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Bioorganic Chemistry of the Induction of Potato Tuber Formation: A Review

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ABSTRACT

In the potato (*Solanum tuberosum*), tuberization is a complex developmental process, leading to the formation of a specialized storage organ by the differentiation of the underground stolon. Tuberization begins with the perception of environmental signals in the leaves. Under short day (SD) conditions and cool temperatures, potato plants produce tubers, while they remain in a vegetative stage under long day (LD) conditions or high temperatures. Environmental signals exercise influences on the control of potato tuberization via several plant hormones and other endogenous factors. Tuberonic acid glucoside (TAG) isolated from the leaves of potato plants is the proposed as specific tuber-inducing substance. Tuberonic acid (TA) and jasmonic acid (JA), which are closely related compounds to TAG, have also showed strong activities on tuber induction. The generation of TAG is associated with the linolenic acid (LA) cascade. In the LA cascade, JA is biosynthesized from 13(*S*)-hydroperoxylinolenic acid (HPOT), catalyzed by lipoxygenase (LOX) as an initial enzyme, and then JA is metabolized to TA and finally converted into TAG. In the present review, which is aimed at elucidating the mechanism of potato tuber induction by means of temperature and LOX derivatives, low temperature was favorable for tuber induction of potato, and LOX activity appeared high level at the initial stage of potato tuberization and was stimulated by low growing temperature. In addition, the high endogenous levels of JA, TA, and TAG were observed at low temperature suggesting that the increase in LOX, which is activated by low temperature, results in large amounts of endogenous JA, TA and TAG, which play a crucial role in potato tuber induction. On the other hand, inhibitory effect for tuber induction under unfavorable environmental conditions could be recovered partially by the treatment of theobroxide, an exogenous tuber-inducing compound.

Keywords: lipoxygenase, hydroperoxylinolenic acid, jasmonic acid, tuberonic acid, tuberonic acid glucoside, theobroxide
Abbreviations: **ABA**, abscisic acid; **AOC**, allene oxide cyclase; **AOS**, allene oxide synthase; **GA**, gibberellin; **GC-SIM-MS**, gas chromatography-selected ion monitoring-mass spectrometry; **HPOT**, hydroperoxylinolenic acid; **JA**, jasmonic acid; **LA**, linolenic acid; **LD**, long day; **LOX**, lipoxygenase; **Me-JA**, methyl jasmonate; **OPDA**, 12-oxo-phytodienoic acid; **SD**, short day; **SHAM**, salicylhydroxamic acid; **TA**, tuberonic acid; **TAG**, tuberonic acid glucoside

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INTRODUCTION

The potato (*Solanum tuberosum*) tuber results from a process named tuberization, which is the formation of a specialized storage organ by the differentiation of the underground stolon (Taylor *et al*. 1992). Tuberization begins with the inhibition of the longitudinal growth at the stolon tip followed by a swelling at the subapical region (Cutter 1978). Subsequently, vigorous thickening growth due to cell division and expansion occurs (Xu *et al*. 1998b). Thereafter, biochemical changes, including accumulation of starch and formation of storage proteins, occur in growing tubers (Appeldoorn *et al*. 1997).

Tuberization is very much influenced by environmental signals and regulated by several plant hormones. Potato plants produce tubers under short day (SD) photoperiods and low temperatures, but they do not form tubers under long day (LD) conditions (Hussey and Stacey 1984). Likewise, potatoes do not form tubers at higher temperature even if SD conditions are satisfied (Ewing and Struik 1992).

The effects of typical hormones on tuberization are well reviewed in the literature (Wareing and Jennings 1980; Melis and van Staden 1984; Vreugdenhil and Struik 1989; Ewing 1995). Gibberellins (GAs) are well-known as inhibitors of tuberization. Exogenous treatments of GAs resulted in tuber inhibitions in systems using whole plants (Okazawa 1960), plantlets *in vitro* (Hussey and Stacey 1984) and excised sprouts cultured *in vitro* (Koda and Okazawa 1983a).

