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ORIGINAL ARTICLE

Auxin synthesis gene *tms1* driven by tuber-specific promoter alters hormonal status of transgenic potato plants and their responses to exogenous phytohormones

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Abstract

Key message **Ectopic auxin overproduction in transgenic potato leads to enhanced productivity accompanied with concerted and occasional changes in hormonal status, and causing altered response of transformants to exogenous auxin or cytokinin.**

Abstract Previously, we generated potato transformants expressing *Agrobacterium*-derived auxin synthesis gene *tms1* driven by tuber-specific patatin gene promoter (B33-promoter). Here, we studied the endogenous hormonal status and the response to exogenous phytohormones in *tms1* transformants cultured in vitro. Adding indole-3-acetic acid (IAA) or kinetin to culture medium affected differently tuberization of *tms1*-transformed and control plants, depending also on sucrose content in the medium. Exogenous phytohormones ceased to stimulate the tuber initiation in transformants at high (5–8%) sucrose concentration, while in control plants the stimulation was observed in all experimental settings. Furthermore, exogenous auxin partly inhibited the tuber initiation, and exogenous cytokinin reduced the average tuber weight in most transformants at high sucrose content. The elevated auxin level in tubers of the transformants was accompanied with a decrease in content of cytokinin bases and their ribosides in tubers and most shoots. No concerted changes in contents of abscisic, jasmonic, salicylic acids and gibberellins in tubers were detected. The data on hormonal status indicated that the enhanced productivity of *tms1* transformants was due to auxin and not mediated by other phytohormones. In addition, exogenous cytokinin was shown to upregulate the expression of genes encoding orthologs of auxin receptors. Overall, the results showed that *tms1* expression and local increase in IAA level in transformants affect both the balance of endogenous cytokinins and the dynamics of tuberization in response to exogenous hormones (auxin, cytokinin), the latter reaction depending also on the carbohydrate supply. We introduce a basic model for the hormonal network controlling tuberization.

Keywords Tuberization · Potato · Auxin · Cytokinin · Phytohormones · Transformants · Hormonal status · *Solanum tuberosum*

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Introduction

Tuber formation in potato is a complex process regulated by a number of external (temperature, lighting, mineral supply) and internal (hormonal status, carbohydrate supply) factors (Jackson 1999). The influence of the external

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factors is largely based on their impact on the internal regulatory systems, especially the hormonal one (Vreugdenhil and Struik 1989; Ewing 1995; Prat 2004; Sarkar 2008; Aksenova et al. 2012, 2014). To date gibberellins were shown to normally inhibit tuberization whereas some of the other known phytohormones (cytokinins, abscisic acid, jasmonic acid) stimulated tuber formation in several experiments. Auxin is one of the most important plant hormones, but its role in the tuberization has long been unclear. Exogenous auxin stimulated tuber formation in many potato varieties (Ito and Kato 1951; van Schreven 1956; Harmey et al. 1966; Aksenova et al. 2000; Romanov et al. 2000). It was reported that the effect of auxin depends on its concentration (Kumar and Wareing 1974), light quality (Aksenova et al. 1994), endogenous auxin-to-cytokinin ratio (Macháčková et al. 1997; Sergeeva et al. 2000), carbohydrate supply (Aksenova et al. 2000) and potato genotype (Romanov et al. 2000). According to other reports, the level of endogenous IAA in stolons increased prior to tuber initiation, and further decreased remaining low throughout the period of tuber growth (Obata-Sasamoto and Suzuki 1979; Koda and Okazawa 1983). Transcriptomic studies revealed changes in the expression of auxin-responsive and signaling genes during the tuber initiation. In particular, the transition from longitudinal to transverse cell divisions at swelling stolon tips was accompanied by several fold decrease in the expression of auxin transcription factor *ARF6* (Faivre-Rampant et al. 2004). In accordance with these data, it was shown that the expression of a number of auxin-dependent genes in potato subsp. *andigena* decreased in the early period of tuber initiation when stolon tips begin to swell (Hannapel et al. 2004; Hannapel 2007). However, a more detailed analysis demonstrated that the expression of many auxin-responsive genes increased in stolons rapidly during tuber initiation (Kloosterman et al. 2005, 2008). Among these genes were *StPIN* (auxin transport), *StYUC* (auxin biosynthesis), *arcA*-like genes and others. Using LS-MS/MS analysis, a dramatic increase in auxin content in stolon tips prior to tuber swelling was found (Roumeliotis et al. 2012). Taken together, these results pointed to an important role of auxin in the initiation and growth of potato tubers.

GM plants represent a useful tool for studying the biological role of bioactive compounds. Recently, we have been able to transform potato with an agrobacterial auxin biosynthesis gene *tms1* under control of the tuber-specific patatin gene class I promoter (B33-promoter) (Kolachevskaya et al. 2015). When grown in culture, these transformants were characterized by the enhanced level of auxin in tubers and increased productivity. However, the status of other phytohormones and their perception systems in *tms1* transformants was not studied, so the possibility of indirect action of auxin through some other plant hormone was not excluded. This has prompted us to conduct a special study of the hormonal systems of the transformants in comparison with control (non-transformed) plants. The obtained results showed that *tms1* expression and auxin overproduction in tubers led to the reduced positive effect of both exogenous auxin and cytokinin on tuber formation, i.e., the plant response to hormone treatment was altered. The concerted changes of endogenous hormonal status in transformants were recorded. These changes might be responsible for the altered response of *tms1*-transformed plants to exogenous phytohormones. New data were obtained supporting tight metabolic relations between cytokinin bases and their ribosides in potato. The ability of cytokinin to enhance auxin signaling by increasing the expression of auxin receptor genes was disclosed. Altogether, these findings demonstrate the complex interplay between different hormonal and carbohydrate systems in potato, where auxin–cytokinin crosstalk is crucial in controlling tuberization.

Materials and methods

Plant growth conditions

The study was performed with potato plantlets (*Solanum tuberosum* L.) cv. Désirée belonging to mid-maturity group (http://www.europotato.org/). Plants were propagated by single-node stem cuttings and grown in vitro on standard Murashige–Skoog (MS) medium solidified with 0.8% (w/v) agar and supplemented with 2% sucrose as described previously (Aksenova et al. 2000; Romanov et al. 2000). Uniform single-node cuttings from the middle part of the plants were used in tuberization experiments. Cuttings were cultivated in vitro on the same medium but with different sucrose content: 3, 5 or 8% (w/v), and supplemented or not with IAA (1 mg L^{-1}) or kinetin (1 mg L^{-1}). Plants were grown under long-day conditions (LD, photoperiod 16 h/8 h) at 24/22 °C during the day/night. The number of plantlets with tubers was recorded every week and the raw weight of tubers was determined at the end of experiments (6–7 weeks). All experiments were carried out at least in triplicate, with 15 plants per replicate; graphs and tables show mean values with standard deviations.

Phytohormone content determination

Isolated organs (tubers, upper parts of shoots or leaves and stems separately) from 4-week-old axenic plants grown on hormone-free media containing 5% sucrose were used to determine endogenous phytohormone levels.

Determination of IAA, JA, abscisic acid (ABA) and salicylic acid (SA)

1 mL of ice-cold 10% methanol (MeOH) extraction solution acidified with 0.05% formic acid was added to fine-homogenized lyophilized samples (7 mg) containing internal standards labeled with stable isotopes: $^{13}C_6$-IAA (10 pmol), $^{2}H_6$-JA (10 pmol), $^{2}H_6$-ABA (5 pmol), $^{2}H_4$-SA (15 pmol). Samples were homogenized using an MM 301 vibration mill (Retsch GmbH and Co. KG, Germany) at a frequency of 27 Hz for 3 min, sonicated and extracted for 25 min at 4 °C. After centrifugation (10 min, 14,000 rpm, 4 °C), the supernatant was collected and pellet was re-extracted with 1 mL of the extraction solution. Combined supernatants were purified on Oasis® HLB column (1 cc/30 mg, Waters Co., Milford, MA, USA) according to Floková et al. (2014). Eluates were dried in a Speed-Vac concentrator (Savant SC210A, Thermo, USA) and reconstituted in a small volume of mobile phase acetonitrile:15 mM formic acid (15/85, v/v) prior to UHPLC–MS/MS analysis. Phytohormones were analyzed using an Acquity UPLC® System (Waters, USA) coupled to a triple quadrupole mass spectrometer Xevo™ TQ S (Waters MS Technologies, Manchester, UK) with an electrospray interface (ESI). Compounds were separated on reversed-phase column (Acquity UPLC® CSH™ C18; 2.1 × 100 mm; 1.7 µm; Waters, Ireland) by gradient elution over 14 min using 15 mM formic acid/water (A) and acetonitrile (B) (Floková et al. 2014, with modifications). The column eluate was introduced in the ESI source of the tandem mass spectrometer Xevo TQ S, operating in multiple ion monitoring (MRM) mode. The MS/MS conditions were optimized as follows: source/desolvation temperature, 120/550 °C; cone/desolvation gas flow, 150/650 L h^{-1}; capillary voltage, 3 kV; cone voltage, 23–30 V; collision energy, 20–28 eV; collision gas (argon) flow, 0.24 mL min^{-1}. The MassLynx™ software (version 4.1, Waters, USA) was used to operate the instrument, acquisition and processing of MS data.

