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ORIGINAL PAPER

Phospholipid and phospholipase changes by jasmonic acid during stolon to tuber transition of potato

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Abstract Potato tuber formation starts with the stolon swelling and is regulated by jasmonates. The cascade of events leading to tuber formation is not completely understood. The aim of this study was to evaluate phospholipid composition and phospholipase activities during four stages of stolon-to-tuber transition of *Solanum tuberosum* L., cv. Spunta, and involvement of phosphatidic acid (PA) in stolon cell expansion during early stages. Effects of jasmonic acid (JA) treatment on phospholipid content and activation of phospholipase D (PLD) (EC 3.1.4.4) and phosphatidylinositol-4,5-bisphosphate-specific phospholipase C (PIP_2-PLC) (EC 3.1.4.3) were studied in the early stages (first stage, hooked apex stolon; second stage, initial swelling stolon) of tuberization. All the phospholipid species identified, phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), PA, and cardiolipin (CL), decreased as tuber formation progressed. PLD and PLC were activated in control tissues at an early stage. JA treatment caused a decrease of PC and PS in first stage stolons, accumulation of PA in second stage stolons, and modification of PLD and PLC activities. PA increased stolon cell area in the first and second stages. These findings indicate that phospholipid catabolism is activated from the early stages of tuber formation, and that JA treatment modifies the pattern of phospholipid (PC, PS, and PA) composition and phospholipase (PLD and PLC) activity. These phospholipids therefore may play a role in activation of an intracellular mechanism that switches the developmental fate of stolon meristem cells, causing differentiation into a tuber.

Keywords Jasmonic acid · Phospholipids · Phospholipases · *Solanum tuberosum* · Tuberization

Abbreviations

CL	Cardiolipin
JA	Jasmonic acid
PA	Phosphatidic acid
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine

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PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PIP_2	Phosphatidylinositol-4,5-bisphosphate
PIP_2-	Phosphatidylinositol-4,5-bisphosphate
PLC	specific phospholipase C
PS	Phosphatidylserine
PLD	Phospholipase D
	All solvent proportions are by volume

Introduction

Tuberization refers to the alteration of stolon growth direction from an axial to a radial direction. Cell division and cell expansion are involved in tuber development. The growth of in vivo tuber occurs initially in the pith and cortex, and then predominantly in the perimedullary zone. The pith cells are large in the early stage of tuber formation whilst the perimedullary cells are not growing. Cell enlargement in the pith and cortex continue throughout the whole process of tuber growth (Xu et al. 1998).

For these events, cells require structural lipids for synthesis of new membranes, and lipid messengers for transduction of various hormonal and environmental signals. Thus, phospholipids provide not only the structural base of cell membranes, but also rich resources for generating cellular regulators (Wang et al. 2002).

Hydrolysis of phospholipids by phospholipases is often the first step in generating lipid and lipid-derived messengers. These enzymes are grouped into four major classes according to the site of lipid hydrolysis (Wang 2001): phospholipase D (PLD), phospholipase C (PLC), phospholipase A_2 (PLA_2), and phospholipase A_1 (PLA_1). Activation of phospholipases has been linked to various signalling processes in plants, such as hormonal and stress responses (Chapman 1998; Munnik et al. 1998; Wang 2001).

Phosphatidic acid (PA), a product of PLD, is considered a second messenger in a wide variety of hormonal treatments (ethylene, abscisic acid), stress mechanisms such as wounding, osmotic, oxidative stress (Munnik 2001), and aluminium toxicity (Martínez-Estévez et al. 2003). Potocký et al. (2003) reported that PLD and PA have a role in the polarised cell expansion leading to pollen tube growth.

During growth of tuber cells a new set of proteins, e.g. patatin and proteinase inhibitors, are expressed (Prat 2004). The main protein of tubers is a 40 kDa glycoprotein, patatin, that accounts for up to 40% of total soluble protein (Racusen and Foote 1980). Patatin, previously termed tuberin, is a vacuolar nonspecific lipid acyl hydrolase present in tubers of Solanaceous plants. Patatin also shows combined PLA_1 and PLA_2 activity (Hirschberg et al. 2001; Ryu 2004; Senda et al. 1996; Shewry 2003). Patatin-like proteins with PLA_2 activity were proposed to be involved in the oxylipin pathway, releasing linolenic acid from membranes, an initial reaction for generation of metabolites such as jasmonic acid (JA) and its derivatives (Jung and Kim 2000; Senda et al. 1996).