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Invited Review

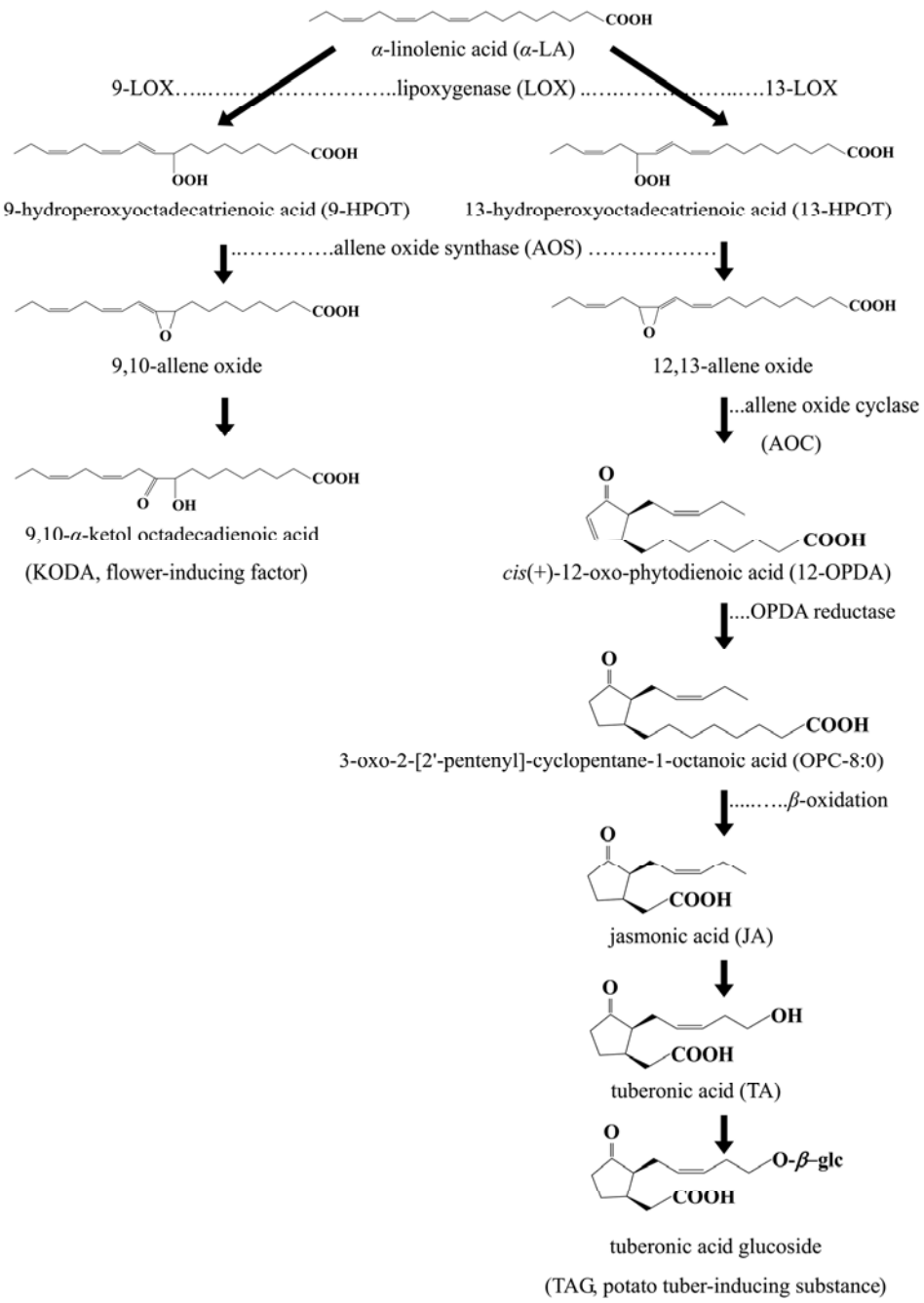

Fig. 1 The linolenic acid cascade.

It is suggested that unfavorable environmental conditions for tuberization, i.e., LD, low irradiance, high temperatures and high nitrogen supplication, are related to high levels of GA activity (Woolley and Wareing 1972; Railton and Wareing 1973; Krauss and Marschner 1982; Menzel 1983).

The existence of a specific potato tuber-inducing substance, which is produced in leaves during SD conditions and transported to the top of the stolon, has been proposed by grafting and other experiments (Gregory 1956; Kumar and Wareing 1973). In subsequent experiments, this presumed tuber-inducing substance was isolated from leaflets of potato plants using the bioassay of a potato single-node stem segment culture and the structure of this active compound was identified to be 12-hydroxyjasmonic acid glucoside (tuberonic acid glucoside, TAG) (Koda *et al*. 1988; Yoshihara *et al*. 1989). 12-Hydroxyjasmonic acid was later named as tuberonic acid (TA). Both TA and its glucoside (TAG) are structurally and biosynthetically related to jasmonic acid (JA). [2-^{14}C] JA applied on potato leaves is metabolized to TAG within 2 weeks and transferred to the stolons and other plant parts (Yoshihara *et al*. 1996).

JA is supposed to counteract the effects of GA (van den Berg and Ewing 1991). At an early stage of potato tuberization, radial cell expansion of stolons occurs (Koda and Okazawa 1983b), and in response to JA, the cells of potato tuber tissue expand as a consequence of water uptake (Takahashi *et al*. 1994). In addition, a large amount of methyl jasmonate (Me-JA), a volatile derivative of JA, has been detected in plant species (Mithöfer *et al*. 2005). JA and Me-JA are involved in various morphogenic events such as tuberization, bulb formation (Koda 1997), senescence (Ueda and Kato 1980), wounding (van den Berg and Ewing 1991), coiling (Weiler *et al*. 1993) and various abiotic stresses (Creelman and Mullet 1995).