Determination of cytokinins

Determination of cytokinins was carried out according to Dobrev and Kamínek (2002) with modifications. Extraction was performed in 1 mL of ice-cold mixture of methanol/water/formic acid (15/4/1, v/v/v), containing labeled internal standards of cytokinins ($^{2}H_5$-tZ, $^{2}H_5$-tZR, $^{2}H_5$-tZ9G, $^{2}H_3$-DZ, $^{2}H_3$-DZR, $^{2}H_6$-iP, $^{2}H_6$-iPR, $^{2}H_6$-iP9G) at concentration 10^{-8} M. The extraction solvent was added to fine-homogenized lyophilized samples (20 mg), sonicated for 5 min, rotated for 30 min at 4 °C, and centrifuged (10 min, 14,000 rpm, 4 °C). The supernatant was collected and pellet was re-extracted with the same extraction solvent. Pooled supernatants were purified from lipids and pigments using GracePure^{TST} SPE C18-Max column (Grace/Alltech, USA), flow through fractions were evaporated to water phase in a Speed-Vac concentrator (Savant SC210A, Thermo, USA) and filled to 2 mL by 1 M formic acid. Cytokinins were pre-concentrated on mixed mode Oasis® MCX reverse-phase cation-exchange SPE column (Waters, Milford, MA, USA). Cytokinin nucleotides were eluted in first fraction by 0.35 M NH_4OH, followed by elution of ribosides and bases using 0.35 M NH_4OH in 60% methanol/water (v/v). Eluates were evaporated till dryness and dissolved in 30 µl of the initial mobile phase used for quantitative analysis. Cytokinin metabolites were analyzed by ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) employing an Acquity UPLC® System (Waters, USA) linked to a triple quadrupole mass spectrometer Xevo™ TQ MS (Waters MS Technologies, Manchester, UK) with an electrospray interface (ESI). Separation was achieved on reversed-phase column (Acquity UPLC® BEH C18, 1.7 µm, 2.1 × 150 mm, Waters) by 24-min binary gradient consisting of methanol (A) and 15 mM ammonium formate pH 4.0 (B) (Svačinová et al. 2012, with modifications). The effluent was introduced in the ESI source of the tandem mass spectrometer Xevo TQ MS. Cytokinin determination and quantitation was performed by multiple ion monitoring (MRM) of precursor $[M + H]^+$ and appropriate product ion. The optimized settings of MRM experiment were as follows: source/desolvation temperature, 120/550 °C; cone/desolvation gas flow, 70/1000 L h^{-1}; capillary voltage, 3 kV; cone voltage, 20–35 V; collision energy, 15–25 eV; collision gas (argon) flow, 0.2 mL min^{-1}. Data were processed using The MassLynx™ software with TargetLynx™ program (version 4.1, Waters, USA).

Determination of gibberellins (GAs)

Gibberellins were quantified according to the modified method of Urbanová et al. (2013). 1 mL of 80% acetonitrile with 5% formic acid and mixture of deuterium-labeled internal standards: $[^{2}H_2]GA_1$, $[^{2}H_2]GA_3$, $[^{2}H_2]GA_4$, $[^{2}H_2]GA_7$, and $[^{2}H_2]GA_{20}$ (10 pmol of each compound) were added to fine-homogenized lyophilized samples (10 mg). After sonication, rotation for 12 h at 4 °C, and centrifugation (10 min, 17,000 rpm, 4 °C), the supernatant was collected and pellet re-extracted for 1 h at 4 °C with 1 mL of the extraction solvent. Pooled supernatants were dried in a Speed-Vac concentrator (Savant SC210A, Thermo, US) up to liquid phase, reconstituted by 5% of NH_4OH (v/v) and loaded on Oasis® MAX column (6 cc/150 mg, Waters Co., Milford, MA, USA). Compounds

were eluted with 0.2 M formic acid in acetonitrile and eluates were dried *in vacuo*. Samples were reconstituted in 60 µL of mobile phase (initial conditions) and injected onto reversed-phase Acquity UPLC CSH C18 column (100 × 2.1 mm, 1.7 µm; Waters) of Acquity UPLC™ I-Class Core System (Waters, USA). GAs were separated isocratically for 1 min at 80% of 10 mM formic acid/water (A, v/v) and 20% of methanol (B) at a flow rate 0.35 mL/min, and then with consecutive linear gradient to 70 and 90% of B over 9 min. Column was thermostated at 50 °C. The effluent was introduced into the electrospray ion source of Xevo® TQ S triple quadrupole mass spectrometer (Waters MS Technologies, UK). The capillary voltage was set on 3 kV. The source block/desolvation temperature was 120 °C/550 °C with desolvation gas flow 650 L h^{-1}. Compounds were quantified by multiple ion monitoring mode (MRM) with settings outlined by Urbanová et al. (2013).

Analysis of transgene expression

The construction of B33-*tms1*-containing vector and potato transformation was described earlier (Kolachevskaya et al. 2015). Leaves, tubers, roots or two upper internodes of shoots from 4-week-old axenic plants grown on hormone-free media containing 5% sucrose were used. Total RNA was extracted from 200 to 300 mg of fresh tissues by means of TRIzol method (Invitrogen) and treated with DNase I. cDNA was synthesized on the RNA template with M-MuLV reverse transcriptase (Fermentas) according to the manufacturer protocol. The absence of genomic DNA in cDNA samples was confirmed by PCR with primers on intron-containing fragment of *patatin* gene (GenBank accession no. NC003065) (see primer sequences in Supporting Information Table S1).

The expression of the selected genes (*tms1*, genes for auxin perception and for GA synthesis/metabolism) was determined using quantitative real-time PCR (qRT-PCR). The primers for the presumable auxin receptor genes are shown in Supporting Information Table S1; the primers for genes encoding GA biosynthetic/catabolic enzymes were designed in Lulai et al. (2016). The conditions for qRT-PCR were as follows: pre-denaturation at 95 °C for 60 s, followed by 35 cycles of denaturation at 95 °C for 50 s, annealing at 60 °C for 20 s and the final extension step at 72 °C for 30 s. Amplicon quality was controlled through DNA melting. DNA sequences encoding potato elongation factor 1-a (ef1a) (GenBank accession no. AB061263) and actin (GenBank accession no. X55749) were used as reference genes (Nicot et al. 2005). Each data record of transcript content represents mean value (±SD) of three technical replicates.

Statistics

The experiments were performed in 2–4 biological replicates; graphics and tables show the mean values with standard errors. The significance of differences was assessed by the statistical software using *t* test. Deviations of the mean values (SE) in qRT-PCR did not exceed 8–9% on average.

Results

The *tms1* expression alters the effect of exogenous hormones on tuberization dynamics in transformed potato plants

It was previously shown that introducing exogenous phytohormones (auxin, cytokinin) at physiological concentrations into the culture medium change the morphogenesis of potato plants promoting tuber formation. Generally, administration of auxin (IAA) to the medium resulted mostly in a tuber weight gain whereas addition of cytokinin (kinetin) led to a tuber number gain (Aksenova et al. 1999, 2000). The magnitude of these effects depended on the sucrose content in the medium and the potato genotype (Romanov et al. 2000). Therefore, of particular interest was to test the response of *tms1* transformants to exogenous plant hormones: auxin and cytokinin. In experiments, potato plants were grown on media containing auxin (1 mg/L IAA) or cytokinin (1 mg/L kinetin), the tuber number was recorded each week and after 7 weeks the tuber biomass was determined. Four independent lines of B33-*tms1* transgenic potato (A1-2, A4-7, A4-8 and A4-24) were used, and the auxin overproduction in tubers of these transformants was confirmed. Yet these lines are distinct regarding some properties. Particularly, line A4-24 is featured by the lowest level of *tms1* expression and auxin content (Supporting Information Fig. S1) (Kolachevskaya et al. 2015).