A PLA_1 called DAD1 protein (Defective in Anther Dehiscence1 protein), catalyzing the initial step of JA biosynthesis, is involved in anther dehiscence, pollen maturation, and flower opening of *Arabidopsis* (Ishiguro et al. 2001). Lipases other than DAD1 involved in jasmonate biosynthesis have not been identified, and the mechanisms and signals that trigger lipase activation and release of jasmonate precursors are also elusive (Schaller et al. 2005).

Jasmonates have been clearly implicated in many developmental processes including tuber formation (Abdala et al. 1996, 2002; Cenzano et al. 2003; Jackson 1999; Koda 1997). Various octadecanoids and derived compounds were identified in potato leaves (Helder et al. 1993; Koda et al. 1988), and the isolation of 12-OH-JA and 11-OH-JA in stolons and young tubers was recently reported by Cenzano et al. (2006). These authors found variations of JA, 12-OH-JA and 11-OH-JA content between the apical region of stolons, and stolons at different stages of *Solanum tuberosum* tuber formation.

Experiments in vitro revealed that direct application of JA, its methyl ester (JAME), tuberonic acid (TA or 12-OH-JA), or its glucoside (TAG) promotes tuberization (Koda et al. 1991). These compounds are presumed to promote tuberization by disrupting cortical microtubules of stolon meristem cells, and by allowing lateral expansion of these cells (Matsuki et al. 1992; Shibaoka 1994), which blocks the effects of gibberellins on microtubule orientation.

Exogenous application of JA to the stolon apex caused remarkable morphological changes during early stages of development, i.e., increased meristem thickness by cell expansion, reduced leaf primordial

length, and early vascular tissue differentiation, particularly of xylem elements (Cenzano et al. 2003).

During tuber formation, changes in phospholipids are necessary for new membrane synthesis. However, there is little knowledge regarding phospholipid modifications in this process, or the signal transduction pathway triggered by JA, a strong hormonal effector of tuber formation. The finding that JA produces an increase of PA in swelling stolons, and induces cell expansion during early stages of tuberization (Cenzano et al. 2003), suggests a common signal transduction pathway mediated by JA and PA that leads to tuber formation. The purposes of the present study were to determine (i) phospholipid composition during stolon-to-tuber transition, (ii) activity of PLD and PLC in second stage stolon, (iii) involvement of PA in cell expansion, and (iv) effect of JA on phospholipid changes and phospholipase activation in early stages of tuberization.

Materials and methods

Plant material

Potato tubers (*Solanum tuberosum* L., cv. Spunta) were planted in soil in a greenhouse under controlled environmental conditions (15 h photoperiod; 30°C/12°C day/night temperature at planting; 35°C/18°C day/night at tuber set). Plants were harvested between 8 and 10 weeks after planting, and organs were collected: first stage of tuberization (hooked apex stolons), second stage (initial swelling stolons), third stage (advanced swelling stolons), and fourth stage (tubers of 1.2 cm length). Each sample replicate consisted of eight stolons for the first and second stages, and six for the third and fourth stages. Phospholipase analyses were performed using plant material corresponding to the second stage, harvested in two consecutive years.

Tissue stabilization and JA treatment

Stolon sections of 1 cm length corresponding to the first and second stages were excised and divided into small pieces. Samples of 500 mg FW were incubated in 10 ml buffer (50 mM Na succinate, 5 mM KCl, 1 mM EDTA, 0.25 M sucrose, pH 6.5) at 25°C and 100 rpm for 15 min to stabilize the system prior to assays. Time 0 was defined after tissues stabilized in incubation buffer for 15 min. The tissue was incubated in incubation buffer and after 15 min 1 μM JA dissolved in buffer was applied for 7, 15, 30, or 60 min. After treatment, samples were filtered on filter paper, and washed three times with cold incubation buffer to eliminate excess hormone.

Phospholipid determination

Total lipids were extracted from washed tissues by the method of Bligh and Dyer (1959), dried under nitrogen stream, and redissolved in chloroform/methanol (9:1) (Racagni et al. 1992).