TAG, a tuber-inducing substance, is biosynthesized by a so-called linolenic acid (LA) cascade (**Fig. 1**). In plants, LA is initially oxygenated to form 9(*S*)- hydroperoxylinolenic acid (HPOT) or 13(*S*)-HPOT by lipoxygenase (LOX, EC 1.13.11.12) and then further metabolized into a number of biologically active compounds (Feussner and Wasternack 2002). JA is synthesized from 13(*S*)-HPOT by consecutive actions of allene oxide synthase (AOS), allene oxide cyclase (AOC), reductase, and *β*–oxidative enzyme (Siedow 1991). Next, JA is metabolized to TA and finally converted into TAG which has been identified as the endogenous tuber-inducing substance of potato (Yoshihara *et al*. 1989).

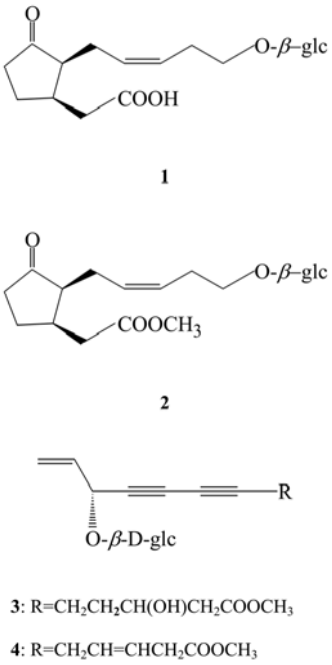

Fig. 2 Endogenous tuber-inducing substances of tuberous plants. 1 is isolated from potato and 2-4 are isolated from Jerusalem artichoke.

On the other hand, theobroxide (**Fig. 3, 6**), isolated from the culture filtrate of the fungus *Lasiodiplodia theobromae*, has been identified as a natural tuber-inducing compound in potato (Nakamori *et al*. 1994). Theobroxide strongly induces potato tuberization *in vitro* and *in vivo* under non-inductive photoperiod conditions (Nakamori *et al*. 1994; Yoshihara *et al*. 2000). Interestingly, a number of studies show the close relationship between theobroxide and JA in potato tuberization. Tuber induction of potato, induced by theobroxide, is correlated with the stimulation of JA and TA syntheses and enhances the activity of LOX, a key enzyme for JA biosynthesis (Gao *et al*. 2003). Their successive study showed that theobroxide might play a role in the swelling of microtubers formed *in vitro* in a similar manner as that of JA, suggesting that theobroxide may be a trigger of JA production (Gao *et al*. 2005). In addition, a JA biosynthesis inhibitor, salicylhydroxamic acid (SHAM), suppresses the inductive effect of theobroxide on potato tuberization and reduces the endogenous content of JA and TA (Gao *et al*. 2003).

In order to establish the mechanisms of tuberization, in this review, temperature is selected as the environmental variable, and its influences are systematically discussed with LOX derivatives and various enzymes in LA cascade. In addition, the role of exogenous treatments of theobroxide in overcoming unfavorable environmental conditions is taken into account.

ENVIRONMENTAL FACTORS

Potato tuberization begins with a sensing of the environmental signals, followed by the generation of a signal (known as tuberigen) in the leaves (Gregory *et al*. 1956). Then, the generated signal is transported successively to a distant organ, such as the stolon tips, at which tuber formation is induced in response (Thomas 1998). Among various environmental cues, photoperiod is one of the most important factors affecting tuberization. Under SD conditions, potato plants produce tubers, but they remain in a vegetative stage under LD conditions (Ewing and Struik 1992). However, the critical night length for tuberization and the strength of the photoperiodic response varies with genotypes (Snyder and Ewing 1989). For example, potato species such as *S. demissum* and *S. tuberosum* ssp. *andigena*, which are often used in experiments with photoperiodic effects on tuberization, require definite day lengths, 12 h or less, to tuberize and they will not tuberize when day length exceeds a critical threshold (Ewing and Struik 1992).

In addition to excessive day length, light exposure in the middle of the dark period (termed night break) also inhibits tuber formation (Jackson 1999). It has been reported that tuberization in *S. tuberosum* cv. 'Arran Pilot' was delayed by interruption of the long dark period and tuber initiation of *S. demissum* was blocked completely by night break (Slater 1963). Many studies have suggested that phytochrome may be involved in potato tuberization since it has been implicated in many photoperiodic reactions. In an experiment that reversed the inhibitory effect of red light by far red light treatment, Batutis and Ewing (1982) provided evidence that phytochrome is involved in the regulation of potato tuberization. Later, Jackson *et al*. (1996) determined that phytochrome B is required for the photoperiodic control of potato tuberization by generating transformants of *S. tuberosum* ssp. that produced much lower levels of phytochrome B protein than normal. They speculated that phyto-

