Plant Growth Regul (2008) 54:189–193
DOI 10.1007/s10725-007-9243-6

BRIEF COMMUNICATION

The effects of IAA and tetcyclacis on tuberization in potato (*Solanum tuberosum* L.) shoot cultures in vitro

Ivana Dragićević · Radomir Konjević · Branka Vinterhalter · Dragan Vinterhalter · Mirjana Nešković

Received: 20 July 2007 / Accepted: 23 November 2007 / Published online: 12 December 2007

Abstract Potato (*Solanum tuberosum* cv. Désirée) shoots grown in vitro in continuous darkness or in long days (LDs), were used to investigate indole-3-acetic acid (IAA) effects on stolon initiation and tuber formation, combining IAA with increased or decreased gibberellin levels. An increased gibberellin (GA) level was achieved by the applying 1 μM GA_3, while decreased gibberellin level was presumably realized by the adding 3 μM tetcyclacis (Tc). About 15% of potato shoots developed stolons both in LDs and in darkness. Stolon initiation was stimulated by GA_3 in darkness and by Tc in LDs. Tuber formation was strongly inhibited in LDs and by GA_3 both in light and darkness, but stimulated in darkness at low GA level. Exceptionally, tuber formation occurred in LDs at the highest Tc concentrations, in about 25% of explants. Indole-3-acetic acid alone stimulated stolon formation in LDs, both in the presence or absence of GA_3. IAA alone also stimulated tuber formation in dark-grown shoots, but could not overcome the inhibitory effect of LDs. Indications that, depending on their concentration ratio, IAA may interact with GA_3 in different tuberization phases, have been discussed.

Keywords GA_3–IAA interaction · Gibberellins · Indole-3-acetic acid · Stolon initiation · Tuber formation

Introduction

Tuberization process in potato consists of several phases (stolon initiation and elongation, tuber induction and formation), which are under a complex control of various endogenous and environmental factors, though gibberellins and day length play the key roles in these processes (Vreugdenhil and Struik 1989; Ewing 1995; Jackson 1999; Fernie and Willmitzer 2001). Gibberellins and LDs are non-inductive factors for the complete tuberization process. However, they may have different effects in separate steps of tuberization. Early steps (i.e. formation of stolons) may be promoted by GA_3 in darkness, or in LDs at very low GA level, while later steps (i.e. tuber formation) are strongly inhibited by GA_3 and occur only in darkness or in short days. It appears, however, that the fine tuning of tuber formation may partly be mediated by other plant growth regulators, such as auxins (Vreugdenhil and Struik 1989; Xu et al. 1998; Romanov et al. 2000; Zhang et al. 2005).

Radomir Konjević—Deceased in July 2006.

I. Dragićević · R. Konjević · M. Nešković
Faculty of Biology, Institute of Botany, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia

I. Dragićević (✉) · R. Konjević · B. Vinterhalter · D. Vinterhalter · M. Nešković
Institute for Biological Research "Siniša Stanković", Bul. Despota Stefana 142, 11060 Belgrade, Serbia
e-mail: dragicev@ibiss.bg.ac.yu

The objective of this study was to investigate the possible interaction of auxins and gibberellins in stolon initiation and tuber formation in potato shoot cultures. Therefore, we examined IAA effects combined with increased or reduced gibberellin levels, in tuber inductive (darkness), or non-inductive (long days) conditions.

Material and methods

The potato cultivar used in our experiments was *Solanum tuberosum* ssp. *tuberosum* L. cv. Désirée. Elite tubers were obtained from PKB Agroeconomic Institute, Belgrade. Shoot cultures were established from sprouts cultured in vitro on the basal medium (BM), consisting of mineral salts (Murashige and Skoog 1962), 0.7% agar, 3% sucrose, and supplemented with 100 mg l^{-1} *myo*-inositol, vitamines (Linsmaier and Skoog 1965), and 0.2 mg l^{-1} (0.89 μM) 6-benzylaminopurine, BAP (Sigma Chemical Co.). Shoot culture clone PKB3, confirmed by Elisa testing (Potato research center Guča) as virus-free, was used in all subsequent investigations.

