. (1977) reported a favourable effect of another auxin-β-indolebutyric acid which had not been tested in our trials.

The cytokinins K, BAP, at the lowest concentration tested (0.1 μM) are sufficient for shoot development. The inhibitory effect of higher K concentrations was balanced by IAA addition. Westcott et al. (1977) found an inhibitory effect of cytokinin-rich media on the growth of tetraploid clones of *Solanum tuberosum*, while high cytokinin (> 10 μM) favoured meristem development in wild triploid and pentaploid species, BAP inducing multiple shoot development. These observations are comparable to the development of isolated shoot tips of tomato which can also tolerate high cytokinin levels (Kartha et al., 1977; Novák & Mašková, 1979). In *Solanum tuberosum* multiple shoot formation was observed on the media with 5 μM K and 5 and 10 μM IAA. Cytokinins induce multiple shoot formation by suppressing apical dominance of the main meristem (Hussey, 1976).

A favourable effect of GA_3 on the development of isolated potato meristem tips has been described by a number of authors (cf. Morel, 1975). Pennazio & Redolfi (1973) reported a striking increase in shoot tip rooting on MS medium with GA_3. Our results prove a favourable effect of GA_3 on multiple shoot formation even at low concentrations of K and IAA (1 μM).

Quak (1961) and Mellor & Stace (1977) observed varietal differences in the degree of in vitro growth and development of potato meristem tips. Our results are in agreement with these conclusions. Varietal differences have also been found in callus initiation and growth from various explants (to be published). The genetic aspects of growth and development of various types of potato tissue cultures needs more study (Simon & Péloquin, 1977).

Fig. 4. Schematic representation for the maintenance, multiplication, and virus eradication of potato germplasm of cultivars.



Heat treatment - *Hitzebehandlung - Traitement thermique*
Propagation - *Fortpflanzung - Propagation*
Virus detection - *Virusnachweis - Détection des virus*
Multiple shoot - *Mehrfacher Trieb - Pousses multiples*
Single shoot - *Einfacher Trieb - Pousse unique*

Abb. 4. Schematische Darstellung der Erhaltung, Vermehrung und Virusbefreiung von Kartoffelkeimplasma oder Züchtungen.
Fig. 4. Représentation schématique de la maintenance, de la multiplication et de l'éradication des virus des plasmas de germes ou des variétés.

Multiple shoot formation and continuous plant propagation under sterile conditions on a simple medium could be used for maintenance and multiplication of potato germplasm and cultivars (Fig. 4). The results of this work follow the project of maintaining the collection of primitive cultivars and wild species at the International Potato Centre in Peru through tissue cultures (Westcott et al, 1977; Henshaw, 1978). Similar methods of maintaining potato clones are proposed by Wang (1977, 1978). Moreover, it may be proposed to supplement in vitro plant propagation methods described with the procedures of virus eradication (heat treatment). Repeated in vitro cultivation (Svobodová, 1966; Ingram, 1973) is suitable for virus infection dilution.

Unlike genetically unstable callus or cell cultures the proposed system has an advantage of a lower risk of genetic changes in the cloned material (Denton et al., 1977).

Zusammenfassung

Einfluss von Wachstumsregulatoren auf die Entwicklung von Meristemspitzen und die Vermehrung in vitro von Solanum tuberosum L.-Pflanzen