Phospholipids were separated by thin layer chromatography (TLC) on sheets of silica gel 60 (Merck; thickness 200 μm) pre-treated with 1% potassium oxalate, 2 mM EDTA in methanol/water (2:3). Plates were activated prior to use for 60 min at 110°C and cooled at room temperature. Phospholipids were separated using the solvent system chloroform/methanol/acetone/acetic acid/water (40:14:15:12:7). Developed chromatograms were stained with phosphomolybdic acid (Kirchner 1978). Phospholipids were identified by comparing retention factor (Rf) with those of commercial standards (Sigma, Aldrich, USA). Spots were scraped off the plates, and phosphorus from each phospholipid species was quantified by comparison with potassium phosphate monobasic solutions at various concentrations. Fractions were mineralized with perchloric acid at 180°C. Then, 2 ml 1% ammonium molybdate, 200 μl Fiske–Subbarow reactive, and water were added to each sample, boiled for 10 min, and cooled for 15 min at 0°C. Phospholipid concentration was quantified based on phosphorus content of the various species, measured spectrophotometrically at 830 nm (Fiske and Subbarow 1925; Racagni et al. 1992).

PLD assay (EC 3.1.4.4)

PLD activity was determined in vitro, using [methyl-^{3}H]-PC as substrate (Novotná et al. 2003). [^{3}H]-Choline produced by hydrolysis of [^{3}H]-PC was quantified in a reaction mixture (100 μl) containing a buffer solution (100 mM MES pH 5.5, 10 mM $CaCl_2$, 2 mM $MgCl_2$, 80 mM KCl, 1 mM EGTA) and 408 μM [^{3}H]-PC (436.8 Bq). The reaction was performed for 10 min at 37°C, and stopped with 100 μl of 1% (w/v) bovine serum albumin (BSA) and

250 µl of 10% (w/v) trichloroacetic acid (TCA). The precipitate was removed by centrifugation (13,000*g* for 10 min), and supernatants were collected for quantification of released [^{3}H]-Choline using liquid scintillation cocktail Eco Lume (ICN) in a scintillation counter (Beckman Coulter LS6500).

PLC assay (EC 3.1.4.3)

[^{3}H]-IP_3 produced by hydrolysis of [^{3}H]-PI-4,5-bisphosphate (PIP_2) was measured as described by Hernández-Sotomayor et al. (1999), in a reaction mixture (50 µl) that contained 35 mM NaH_2PO_4 (pH 6.8), 70 mM KCl, 0.8 mM EGTA, 0.8 mM $CaCl_2$ (25 µM final Ca^{2+} concentration), 200 µM [^{3}H]-PIP_2 (~333 Bq), and 0.08% deoxycholate. After incubation for 10 min at 30°C, the reaction was stopped, precipitates removed, and supernatants collected for quantification of released labelled compound as described in the preceding section.

Phosphatidic acid treatment

Phosphatidic acid (1,2-Di (*cis*-9-octadecenoyl)-sn-glycerol 3-phosphate sodium salt, (Sigma, Aldrich, USA) was prepared in liposome form as follows: PA alone or mixed with dioleoylphosphatidylethanolamine (Sigma, Aldrich, USA) in a molar ratio of 39:1 were made up in chloroform/methanol (9:1) and dried under N_2 stream. Then, buffer (50 mM Na succinate, 5 mM KCl, 1 mM EDTA, 0.25 M sucrose, pH 6.5) was added and dispersed by sonication in a water bath for 30 s, alternating with 30 s vortexing and 30 s ice cooling. This procedure was repeated six times. Resulting liposomes were immediately used for PA treatment. Exogenous PA enters the cell possibly via endocytosis and it has been suggested that it is relocalizated into endomembrane compartments and finally to secretory vesicles (Potocký et al. 2003). First and second stages stolons were treated with 5 or 10 µM PA in triplicate. For control treatment liposomes deficient in PA (with dioleoylphosphatidylethanolamine) were used. All samples were incubated in shaker for three days at 25°C.

Histological studies for evaluation of PA effect on cell expansion

Stolon sections were fixed in FAA (ethanol/water/formaldehyde/acetic acid, 50:35:10:5), dehydrated in a graded series of ethanol and ethanol/xylol, and embedded in Histowax (D'Ambrogio de Argüeso 1986). Longitudinal microtome sections (5 µm) were obtained using a Microm HM 310 microtome, stained with a Safranine-Hematoxiline-Fast Green combination (Johansen 1940; O'Brien and Mc Cully 1981) and mounted in Depex. Sections were examined using a Zeiss "Axiophot" microscope (Carl Zeiss, Germany) equipped with AxioCam System.

Two zones were defined for evaluation of cell expansion: Zone I, corresponding to central mother cells situated immediately under the tunic; and Zone II, corresponding to rib meristem cells (Fig. 4).

