J Plant Growth Regul (1994) 13:147–152

Journal of
Plant
Growth
Regulation

Morphogenesis of Potato Plants In Vitro. II. Endogenous Levels, Distribution, and Metabolism of IAA and Cytokinins

Lydiya I. Sergeeva,[1,*] Ivana Macháčková,[2] Tatyana N. Konstantinova,[1] Svetlana A. Golyanovskaya,[1] Josef Eder,[2] Oksana O. Zaltsman,[1] Jan Hanuš,[2] and Nina P. Aksenova[1]

[1]Institute of Plant Physiology, Russian Academy of Sciences, Moscow, Russia; and [2]Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Praha, Czech Republic

Received January 20, 1994; accepted April 27, 1994

Abstract. The levels of endogenous IAA and cytokinins (zeatin, zeatin riboside, isopentenyladenine, and isopentenyladenosine) were determined in potato plants cultured in vitro under red light (R) and blue light (B) on medium with or without hormones. On medium without hormones in B, plants contained much higher cytokinin levels, particularly in leaves and roots, and also slightly elevated IAA levels. Kinetin in the medium in B changed the distribution of cytokinins and significantly increased IAA level in roots. In R, the presence of kinetin led to an increased cytokinin level in the whole plant, while the IAA level was slightly lower. IAA in the medium in B decreased cytokinin level in all plant parts, while the IAA level did not change significantly. In R, the presence of IAA in the medium led to a moderate increase of CK level and to a significant increase in IAA level, especially in roots. Uptake of 1-^{14}C-IAA and of ^{3}H-zeatin was generally higher in B than in R. Higher percentage of IAA taken up in B was converted to conjugates in the roots. Metabolism of ^{3}H-zeatin was similar in R and B with only slight differences in metabolite amounts.

Thus, in all experimental situations in which tuber formation was stimulated, IAA level in roots and stolons rose significantly, stressing the importance of an IAA gradient for tuber formation.

Aksenova et al. (1994) demonstrated that in potato plants cultured in vitro under red light (R), formed long, thin stems with stunted leaves, and, in general, no tubers were formed, whereas under blue light (B), stems remained short, thick with large leaves, and some microtuber formation occurred. IAA applied to the medium in R increased fresh weight, reduced stem length, and significantly promoted tuber formation, whereas in B it had only a slight effect. Kinetin (K), on the other hand, was found to be more active in B, where it strongly increased fresh weight and tuber formation. This investigation was undertaken to determine whether these morphogenetic effects of red and blue light and of exogenous hormones were mediated by changes in the phytohormone balance. We analyzed the levels of free IAA and cytokinins (CK) [zeatin (Z), zeatin riboside (ZR), isopentenyladenine (iP), and isopentenyladenosine (iPA)] in individual organs of in vitro-grown potato plants, both under R and B, in some experiments with darkening of shoots. Uptake and distribution of labelled IAA and Z and their metabolism in R and B were also studied.

Materials and Methods

Plant Material

In vitro culture of potato (*Solanum tuberosum* L., cv. Miranda) was derived from and propagated through stem cuttings. For growing and lighting conditions, see Aksenova et al. (1994). Material for analyses was taken from plants of different ages (see Results); leaves, stems, and roots with stolons were harvested and analyzed separately. While harvesting, the plant parts were immediately frozen in liquid nitrogen, lyophilized and kept dry in a refrigerator at 4°C until analysis.

Determination of Free IAA

Extraction by 80% methanol, followed by purification with diethyl ether partitioning and Polyclar AT columns, and determination by HPLC with fluorimetric detection were performed as

* Author for correspondence.

described by Eder et al. (1988) and Štiller et al. (1992). 1-^{14}C-IAA (Amersham, spec. act. 57 MBq · mmol^{-1}, actual activity 3 kBq) was used as internal standard to determine recovery.

Determination of Cytokinins

Cytokinins were extracted by 80% methanol, the extract was purified on phospho- and DEAE-cellulose columns and SeP-Pak C-18 cartridges, and was separated into fractions containing Z, ZR, iP, and iPA on HPLC. The content of cytokinins in individual fractions was determined by ELISA (Macháčková et al. 1993). ^{3}H-m-Hydroxybenzyladenosine (prepared by alkylation of ^{3}H-adenosine by 3-acetoxybenzylbromide, spec. act. 1.2 TBq · mmol^{-1}, actual act. 6 kBq) was used to determine recovery.

