Plant Signaling & Behavior 7:10, 1360-1363; October 2012; © 2012 Landes Bioscience

A crosstalk of auxin and GA during tuber development

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Several hormones have been studied for their effect on tuber initiation and development. Until recently, the hormone with the most prominent role in tuber initiation was attributed to GA. Genes involved in GA degradation do exhibit an upregulated profile during early stages of tuber development, leading to a rapid decrease of active GA content, thereby facilitating stolon-tip swelling. While GA is known to be involved in shoot and stolon elongation, the development of the new organ requires changes in meristem identity and the reorientation of the plane of cell division. In other developmental processes, such as embryo patterning, flower development and lateral root initiation auxin plays a key role. Recent evidence on the involvement of auxin in tuber formation was providedby the measurement of auxin content in swelling stolons. Auxin content in the stolon tips increased several fold prior to tuber swelling. In vitro tuberization experiments with auxin applications support the role of auxin during tuber initiation. Taken together, it is becoming clear that the initiation and induction of tubers in potato is a developmental process that appears to be regulated by a crosstalk between GA and auxin.

Keywords: potato, tuberization, auxin, gibberellic acid

Submitted: 07/03/12

Accepted: 07/17/12

http://dx.doi.org/10.4161/psb.21515

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Addendum to: Roumeliotis E, Kloosterman B, Oortwijn M, Kohlen W, Bouwmeester HJ, Visser RG, Bachem CW. The effects of auxin and strigolactones on tuber initiation and stolon architecture in potato. J Exp Bot. 2012 Jul;63(12):4539–47; PMID: 22689826; DOI: 10.1093/jxb/ers132

Several plant hormones have been studied for their effect on tuber initiation. The class of plant hormones that has been studied most extensively is the group of Gibberellic Acids (GAs). More than 120 GAs have been identified in plants, but only GA_1 and GA_4 are biologically active. GAs have an inhibitory role on tuber induction.[1-3] Analysis of the endogenous GA levels show that after tuber induction, content of the active GAs in the swelling stolon tips is depleted,[4,5] while application of active GAs inhibits initiation of tuberization.[4,6] Several GA biosynthesis genes have been identified in potato and their role in tuber initiation has been studied. The expression of *StGA2ox1*, which is involved in GA degradation, is induced prior to stolon swelling.[7] In agreement with these results, StGA20ox1 (active GAs biosynthesis) overexpression or antisense potato plants delayed or advanced the time point of tuberization, respectively.[8] In addition, antisense transgenic plants for the StGA2ox1 gene had increased levels of GA_{20} that is an inactive form of GA, reduced stolon growth and earlier in vitro tuberization. In contrast, overexpression of StGA2ox1 delays in vitro tuberization and alters tuber morphology.[7] StGA3ox2 has been used in constructs with leaf, tuber specific or constitutive overexpression with the CaMV 35S promoter. The leaf specific expression of *StGA3ox2* and the 35S overexpression resulted in earlier tuberization, in contrast to the tuber specific expression that had slightly delayed tuberization.[9] The different phenotypes observed when the *StGA3ox2* gene expression is driven by the leaf or the tuber specific promoter can be explained by different transport of the various GAs. The main transporter in pea was shown to be GA_{20},[10] a precursor of GA_1. Therefore, overexpression of *StGA3ox2* in the leaves would convert GA_{20} to GA_1 in the leaves more vigorously, resulting in lower GA_{20} being transported to the stolon tips. As a consequence, reduced GA_{20} availability in the stolon tips will result in reduced GA_1 content that can lead to earlier tuberization.

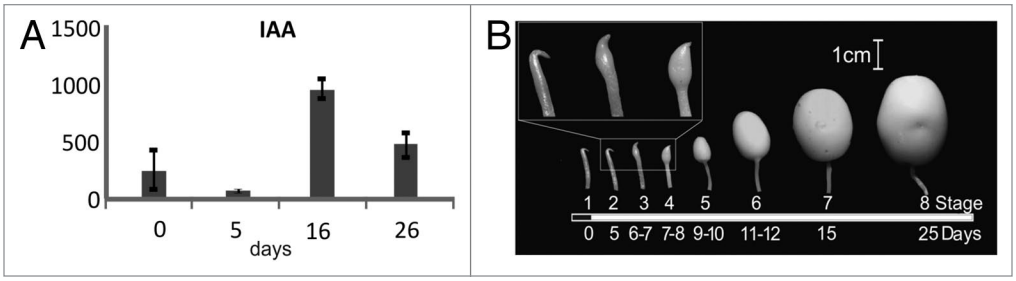

Figure 1. (A) Concentration of free IAA the stolon rip. Plants were grown for 9 weeks under non-inductive conditions, before switch to inductive short day conditions. IAA and OxIAA concentrations are in pmol per gram of fresh weight. Samples were harvested under long day conditions (LD day 0) just before switching to inductive short days and after 5, 8 and 26 d in short day conditions (SD day 5, 8 and 26 respectively). Error bars represent standard error of the mean of two replicated measurements. (B) Representation of stages of tuberization before and after transition to inductive conditions (black section of the bar represents SD and white section represents LD). The days of growth are shown below. The inset in the top left, is a 3 × magnification of stages 2–4.