The transformants exhibited certain differences from control plants in their response to exogenous phytohormones. Both hormones stimulated the tuber formation in non-transformed plants but only kinetin brought the proportion of plants with tubers up to 100%. At low (3%) sucrose concentration in the medium adding IAA stimulated tuberization in both control and transformed plants, though by the 7th week the tuber numbers in transformants on media with IAA and without hormones had leveled off. Kinetin increased the fraction of plants with tubers versus media without hormones or supplemented with auxin by 15–50% by the end of culturing (Fig. 1). In transformants with pronounced tms1-phenotype, IAA markedly accelerated the tuberization, which surpassed not only that of

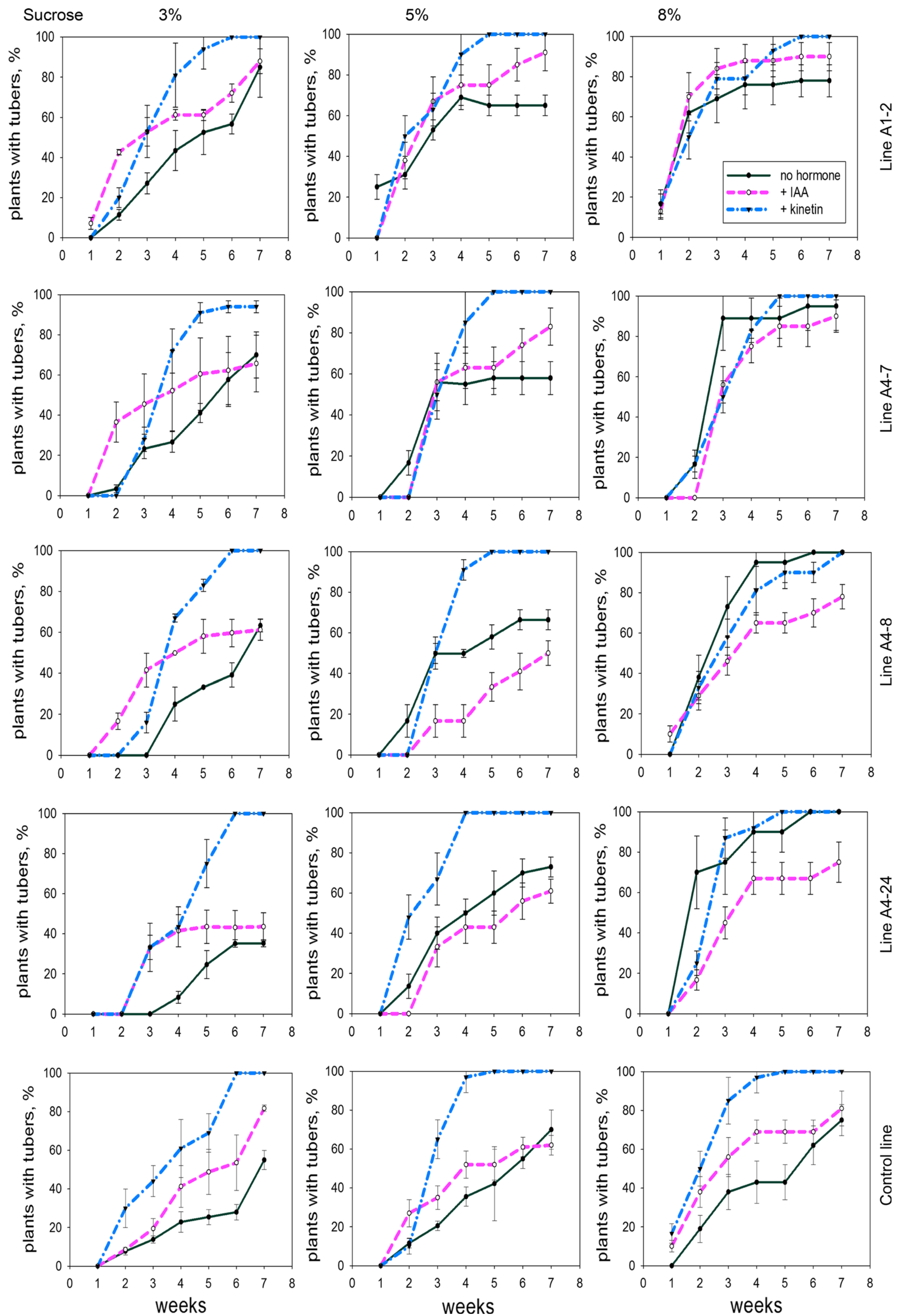

Fig. 1 Effects of exogenous IAA or kinetin on the tuberization dynamics in B33-*tms1* transgenic and control potato plants on media with different (3, 5, 8%) sucrose content

plants on hormone-free medium but also the plants on medium supplemented with kinetin. This promoting effect of IAA was observed during the first 2–3 weeks of cultivation; thereafter, the upregulation by cytokinin became dominating. In control plants, on the contrary, the kinetin effect dominated over the entire cultivation period. The line A4-24 with weak tms1-phenotype occupies an intermediate position in this respect.

IAA addition to the medium containing 5% sucrose still provided a certain stimulating effect on tuberization dynamics (acceleration during first 3 weeks) in control plants, but significantly delayed tuber formation in all transformed lines in the 1st week (Fig. 1). Adding kinetin to the medium with 5% sucrose generally strengthened the tuberization in all lines tested. The stimulation of tuberization by auxin in the control plants in the 2nd week significantly exceeded the stimulation by cytokinin. By contrast, in transformants the kinetin effect in the first weeks of culturing on 5% sucrose (unlike plants on 3% sucrose) was not only non-inferior to the effect of auxin (lines A1-2, A4-7), but even significantly superior to it (lines A4-8, A4-24).

Increasing sucrose concentration in the medium up to 8% significantly enhanced the tuberization in all lines on hormone-free medium versus the low (3%) sucrose concentration. Added IAA contributed to strengthening the tuberization in control plants from the 1st and until the 7th week of culturing. In contrast, among transformed lines exposed to IAA, a limited tuber gain (from the 3rd week) was observed only in plants of A1-2 line, while plants of other lines had less tubers than plants on hormone-free medium throughout the whole cultivation period. The number of tubers in transformed plants grown on hormone-free medium was notably higher than in control plants grown on media without hormones or with IAA addition. Lines A1-2 and A4-7 retained the advantage in the number of tubers also when grown on IAA-containing medium (Fig. 1).

The addition of kinetin to the medium with 8% sucrose stimulated tuber formation in control plants starting from the 1st week, and it led to 100% tuberization in plants by the 4th week. Still, tuberization was anyhow inhibited in transformed lines, and tubers appeared in 100% transformants later than in control plants under the same conditions (Fig. 1).

Effects of exogenous phytohormones on the tuber biomass were clearly dependent on sucrose content in the medium (Fig. 2a). At 3% sucrose both hormones increased average tuber mass to a similar degree (by 52–80%). At 5% sucrose, the positive effect of IAA on tuber weight was markedly reduced in transformants but not in control plants; kinetin lowered (by 36–53%) tuber mass in all lines except for the control one. At 8% sucrose both hormones tended to reduce the average tuber weight in transgenic lines, kinetin being particularly suppressing in lines A1-2 (by 64%) and A4-7 (by 69%). The control plants, on the contrary, responded to hormone administration by increasing tuber mass (by 42–43%).

Thus, measuring tuber mass provided additional evidence that the expression of the B33-*tms1* construct alters potato plant responses to exogenous phytohormones: auxin and cytokinin. Exogenous hormones were shown to have a similar effect on tuber mass in control and *tms1*-transformed plants only at low (3%, threshold for tuberization) level of carbohydrate supply. By increasing the sucrose content in a medium first exogenous kinetin and then IAA reduced the average tuber weight in transformants but not in control plants. Although the results of different lines were not absolutely identical, this general trend prevailed.

However, these alterations (delay of initiation and slowing growth of tubers) occurred in *tms1* transgenic plants that had the significant initial advantage in these two parameters over control plants. As a result, the productivity of the transgenic lines A1-2 and A4-7 was higher than that of control plants, not only on the hormone-free medium, but also, although to a lesser extent, on the IAA-supplemented medium (at 3–5% sucrose) or on the kinetin-supplemented medium (at 3% sucrose) (Fig. 2b). The tuber yield in the transformants grown on the hormone-free medium with 8% sucrose greatly exceeded the yield in control plants. Upon phytohormone addition to high-sucrose media the tuber yields in transgenic and control plants tended to level off.

Thus, in experiments with exogenous phytohormones the differences between *tms1*-transformed and non-transgenic (control) plants in response to auxin or cytokinin (tuberization rate, tuber mass and yield) became clearly apparent.

Changes in the content of endogenous cytokinins in *tms1* transformants

Results derived from experiments with exogenous phytohormones demonstrated the altered response of B33-*tms1* transgenic plants to auxin (which was expected) as well as to cytokinin, indicating the change not only of auxin, but also of cytokinin signaling system in transformants. In this regard, the possibility remained that the effect of ectopic auxin on tuberization was completely or partially mediated by its influence on cytokinin signaling, thus cytokinins were presumably playing a role of the principal effectors. This prompted us to undertake a detailed analysis of the content of different forms of cytokinins, focusing on active (bases) and transport (ribosides) forms, in the transgenic and control lines of potato.