Studies of the Uptake and Metabolism of 1-^{14}C-IAA

Plants of different age were transferred from agar to a solution containing 1-^{14}C-IAA (Amersham, spec. act. 57 MBq · mmol^{-1}, actual activity 1.6 kBq · ml^{-1}) with cold IAA in the total concentration of 1 mg · l^{-1} and kept in this solution under the respective light conditions (only roots were in contact with the solution). After 3 h, the roots were thoroughly washed, frozen, and homogenized in liquid nitrogen, extracted with 80% methanol, total radioactivity counted, then analyzed by thin layer chromatography on silica gel G in the solvent system isopropanol/ammonia/water (85:15:5) and detected radiometrically. The identity of the metabolites was determined by cochromatography with authentic standards and, in the case of IAAGlu, by hydrolysis and chromatographic behavior of the products.

Studies of the Uptake, Distribution, and Metabolism of ^{3}H-Zeatin

The method used was similar to that described above for the labelled IAA, and only the exposition to labelled solution under the respective light conditions was 5 and 20 h. Specific activity of labelled zeatin (prepared by alkylation of ^{3}H-adenosine with 4-tert-butoxy-3-methyl-trans-but-2-enylbromide followed with Dimroth rearrangement and hydrolysis of tert-butoxy group) was 1.6 TBq · mmol^{-1}, actual activity 5 kBq · ml^{-1}. Cold Z was added to the final concentration 10^{-6} mol · l^{-1}. After the incubation period, the roots were thoroughly washed, and plants were divided into leaves, roots, and upper and lower stem halves. Plant material was dried at 95°C, combusted in the stream of oxygen at 600°C, and the arising ^3H_2O was trapped in the dioxane scintillator and counted. For checking the metabolites, separated roots and shoots were extracted as described by Noodén and Letham (1984). The extract was evaporated, dissolved in 80% methanol, purified on a Sep-Pak C-18 cartridge, and then applied to an HPLC column used for cytokinin separation (Macháčková et al. 1993). Next, 1-min fractions were collected, and after they were added to a scintillator, their radioactivity was counted. Identity of metabolites was determined according to the mobility of authentic standards. All experiments were repeated twice. The absolute values of both endogenous levels and of uptake and distribution differed, but the differences between individual organs and R and B were similar. Results of one experiment of each type are given; SE represents the error in the frame of one experiment.

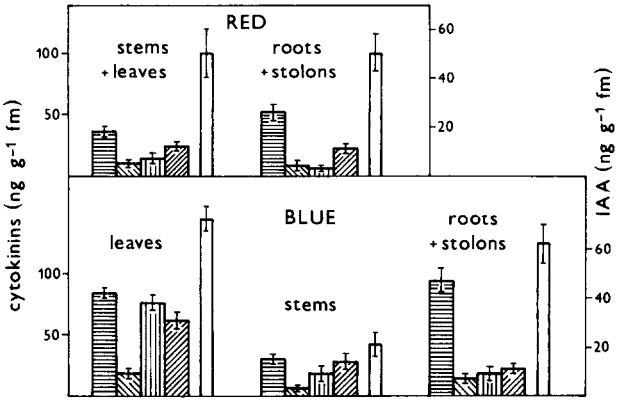

Fig. 1. Levels of free IAA and individual cytokinins in leaves, stems, and roots (with stolons, if present) of potato plants grown in vitro under red or blue light during an 18-h photoperiod for 40 days on medium without hormones. (□) IAA; (▤) zeatin riboside; (▧) zeatin; (▥) isopentenyladenosine; (▨) isopentenyladenine.

Results

Levels of Free IAA and Cytokinins in R and B

Plants under R had much lower cytokinin content in both shoots and roots than in plants under B [Fig. 1(a)]. The highest CK level was observed in B in leaves, being much higher there than in roots or stems, whereas in R, the levels in shoots and in roots were similar (Fig. 1). IAA levels were comparable in roots under R and B; a slightly higher level was found in shoots in R than in B (Fig. 1). The ratio total cytokinins/IAA was much higher in all plant parts in B than in R, the highest being in leaves and stems (3.4 and 3.9, respectively).