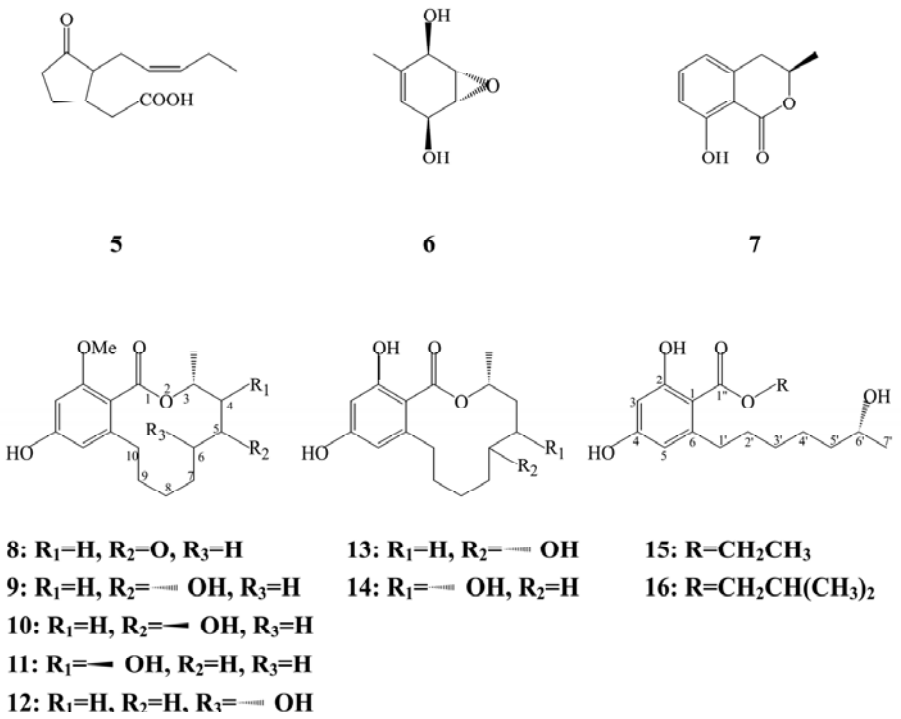

Fig. 3 Potato microtuber forming substances from *Lasiodiplodia theobromae*.

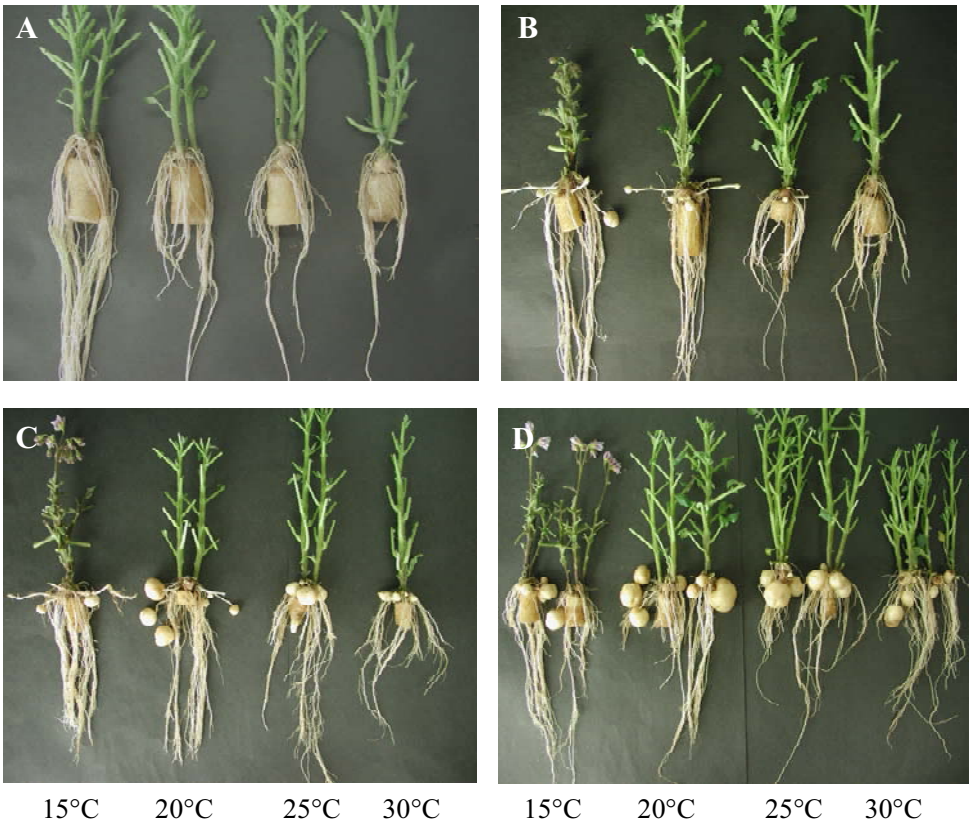

Fig. 4 Temperature effect on the tuberization of potato. Pictures were taken at 1 week (**A**), 2 week (**B**), 3 week (**C**), and 4 week (**D**) after temperature treatments. Reprinted from **Nam KH, Minami C, Kong F, Matsuura H, Takahashi K, Yoshihara T** (2005) Relation between environmental factors and the LOX activities upon potato tuber formation and flower-bud formation in morning glory. *Plant Growth Regulation* **46**, 253-260, with kind permission of Springer Science and Business Media, ©2005.

chrome B is probably not involved in the induction of tuberization, but rather involved in a negative regulatory mechanism that prevents tuberization in a non-inductive photoperiod, LD or SD with night break.