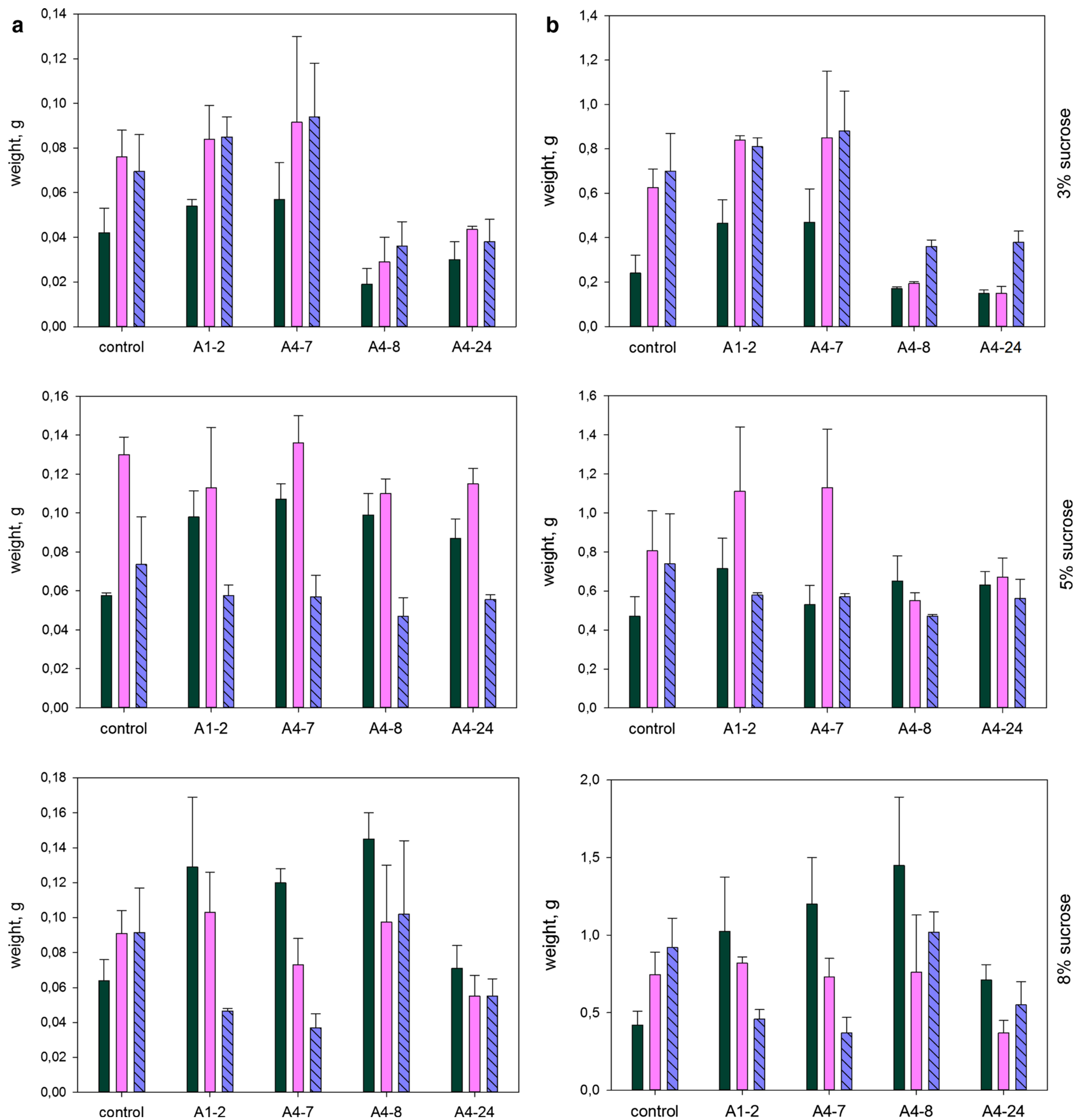

Fig. 2 The average raw weight per tuber (**a**) and the productivity (**b**) of B33-*tms1* transgenic and control potato plants on media with different (3, 5, 8%) sucrose content. *Dark*, *light* and *hatched bars* (from *left* to *right*) correspond to plants grown on hormone-free-, IAA-containing- or kinetin-containing media, respectively

Genuine cytokinins are cytokinin bases (Lomin et al. 2015), among which *trans*-zeatin (tZ) and isopentenyl adenine (iP) are typically the most active, whereas *cis*-zeatin (cZ) is the least active. *N*9-ribosides (R) of these bases are considered to be transport forms moving either via xylem (mainly tZR) from root to shoot, or via phloem (mainly iPR) to all parts of the plant (Hirose et al. 2008). The transport forms were assumed to be somehow "activated" in target organs (Lomin et al. 2015; Osugi and Sakakibara 2015). We have identified in potato plants three main cytokinins, tZ, iP and cZ, as well as their ribosides, and determined the content of these compounds in tubers and shoots (Fig. 3). The content of active cytokinins per gram dry weight was generally higher in shoots than in

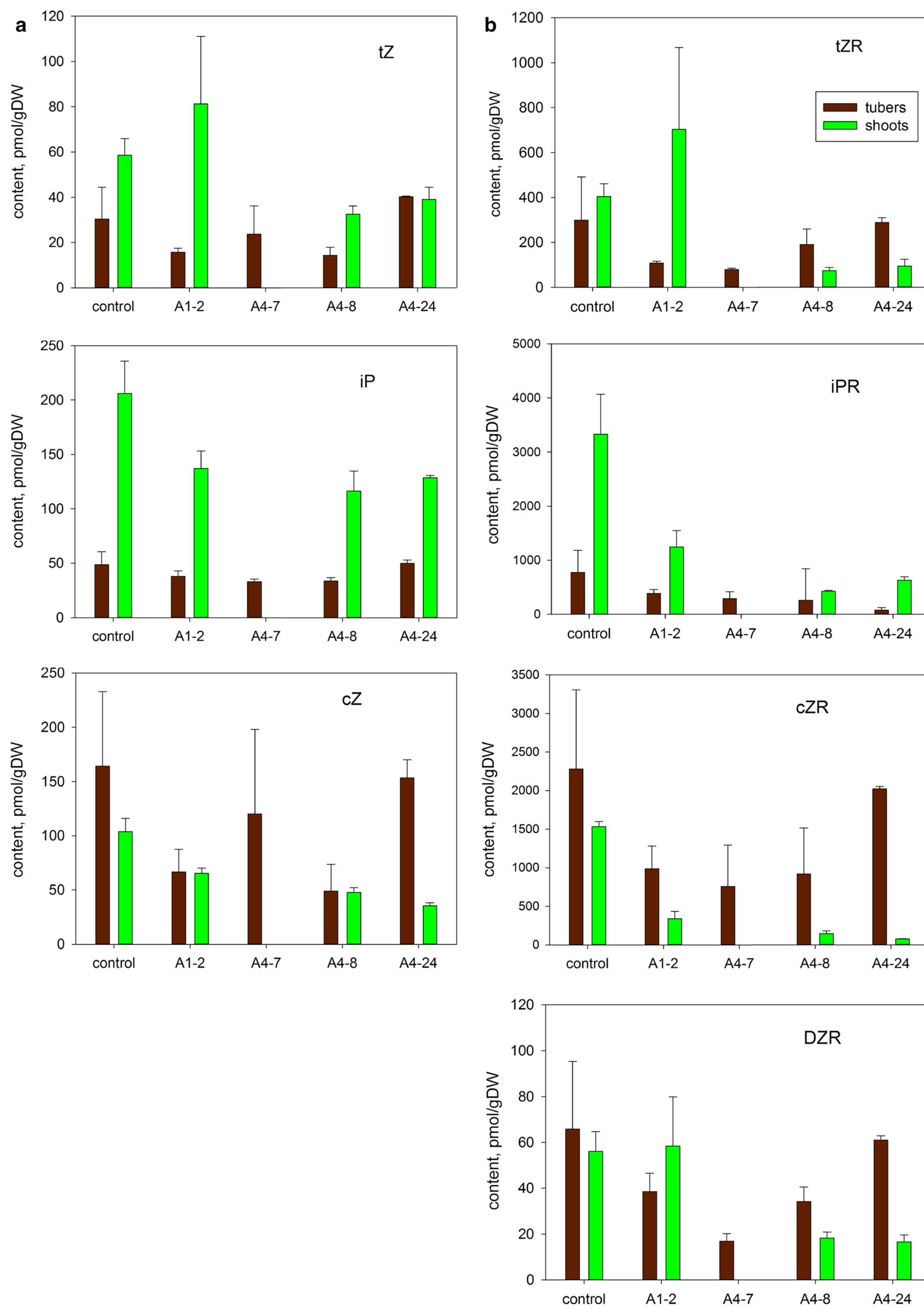

Fig. 3 The content of cytokinin bases (**a**) and ribosides (**b**) in tubers (*dark bars*) and shoots (*light bars*) of 4-week-old B33-*tms1* transgenic and control potato plants grown on medium with 5% sucrose (data on shoots of the line A4-7 were missing)

tubers. At the same time the reduction of tZ, cZ and iP was generally noted in the lines markedly overproducing auxin (lines A1-2, A4-7, A4-8). Cytokinin ribosides behaved similarly, their content decreased significantly in all plant lines strongly expressing the transgene.