Darkening the roots in B significantly increased cytokinin level in roots, and decreased that of leaves, and affected IAA levels in the opposite way, that is, it was decreased in roots and rose in leaves. The ratio of CK/IAA thus sank in leaves to 1.2 and rose in roots to 4.3. Darkening the shoots increased the levels of CK in them, thus increasing the CK/IAA ratio in both shoots and in roots (Fig. 2).

Levels of IAA and Cytokinins in R and B after IAA or Kinetin Application

Both IAA and kinetin applied to the medium for the whole cultivation period affected the levels of endogenous IAA and CK differently in R and in B. IAA in B, where it had only negligible morphoge-

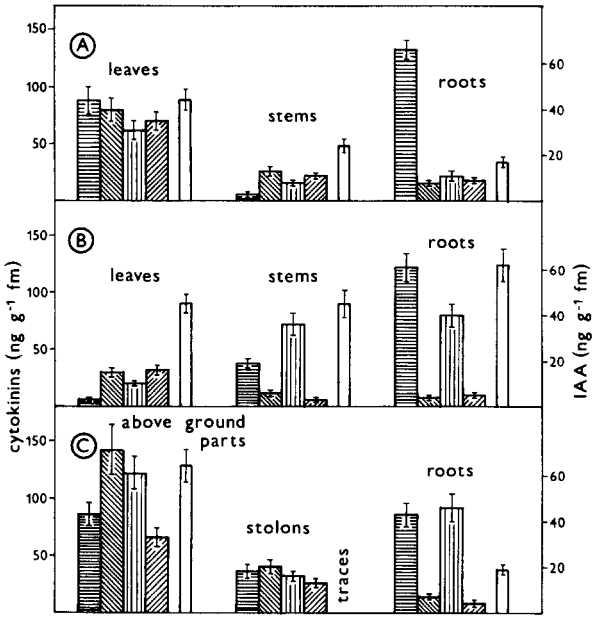

Fig. 2. Levels of free IAA and individual cytokinins in leaves, stems, and roots (with stolons, if present) of potato plants grown in vitro under blue light during an 18-h photoperiod for 40 days with different parts of the plants covered. (A) Whole plant illuminated; (B) roots covered; (C) shoots covered. (□) IAA; (▤) zeatin riboside; (▧) zeatin; (▥) isopentenyladenosine; (▨) isopentenyladenine.

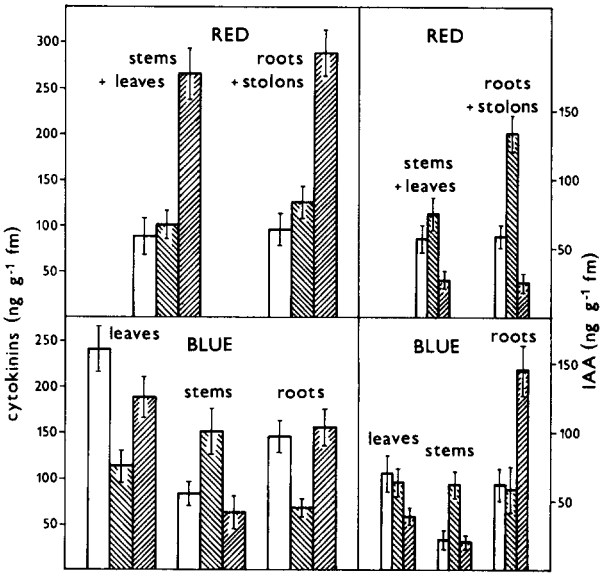

Fig. 3. Levels of free IAA and total free cytokinins in leaves, stems, and roots (with stolons, if present) of potato plants grown in vitro under red or blue light during an 18-h photoperiod for 40 days on medium with IAA or kinetin (1 mg · L^{-1}). (□) control without hormones; (▧) IAA; and (▨) kinetin. The values to the left of the dividing line should be viewed from the left *y* axis, the values to the right of the line should be viewed from the right *y* axis.