Every 30 days shoot cultures were propagated by culturing single-node stem cuttings on BM without BAP. For all experiments, single-node cuttings (with one leaf) were placed on BM supplemented with growth regulators: indole-3-acetic acid, IAA (Sigma Chemical Co.), gibberellic acid, GA_3 (Sigma Chemical Co.), or growth retardant tetcyclacis, Tc (5-(4-chlorophenyl)-3,4,5,9,10-pentaaze-tetra-cyclo-5,4,1,0,0-dodeka-3,9-dien, BASF 106 W (BASF, Mannheim). Gibberellic acid was applied to the medium after filter sterilization (Millipore filters, pore size 0.2 μm). In the first set of experiments, single-regulator treatments consisted of adding to the medium IAA, GA_3 or Tc in different concentrations (0.01, 0.1, 1.0, and 10 μM), in order to determine their basic effects on stolon initiation and tuber formation. According to these results, suitable concentrations of GA_3 and Tc for the study of their interaction with 0.01–10 μM IAA were chosen. Increased GA level was achieved by applying 1 μM GA_3, selected as the lowest tested concentration that totally inhibited tuber formation. Decreased gibberellin level was achieved by adding 3 μM Tc, that reduced shoot length to 1/4 of control length and strongly induced tuber formation. After 30 days the

Table 1 Effects of applied PGRs on potato shoot elongation in long day (LD) or in continuous darkness (CD) after 30 days

Average shoot length ± S.E. (mm)																
Applied PGR	Concentration (μM)															
	0		0.01		0.1		1.0		3.0		10					
	LD	CD	LD	CD	LD	CD	LD	CD	LD	CD	LD	CD	LD	CD	LD	CD
GA_3	75.2 ± 1.3	78.5 ± 2.8	101.4 ± 3.8	105.3 ± 8.7	102.0 ± 5.5	108.5 ± 8.2	118.0 ± 6.4	61.7 ± 2.9	–	–	115.6 ± 8.6	57.8 ± 3.0				
Tc	75.2 ± 1.3	78.5 ± 2.8	44.5 ± 3.1	51.1 ± 7.2	36.5 ± 2.1	47.3 ± 6.9	38.6 ± 3.6	9.9 ± 2.8	18.8 ± 2.6	4.5 ± 1.0	4.9 ± 0.6	2.1 ± 0.3				
IAA	75.2 ± 1.3	78.5 ± 2.8	83.3 ± 4.9	75.3 ± 7.0	103.3 ± 4.9	96.3 ± 7.2	92.5 ± 5.3	56.9 ± 7.9	–	–	81.3 ± 5.5	38.8 ± 10.7				

percent of shoots with stolons and tubers were determined.

Since a great diversity concerning gravitropic orientation of axillary organs was observed, and axillary shoots could be mistaken for true stolons, the angle between the main shoot axis and the axillary organ was taken as the basic criterion for distinction. Therefore, all axillary organs growing under the angle greater than 45° to the main shoot axis above the node were designated as stolons. Sessile tubers were excluded from calculations for stolons, since tuberization happened without stolon formation and elongation.

Shoot cultures were grown at 25 ± 2°C, in darkness, or in long days (LDs) of 16 h photoperiods ("Tesla" white fluorescent lamps, 65 W, 4,500 K; light flux of 45.5 μmol m^{-2} s^{-1}). Data presented in figures are the means ± S.E. of three independent experiments (at least 30 replicates per experiment).