Numerical data on changes in the cytokinin content in transformants relative to non-transgenic potatoes are displayed in the color table (Supporting Information Fig. S2a,b). 41 pairs (26 significant + 15 formally non-significant) out of 49 compared pairs showed a clear decrease in the content of active and transport cytokinin forms. The statistically significant reduction averages 62.6% and formally non-significant amounts to 43.1%. Overall more than twofold (55.4%) drop in the content of active and transport cytokinin forms in plants markedly overproducing auxin can be stated. The amplitude of riboside content changes (52.8–87.9%) exceeded that of base content changes (28.3–52.4%), especially in shoots. Of the six cases of no change (difference less than 10%) in the cytokinin content, five corresponded to tubers from the line A4-24 which differed by the least pronounced tms1-phenotype (Kolachevskaya et al. 2015) (Supporting Information Fig. S1). Among all 49 pairs of comparison, only two pairs showed cytokinin level rise in transformants (shoots of line A1-2), but even this rise was not statistically significant. Thus, the common response of cytokinin regulation system on the introduction of B33-*tms1* construct in potato was an approx. twofold reduction of cytokinin content, both in base and riboside forms, occurring in tubers as well as in most of shoots. A general correlation was observed between the level of transgene expression and the degree of its impact on the cytokinin content. Given that cytokinins stimulate tuber formation at least within this experimental design (Aksenova et al. 2000; Romanov et al. 2000), we may conclude that the promotive effect of auxin on tuber formation is not mediated by cytokinins.

Taking advantage of the series of independent transformed lines, we evaluated the degree of correlation between particular cytokinin contents in different organs/lines of potato, by determining the correlation coefficients (CC) in all possible (21) comparison pairs (Supporting Information Fig. S2c). The highest CC corresponded to pairs cZ–cZR (CC = 0.92), tZ–tZR (CC = 0.91) and iP–iPR (CC = 0.82). This indicates that the components of each pair are closely metabolically related and support the formation of active cytokinin bases predominantly from the corresponding ribosides (Sakakibara 2006). At the same time cZ had an extremely low degree of correlation (de facto a lack of it) with tZ and iP, and cZR showed lack of correlation also with both tZR and iPR. These results suggest that the biosynthesis/metabolism of *cis*-zeatin and its riboside is not related to the biosynthesis/metabolism of *trans*-zeatin, isopentenyladenine and their ribosides in potato. A fairly high degree of correlation (CC = 0.74–0.78) of pattern of dihydrozeatin riboside (DZR) contents was shown with patterns of cZ and such ribosides as tZR and cZR. These correlations may point to a possible source of DZR formation in a cell.

To verify and validate the estimates of the foregoing correlation degrees further, we performed a similar analysis but based on changes in the cytokinin content in different transgenic lines versus non-transgenic one (Supporting Information Fig. S2c). This independent verification confirmed the high correlation degrees between cytokinin bases and corresponding ribosides: CC of pairs tZ–tZR, iP–iPR and cZ–cZR were equal to 0.93, 0.97 and 0.73, respectively. Furthermore, a high correlation degree of DZR pattern with tZR (CC = 0.92) and cZ (CC = 0.74) but not with cZR (CC = 0.53) patterns was confirmed. Other high correlations emerged in the lower left part of the table in Fig. S2c (pairs cZR–iP; cZR–iPR; DZR–tZ) were compromised by small CC of the same pairs in the upper right part of the table. Based on these data, the most plausible substrate for DZR, at least in the potato, appears to be tZR, though the involvement of cZ-type cytokinins is not excluded.

Cytokinin enhances the expression of potato genes encoding orthologs of auxin receptors

The eventual causes of the inhibitory effect of cytokinin on tuberization in *tms1* transformants at high (5–8%) sucrose content in the medium remain unclear. Obviously, this phenomenon cannot be explained simply by a cytokinin excess in transformants over the control plants since, unlike auxin, the level of endogenous cytokinins in transformants is lower than in non-transgenic plants. Some data indicated that this inhibition might be associated with auxin signaling. The work on Arabidopsis showed that cytokinins stimulate auxin biosynthesis by activating the expression of the appropriate biosynthetic genes (*CYP79B2, CYP79B3, CYP83B1, NIT3*, etc.) (Jones et al. 2010). Earlier an increase in auxin content under the influence of excessive cytokinin was found in potato (Macháčková et al. 1997).

Thus, exogenous cytokinin raises the auxin level apparently equally in both control plants and transformants, but in the latter this level is already elevated due to *tms1* activity. The high sucrose concentration further activates the patatin promoter which drives the expression of *tms1*. As a result, the total concentration of endogenous auxin in transformants may well go far beyond the physiological optimum, that in its turn is fraught with the switching the sign of cytokinin effect from positive to negative.

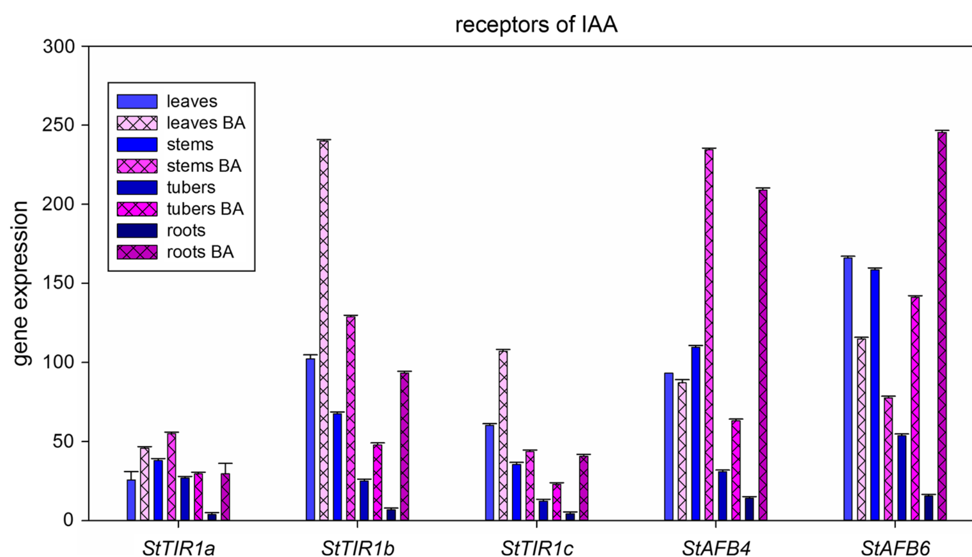

Fig. 4 Effect of cytokinin treatment on the expression of genes encoding orthologs of auxin receptors in potato. 4-week-old non-transgenic plants grown on medium with 5% sucrose were treated with cytokinin (1 μM benzyladenine) for 1 h. Potato organs were sampled at due time, their RNA was extracted, treated by reverse transcriptase and analyzed via qRT-PCR using potato elongation factor 1-α (ef1α) as the normalizing gene. *Hatched* and *non-hatched bars* correspond to cytokinin-treated and mock-treated plants, respectively

The signaling intensity of any hormone is known to depend not only on its own dose but also on functioning of the sensing apparatus of the cell, where receptor proteins play a key role. We have identified five genes in potato genome orthologous to Arabidopsis genes encoding auxin receptors. Among them, three genes, named *StTIR1a*, *StTIR1b* and *StTIR1c*, are closely related to the receptor gene *TIR1* of Arabidopsis, whereas *StAFB4* and *StAFB6* are most structurally similar to receptor groups *AFB4* and *AFB6*, respectively (Supporting Information Fig. S3; Table S2).

To assess the likelihood of cytokinin impact on the auxin perceiving apparatus of potato cells, we have analyzed the expression of genes for the putative auxin receptors in four potato organs by qRT-PCR. Measurements revealed that the expression of genes encoding the likely auxin receptors in potato was largely stimulated by cytokinin (Fig. 4). This stimulation was evident to a greater degree in the roots (all 5 genes) and tubers (all except *StTIR1a*). The stimulation in the above-ground organs was also noticeable, but less pronounced: the expression of *StTIR1a*, *StTIR1b*, *StTIR1c* in leaves, and of *StTIR1b* and *StAFB4* in stems was upregulated by cytokinin. Similar results were obtained at a lower concentration of sucrose (not shown). This indicated that cytokinins may enhance the auxin signaling by two major mechanisms: increasing the concentration of IAA as well as the sensitivity of cells to this hormone.

The content of non-target endogenous phytohormones in *tms1*-transformed potato plants

It is known that many plant hormones take part in the regulation of the tuberization process in potato (for recent reviews, see Aksenova et al. 2012, 2014). Therefore, the possibility remained that the effect of auxin in *tms1* transformants was mediated, either totally or at least partly, if not by cytokinins, then by some other hormones.