netic effects (Aksenova et al. 1994), decreased CK level, namely in roots, and increased IAA level in stems. The CK/IAA ratio was reduced thus in the whole plant (Fig. 3). Kinetin, the most important effect of which in B was increased fresh weight and enhanced tuber formation, brought about a redistribution of CK: a drop in roots and a rise in stems (Fig. 3). Also, IAA changed its distribution patterns to very high levels in roots and low ones in leaves and stems (Fig. 3). The CK/IAA ratio in roots changed from 2.4 to 1.1. In R, IAA brought about an increased CK level in roots (by ca. 45%) and a significant rise in IAA level, especially in roots (ca. three times). Thus, the CK/IAA ratio in roots in R changed from 1.8 to 0.8 after IAA application. Kinetin in R had significantly increased CK level in both shoots and roots. On the other hand, IAA level in the whole plant was lowered, thus changing the CK/IAA ratio in roots from 1.8 to 12.4 (Fig. 3).

Uptake and Metabolism of Labelled IAA

The uptake of 1-^{14}C-IAA was higher in all three age categories in B as compared to R (Table 1). The percentage remaining as IAA in roots was the same both in R and B in young plants, but for older ones, in the period when the stolons are formed, more IAA remained unchanged in R. Two main IAA metabolites were identified on the basis of TLC with authentic standards, and, on the basis of hydrolysis experiments: indoleacetyl glucose (IAGlu) and indoleacetyl aspartic acid (IAAsp). In R, more IAGlu was formed, whereas in B, the greater part of conjugates was represented by IAAsp (Table 1).

Uptake, Distribution, and Metabolism of Labelled Zeatin

Uptake of ^{3}H-zeatin was higher in B than in R by about 41% (data not given) in both age categories. In B, higher activity remained in roots than that in R and from the activity in the shoot higher part accumulated in the upper part of the stem in B, whereas in R, the higher part of the activity accumulated in the lower part of the stem (Table 2). The main metabolites of zeatin were adenosine, zeatin-O-glucoside, and adenine; small amounts of Z and ZR were also detected, and, finally, two unknown metabolites with relatively high-retention time were found. No large differences were found in zeatin metabolism in B and R (Table 3). Similar results

Table 1. Uptake and conjugation of applied 1-^{14}C-IAA in roots of potato plants of different age cultured in vitro under R and B.

Light	Plant age (days)	Uptake (dpm · g^{-1} d · wt · 10^3)	Conjugates formed (% taken up activity)	IAGlu (% taken up activity)	IAAsp (% taken up activity)
R	20	440 ± 11	82 ± 9	36 ± 3	20 ± 2
	30	352 ± 21	45 ± 4	ND	ND
	60	227 ± 21	39 ± 4	ND	ND
B	20	501 ± 26	78 ± 8	18 ± 2	43 ± 4
	30	432 ± 12	80 ± 8	ND	ND
	60	257 ± 16	82 ± 8	ND	ND

ND = not determined.
Note. Labelled IAA was applied in solution with cold IAA in the final concentration 1 mg · L^{-1}, actual activity was 1.6 kBq · ml^{-1}. Incubation lasted for 3 h.

Table 2. Distribution of radioactivity in potato plants cultivated in vitro in R and B 20 h after application of ^{3}H-zeatin together with cold zeatin in final concentration 10^{-6} mol · L^{-1}.

Light	Plant age (days)	Roots (% taken up activity)	Activity upper stem part (% of the activity in the shoot)	Lower stem part (% of the activity in the shoot)
B	20	84 ± 8	58 ± 6	42 ± 4
B	40	82 ± 9	52 ± 5	48 ± 5
R	20	76 ± 8	44 ± 5	56 ± 6
R	40	70 ± 7	40 ± 4	60 ± 7

Note. After the end of incubation plants were dried, combusted in the stream of oxygen and ^{3}H in the form of ^3H_2O was trapped in scintillator.

Table 3. Amounts of ^{3}H-zeatin metabolites (in % of taken up activity) in potato plants cultivated in vitro in R and B after 20 h incubation.