Statistical analysis

Phospholipid determination was statistically analyzed by ANOVA Test. Data were subjected to Tukey HSD Test using Statistica Program (1999) Software. Numbers of independent experiments performed for lipid analyses are indicated in each figure. For phospholipase analyses, three replications were performed. Cell areas were evaluated using three independent repetitions; each repetition corresponded to the average of 20 cell area measurements using Axio Vision 4.2 Software. Each cell area zone was analyzed using ANOVA; a posteriori comparison of means was made using Tukey HSD Test. Results were processed with Statgraphics Plus Version 3.1 Software.

Results

Phospholipid determination in ontogenetic stages of stolon-to-tuber transition

Several phospholipid species, including phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidic acid (PA), and cardiolipin (CL), were identified during the transition of stolon into tuber. Retention factors (Rf) for the phospholipid species in the solvent system used were 0.40 (PI), 0.44 (PS), 0.49 (PC), 0.56 (PE-PG), 0.69 (PA), and 0.85 (CL) (Fig. 1a). In control tissues (non JA-treated), phosphorus content in each phospholipid species decreased as tuber formation progressed. PA was the most abundant phospholipid in all stages of tuber

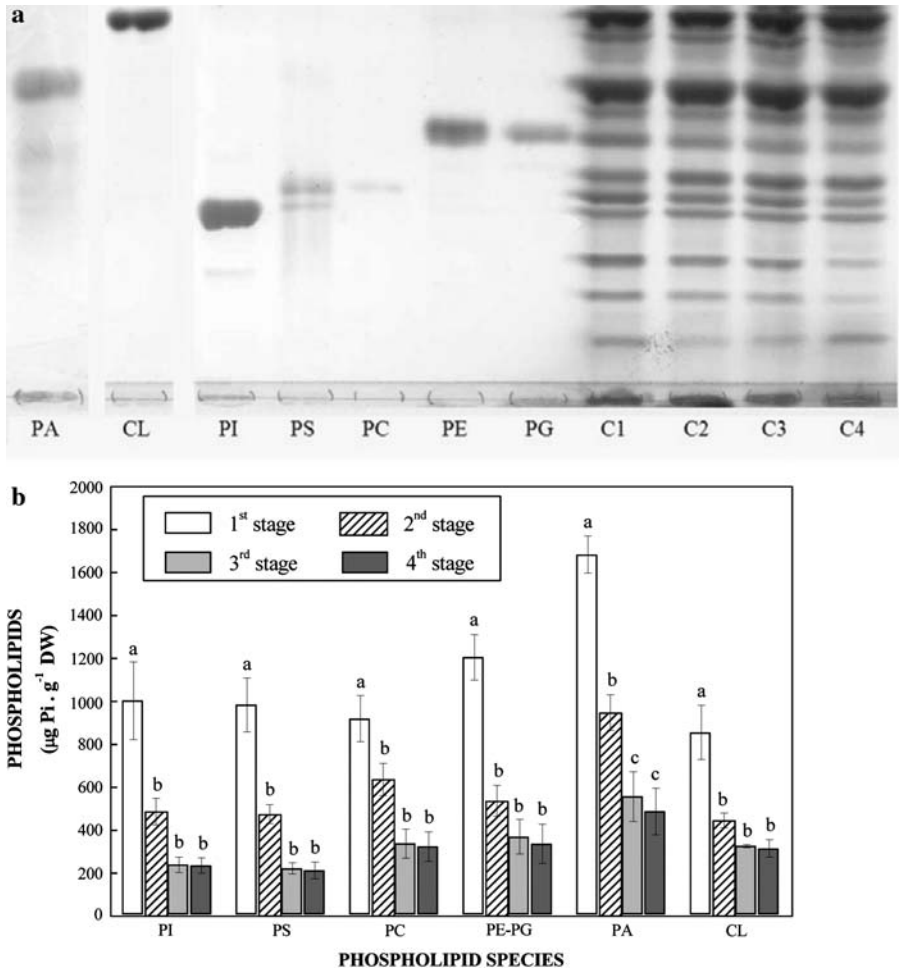

Fig. 1 Changes in phospholipid content of stolon and tuber tissues during tuberization. (**a**) TLC plate with phospholipid standards and stolon and tuber samples; (**b**) Phospholipid content. The phosphorus moiety of phospholipids was used for quantification. Phospholipids were extracted from control (non-jasmonic acid-treated) tissues, analysed by TLC, and content of individual phospholipid species was determined. Phosphatidic acid (PA), cardiolipin (CL), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine/phosphatidylglycerol (PE/PG), first stage control (C1), second stage control (C2), third stage control (C3), and fourth stage control (C4). Mean values ± SE ($n = 8$). Letters indicate values that differ significantly at $P < 0.05$

formation analysed, followed by PE-PG, PI, PS, PC, and CL (Fig. 1b). Level of all species was higher in the first stage than in later stages. Significant differences among the first, second, and third stages were observed only for PA.