Temperature is also a major environmental factor controlling potato tuberization. Low temperatures are very favorable for tuber induction, while high temperatures exert negative influences (Ewing 1981; Ewing and Struik 1992). Tuberization is inhibited under cool air temperature and warm soil temperature condition, but this was not attributable to the failure of the production of a tuber-inducing stimulus in leaves (Reynolds and Ewing 1989). At the high soil temperature, the produced stimulus was transported through the stolons, but the stolons were prevented from developing into tubers. By controlling the temperature of parts of the plants such as shoots, roots, stolons, and tubers independently, it was confirmed that high temperatures in the shoots exerted the most serious inhibitory effect on tuber induction (Ewing and Struik 1992). While slightly increased temperatures of stolons and tubers showed no particular effect, high temperature of roots resulted in a minor negative effect.

In our recent experiments, the effects of various growing temperature treatments (15, 20, 25 and 30°C) on tuberization using 2-week-old potato plants were examined. Our findings suggested that low temperature (15°C) is suitable for tuber induction, while relatively high temperature (20~25°C) promotes tuber growth. However, high temperature (30°C) is inhibitory for tuberization in both tuber induction and growth, although the inhibitory effect is much greater in tuber induction (Nam *et al.* 2005) (**Fig. 4**).

In addition to SD and low temperature, other environmental factors such as high light intensity or low nitrogen level also promote induction of potato tuber formation (Werner 1934; Krauss 1985; Struik 1986; Demagante and van der Zaag 1988).

PLANT HORMONES AND TUBER INDUCING SUBSTANCES

Specific environmental signals are known to control tuberization of potato via several plant hormones. GAs are well-known to have an inhibitory effect on tuber induction (Okazawa 1960; Koda and Okazawa 1983a; Hussey and Stacey 1984). Exogenous application of GA to potato stems promoted stolon elongation and suppressed tuber formation (Smith and Rappaport 1969; Kumar and Wareing 1972; Vreugdenhil and Helder 1992). It was also reported that the endogenous level of GA was high during stolon elongation and declined when stolon tips started to swell under inducing conditions, whereas a high level was maintained under non-inducing conditions (Pont-Lezica 1970; Koda and Okazawa 1983b; Xu *et al*. 1998a). In contrast, treatments with the GA-biosynthesis inhibitors, such as ancymidol and tetcyclacis, stimulated tuber induction (Perl *et al*. 1991; Vreugdenhil *et al*. 1994). Many reports have suggested that unfavorable environmental conditions for tuberization are correlated with high levels of GA activity (Woolley and Wareing 1972; Railton and Wareing 1973; Krauss and Marschner 1982; Menzel 1983). In an experiment using *S. tuberosum* ssp. *andigena*, the levels of GA declined after transfer from LD or night break conditions to SD (Machackova *et al*. 1998) and negative influences of LD conditions on tuberization were improved by a partial block in its the GA biosynthetic pathway (van den Berg *et al*. 1995a, 1995b). Another report showed that photoperiod-dependent tuberization is mediated by GA application, which prevents or delays tuberization under inducing SD conditions. However, the application of ancymidol, an inhibitor of GA biosynthesis will allow tuberization in non-inducing LD (Jackson and Prat 1996). Moreover, Menzel (1980, 1983, 1985) demonstrated that the inhibitory effects of high temperature on tuberization might also be mediated through increased GA levels.

The effects of abscisic acid (ABA) on potato tuberization have been well documented in the literature. Application of exogenous ABA promoted tuberization in whole plants, stem cuttings, and stolon tips (El-Antably *et al*. 1967; Biran *et al*. 1972, 1974; Krauss and Marschner 1976). Other effects of ABA include increased numbers of tubers, earlier initiation of tubers, and the formation of sessile tubers (Abdullah and Ahmad 1980; Menzel 1980). However, other experiments with cultured stolons and sprouts showed that ABA suppresses potato tuberization (Palmer and Smith

1969b; Hussey and Stacey 1984). The effects of applied ABA depended on variety and concentration (Palmer and Smith 1969b; Hussey and Stacey 1984). Endogenous levels of ABA were increased under tuber-inducing conditions and decreased when nitrogen was supplied during tuber formation (Krauss and Marschner 1982; Marschner *et al*. 1984). Besides, ABA is generally believed to reduce GA-promoted processes during plant development. In contrast to GA, the exogenous application of ABA reduced stolon elongation and promoted tuber formation in potato (Okazawa and Chapman 1962; Xu *et al*. 1998a). Menzel (1980) reported that tuberization was delayed under high temperatures and high levels of GA, but partly reversed by ABA. This indicates that temperature exerts its influence by altering the balance between the levels of endogenous GA and ABA. Xu *et al*. (1998a) suggested that ABA stimulates tuberization by counteracting GA, and that sucrose regulated tuber formation by influencing GA levels.