Light	Plant part	Adenosine	Zeatin O-glucoside	Adenine	Zeatin riboside	Zeatin	X_1	X_2
R	Shoot	28	40	16	9	4	tr	4
R	Root	33	43	11	6	2	2	4
B	Shoot	38	30	17	10	3	tr	2
B	Root	40	31	12	8	2	2	4

tr = traces.

were obtained with isopentenyladenine (results not shown).

Discussion

In plants grown in R, relatively low levels of CK and intermediate levels of IAA with almost no differences between shoots and roots were detected. In B, plants contained much higher levels of both CK and IAA, more CK and IAA being located in shoots (namely, in leaves). The lower IAA level in R is in accordance with literature data (e.g., Jones et al. 1991, Behringer et al. 1992). (See discussion in the preceding article (Aksenova et al. 1994.) But in spite of the lower IAA level in R compared to that of B, the longitudinal growth was greater in R (fresh mass was the same in both light treatments). Moreover, in R, added IAA caused shortening of the stem, while the level of endogenous IAA increased. One of the possible explanations is that the endogenous level of IAA is sufficient to sustain growth even without IAA added to the medium. We may also envisage the involvement of gibberellins, the level of which is known to be increased by R (O'Brien et al. 1985). A higher level of CK in B was already reported for *Amaranthus caudatus* by

Table 4. Free IAA level in the individual organs of potato plants grown in vitro under red or blue light at 18 h photoperiod for 40 days on medium without hormones (C) or with IAA or kinetin (1 mg $\cdot L^{-1}$).

		IAA level (ng $\cdot g^{-1}$ f $\cdot$ wt)			Tubers (g)		
		Treatment			Treatment		
Light	Plant organ	C	IAA	K	C	IAA	K
R	leaves + stems	54.2	78.6	28.4			
R	roots + stolons	56.7	134.3	26.5	0	3.05	0.58
B	leaves	71.3	62.8	36.3			
B	stems	19.4	59.7	18.2			
B	roots + stolons	48.1	55.2	146.5	0.64	0.52	1.63

Note. Fresh matter of the tubers formed on 10 plants under different treatments is also given.

Obrenovic (1980) and in some other plants (see review by Vince-Prue 1985).

When we compare the conditions under which either light or hormones enhanced the root/shoot ratios (always roots with stolons/stems with leaves) and tuber formation, we may generalize that in these situations (IAA in R, kinetin in B, and, to a lesser extent, kinetin in R and darkening of roots in B) the IAA level in roots and stolons rose significantly, creating thus an evident gradient of IAA from the shoot to the root (Table 4). No such generalization can be made for the CK level (in B it was decreased by IAA, in R it was increased by kinetin treatments which did not show any significant morphogenetic changes).

These results stress the possible importance of an IAA gradient for tuber formation and they show no significant change of this process due to changes in the CK level. However, it should be noted that under all conditions where tubers were formed, there was also a relatively high level of CK in roots. We may also envisage the importance of a CK/IAA ratio for the respective morphogenesis. There are many reports on stimulatory role of cytokinins on tuber formation (e.g., Palmer and Smith 1969, 1970, Mauk and Langiller 1978). But in these reports CK were applied to in vivo plants either at the time of tuber initiation or shortly prior to it. In our case, in vitro plants were in contact with kinetin during the entire growth period. Moreover, the regulators applied to the medium at the beginning of the culture interval can be depleted before the end of this interval. For example, we have observed that IAA disappeared very quickly from the medium without plants: within 1 week IAA level decreased to 6% of original value (data not shown). The regulators taken up are rapidly metabolized as was shown also in our experiments. This means that regulators added to the medium act first of all in the beginning of subculture interval, and their level both in the medium and in the plants may be very low at the time of tuber initiation.

On the other hand, the role of IAA in tuber formation is not so surprising. IAA is believed to increase sink capacity of, for example, fruits (Bangerth 1984), and thus, high IAA level in roots (as well as increased root/shoot ratio as discussed in the preceding article) (Aksenova et al. 1994) may increase the percentage of sucrose remaining therein, assimilates going to roots, and thus stimulates tuberization.