Results and discussion

When single-node cuttings were put on the medium, axillary buds started growing after about 2 days, and produced shoots, which gave rise to stolons or tubers, depending on the applied growth regulators and light conditions. These treatments affected the main shoot elongation in a predictable way (Table 1). Gibberellic acid and IAA stimulated stem elongation, whereas in the presence of Tc the stem length was drastically reduced. Unexpectedly, in shoot cultures grown in darkness at 1 and 10 μM GA_3 elongation was arrested due to apical necrosis. Instead, the growth

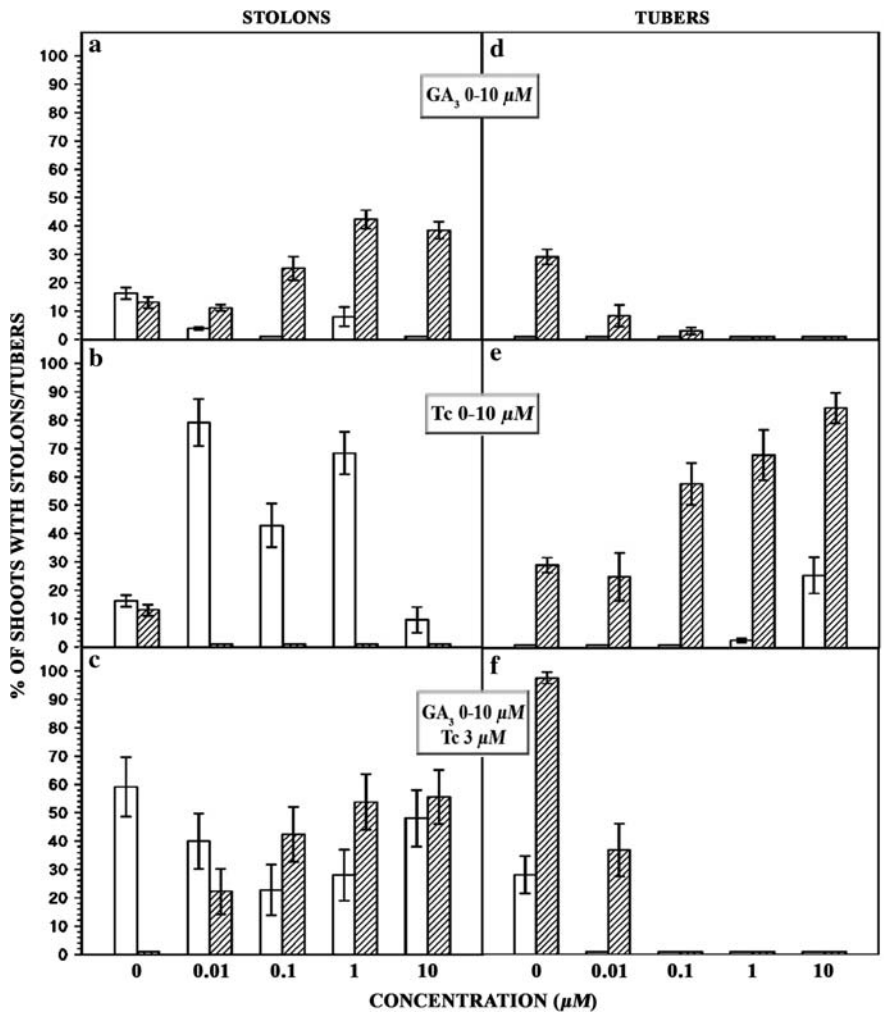

Fig. 1 Effects of gibberellic acid, tetcyclacis and their combinations on stolon initiation and tuber formation in potato shoots grown in long days (□) or continuous darkness (▨) for 30 days. (**a**, **d**) 0–10 μM gibberellic acid (GA_3), (**b**, **e**) 0–10 μM tetcyclacis (Tc), and (**c**, **f**) 0–10 μM gibberellic acid + 3 μM tetcyclacis. Line bars indicate standard errors of the means

of axillary branches was promoted. With this explanation in mind, the data in Table 1 can be taken as evidence that IAA, GA_3, Tc, and light conditions are acting in potato shoot cultures in an expected manner.

