Potato Research 42 (1999) 471 – 481

Gibberellins and tuberization in potato

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Additional keywords: *Solanum tuberosum*, *S. andigena*, *S. demissum*, hormones, regulation, starch accumulation, tuber formation

Summary

The evidence for a role of gibberellins in the regulation of potato tuber formation is reviewed. Endogenous gibberellin levels in plants are high under non-inducing conditions and decrease under inducing conditions. Exogenously applied gibberellins inhibit tuber formation, whereas applying inhibitors of gibberellin biosynthesis has the opposite effect. Cellular events involved in tuberization, viz., cell division, cell enlargement and orientation of micro-tubules, are also reviewed. Based on available evidence, a major regulatory role of gibberellins is suggested. However, it is also argued that tuber formation is not simply regulated by gibberellins acting as the sole signal between above-ground and below-ground parts, since stolon tips are able to synthesize their own gibberellins, and the phenotype of phytochrome B-antisense plants cannot be explained only by altered levels of GAs.

Introduction

The formation of vegetative storage organs in plants, viz. tubers and bulbs, is often influenced by environmental conditions, the most prominent factor being daylength. Since we can envisage the perception of daylength to occur only in the above-ground parts of the plants, some kind of signalling between above-ground and below-ground parts should occur. Hormones have often been suggested to play such a role in the regulation of tuber and bulb formation. Overviews of the possible role of various hormones are given in several recent reviews (Vreugdenhil & Struik, 1989; Ewing & Struik, 1992; Ewing, 1995). All classes of hormones were found to have some effect on one or more of the various aspects of plant development, eventually leading to the formation of tubers. One of the most likely candidates to play a major role is gibberellin (Xu et al., 1998a; Jackson, 1999).

The aim of this review is to summarize available data on the role of gibberellins (GAs) in tuber formation and especially with potatoes. We will describe data on endogenous GAs, effects of exogenously applied GAs and cellular events involved in tuber formation and concomitant starch accumulation that are likely to be regulated by GAs. Finally, we will investigate whether GAs act as the sole signal between above-ground and below-ground plant parts with regard to tuber formation.

Endogenous GAs

If gibberellins are important regulators, their endogenous level is expected to be influenced by environmental conditions affecting tuber formation. Most data obtained

so far are from experiments in which temperature and/or daylength were varied, and the levels of GAs were subsequently analyzed using paper chromatography and bioassays. Only recently, more reliable physico-chemical methods such as HPLC and GC-MS have been used to assess gibberellins in relation to tuber formation.

Bioassays. Most reports describe levels of gibberellin-like substances in leaves or whole shoots of plants grown under either long-day (LD) or short-day (SD) conditions. The activities of GA-like substances were reported to be high under LD conditions, and to decrease under inducing conditions (Pont Lezica, 1970; Railton & Wareing, 1973; Kumar & Wareing, 1974). Machackova et al. (1998) determined GA levels in *S. tuberosum* ssp. *andigena* grown under LD, SD, or SD with a brief interruption of the dark period. Plants grown under the latter conditions were in many aspects intermediate between LD- and SD-grown plants. LD-plants did not tuberize, and the GA level in these plants was higher in leaves, stems and combined roots and stolons, as compared with SD plants which formed tubers. Preventing tuberization by night break resulted in higher levels of GA except in roots+stolons.

Less information is available on GA levels in stolon tips. Only Okazawa (1967) and Koda & Okazawa (1983b) analyzed various stages of elongating and swelling stolons. In elongating stolons, the level of GA-like substances was high, and this level decreased when stolons began to swell. Because of the limited number of data, it is impossible to conclude whether changes in GA levels in stolon tips precede tuber formation, or are a result of this process.

Krauss (1981) determined the level of GAs in relation to the growth rate of individual tubers. However, no correlation between these two parameters was found, suggesting that GAs do not regulate tuber growth. The physiological disorder 'little potato', in which physiologically old seed tubers directly form new tubers, was associated with differences in GA levels between old and new tubers. The new 'little potatoes' have higher GA levels than the seed tuber on which they grow (Wurr et al., 1980).

New methods. Jones et al. (1988) were the first to analyze gibberellins in potato sprouts by GC/MS. Both GA_1 and GA_{20} were detected but no analysis was done on GA levels in relation to tuber induction.