JA effect on phospholipid content in early stages of tuber formation

The phospholipid content of control (non-treated) and JA-treated tissues of first and second stages is shown in Fig. 2. In control tissues, content of PC, PS and PA did not change between time 0 and 7 min. PC in general did not change as a function of incubation time (Fig. 2a), whereas first stage PS decreased at 30 min similar to the response to JA treatment (Fig. 2b). Moreover, PA is less at 60 min compared to 0–15 min both in controls and JA treatment (Fig. 2c).

JA treatment modified PC and PS content in tissues of first stage tuberization, i.e., it decreased PC 41% at 30 min and 34% at 60 min relative to control tissue (Fig. 2a), and decreased PS 39% at 30 min and 52% at 60 min (Fig. 2b). The only phospholipid modified by JA treatment in second stage tuberization was PA, which increased 41% relative to control at 30 min (Fig. 2c). Neither PC nor PS was modified by JA in the second stage (Fig. 2a, b). Other phospholipids examined were not affected by JA treatment in the first or second stage (data not shown).

JA effect on phospholipase activities

The early stages of tuberization involve mainly morphological and histological changes, with PC and PS decrease in the first stage and PA increase in the second stage. We compared phospholipase

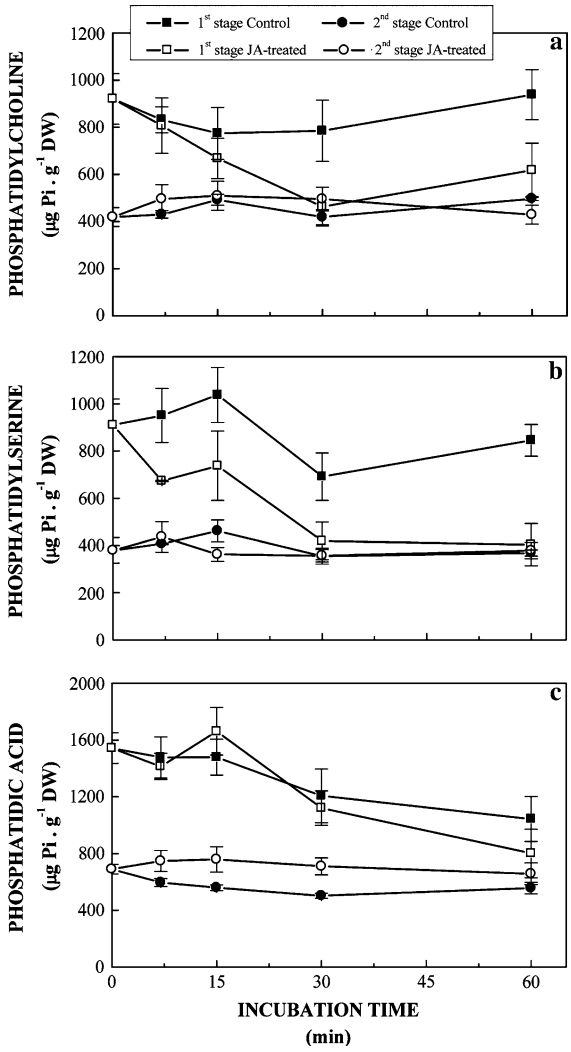

Fig. 2 Effect of jasmonic acid (JA) treatment on phospholipid content of stolon during first and second stages of tuberization. (**a**) Phosphatidylcholine; (**b**) phosphatidylserine; (**c**) phosphatidic acid. Control (non-JA-treated) and JA-treated tissues were incubated for various times as indicated. Phospholipids were extracted from tissues and analysed by TLC; phospholipid content was determined for each incubation time. Mean values ± SE ($n = 6$)

activities in control and JA-treated tissues of the second stage.