Von vier Kartoffelsorten (Blanik, Cira, Nora und Radka) wurden die Meristemspitzen entnommen. Der Nährboden enthielt Makro- und Mikroelemente gemäss Murashige & Skoog (1962), Vitamine entsprechend B-5 (Gamborg et al., 1968), 100 mg/l Inositol, 30 g/l Saccharose und 8 g/l Agar. Wir untersuchten die Wirkung von β-Indolessigsäure (IAA), α-Naphytlessigsäure (NAA), Kinetin (K), 6-Benzylaminopurin (BAP) und Gibberellinsäure (GA_3). Auf den Nährböden mit IAA und NAA wurde mangelhaftes Triebwachstum und unvollständige Blattentwicklung (Abb. 1) festgestellt; die Pflanzen konnten nicht in Erde versetzt werden. K (0.1 μM) induzierte vollständige Pflanzenentwicklung (Tabelle 1), während BAP (0,1 μM) die Triebenentwicklung anregte, worauf die Bewurzelung auf Nährboden mit 0,1 μM IAA (Abb. 2) erfolgte. Alle GA_3-Konzentrationen hatten einen vorteilhaften Einfluss auf die Entwicklung von Pflänzchen aus Meristemspitzen (Abb. 1 und Tabelle 1). Optimales Wachstum und rasche Entwicklung der Pflanzen aller vier Sorten wurde mit dem Nährboden mit IAA + K + GA_3 erreicht. Mehrfache Triebbildung erfolgte auf den Medien mit erhöhten GA_3-Konzentrationen (5 und 10 μM), speziell bei den Sorten Cira und Blanik (Abb. 3). Die Pflanzen aus der Meristemspitzenkultur konnten unter sterilen Bedingungen in Perlit, das mit der Hoagland's-Lösung und mit einem Zusatz von 0,1 μM IAA gesättigt war, fortlaufend vermehrt werden. Die Pflanzen entwickeln sich aus Achselknospen. Ein Steckling aus einem Meristem ist fähig, 5-8 Pflanzen in zwei Monaten hervorzubringen. Der Artikel beschreibt das System der mehrfachen Triebbildung und die laufende Fortpflanzung in vitro zur Erhaltung und Vermehrung von Kartoffel-Keimplasma (Westcott et al., 1977) und virusfreien Züchtungen (Abb. 4).

Résumé

L'effet des régulateurs de croissance sur le développement du méristème apical et sur la multiplication in vitro de Solanum tuberosum L.

Des méristèmes apicaux ont été prélevés sur quatre variétés de pommes de terre: Blanik, Cira, Nora et Radka. Le milieu de culture contenait des macro- et microéléments d'après les indications de Murashige & Skoog (1962), des vitamines B-5 (d'après Gamborg et al., 1968), 100 mg/l d'inositol, 30 g/l de sucrose et 8 g/l d'agar. Nous avons étudié l'action de l'acide β-indoleacetic (IAA), de l'acide α-naphtylacétique (NAA), de la kinétine (K), de la 6-benzylaminopurine (BAP) et de l'acide gibberellic (GA_3). Sur les milieux contenant de l'IAA et du NAA, nous avons observé un développement imparfait des pousses, et un développement partiel des feuilles (fig. 1); les plantes ne pouvaient pas être transplantées en terre. K (0,1 μM) a induit un développement complet des plantes (tableau 1) tandis que le BAP (0,1 μM) a stimulé le développement des pousses suivi de leur enracinement dans le milieu contenant 0,1 μM d'IAA (fig. 2). Toutes les concentrations de GA_3 ont eu un effet bénéfique sur le développement des plantules issues de méristèmes apicaux (fig. 1 et tableau 1). La croissance optimale et le développement rapide des plantes des 4 variétés ont été observés sur le milieu contenant IAA + K + GA_3.

Des pousses multiples se sont formées sur les milieux contenant des concentrations élevées en GA_3 (5 et 10 μM), notamment sur les variétés Cira et Blanik (fig. 3). Les plantes obtenues à partir de culture de méristèmes apicaux ont pû être continuellement propagées en conditions stériles dans de la perlite saturée d'une solution de Hoagland avec addition de 0,1 μM de IAA. Les plantes se développent à partir des bourgeons axillaires; une plantule d'origine méristématique est capable de donner naissance à 5 à 8 plantes en 2 mois. L'article décrit le système de formation des pousses multiples et la propagation continue in vitro pour la maintenance et la multiplication de variétés de pommes de terre indemnes de maladies (Westcott et al., 1977) et de virus (fig. 4).

References

Chromova, L. M., L. N. Trofimec & V. A. Knjazev, 1977. The influence of growth regulators on morphogenesis of cultured apical meristem. *Nauchnye Trudy Nikch (Kiev)* 30: 19-25. (in Russian).

Denton, I. R., R. J. Westcott & B. V. Ford-Lloyd, 1977. Phenotypic variation of *Solanum tuberosum* L. cv. Dr Mc Intosh regenerated directly from shoot-tip culture. *Potato Res.* 20: 131-136.