Van den Berg et al. (1995a,b) used a dwarf *S. andigena* line to analyze GA metabolism, and the possible role of daylength in regulating tuber formation via GA biosynthesis. When ^{14}C-GA_{12}-aldehyde or ^{14}C-GA_{12} were applied to isolated shoot tips, a series of GAs was detected after a 6 or 24 h incubation period. GAs were identified based on co-elution with authentic standards, viz., GA_{53}, GA_{44}, GA_{19}, GA_{20}, GA_1, GA_8. This series of GAs indicates the presence of the early-13-hydroxylation pathway in potato shoots. The dwarf plants, which tuberize under LD conditions, contained much less GA_1 than wildtype plants grown under similar non-inducing conditions. These dwarfs appeared to be blocked early in the GA biosynthesis pathway, viz., in a step prior to GA_{12}. When non-dwarf *S. andigena* plants were grown under SD conditions, the level of GA_1 was almost three times

lower than under LD conditions (van den Berg et al., 1995b).

We have analyzed GAs in another strictly daylength dependent potato species, *S. demissum*. Based on coelution with standards, GA_1, GA_{20}, GA_4 and GA_9 were detected in mature leaves. Table 1 shows preliminary data of GA levels under inducing and non-inducing conditions. A major decrease is observed in GA_1 under SD conditions. Changes in the other GAs, if any, were much smaller.

Table 1. Levels of gibberellins in leaves of *Solanum demissum*, grown under short day (SD, 8 h) or long day (LD, 16 h) photoperiod. Samples were taken from mature leaves after 6-14 days at either LD or SD conditions, and analyzed as described in Xu et al. (1998).

	Gibberellin level (ng g^{-1} fresh weight)	
	Long days	Short days
GA_1	147	27
GA_{20}	53	33
GA_4	13	15
GA_9	15	22

The above-mentioned data were obtained on leaves or shoots, and the GAs detected here might play a role in signal transduction from shoot to stolon tip. Xu et al. (1998a) analyzed GAs in tuberizing and control stolons using single-node cuttings growing in vitro. Tuber formation was manipulated via sucrose levels of the medium, with high levels being inductive. In stolons and young tubers the same types of GAs were found as described for leaves. Tuber induction by increased levels of sucrose resulted in a marked decrease in gibberellin levels, most noticeably in GA_1. This decrease was observed before visible swelling occurred. Furthermore, the levels of GA_1 in different parts of elongating and swelling stolons were determined. The tip of elongating stolons had the highest GA_1 level, up to ten times higher than in the more basal part of the stolon. Tips of stolons just before visible swelling had much lower GA_1 levels, and these levels dropped further in young tubers.

Exogenously applied GAs

In this section we will describe effects of exogenously applied GAs only on morphological events, i.e. stolon growth and swelling of stolons, since the cellular aspects will be dealt with in a separate section.

Whole plants and cuttings. The effects of exogenously applied GAs on the growth and tuberization of whole potato plants have been studied since 1950s. As compared with other phytohormones (i.e. cytokinins or IAA) whose application had contradictory effects, treatment of whole potato plants or cuttings by GAs gave unambiguous results.

GA treatment promoted shoot growth in whole plants or leaf-bud cuttings (Menzel, 1980). Flower buds, that usually abort under SD conditions, developed

normally and formed fruits after 6-day applications of GA (spraying on the foliage) at the beginning of the inductive period (Markarov, 1990).

The importance of GAs in stolon initiation and the maintenance of diageotropic growth was demonstrated in experiments where application of gibberellins promoted stolon formation and elongation (Smith & Rappaport, 1969; Kumar & Wareing, 1972; Woolley & Wareing, 1972). GA sprayed on the leaves resulted in long stolons under both SD and LD conditions (Hammes & Nel, 1975).

Application of GA to shoots had an inhibitory effect on tuber formation, i.e., tuber formation was prevented, inhibited or delayed (Lippert et al., 1958; Okazawa, 1960; Lovell & Booth, 1967; Menzel, 1980; Yanina et al., 1990). Tizio (1971) showed that different kinds of gibberellins (GAs 1, 3, 4, 5, 7, and 9) had a similar effect: they all prevented tuber formation on stem cuttings. However, under inductive (short day) conditions, applications of GA does not completely prevent tuber formation of whole plants, although the number and total mass of tubers per plant decreases by 30–50% (Hammes & Nel, 1975; Markarov, 1990).