According to the literature data, abscisic acid (ABA) and jasmonic acid (JA) rather contribute to the formation of tubers, although they are not, most probably, the key factors in this process (Aksenova et al. 2012, 2014). In our study, we determined the content of ABA, JA and also salicylic acid (SA) in tubers of *tms1* transformants in comparison to non-transgenic potato plants (Table 1). Data on ABA showed no regular changes correlating with the intensity of *tms1* transgene expression and the ectopic increase in IAA level. It was not an increase but rather a decrease (by 15–16%, statistically non-significant) in ABA content that was observed in tubers of the clone A1-2, which was characterized by the strong transgene expression and enhanced tuberization (Kolachevskaya et al. 2015). The relatively high ABA content in tubers was found in plants of the A4-24 line featured by the least pronounced tms1-phenotype. On the whole, the magnitude of increase in ABA content in tubers of transformants (relative to control plants) was rather small and statistically non-significant, the maximal gain in lines strongly expressing *tms1* was equal to only 38% (clone A4-7). All these results argue against the systematic participation ABA in the *tms1*-induced enhancement of tuberization in transgenic potato lines.

The similar situation was evident for the content of JA and SA in the tubers: variations in the content of these plant hormones were rather small and oppositely directed, depending on the plant lines (Table 1). The absolute level of JA was low (36–77 pmol/g DW), by 15- to 70-fold and 200- to 1300-fold inferior to ABA and SA contents, respectively. The content of JA in transformants seemed to be slightly increased compared to control plants though for

Table 1 Contents of non-target phytohormones in tubers of B33-*tms1* transgenic and control potato lines culturing in vitro at DD on a medium containing 5% sucrose

Line	Phytohormone content (pmol/g dry weight)		
	Abscisic acid (ABA)	Jasmonic acid (JA)	Salicylic acid (SA)
A1-2	1411 ± 234	53 ± 39	46597 ± 8037
A4-7	2546 ± 991	43 ± 14	36923 ± 8950
A4-8	1933 ± 310	59 ± 2	17502 ± 1245
A4-24	2191 ± 333	77 ± 32	14909 ± 1441
Control	1759 ± 416	36 ± 5	30521 ± 3092

two lines (A1-2 and A4-7) this difference was statistically non-significant and the highest JA content occurred in line A4-24, with the weakest tms1-phenotype. As for SA, its levels were reduced in transgenic lines A4-8 and A4-24 but enhanced in lines A1-2 and A4-7 with pronounced tms1-phenotype, although this increase was small in amplitude and statistically non-significant.

Gibberellins (GAs) are known as negative regulators of potato tuber formation, so the effect of *tms1* expression on GA regulatory system was of particular interest. We have determined the content of biologically important forms of GAs in the lines A1-2 and A4-8 in comparison with the control line. In potato organs, we were able to identify two active gibberellins, GA_1 and GA_3, as well as GA_{20} which is a direct precursor of both active GAs (Hedden and Thomas 2012). Also GA_8, an inactive product of GA_1 catabolism, was found. All detected GAs were in low concentrations, without any marked differences between control and transgenic lines (Fig. 5). As a rule, the highest GA content was observed in leaves and the lowest in tubers. A tendency of enhancing the GA_3 content in stems and leaves of transgenic plants versus control ones was compromised by a large deviation range of GA content values. So, no concerted changes in GA content in different potato organs were revealed as a result of B33-*tms1* transgene expression.

To get deeper insight into the biochemical processes underlying GA metabolism in transgenic and control lines, we have analyzed the expression of genes encoding GA biosynthetic/catabolic enzymes in different organs of potato plants. The selected enzymes catalyze the final steps of GA biosynthesis and catabolism: GA20 oxidase (GA20ox) the conversion $GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow$ - GA_{20}; GA3 oxidase (GA3ox) the conversion of GA_{20} to active GA_1 and GA_3, while GA2 oxidase (GA2ox) catalyzes the oxidative inactivation of active GAs and their precursors (Hedden and Thomas 2012). Each enzymatic activity is encoded in potato by a small gene family (Lulai et al. 2016), so we have determined the expression level of three homologous genes belonging to each family, in three organs (leaves, stems and tubers) of the transgenic (A1-2, A4-8) and control lines. The results were essentially in agreement with the GA content data: the expression level of the overwhelming majority of genes was negligible if any (Fig. 6a). The average expression level of GA metabolic genes corresponded to approx. 5 arbitrary units which was inferior to most auxin receptor gene expression by approx. 10- to 50-fold. Some genes were not expressed in all or most tested organs, namely *GA3ox2*, *GA2ox3*, *GA3ox3*, *GA20ox4*. Only rare concerted differences in gene expression between transgenic and control lines (*GA20ox2* and *GA2ox3* in stems; *GA3ox1* in tubers) were observed (at a very low level). Among them the rise in expression of *GA20ox2* and *GA2ox3* in the same organ (stem) will eventually neutralize each other.

To test whether the selected genes for GA biosynthesis/catabolism are regulated by auxin, we have undertaken a study of gene expression in plants treated with exogenous IAA. This experiment confirmed that the background level of GA metabolic gene expression was very low, not exceeding 5–10 arbitrary units (Fig. 6b). However, exogenous auxin administration upregulated the expression of some genes, namely *GA3ox1*, *GA2ox1* (in most organs) and *GA20ox2* (in leaves and stems). These data proved that GA biosynthesis is indeed controlled by auxin raising the active GA content, especially in leaves where no auxin-dependent increase in the expression of catabolic *GA2ox* genes was observed.

Thus, the analysis of the hormonal status of *tms1* transformants revealed some concerted changes (auxin, cytokinins) typical for the majority of transgenic lines displaying a distinct tms1-phenotype, as well as occasional changes (JA, SA) in individual lines. The systematic changes which were reproducible in independent lines of transformants were revealed, particularly a sharp increase in the IAA content in tubers and an approx. twofold reduction of active cytokinins and cytokinin ribosides in tubers and in (most of) shoots. It is obvious that the observed concerted changes in cytokinins are consequences of the increase in the auxin level in transformants.

Peculiar features of individual lines might serve differences in tZR concentration ratios between shoots and tubers, for example, in the line A1-2 this ratio was equal to 6.5 whereas in the line A4-8 to only 0.4. The lines also vary in the parameters such as SA concentrations, ratios between contents of different phytohormones, hormone distribution between organs, etc. Non-concerted

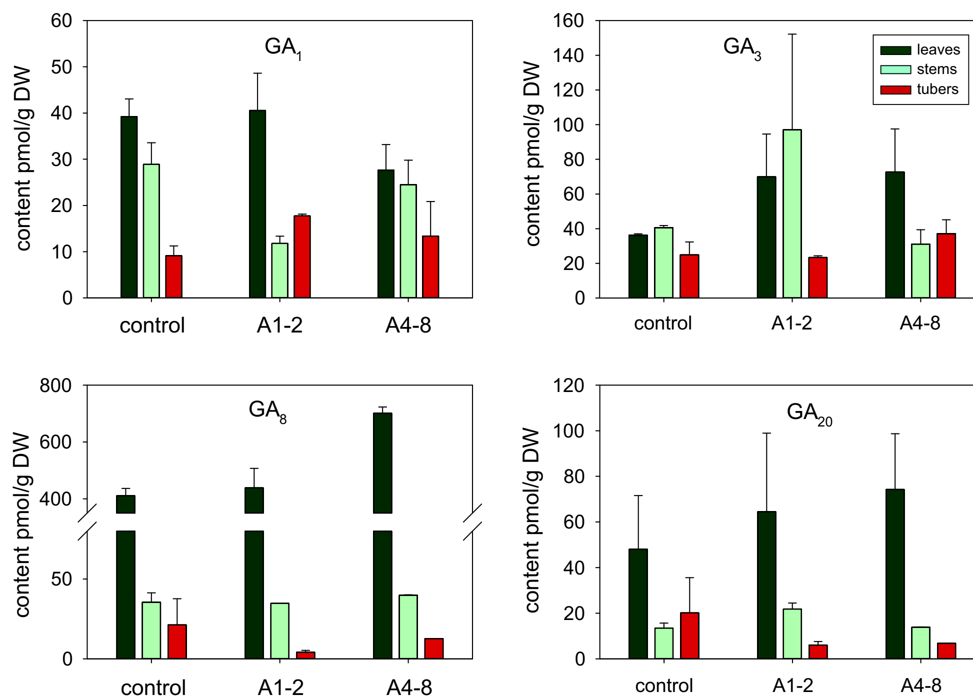

Fig. 5 The content of detected gibberellins in different organs (leaves, stems, tubers) of 4-week-old B33-*tms1* transgenic and control potato plants grown on medium with 5% sucrose

(occasional) changes in hormonal status detected in the independent transformed lines evidently represent individual properties of these lines (eventually depending on the transgene insertion site), and may be responsible for their tuberization peculiarities.

Discussion

The disclosure of the regulatory mechanisms that govern the development of plants one way or another is one of the key challenges in plant biology. This is crucial not only for basic research, but also with regard to food crops of great practical importance. The modern approaches provide an opportunity to better understand the details of the intimate processes that occur in plants, such as biosynthesis, signaling and interaction of plant hormones. These approaches include the use of GM plants harboring transgenes for phytohormone synthesis or catabolism. Among the generated transformants, those acquiring traits of improved productivity can serve as a source for developing new crop varieties.