Gamborg, O. L., R. A. Miller & K. Ojima, 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* 50: 151-158.

Gamborg, O. L. & L. R. Wetter (Eds.), 1975. Plant tissue culture methods. National Research Council of Canada, Praire Regional Laboratory, Saskatoon, Saskatchevan, Canada, 109 pp.

Gregorini, G. & R. Lorenzi, 1974. Meristem-tip culture of potato plants as a method of improving productivity. *Potato Res.* 17: 24-33.

Henshaw, G. G., R. J. Westcott & W. M. Roca, 1978. Tissue culture methods for the storage and utilization of potato germplasm. 4th International Congress of on Plant Tissue and Cell Culture, (1978), University of Calgary, Canada. Abstract No 1204.

Hussey, G., 1976. In vitro release of axillary shoots from apical dominance in monocotyledons plantlets. *Ann. Bot.* 40: 1323-1326.

Ingram, D. S., 1973. Growth of plant parasites in tissue culture. In: H. E. Street (Ed.), Plant tissue and cell culture, Blackwell, Oxford, pp. 392-421.

Kartha, K. K., S. Champoux, O. L. Gamborg & K. Pahl, 1977. In vitro propagation of tomato by shoot apical meristem culture. *J. Am. Soc. hort. Sci.* 102: 346-349.

Lam, S. L., 1975. Shoot formation in potato tuber disc in tissue culture. *Am. Potato J.* 52: 103-106.

Mellor, F. C. & R. Stace-Smith, 1977. Virus-free potatoes by tissue culture. In: J. Reinert & Y. P. S. Bajaj (Eds.), Applied and fundamental aspects of plant cell, tissue, and organ culture. Springer Verlag, Berlin-Heidelberg-New York, pp. 616-635.

Morel, G., 1975. Meristem culture techniques for the long-term storage of cultivated plants. In: O. Frankel & J. G. Hawkes (Eds.), Crop genetic resources for today and tomorrow. University Press, Cambridge, pp. 327-332.

Morel, G. & C. Martin, 1955. Guérison de pommes de terre atteintices de maladies a virus. *C.r. Acad. Sci., Paris* 41: 472-474.

Murashige, T. & F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Pl.* 15: 473-497.

Novák, F. J. and I. Mašková, 1979. Apical shoot tip culture of tomato. *Scientia Hort.* 10: 337-344.

Pennazio, S. & P. Redolfi, 1973. Factors affecting the culture in vitro of potato meristem tips. *Potato Res.* 16: 20-29.

Petrů, E., 1976. Docílení viruprosté sadby metodou meristémových kultur. In: J. Zadina & E. Jermoljev (Eds.), Šlechtení bramboru. Academia, Praha, pp. 327-332.

Quak, F., 1961. Heat treatment and substances inhibiting virus multiplication in meristem culture to obtain virus-free plants. *Adv. Hort. Sci. Appl.* 1: 144-148.

Simon, P. W. & S. J. Péloquin, 1977. The influence of parental species on the origin of callus in another culture of *Solanum* hybrids. *Theor. appl. Genet.* 50: 53–56.
Svobodová, J., 1966. Elimination of viruses by means of callus tissue culture. In: A. B. R. Beenstra & J. Dykstra (Eds.), Virus in plants. John Wiley, New York-London, pp. 48–53.
Wang, P. J., 1977. Regeneration of virus-free potato from tissue culture. In: W. Barz, E. Reinhard & M. H. Zenk (Eds.), Plant tissue culture and its bio-technological application. Springer Verlag, Berlin-Heidelberg-New York, pp. 386–391.
Wang, P. J., 1978. Gene preservation and foundation stock seed production of potato in vitro. 4th International Congress on Plant Tissue and Cell Culture (1978), University of Calgary, Canada. Abstract No 1205.
Westcott, R. J., G. G. Henshaw & W. M. Roca, 1977. Tissue culture storage of potato germplasm: Culture initiation and plant regeneration. *Pl. Sci. Lett.* 9: 309–315.