GA_3 treatment of stem segments with leaves stimulated stem growth and resulted in a high level of endogenous, free GAs, and a low level of conjugated GAs. The glucose content in these stems and leaves was lower than in control plants (Simko, 1994). By contrast in tuber tissue, GA_3 treatment increased reducing sugar content and reduced sucrose content (Mares et al., 1981).

Jackson et al. (1996) showed that the antisense phytochrome B transformants form tubers in LD, implying that PhyB is involved in a regulatory pathway that prevents tuberization in non-inductive photoperiods. Gibberellins have also been implicated in the inhibition of tuberization of wild-type plants in long days. In an attempt to understand the relationship between phytochrome B and gibberellins, Jackson & Prat (1996) investigated the effect of applications of GA and an inhibitor of gibberellin biosynthesis, ancymidol, on wild-type and phytochrome B-antisense (*Solanum tuberosum* ssp. *andigena*) plants. Stem elongation, chlorophyll levels and the tuberization response were analysed. Phytochrome B-deficient plants showed signs of increased gibberellin levels or responsiveness, which may contribute to their elongated growth and reduced chlorophyll levels. The results showed that some phenotypes of the phytochrome B-antisense plants, i.e. increased stem length and reduced chlorophyll, can be mimicked by treating wild-type plants with GA. However, another phenotype, i.e. tuberization response in long days, is mimicked by application of GA biosynthesis inhibitor ancymidol to wild-type plants, thus appearing to be the result of a reduction in the gibberellin levels. Nevertheless, PhyB-deficient plants differ from ancymidol-treated wild-type plants in that they are not dwarfs. Thus, the authors believe that tuberization of the antisense plants in long days does not appear to be the result of a general reduction in gibberellin level. This apparent contradiction could be explained if different gibberellin species control stem elongation and tuberization in potato. Alternatively, tuberization may be controlled by a balance of positively and negatively acting hormones, where GAs would act as the negative effectors.

In vitro cultures. Growing potato tubers in vitro is of interest both for commercial production and for research purposes. Formation of tubers on in vitro plantlets is possible without the addition of hormones to the medium (Garner & Blake, 1989). In all studies reported so far, GAs inhibited tuber formation in vitro (e.g. Okazawa, 1967; Hussey & Stacey, 1984; Butenko, 1990; Xu et al., 1998a). Depending on their concentration, GAs completely inhibited, reduced or delayed tuberization in different in vitro systems: (1) GA_3 inhibited tuberization of intact plantlets grown in the light on media with high sucrose concentrations (Hussey & Stacey, 1984); (2) GA_3 decreased tuber formation in darkness on stem pieces or excised buds taken from plants that had been given photoperiodic induction (Harmey et al., 1966); (3) Application of GA_3 strongly inhibited tuberization of sub-cultured sprouts (Garcia-Torres & Gomez-campo, 1973) or stolons (Koda & Okazawa, 1983a) in darkness. The inhibiting effects could be partly overcome by abscisic acid (Koda & Okazawa, 1983a; Xu et al., 1998a). The latter authors also showed that with increasing concentration of GA in the medium, stolon elongation was stimulated and tuberization was progressively inhibited. Various types of growth regulators acting on GA-biosynthesis had a positive effect on numbers of tubers formed in vitro (Simko, 1993; Harvey et al., 1991; Levy et al., 1993; see also below).

GA-biosynthesis inhibitors

To assess the possible role of gibberellins in the regulation of tuber formation, growth regulators, known to inhibit the biosynthesis of GAs, have been used widely. Chlormequat (CCC or (2-chloroethyl)-trimethylammonium chloride) blocks GA synthesis early in the pathway, before ent-kaurene (Rademacher, 1999). This compound is able to counteract the inhibiting effects of high temperature on tuber formation in whole plants, the effect of CCC depending on plant age (Menzel, 1980, 1985). When non-induced LD plants were pre-treated with chlormequat, cuttings taken from the plants formed tubers, in contrast to cuttings taken from control plants (Langille & Hepler, 1992). In in vitro experiments it also stimulated tuber formation, especially when combined with a cytokinin (Hussey & Stacey, 1984). However, tuber weight was reduced by adding chlormequat to the medium (Harvey et al., 1991). Sometimes the addition of chlormequat together with benzyladenine to the medium, either had no effect or significantly reduced the weight of microtubers per shoot (Leclerc et al., 1994).