Stolons arose mainly on the basal nodes and very rarely on middle ones. During our experimental period of 30 days, stolons remained without tubers in non-inductive conditions. Since Désirée is a facultative SD cultivar, tubers may occur even in non-inductive conditions after about 6 weeks. Tubers were observed as swellings along the shoots; they were mainly formed in lateral position just above the medium. On a regulator-free control medium, about 15% of the shoots developed stolons both in LDs and in darkness (Fig. 1a, b). Long days in combination with elevated GA level in the medium suppressed stolon initiation, but in darkness their initiation was stimulated by GA_3 (Fig. 1a). The decrease of endogenous GAs strongly stimulated stolon initiation, but only in LDs, and completely blocked that process in darkness (Fig. 1b), Tuber formation was inhibited by GA_3 (Fig. 1d) and stimulated by Tc (Fig. 1e), in proportion to their concentrations. Tubers were sessile and formed exclusively in darkness (Fig. 1d, e), with one exception. Namely, at higher concentrations of Tc (1 and 10 μM), the inhibitory effect of LDs on tuber formation was counteracted and tubers appeared in about 25% of shoots (see also Fig. 2f, with 3 μM Tc). In further experiments Tc and GA_3 were added together, in an attempt to reverse Tc

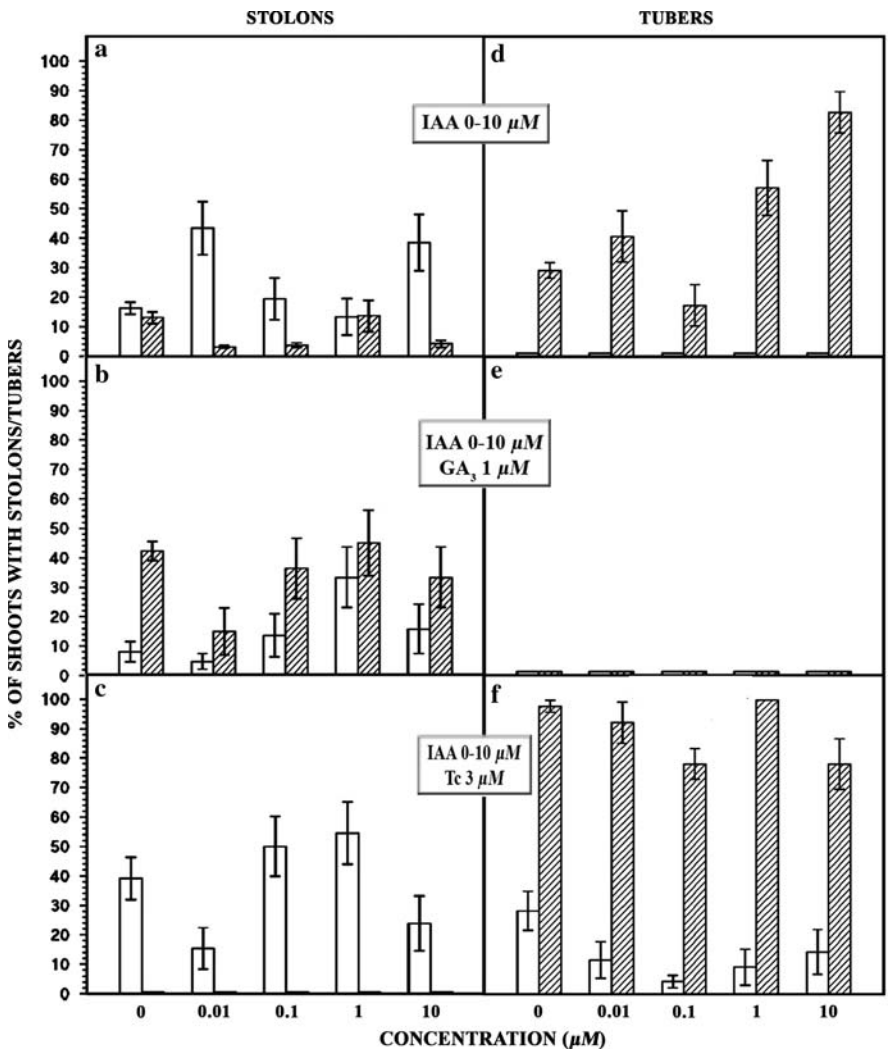

Fig. 2 Effects of indole-3-acetic acid and its combinations with 3 μM tetcyclacis and 1 μM gibberellic acid on stolon initiation and tuber formation in potato shoots grown in long days (□) or continuous darkness (▨) for 30 days. (**a**, **d**) 0–10 μM indole-3-acetic acid (IAA), (**b**, **e**) 0–10 μM indole-3-acetic acid + 1 μM gibberellic acid (GA_3), and (**c**, **f**) 0–10 μM indole-3-acetic acid + 3 μM tetcyclacis (Tc). Note that treatment with 1 μM IAA + 3 μM Tc resulted in tuber formation in 100% of shoots, in all three experiment replications

action. Stolon initiation in darkness was restituted by GA_3, but in LDs the reversal of stolon initiation was not complete (Fig. 1c). The experiments aimed at assessing the reversal of Tc effect on tuber formation were pointless (Fig. 1f), since added GA_3 would inhibit tuberization anyway. The formation of tubers in LDs perhaps indicates that some side effects of Tc occur, restricting stolon initiation and enabling earlier formation of tubers, if the GA level is low. The possibility that Tc may affect the synthesis of a substance other than GA should be further studied.