In addition to GAs, several other plant hormones have been studied for their effect on tuber initiation. Exogenous application of cytokinin (zeatinriboside) to an in vitro tuberization system, resulted in increased tuber formation.[11] In addition, plants overexpressing a cytokinin biosynthesis gene (*isopentenyltransferase* gene-*ipt*) yielded more tubers with reduced tuber weight and nitrogen content,[12] suggesting that cytokinins may act more on promoting stolon branching than on tuber induction. Abscisic acid (ABA) applications on in vitro tuberization systems have produced contradictary results. Koda and Okazawa[6] reported that application of ABA in an in vitro tuberization system with 2% sucrose resulted in slight swellings in the sub apical region that did not develop into proper tubers. In contrast, Xu et al.[4] reported that ABA application resulted in higher frequencies of tubers only in 1% sucrose, but estimation of the ABA-like substances in tuberizing explants were not different compared with non tuberizing explants.

Other hormones such as jasmonic acid (JA) have also been implicated in the initiation of tuberization. Application of JA in in vitro explants enhanced tuberization.[13] In addition, an important increase in JA content was noticed at tuber set, but in tubers no changes in the content of JA was noticed.[14] Nevertheless, JA application experiments showed promotion or inhibition of tuberziation, depending on the concentrations applied.[2]

Auxin plays a very important role in almost all developmental events in a plant that require changes in meristem identity (reviewed in reference 15). Cytological studies in the stolon tip revealed that upon tuber induction the apical meristem ceases cell division and the plane of cell division in the sub-apical medullary region changes from a lateral to a longitudinal thereby terminating stolon elongation and resulting in swelling.[16]

Recently, Dhonukshe et al.[17] showed that the re-orientation of the plane of cell division of Arabidopsis stem cells is auxin dependent, demonstrating a possible auxin mediated mechanism that regulates changes in the orientation of cell division in plants. Therefore, changes in the orientation of cell division that results in swelling of the stolon tip are compatible with a role for auxin in tuber development.

Early experiments with applications of auxin in in vitro tuberization systems did not provide a conclusive link between auxin and tuber development.[4,5] The advent of the genomic era in biology provided new tools to study tuber initiation and development though the study of gene expression and function. The differential expression of an auxin response factor gene showing a peak in expression just after tuber initiation provided a first indication that auxin plays a role in tuber initiation.[18] A more comprehensive expression study using a microarray approach provided a much more detailed picture of the transcriptome wide regulation of genes during tuber initiation. This microarray experiment revealed that a large number of auxin related genes had a differential expression profile during early events in tuber development.[19] Examples of such genes are two *PIN*-like

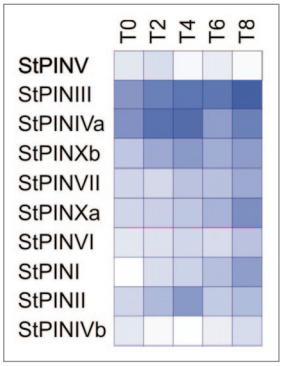

Figure 2. Heat map of expression of the StPINs in stages T0 to T8 of the developmental series, 0 to 8 d after induction to tuberize, shades of blue represent fold increase in the expression of the corresponding gene and white indicates the lowest expression detected. Lowest expression is detected for *StPINVb* at stage T4 [C(t) = 36.29], and highest expression is detected for *StPINIII* at stage T8 [C(t) = 25.14].

genes, an *adr11-2* (auxin downregulated) and an *acrA-like* (auxin regulated gene containing a GTP-binding site) genes. In Arabidopsis, transcript levels of *adr* genes were shown to be downregulated in presence of auxin,[20] while in tobacco, *acrA* expression levels are upregulated after auxin application.[21] In potato, transcript levels of the *StPIN-like* and the *acrA-like* gene exhibited a peak in expression after tuber initiation, while the *adr-like* gene was downregulated. These expression profiles indicate that auxin levels are likely to increase during early stages of tuber development. Scoring of auxin content in the stolon (**Fig. 1**) reveal that after tuber initiation, auxin content indeed increases