Growth retardants with a nitrogen-containing heterocycle, e.g. ancymidol, paclobutrazol or tetcyclasis, inhibit GA biosynthesis by blocking the conversion from ent-kaurene to ent-kaurenoic acid (Rademacher, 1999). These compounds are in general more potent than chlormequat. Paclobutrazol inhibits stem elongation and stimulates tuber formation in whole plants (Balamani & Poovaiah, 1985) and in vitro plantlets (Simko, 1993). Ancymidol and tetcyclasis were also reported to enhance tuber formation in vitro (Levy et al., 1993; Vreugdenhil et al., 1994). Using the latter compound, recalcitrant potato lines, e.g. a cross between a tuberizing and a non-tuberizing species, could be forced to form tubers (Vreugdenhil et al., 1994). Contrary to the effects of chlormequat, ancymidol and paclobutrazol did not reduce final tuber weight (Harvey et al., 1991).

Specificity of the effects of growth retardants was shown by simultaneous addition of exogenous GA to plants or in vitro cultures: tuber formation was blocked and the development of shoots was stimulated (Vreugdenhil et al., 1994; Balamani & Poovaiah, 1985).

Cellular events regulated by GA

Cell division. Tuber formation and subsequent tuber growth are the result of cell divisions and cell enlargement. The exact timing of both events have been discussed by Xu et al. (1998b). Under tuber-inducing conditions the plane of cell division changes from radial to longitudinal. Radial cell divisions, resulting in elongation of the stolon, occur mainly in the apical zone whereas longitudinal divisions result in swelling of the stolon and occur in the subapical region (Xu et al., 1998b). It is likely that GA plays a role in this switch in the plane of cell division, although this has not been proved directly.

Sanz et al. (1996) showed that in vitro developing buds elongated in the presence of GA, partly due to cell elongation. In the absence of GA, but induced to tuberize due to high sucrose, the buds started to swell, partly due to cell widening. The direction of cell expansion is known to be regulated by the cortical microtubules. Sanz et al. (1996) showed that under inducing conditions (in vitro, without GA) the cortical microtubules reorient in such a way as to allow expansion in radial direction. In later stages of tuber development the orientation of the microtubules is such that isodiametric expansion is possible. In the presence of GA the orientation does not alter, and the cell can only expand longitudinally. Similar results were described by Fujino et al. (1995). These results suggest that under non-inducing conditions cell divisions in stolons mainly occur in the apex, the new cell walls being transversal, and cells expand longitudinally because of the orientation of the cortical microtubules. On tuber induction, cell division in the apical zone stops and a new type of division, with the new wall in the longitudinal direction, starts in the subapical zone. These cells expand in a radial direction, resulting in the formation of the tuber.

Tuber-specific proteins. GA interferes with the accumulation of patatin, a glycoprotein associated with tuberization. To determine the effects of applications of GA_3 on the induction of the accumulation of this major tuber protein, patatin levels were measured in tubers from whole plants and in petioles from single-node cuttings. In both systems GA_3 inhibited the accumulation of patatin and other tuber-specific proteins. This effect appeared to be selective since most of the other proteins were not affected (Hannapel et al., 1985; Park, 1990).

Enzyme activities and starch accumulation. In contrast to stolons and other parts of the potato plant, tubers accumulate huge amounts of starch. Hence, it is to be expected that major changes in carbohydrate metabolism occur, coinciding with or preceding tuberization, and that GAs, as one of the main controlling factors of tuber formation, also have an effect on carbohydrate metabolism.

The growth of potato tubers is completely dependent on imported assimilates.

Import of carbohydrates occurs via the phloem in the form of the di-saccharide sucrose. The imported sucrose first has to be split into hexoses to be used for respiration, the synthesis of starch or the synthesis of cell components. In stolons, hydrolysis occurs via the activity of invertase, most likely in the apoplast. On tuber induction, sucrose was found to be split by the action of the reversible enzyme sucrose synthase, yielding fructose and UDP-glucose (Appeldoorn et al., 1997; Ross et al., 1994; Helder & Vreugdenhil, 1999). Simultaneously the activity of fructokinase increased drastically, removing the fructose to ensure an unidirectional flux of sucrose (Appeldoorn et al., 1997). The resulting hexose-phosphates are used for the synthesis of starch. In this pathway, AGPase and starch-synthesizing enzymes (soluble and granule-bound starch synthases) are important (see apRees, 1992, for details). Starch phosphorylase, an enzyme which plays a role in starch degradation, might also be active in the synthetic direction.