Light quality affected also hormone uptake, distribution, and metabolism. Generally, the uptake from medium was higher in B than in R. Distribution of zeatin that had been taken up was changed only slightly: in B, more Z remained in roots and more activity accumulated in the upper stem part. Also, metabolism of Z was changed only slightly by light quality, but with IAA a more pronounced effect was seen on the type of conjugation. It is difficult to assess on the basis of existing knowledge, if and how these changes may contribute to changed growth and development.

Our results indicate that hormone gradients might play a crucial role in some morphogenetic processes and that light-quality influences plant development, at least partly, via changes in these hormone gradients.

Acknowledgments. The authors thank Prof. G. W. M. Barendse of the University of Nijmegen for critical reading of the manuscript, Dr. M. Strnad for providing antibodies and conjugates for ELISA for cytokinins and Mrs. B. Čermáková for excellent technical assistance and preparation of the manuscript.

References

Aksenova NP, Konstantinova TN, Sergeeva LI, Macháčková I, Golyanovskaya SA (1994) Morphogenesis of potato plants in vitro. I. Effect of light quality and hormones. J Plant Growth Regul 13:143–146

Bangerth F (1984) Wirkung von Phytohormonen und Wachstumsregulatoren bei der Induktion, dem Wachstum und dem Konkurenzverhalten von Tomatenfruchten. Ber Deutsch Bot Ges 97:257–267

Behringer FJ, Davies PJ, Yang P, Law DH (1992) The role of indoleacetic acid in mediating changes in stem elongation of etiolated *Pisum* seedlings following exposure to light. In: Karssen CM, vanLoon LC, Vreugdenhil D (eds) Progress in Plant Growth Regulation. Kluwer Acad Publ House, Amsterdam, pp 437–445

Eder J, Rovenská J, Kutáček M, Čermák V (1988) HPLC analysis of indole compounds in *Agrobacterium tumefaciens* and transformed tobacco cells. In: Kutáček M, Bandurski RS, Krekule J (eds) Physiology and biochemistry of auxins in plants. Academia, Praha. SPB Academic Publishing. The Hague, pp 389–390

Jones AM, Cochran DS, Lamerson PM, Evans ML, Cohen JD (1991) Red-light regulated growth. I. Changes in the abundance of indoleacetic acid and a 22-kilodalton auxin-binding protein in the maize mesocotyl. Plant Physiol 97: 352–358

Macháčková I, Krekule J, Eder J, Seidlová F, Strnad M (1993) Cytokinins in photoperiodic induction of flowering in *Chenopodium* species. Physiol Plant 87:160–166

Mauk CS, Langiller AR (1978) Physiology of tuberization in *Solanum tuberosum* L. Plant Physiol 62:438–442

Noodén LD, Letham DS (1984) Translocation of zeatin riboside and zeatin in soybean explants. J Plant Growth Regul 2: 265–279

Obrenovic S (1980) Study of the blue and red light action in *Amaranthus caudatus* sections. In: DeGreef J (ed) Photoreceptors and Plant Development, Antwerpen Univ Press, Amsterdam, pp 383–392

O'Brien T, Beall FD, Smith H (1985) De-etiolation and plant hormones. In: Pharis RP, Reid DM (eds) Hormonal regulation of development. III. Role of environmental factors. Encycl Plant Physiol, New Ser, Vol 11, Springer Verlag, Berlin Heidelberg New York Tokyo, pp 282–307

Palmer CE, Smith OE (1969) Cytokinins and tuber formation in the potato *Solanum tuberosum* L. Nature 221:279–280

Palmer CE, Smith OE (1970) Effects of kinetin on tuber formation on isolated stolons of *Solanum tuberosum* L. cultured in vitro. Plant Cell Physiol 11:303–314

Štiller J, Našinec V, Svoboda S, Němcová B, Macháčková I (1992) Effects of agrobacterial oncogenes in kidney vetch (*Anthyllis vulneraria* L.). Plant Cell Reports 11:363–367

Vince-Prue D (1985) Photoperiod and hormones. In: Pharis RP, Reid DM (eds) Hormonal regulation of development. III. Role of environmental factors. Encycl Plant Physiol, New Ser, Vol 11, Springer Verlag, Berlin Heidelberg New York Tokyo, pp 308–364