PLD activity in control tissues showed a peak at 7 min with magnitude ~40% higher than basal level (Fig. 3a). PIP_2-PLC showed a later peak, between 15 and 30 min (Fig. 3b). PLD activity in JA-treated tissues was 155% higher than that of control at 7 min. This difference was maintained until 30 min, and PLD activity subsequently decreased until 60 min

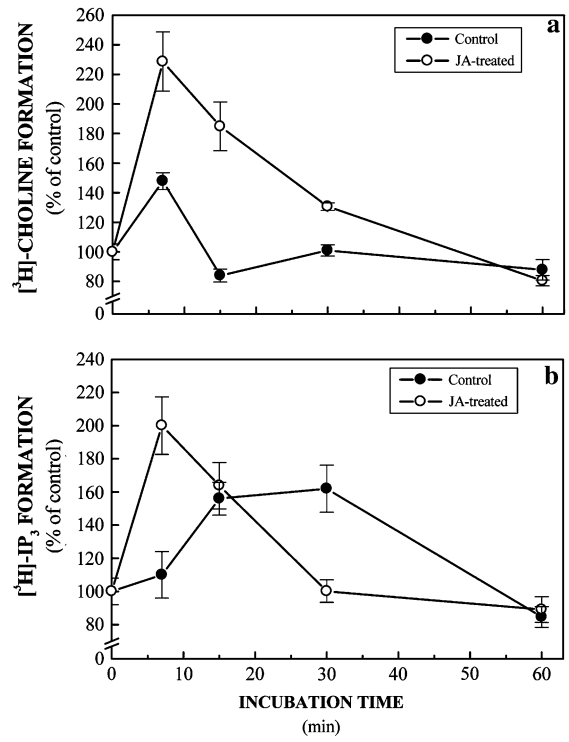

Fig. 3 Phospholipase activities of control (non-jasmonic acid-treated) and jasmonic acid-treated tissues at various times during second stage of tuberization. (**a**) Phospholipase D (PLD) activity; (**b**) phosphatidylinositol-4,5-bisphosphate specific phospholipase C (PIP_2-PLC) activity. Results, expressed as percentage of basal activity (at time zero in the absence of jasmonic acid), were 0.198 and 0.212 nkat mg^{-1} protein for PLD and PIP_2-PLC, respectively

(Fig. 3a). PIP_2-PLC activity in JA-treated tissues was 100% higher than that of control at 7 min, decreased at 30 min reaching control value and was maintained until 60 min (Fig. 3b). The increased activity of both enzymes at 7 min contributed to PA formation. In control tissues, PIP_2-PLC was activated early and then rapidly declined. PLD was also activated early, but showed a more sustained response over the time-course of the experiment.

PA effect on cell expansion

To quantify the effect of PA on cell expansion, and rule out possible increase of cell area by water uptake into cells, stolon diameter was measured before and after incubation with PA. Cell expansion was measured in apical meristem of early stages (less than 0.3 cm in diameter) considering that pith cells first

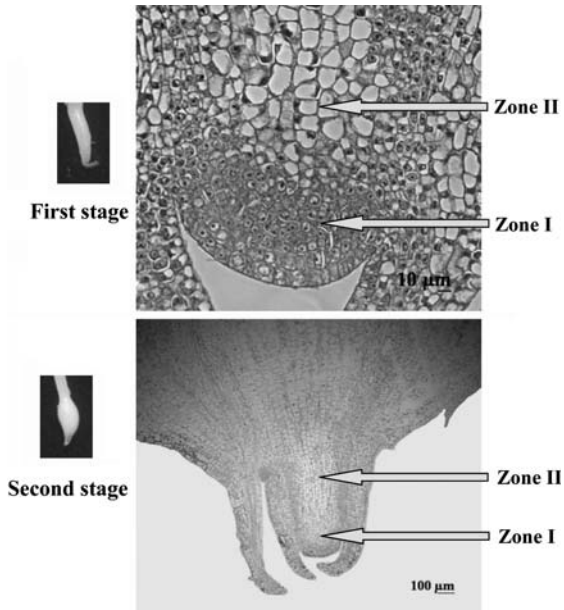

Fig. 4 Photomicrographs of first and second stage of tuberization showing the two zones analized in the corpus. Zone I, corresponding to central mother cells situated immediately under the tunic; Zone II, corresponding to rib meristem cells