The effect of applied IAA on stolon initiation was dependent on light conditions. In LD-grown shoots a stimulating IAA effect on stolon initiation was observed only with the lowest (0.01 μM) and highest (10 μM) concentrations (Fig. 2a). Indole-3-acetic acid applied alone stimulated tuber formation in dark-grown shoots, but could not overcome the inhibitory effect of LDs (Fig. 2d). In combination with 1 μM GA_3 (Fig. 2b), the effect of IAA in LDs was not different from control, except at 1 μM, where increased stolons initiation was observed; hence, a positive interaction of IAA and GA_3 occurred only at their equimolar concentrations. In darkness, GA_3 and IAA kept stolon initiation at the level obtained with GA_3 alone, except at the lowest (0.01 μM) IAA concentration (Fig. 2b). It was not possible to study the interaction of IAA and exogenous GA_3 on tuber formation, since there were no tubers at all (Fig. 2e). When endogenous GA_3 level was decreased (Fig. 2c), the Tc induced stimulation of stolon initiation in LDs was repeatedly demonstrated, except at 0.01 and 10 μM IAA. In the presence of Tc, the IAA effect on tuber formation depended on light conditions (Fig. 2f). In dark-grown shoots, tuber formation was strongly promoted, both in control and IAA-supplemented media (Fig. 2f), occurring on 100% shoots, or nearly so. Decreasing the GA level counteracted the inhibitory effect of LDs on tuber formation; this effect was also evident when IAA was present (Fig. 2f).

Indole-3-acetic acid apparently can modulate both stolon initiation and tuber formation, once they were induced by darkness and low GA content. It is known that plant hormones have multiple actions, which may be limited to specific tissues and developmental stages. Moreover, IAA might be involved in two different phases of stolon initiation, the lowest concentration being optimal for one of them, while in the other phase the highest concentrations is required (Fig. 2a). This could be resolved only in further studies by investigating other parameters of tuber induction and formation. Our results demonstrate that in these studies more attention should be given to factors that may modify the well-known effects of day-length and gibberellins; IAA is certainly one of them.

Acknowledgements We are grateful to Dr. W. Rademacher, BASF, Agricultural Research Center, for the gift of the growth retardant tetcyclacis. This research was supported by the Serbian Ministry of Science and Environmental Protection, grant No. 143026B.

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