It is known that the potato tuberization is a complex multi-step process, which is controlled by both external and internal factors (Chailakhyan 1984; Ewing 1995). The most important among the internal factors are hormones and carbohydrates (Sergeeva et al. 2000; Prat 2004; Sarkar 2008; Aksenova et al. 2012, 2014). In the last decades, the intricate hormone-carbohydrate regulation of tuberization was studied in our laboratory, using as a test system natural and transgenic forms of potatoes cultivated in vitro on media supplemented with plant hormones (auxin, cytokinin) and carbohydrates (sucrose). In this way, both hormones were shown to stimulate tuberization, auxin mainly by increasing tuber mass while cytokinin by tuber number (Aksenova et al. 2000; Romanov et al. 2000). Phytohormone effects were largely dependent on the sucrose content in the medium, in particular, hormonal effects were more pronounced at low (threshold) sucrose concentration. The transformation of potato plants with B33-driven *rolB*-transgene exerting auxin-like effects led to accelerated tuberization and increase in tuber weight, but on the other hand, to weakening of positive effect of exogenous phytohormones on the tuber number at elevated (5–8%) sucrose content in the medium (Aksenova et al. 1999, 2000; Romanov et al. 2000). This finding served as a prerequisite for the design and subsequent generation of transgenic potato expressing auxin synthesis gene under control of the tuber-specific patatin promoter (B33-*tms1* construct) (Kolachevskaya et al. 2015).

Plants with the inserted auxin biosynthesis gene *tms1* demonstrated the same trend as the plants harboring the B33-*rolB* construct did earlier. These *tms1* transformants were featured by accelerated tuberization and increased productivity. Adding IAA or kinetin to the culture medium affected tuberization in *tms1*-transformed and control

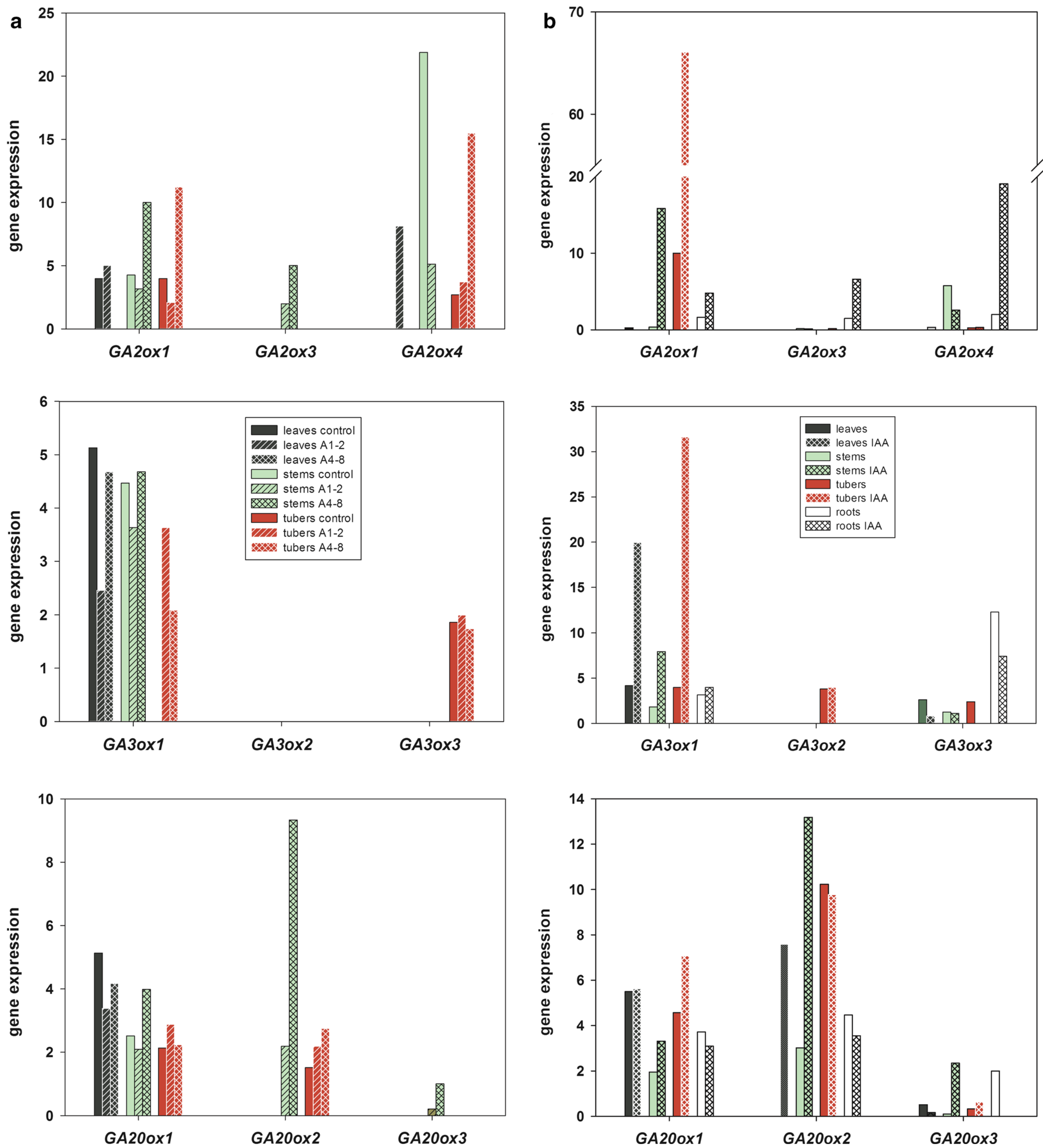

Fig. 6 Relative expression of gibberellin oxidase (*StGAox*) genes. *StGA2ox1*, *3* and *4* genes encode proteins for conversion of GA_{20} (precursor) to GA_{29} and GA_1 (bioactive) to GA_8 (inactive); *StGA20ox1*, *2* and *4* genes encode proteins that sequentially convert GA_{53} to GA_{20}; *StGA3ox1*, *2* and *3* genes encode proteins for conversion of GA_{20} to GA_1 (bioactive) and GA_3 (bioactive). **a** Transgenic and control potato lines. **b** Control line treated with auxin (1 μM IAA) for 1 h. Potato organs were sampled at due time, their RNA was extracted, treated by reverse transcriptase and analyzed via qRT-PCR using potato elongation factor 1-α (ef1α) as the normalizing gene. *Hatched* and *non-hatched bars* correspond to auxin-treated and mock-treated plants, respectively

plants differently, and this effect clearly depended on sucrose content in the medium. Hormones markedly stimulated tuberization in transformants only at low (3%) sucrose concentration in the medium. At the higher (5–8%) sucrose content, the phytohormone effects became first weaker and then even reversed. By contrast, in control

(non-transgenic) plants the stimulation was observed in all experimental settings. The promoting effect of exogenous auxin on tuberization in non-transgenic potato resembled the effect of transgene expression in B33-*tms1* transformants grown on hormone-free medium (see Fig. 1, columns for 3 and 5% sucrose). This was evidence that both exogenous and endogenous auxins acted similarly on the tuber formation in potato.