Cytokinins have been found associated with potato tuberization, but in contrast to GA and ABA, less attention has been paid to cytokinins (Palmer and Smith 1969). Many reports have demonstrated that exogenous application of cytokinins stimulates potato tuberization (Palmer and Smith 1969a; Kumar and Wareing 1974; Hussey and Stacey 1984) and endogenous levels of cytokinins are high in induced tissues (Mauk and Langille 1978; Obata-Sasamoto and Suzuki 1979), implying cytokinins as positive tuber-inducing factors. Langille and Forsline (1974) have reported that the levels of cytokinins were increased temporarily under tuber-inducing environmental conditions such as SD and cool temperatures. Exogenously applied N^6-benzyadenine (BA) extensively induced microtubers on different explants including stolons, shoot cuttings and intact microplantlets (Donnelly *et al*. 2003). Although cytokinin is necessary for tuberization, it is not the only factor regulating tuber induction in potatoes. Since no significant effects of cytokinins on stolon elongation and tuber formation have been reported under inducing or non-inducing conditions, it would seem that cytokinins are not a limiting factor in tuber formation (Xu *et al*. 1998b). Furthermore, it has been verified that cytokinins do not stimulate, but rather suppress tuber growth even at concentrations optimum for *in vitro* tuberization in the dark (Sarker *et al*. 2006). It has also been proposed that cytokinins are less important than GA in relation to potato tuberization (Dimalla and van Staden 1977). Vreugdenhil and Struik (1989) have reported that the response of a stolon to high levels of cytokinin depends on the interaction with other hormones, mainly the levels of GA_3. Exogenous cytokinins decreased tuber growth during JA-induced tuberization in potato (Dermastia *et al*. 1996). Similarly, Sarker *et al*. (2006) reported that exogenous cytokinins antagonize the jasmonate-effect on tuber growth after induction, and the related effects of these two hormones interrelate with the regulation of endogenous sugar and starch levels in tubers depending on the maturing time of the cultivars during potato tuber formation *in vitro*.

It has been established that the hormones, auxin and ethylene, play a minor role on tuberization (Melis and van Staden 1984; Vreugdenhil and van Dijk 1989). Harmey *et al*. (1966) reported that the application of IAA in the tuber-inducing medium led to early tuber initiation. However, Obata-Sasamoto and Suzuki (1979) showed a high level of auxin was present in the stage prior to tuber initiation but its levels declined during tuber development. Probably, auxin plays an important role in stolon orientation and growth. Moreover, its function is pronounced when combined with other hormones (Ewing and Struik 1992). Application of IAA in the presence of GA significantly inhibited the elongation of stolons than under GA conditions alone, and the supplement of IAA in 1% sucrose medium completely blocked the growth of the lateral buds. These findings suggest that IAA indirectly supports tuberization by counteracting the effects of endogenous GA (Xu *et al*. 1998a). IAA, which reduces stolon elongation, stimulated the production of ethylene, an inhibitor of tuber formation (Vreugdenhil and van Dijk 1989).

Application of exogenous ethylene has been found to inhibit potato tuberization in several *in vitro* studies and the addition of an ethylene antagonist accelerated tuberization in potato (Vreugdenhil and van Dijk 1989; Vreugdenhil and Struik 1990). It is certain that ethylene stimulates the production of GA, which is well-known to inhibit tuberization. Although limited evidence is available on the beneficial effect of ethylene on potato tuberization, a probable hypothesis is that ethylene is produced by friction between soil particles and the growing stolon tip, thereby preventing the elongation of stolon (Vreugdenhil and Struik 1989; Vreugdenhil and van Dijk 1989).