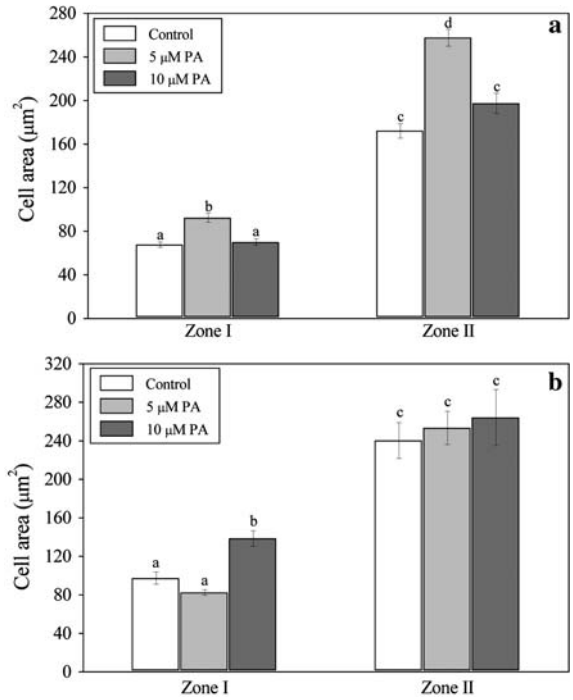

Fig. 6 Phosphatidic acid effect on cell area. (**a**) First stage of tuberization; (**b**) second stage. Mean values ± SE ($n = 3$)

enlarge and then divide longitudinally. Two zones were delimited in the corpus: Zone I, corresponding to central mother cells situated immediately under the tunic; and Zone II, corresponding to rib meristem cells (Fig. 4). Photomicrographs of the first stage of tuberization show that 5 μM PA produced marked cell expansion in Zones I and II compared to control whereas 10 μM PA did not increase cell area (Fig. 5). In the second stage, 5 μM PA did not produce cell expansion in Zones I and II compared to control. 10 μM PA caused an increase in cell area only in Zone I (data not shown).

The increase in cell expansion caused by 5 μM PA in the first stage was statistically significant ($P < 0.05$) in Zones I (36.34%) and II (49.72%) (Fig. 6a). In the second stage, 10 μM PA caused a significant increase in cell area in Zone I (42.36%) (Fig. 6b).

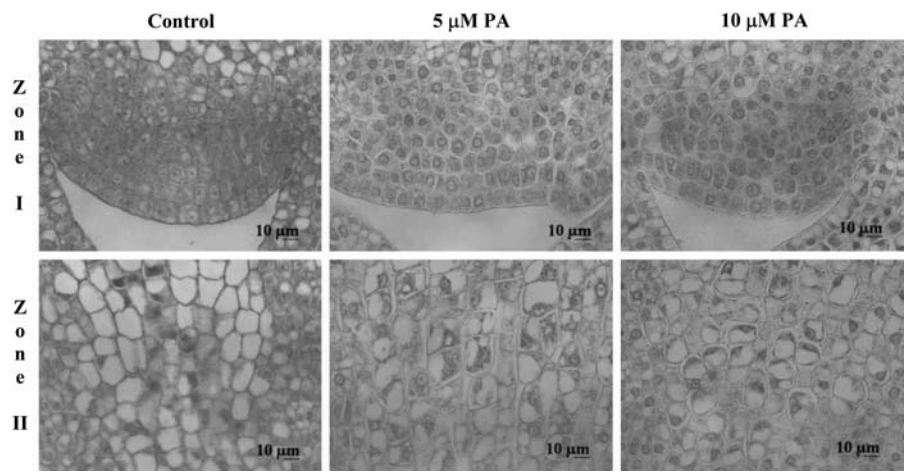

Fig. 5 Phosphatidic acid (PA) effect on cell expansion at first stage of tuberization. Treatments (control, 5 μM PA and 10 μM PA) in columns and Zones (I and II) in rows. Liposomes containing PA were used for 3 days treatment. Exogenous PA possibly enters to the cells via endocytosis. Bright field images of longitudinal sections were taken using Zeiss Axiophot microscope equipped with AxioCam System

Discussion

Phospholipid hydrolysis is triggered by various cellular and environmental cues, and is involved in many cellular processes through generation of signal messengers, cytoskeletal rearrangement, vesicular trafficking, secretion, membrane remodelling, and lipid degradation (Wang 2002).

In tuber formation, the most dramatic changes at the cellular level occur during the early stages of the stolon-into-tuber transition, and are accompanied by a decrease in all the phospholipid species analyzed. Level of endogenous JA also decreases as tuberization progresses (Cenzano et al. 2006).

Although no reports could be found describing PA as major component of any endomembrane structure, an unexpected result reported here is the high level of PA in the stages of stolon to tuber transition. The high PA level may be thought to have occurred as an artefact during the extraction procedure; if this had been the case, it could have been expected a similar extent of phospholipid degradation in all samples. However, in support of our results we found a PA sustained decrease with the advance of tuber formation. Our results are in agreement with Regente et al. (2008), who informed a high level of PA in extracellular washing fluids of sunflower seeds measured by electrospray ionization tandem mass spectrometry (ESI-MS/MS). However, our finding is distinct from Dobson et al. (2004) study who found PA as a minor phospholipid in mature potato tubers stored at different weeks postharvest.