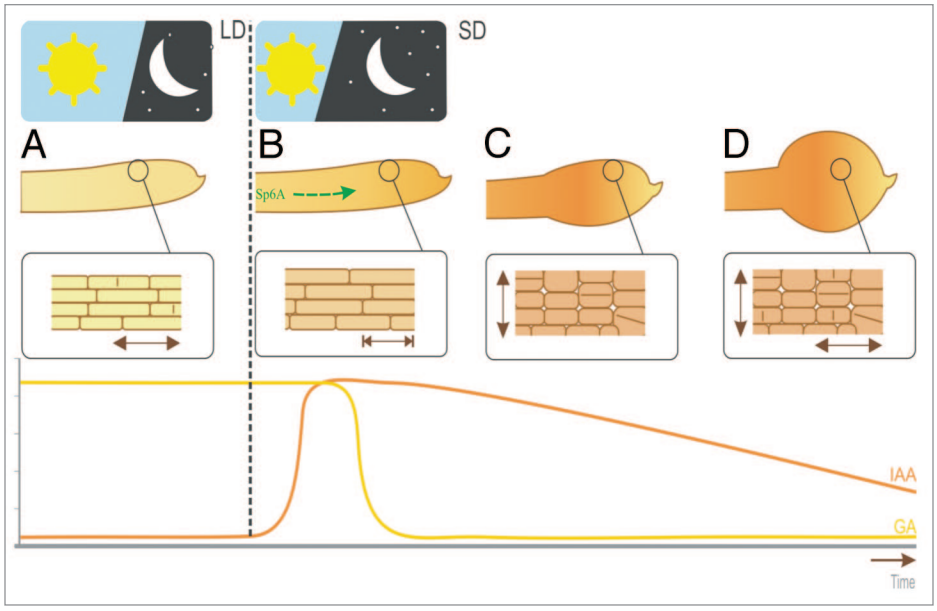

Figure 3. Changes in the GA and IAA content in the stolon tip. GA content is initially high and IAA content is low under non-inductive long day conditions. The stolon is elongating longitudinally (A). The black dotted line represents switch to tuber inductive long days. StSP6A transcript (green arrow) is the mobile signal that reaches the stolon tip. Elongation of the stolon is terminated (B), and GA content drops while IAA content is increased. Tuber swelling is achieved initially by cell transversal division (C) and finally by random cell divisions (D).

in the stolon tip and in the region just proximal to the site of swelling.

Furthermore, the analysis of auxin biosynthesis genes, as well as of *PIN* family of genes that are involved in auxin transport, verified that the at least one auxin biosynthesis gene, named *StYUC-like1,* is upregulated during early stages of tuber development. In addition, auxin transport assays revealed that IAA is directionally transported from the distal stolon apical meristem (STAM) to the proximal region of the stolon, strengthening the hypothesis that the stolon tip is a possible site of auxin biosynthesis.[22] Expression studies of the *StPIN* genes in potato in early stages if tuber initiation as described by Kloosterman et al.[19] revealed that several genes exhibit an peak in expression profile after tuber induction (**Fig. 2**). These results, combined with the known role for auxin in the development of other meristems, provides strong evidence for a role of auxin and auxin distribution in tuberization and more specifically in the events that take place after tuber initiation.

Our findings on auxin content together with the known role for GAs in tuber initiation and development, allows us to describe the physiological model for the combined action of GAs and auxin during early stages of tuberization. GAs and auxin seem to be involved in tuber development in two consecutive stages (**Fig. 3**). At the stolon growth phase, GA content is relatively high and is mediating stolon elongation. The plain of cell division remains transversal during this phase. Auxin content is relatively low and the role of auxin is to maintain stolon apical dominance. Short day conditions induce tuber initiation, and the mobile signal StSP6A is produced and transported from the aerial parts of the plant to the stolon tips.[23] When the StSP6A protein reaches the stolon tip, tuber formation is induced. Concomitantly, GA levels are rapidly reduced and auxin content peaks. This occurs together with termination of longitudinal stolon growth, change of the plane of cell division and swelling of the stolon to form the tuber. GA content is a very important switch in this developmental event. When GA degradation is hindered, a delay in the tuber initiation can occur, while greater GA degradation produces earlier tuberization.[7]

At the tuber swelling stage, GA content has been degraded, while IAA content remains high and slowly decreases over time, correlated with a peak in the expression of a *StYUC* gene. It is during this stage, that changes in the orientation of cell division in the tuber take place initiating tuber growth. In vitro experiments with auxin application also point out the importance of auxin in tuber initiation.[22] If auxin levels remain high by continuous application of auxin to the stolon tip, tuber formation is inhibited. On the other hand, a single auxin pulse in vitro stimulates tuberization in comparison to the controls.[22] Therefore, the auxin peak that was found in vivo, is an important factor for a stolon to start swelling. The recent finding that the transcription factor KANADI interacts with FT in rice,[24] provides a possible mechanism for the dove-tailing of the light-regulation pathway with the auxin control of cell division in plants. Further research will show how this link may function in the potato system.

Based on these results, we conclude that auxin has an important role in tuber development, possibly by regulating tuber growth through mediating meristem identity, cell division and the plane of cell division.

Acknowledgments

E.R. gratefully acknowledges financial support from the Bakalas Foundation, and the Stichting Veenhuizen-Tulpfonds.

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