The existence of a specific potato tuber-inducing substance, which is produced in leaves during SD conditions and transported to the top of the stolon, was postulated by grafting and other experiments (Gregory *et al*. 1956; Kumar and Wareing 1973). Later, many research groups made a great effort to identify this specific tuber-inducing stimulus. In 1988, the occurrence of a tuber-inducing stimulus in potato leaves was confirmed in bioassays using a potato single-node segment culture (Koda and Okazawa 1988; Koda *et al*. 1988; Yoshihara *et al*. 1989) and the active substance isolated from potato leaves (Koda *et al*. 1988). This substance showed tuber-inducing activity *in vitro* at a concentration of 0.01 mg/l (*c* 3×10^{-8} M) and its chemical structure was identified to be 3-oxo-2-(5-β-D-glucopyranosyloxy-2-z-pentenyl)-cyclopentane-1-acetic acid (12-hydroxyjasmonic acid glucoside, named TAG) (Yoshihara *et al*. 1989) (**Fig. 2, 1**). Afterwards, TAG methyl ester and two polyacetylene compounds, methyl *β*–D-glucopyranosyl helianthenate A and B were isolated from the leaves of Jerusalem artichoke (*Helianthus tuberosus* L.) (Matsuura *et al*. 1993) (**Fig. 2, 2-4**). The aglycone of TAG (12-hydroxyjasmonic acid, named TA) was also shown to have strong tuber-inducing activities in potato (Yoshihara *et al*. 1989; Koda *et al*. 1991). Both TA and its glucocide (TAG) are structurally and biosynthetically related to JA. The potato tuber-inducing activity of JA was almost the same as that of TA and TAG (Koda *et al*. 1991). When [2-^{14}C](±) JA was applied on potato leaves, it was metabolized to TAG within 2 weeks and transferred to the stolons and other plant parts (Yoshihara *et al*. 1996).

JA and its methyl ester (MeJA) are ubiquitous in the plant kingdom and it is believed that they induce a wide variety of plant responses (Koda 1992). They are involved in various morphogenic events such as tuberization, bulb formation (Koda 1997), senescence (Ueda and Kato 1980), wounding (van den Berg and Ewing 1991), coiling (Weiler *et al*. 1993) and abiotic stress (Creelman and Mullet 1995). Exogenously applied JA and MeJA induce tuberization of potato stolons, shoot cuttings and plantlets cultured *in vitro* (Yoshihara *et al*. 1989; Pelacho and Mingo-Caster 1991; Koda *et al*. 1991; Ravnikar *et al*. 1992; Pruski *et al*. 2001, 2002). Abdala *et al*. (1996) confirmed the endogenous JA content in roots, stolons and periderm of newly formed tubers. The highest concentration of JA was detected in foliage at the initial growth stages of the potato plants and in roots and stolons at the stage of tuber set. However no changes were observed in tubers between the stages of tuber set and advanced tuberization (Creelman and Mullet 1995; Abdala *et al*. 2000). Besides, a number of reports showed that exogenous application of JA does not affect tuber induction in potato (Helder *et al*. 1993; Jackson and Willmitzer 1994; Sarkar *et al*. 2006). Application of SHAM, an inhibitor of JA biosynthesis, did not prevent tuberization under SD conditions (Helder *et al*. 1993). These results indicate that differences in the levels of JA itself do not control tuberization. It has been suggested that potato tuberization is regulated by a balance between the levels of JA and other hormones. Koda and Kikuta (2001) reported that JA-induced tuberization of potato plants *in vitro* depended on the maturation time of the cultivar. The relative higher JA-response of an early cultivar is assumed to be due to the lower levels of endogenous GAs (Koda 1997; Koda and Kikuta 2001). JA during potato tuberization counteracts the

effect of GA on microtubule orientation (Jackson 1999). JA reversed the inhibitory effect of GA_3 on tuberization of potato shoot cuttings *in vitro*, and the promoting effect of JA on potato tuberization was antagonized in rooted plantlets possessing a high level of endogenous GAs (Castro *et al*. 2000). It has also been observed that JA enhances the growth of potato plantlets *in vitro*, simulating in the ratio of active to inactive cytokinin (Dermastia *et al*. 1994; Koda 1997). Sarkar *et al*. (2006) showed that cytokinins antagonize the jasmonate-effect on tuber growth after induction, although this reversing effect is more clearly with JA than MeJA independently on the cultivars.

MICROTUBER FORMING SUBSTANCES FROM FUNGI AND PLANTS

Lasiodiplodia theobromae is a common pathogenic fungus found in the tropics and subtropics, and its culture filtrate inhibits the growth of higher plants and produces various organic metabolites (Hirai 1938; Aldridge *et al*. 1971). Several potato microtuber inducing substances have been isolated from the culture filtrates of the fungus *L. theobromae* IFO 31059 by bioassay using cultures of single-node segments of potato stem *in vitro*. Nakamori *et al*. (1994) isolated three potato-tuber inducing substances and their structures identified as JA (**Fig. 3, 5**), theobroxide (**Fig. 3, 6**) and mellein (**Fig. 3, 7**). In additional experiments, six lasiodiplodin-related compounds, 5-oxolasiodiplodin (**Fig. 3, 8**), 5-hydroxylasiodiplodins (**Fig. 3, 9**) and (**Fig. 3, 10**), (3*R*),(4*S*) -4-hydroxylasiodiplodin (**Fig. 3, 11**), (3*R*),(6*R*)-6-hydroxy-de-*O*-methyllasiodiplodin (**Fig. 3, 13**), (3*R*),(5*R*)-5-hydroxy-de-*O*-methyl -lasiodiplodin (**Fig. 3, 14**) (Matsuura *et al*. 1998; Yang *et al*. 2000a) and two resorcinol derivates, ethyl (6' *R*)-2,4-dihydroxy-6-(6'-hydroxyheptyl)benzoate (**Fig. 3, 15**) and isobutyl (6' *R*)-2,4-dihydroxy-6-(6'-hydroxy-heptyl)benzoate (**Fig. 3, 16**) (Yang *et al*. 2000b) were isolated as biologically active compounds inducing potato microtuber formation. More recently, (*3R*,6*S*)-6-hydroxylasiodiplodin (**Fig. 3, 12**) was isolated from the culture broth of the Shimokita 2 strain of *L. theobromae* (Li *et al*. 2005).