Is there any evidence for a controlling role of GA in these pathways? Mares et al. (1981) applied GA to growing tubers on intact plants. As a result, the activity of AGPase decreased whereas the activity of starch phosphorylase remained more or less constant. Surprisingly, no major change in starch content in the tubers was observed.

We have used in vitro grown axillary buds extensively as a model system to study changes occurring during tuber formation. In the presence of high sucrose such buds develop into tubers, whereas addition of GA completely prevents tuberization and results in the growth of shoots or stolons (Vreugdenhil et al., 1998). Under inducing conditions (no GA) the activities of invertases and of glucokinase decrease in the buds, and the activities of sucrose synthase, fructokinase, AGPase, starch phosphorylase and granule-bound starch synthase increase (Visser et al., 1994; Appeldoorn et al., 1997; Appeldoorn et al., 1999). The regulation is likely to be at transcriptional rather than translational level (Visser et al., 1994). When GA is added to the medium the afore-mentioned changes do not occur, despite the continuously high level of sucrose in the medium and in the tissue (Vreugdenhil et al., 1998). When studied in more detail, it appeared that the changes in enzyme activities occurred mainly at the site of swelling, and not or to a much lesser extent in the subtending stolon (Vreugdenhil et al., 1998). Fig. 1 shows the localization of the activity of AGPase, a key-enzyme in starch biosynthesis, in developing buds grown in the presence or absence of GA. High activity is found in the swelling part, whereas the subtending stolon has a much lower activity. Also the non-swollen stolon grown in the presence of GA has a lower activity.

The timing of changes in enzyme activities as related to tuber formation and regulated by GA is such that these changes are likely to be the result of an altered morphogenetic pattern rather than causing it.

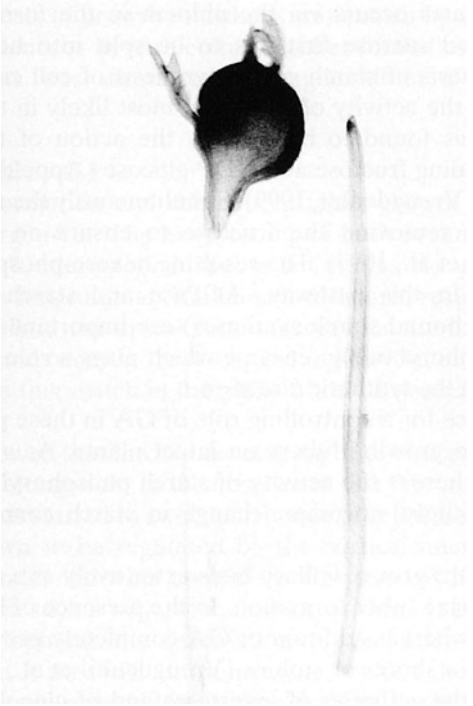

Fig. 1. Activity of ADPglucose pyrophosphorylase (AGPase) in axillary buds of single-node potato stem pieces grown in vitro. Single-node cuttings were grown in vitro for 8 days as described by Appeldoorn et al. (1997) in the presence (right) or absence (left) of gibberellins. Longitudinal sections were assayed for AGPase activity using a coupled enzyme staining, resulting in precipitation of nitro-blue tetrazolium at the sites of activity (modified from Wittich & Vreugdenhil, 1998). Note heavy staining in tuber, and low activity in non-swollen parts.

Conclusions

Gibberellins are major regulators of potato tuber formation. They inhibit tuberization, cause stolons to elongate rather than to swell, inhibit starch accumulation and the synthesis of tuber-specific proteins. The endogenous levels of GAs and especially of GA_1 are low under conditions favouring tuberization, and high under non-inductive conditions.

However, it is still questionable whether GA is the transportable factor that plays a role in signalling between shoot and stolon tips. GA levels decrease in shoots in inducing conditions and also in stolon tips. However, data from Jackson et al. (1996) contradict a simple model involving inhibition of tuber formation under non-inductive conditions by shoot-derived GAs. Stolon tips appear to be able to synthesize their own GAs, depending on conditions (Xu et al., 1998a). It is more likely that a common trigger induced by SD regulates GA levels in shoots, resulting

in a decrease of shoot elongation. Simultaneously this trigger which might be different from GA down-regulates GA synthesis in stolon tips resulting in a cascade of cellular changes such as reorientation of cytoskeleton and redirection of carbon flux; these eventually lead to swelling of the stolon and accumulation of starch.

Acknowledgements

Part of this work was supported by a grant from the International Agricultural Centre, Wageningen.

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