Production of phospholipids, lysophospholipids, and free fatty acids is regulated by the activity of multiple phospholipases (Munnik 2001; Ryu and Wang 1998; Van der Luit et al. 2000; Wang 2000). PLD and PLC are present in a broad range of organisms (bacteria, simple eukaryotes, plants, animals). In plants, other components of phosphoinositide metabolism are found in addition to phospholipases. PLD and PLC are obviously important in plant development, since they are involved in germination, osmotic regulation, pathogen infection, environmental stress response, cellular fusion, and other physiological processes.

In general, lipid changes in plants and the enzymes involved have been exhaustively studied in stress response (Lee et al. 1997), but not in normal physiological processes such as tuberization. Although PLD and PLC are expressed in most plant tissues, little is known about their regulation mechanisms.

PLD and PLC were found to be activated during the second stage of tuber formation, when the major morphological and biochemical changes occur. Thus, activation of specific enzymes for lipid metabolism during tuberization is also important for membrane biogenesis during stolon cell expansion. In view of the observed lipid changes in potato tissues and PLD—PLC activities, lipid metabolism appears to be activated from the early stages of tuber formation. Under certain physiological conditions, these enzymes can respond to specific stimuli, such as JA.

Hormonal signals in morphogenetic processes are common events which require specific target tissues and transduction pathways to exert their action. During tuber formation several hormones, including jasmonates, have been shown to play essential roles.

JA exerts its action through expansion of cells in the stolon apex (Cenzano et al. 2003) and modification of the microtubule rearrangement in suspension cells of potato (Matsuki et al. 1992). Since cell expansion includes membrane remodelling and cytoskeletal rearrangements, we expect that lipid metabolism is involved in tuber formation.

In this study, JA treatment of tissues in early tuberization stages caused a decrease of PC and PS. The only phospholipid increased by JA treatment was PA. JA treatment also enhanced activity of the enzymes PLD and PLC.

We also found that PA induces cell expansion in central mother cells (Zone I) but in those cells that enlarge to form the tuber (Zone II) only 5 μM PA induces cell expansion at first stage. Thus, considering that JA strongly promotes cell expansion (Cenzano et al. 2003) and PA increases cell area, we consider JA as a signal molecule for cell expansion regulation and PA as a mediator for stolon elongation, previous event for tuber formation.

Numerous evidence in the last few years have shown that exogenously applied phospholipids exert strong effects on plant growth and development (Cowan 2006). Although the underlying mechanism of this phenomenon is not known, it reflects that phospholipids added extracellulary are incorporated by the cells or eventually exert their effect in contact with the membrane.

Potocký et al. (2003) proposed a model for the role of PLD and PA in plant cell expansion. In this model,

an elevated concentration of intracellular PA (achieved by external application of PA liposomes to pollen culture) stimulates phosphatidylinositol bisphosphate (PIP_2) production, which initiates formation of protein regulatory complexes that control actin organization needed for cell growth. PLD has been shown to be strongly associated with microtubules and membranes (Lloyd and Hussey 2001), and triggers reorganization of microtubules in plant cells (Dhonukshe et al. 2003).

PA is produced by two routes: one involving PLD, which hydrolyses structural phospholipids such as PC and PE, and the other mediated by PIP_2-PLC, which hydrolyses PIP_2 into two second messengers, diacylglycerol (DAG) and inositol triphosphate (IP_3). DAG is rapidly phosphorylated to PA by DAG-kinase (Munnik et al. 1998). On the other hand, the enzyme phosphatidylserine decarboxylase can catalyze conversion of PS to PE, also a PLD substrate. The decrease of PS observed in control and JA-treated tissues in first stage of tuber formation may result from this pathway.

In coincidence with the lipases activation, PA increase after JA treatment may be due to PC and PS degradation, or to other metabolic routes, e.g., phosphoinositide hydrolysis by PIP_2-PLC enzyme producing DAG as a substrate for PA formation.

Our results indicate that important lipid and related enzyme modifications occur during initiation of tuber formation, and that exogenous JA causes an early decrease of PC and PS and later increase of PA, mediated by PLD and PLC. Thus, exogenous JA modifies phospholipids, including PC, PS, PA, and phospholipases, which may determine initiation of tuber formation. Exogenous PA caused cell expansion; therefore PA appears to be an important signal mediating the role of JA.

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