Earlier, a study was performed on transgenic potatoes expressing *Agrobacterium*-derived *ipt* gene for cytokinin synthesis. The independent lines of *ipt*-transformants had elevated by 1.5- to 4-fold levels of endogenous cytokinins coupled with 1.3- to 2.6-fold increase in IAA content. Among these lines those keeping the cytokinin-to-auxin ratio close to natural one, attained the highest productivity (Macháčková et al. 1997). This pointed at a close relationship (crosstalk) between the content of different plant hormones, which may account for the observed morphogenetic alterations. The present results are in accordance with the foregoing studies. Potato plants expressing the gene for auxin synthesis *tms1* showed, in addition to a local increase in the IAA level, reproducible changes in the cytokinin level too. Unlike cytokinins, the excess of which led to an increased auxin content (Macháčková et al. 1997), IAA excess, on the contrary, led to a decrease in the concentration of active cytokinins and their precursors *N*9-ribosides (the transport forms). Similar trend was observed in both tubers and shoots. The negative effect of auxin on cytokinin content had been observed long ago for a number of model plants (tobacco, tomato, pea, bean) as well as for calluses/cells in culture (Kamínek et al. 1997). A decrease in cytokinin activity in Arabidopsis upon the auxin treatment was also reported (Nordström et al. 2004). As for potatoes, our data on the effect of auxin overproduction on cytokinin and other hormone content, to our knowledge, were gained for the first time. The main reasons for auxin-dependent decrease in the level of cytokinins may be their suppressed biosynthesis and/or enhanced catabolism. Enzymes responsible for cytokinin biosynthesis are IPT; studies on Arabidopsis demonstrated the plurality of *IPT* genes and their organ-specific expression in plants (Kakimoto 2001; Takei et al. 2001; Miyawaki et al. 2004). According to numerous data, in Arabidopsis auxin increased the expression of genes *AtIPT5* and *7* in roots without affecting the *AtIPT1,2,3,9* genes (Miyawaki et al. 2004); reduced expression of *AtIPT3* in the stem, without affecting *AtIPT7* but seemingly enhancing the expression of *AtIPT1* and *5* (Müller et al. 2015); in the whole 7-d-old seedlings reduced the expression of *AtIPT1,3,4,6,7*, while increasing the expression of *AtIPT5* (Di et al. 2016). In other plant species, the potential of auxin to regulate *IPT* gene expression was also shown. In Chinese cabbage (*Brassica rapa* L.), transcripts of *BrIPT3* and *7* in root were reduced by NAA incubation (Ando et al. 2005). In pea nodal stem, auxin reduced the cytokinin content and downregulated two *IPT* genes (*PsIPT1* and *PsIPT2*) (Tanaka et al. 2006). In the rice shoot apex, the expression of *OsIPT2* and *OsIPT4* was clearly, and of *OsIPT7* and *OsIPT8* tentatively inhibited by auxin (Minakuchi et al. 2010). In chrysanthemum (*Dendranthema grandiflorum*), auxin downregulated the biosynthesis of cytokinin in nodes by repressing the activity of *DgIPT3* (Chen et al. 2013). Thus, there is no doubt that auxin is able to influence the cytokinin content by regulating the activity of its biosynthesis genes. Regarding other possible ways of auxin action, namely its impact on the activity of cytokinin-degradation enzymes, much less data are still available. Nevertheless, it was reported on the possibility of activation by auxin of cytokinin oxidase/dehydrogenase (*CKX*) genes in Arabidopsis (Werner et al. 2006) and pea (Shimizu-Sato et al. 2009).

Gibberellins are well-known participants of the hormonal crosstalk regulating tuberization (Xu et al. 1998; Aksenova et al. 2012, 2014); they inhibit tuberization primarily at its early stages (induction, initiation). In the Arabidopsis model, auxin was reported to increase the expression of *GA20oxI* and *GA3oxI* genes, necessary for the synthesis of active GAs, and inhibited the expression of *GA2oxI* gene that causes the GA inactivation (Thomas and Hedden 2006). By contrast, cytokinins were suggested to affect GA biosynthesis in the opposite way, namely by suppressing the expression of *GA20oxI* and *GA3oxI* genes (Brenner et al. 2005). Thus, changes in hormonal status of auxin and cytokinins in *tms1* transformants were theoretically both favorable for the increase in the level of active GAs. In practice, we have failed to find any significant difference in GA content between transgenic and control potato lines. The relatively high level of GA_8 in leaves is indicative of the extensive metabolization of GA_1 therein. In addition, no marked difference between lines was observed in the expression of genes for GA synthesis/catabolism, though treatment of potato plants with exogenous IAA activated some of these genes. The low GA content was consistent with the low expression level of GA synthesis genes. All this means that either the local and moderate increase in IAA content in B33-*tms1* transformants was not sufficient to enhance GA biosynthesis or plant became adapted to such increase after some period and did not react to it any longer. The first explanation is in accordance with the data on potato wounding (Lulai et al. 2016): a moderate increase in auxin concentration in tubers did not influence the GA content therein. As for other non-target phytohormones, no significant concerted changes in the content of ABA, JA and SA in tubers of transformants were recorded in our experiments. Occasional changes in non-target hormone contents may account for some peculiarities of growth and morphogenesis of individual transgenic lines.

Considering the detected changes (or their absence) in non-target hormone contents, we can conclude that none of these hormones mediates the stimulatory effect of auxin on tuberization, so auxin positive effect is due to auxin itself. However, the responsive non-target hormones may account for the counteraction of auxin effects, observed in B33-*tms1* transgenic plants culturing at elevated sucrose contents in the presence of exogenous hormones. The simplified model for the hormone–carbohydrate network which regulates potato tuberization is shown in Fig. 7. The tuberization proceeds through three stages (induction–initiation–growth) which are regulated positively (by auxin and cytokinin) and negatively (by gibberellin) (Aksenova et al. 2012, 2014). The hormonal crosstalk in potato appears to be organized in a way that excessive auxin provokes the drop in cytokinin level and (at high auxin concentration) also GA accumulation. Both changes lead to the inhibition of tuber induction and initiation. The adverse hyperaccumulation of endogenous auxin should primarily occur in B33-*tms1* transgenic plants culturing on media with high sucrose which is the inducer of B33-promoter activity (Rocha-Sosa et al. 1989; Wenzler et al. 1989; Deryabin et al. 2003; Naumkina et al. 2007). This model can explain the difference between transgenic and control plants in their response to auxin treatment. So, due to the specific hormonal crosstalk the non-target hormones can counteract the excessive auxin-induced tuberization by a feedback-like mechanism.

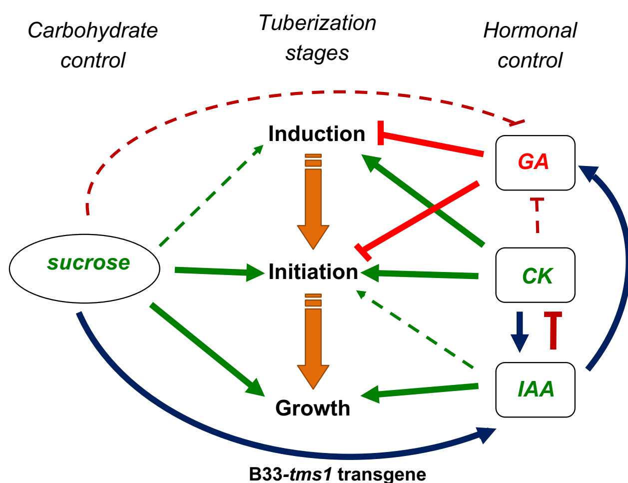

Fig. 7 A schema for multi-hormonal control of potato tuber formation. Tuber induction and initiation are promoted by cytokinins (CK) but inhibited by gibberellins (GA). Tuber growth and eventually initiation are promoted by auxin (IAA). CK upregulate IAA signaling but downregulate GA signaling. IAA acts positively on GA level but negatively on CK level. Sucrose stimulates tuber initiation and growth and eventually also tuber induction by repressing GA action. In B33-*tms1* transgenic plants, sucrose activates B33 promoter leading to endogenous auxin overproduction. *Arrows* indicate positive regulation, and T-bars negative regulation

These data suggest that the altered response of transformants to exogenous kinetin may result from the positive influence of cytokinins on the auxin signaling (Fig. 7). This explanation is largely supported by the literature as well as our data on cytokinin upregulation of potato genes coding for auxin receptor orthologs. Cytokinins are likely to act in two ways: by increasing the level of endogenous IAA and by increasing the cell sensitivity to auxin. As a result, the excessive auxin signaling, caused by cytokinin and high sucrose in the medium, activates the foregoing feedback-like mechanism which changes the sign of the cytokinin effect on B33-*tms1* transgenic plants from positive to negative. So, here just auxin can serve as mediator of the cytokinin-negative regulation of tuberization in transformed plants. Thus, even moderate and quite local change in the concentration of a single hormone (auxin) leads to a significant remodeling of the hormonal complex in the plant, and this fact should be taken into account in the research and breeding practice.

The hormonal regulation system comprises two equally important moieties, namely hormones per se as well as their perception apparatus. The receptor proteins play a key role in the hormone perception. In the present study, we have identified a set of potato genes orthologous to auxin receptor genes from other species. Among these potato genes, three genes are close orthologs of the Arabidopsis *TIR1* gene branch while two other genes are closer to *AFB4* and *AFB6* branches. Each gene was shown to be expressed in main potato organs: leaf, stem, root and tuber, the expression degree depending on the gene and the organ tested. The highest expression occurred in leaves and stems while the least in roots. Interestingly, the expression of putative auxin receptor genes was very sensitive to cytokinin treatment. In most cases, the expression was increased upon cytokinin administration, and the strongest response was detected in roots followed by tubers. Among the genes of putative auxin receptors, the gene *StTIR1b* proved to be the most sensitive to cytokinin; in some aspects it behaved like a gene of primary response to cytokinin. These data suggest a new link between cytokinin and auxin signaling systems in potato, on the level of auxin perception apparatus. This link suggests that cytokinins might promote tuberization, at least partly, by enhancing auxin sensitivity of potato cells. Also this emerged link opens new perspectives for the future studies on hormonal regulation of potato ontogeny.

Author contribution statement GAR and OOK conceived the design of the study. VVA, EBR and YIB produced a vector for potato transformation. OOK, LIS, KF and IAG performed the experimental work, and SNL did the bioinformatics work. GAR supervised the research. GAR and OOK wrote the draft of the manuscript and GAR

is responsible for its final form. All authors read, commented and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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