On the other hand, cucurbic acid and methyl cucurbate isolated from the seeds of *Cucurbita pepo* showed tuber-inducing activity in potatoes, but their activities were somewhat lower than those of JA and MeJA (Fukui *et al*. 1977; Koda *et al*. 1991). Experiments comparing the tuber-inducing activities of JA and related substances indicated that the partial structures are indispensable for the tuber-inducing activity included a carboxyl group or its ester at the C-1 position, a double bond (pentenyl group) in the substituent at the C-2 position, and an oxygen atom at the C-3 position (Koda *et al*. 1991).

LIPOXYGENASE AS THE KEY ENZYME AND LINOLENIC ACID CASCADE PRODUCTS

A potato tuber-inducing substance, TAG, is biosynthesized by a so-called LA cascade (**Fig. 1**). Using α-LA as the initial substrate, molecular oxygen is stereo-specifically introduced into either position carbon 9 or 13 of LA by LOX protein catalysis, leading to either 9- or 13-HPOT, respectively (Howe and Schilmiller 2002; Kongrit *et al*. 2006). 9- and 13-HPOT are substrates for members of the CYP74 family of cytochrome P450, which is a group of enzymes of oxygen-activated reactions, such as AOS of CYP74A, hydroperoxide lyase of CYP74B/C and divinyl ether synthase of CYP74D (Hannemann *et al*. 2007). These enzymes are localized in membranes of chloroplasts (Froehlich *et al*. 2001) and utilize the acyl hydroperoxide of the substrate as oxygen donor and form new carbon-oxygen bonds in the products, which function serve as essential signals for plant mechanical responses (Weiler *et al*. 1993) and some developmental processes (McConn and Browse 1996).

13-HPOT is metabolized to divinyl ether fatty acids (e.g., etherolenic acid) by divinyl ether synthase, to C_6 aldehydes and C_{12} ω-keto-fatty acids by hydroperoxide lyase and 12,13-epoxyoctadecatrienoic acid (allene oxide) by AOS. Since the product of AOS branch is an unstable epoxide intermediate, it is converted either to enantiomerically pure *cis*(+)-12-oxo-phytodienoic acid (12-OPDA), the first cyclic and biologically active compound, by AOC or to a mixture of α- and γ-ketols and racemic *cis*-OPDA spontaneously in the absence of AOC (Hamberg and Fahlstadius 1990; Laudert *et al*. 1997). 12-OPDA is reduced by OPDA reductase yielding 3-oxo-2-[2'-pentenyl]-cyclopentane-1-octanoic acid, which is subsequently transformed to JA by three rounds of β-oxidation (Howe and Schilmiller 2002). Finally, JA is further derived to TA and then TAG, which has been identified as the endogenous tuber-inducing substance of potato (Yoshihara *et al*. 1989).

On the other hand, in the 9-LOX pathway, 9-HPOT experiences an analogous set of catalytic reactions by other isoforms of divinyl ether synthase, hydroperoxide lyase or AOS, resulting in colnelenic acid, 9-oxo-nonanoic acid or 9,10-allene oxide, respectively. Afterwards, 9,10-allene oxide is transformed to 9,10-α-ketol octadecadienoic acid, 10,13-γ-ketol octadecadienoic acid or 10-OPDA via nonenzymatic reactions. 9,10-α-ketol octadecadienoic acid is believed to be involved in inducing factors in flower bud formation (Takimoto *et al*. 1989, 1991, 1994; Yokoyama *et al*. 2000; Yamaguchi *et al*. 2001; Suzuki *et al*. 2003; Yokoyama *et al*. 2005).

Enzyme activities and protein contents

LOX is the first enzyme in the LA pathway and ubiquitous among eukaryotes (Siedow 1991; Porta and Rocha-Sosa 2002). In different plant species, LOXs are present as multiple isoforms or isozymes suggesting that each one may play distinct functions in the plant (Royo *et al*. 1996; Heitz *et al*. 1997; Smith *et al*. 1997; Fischer *et al*. 1999). Many studies have suggested that LOXs play a crucial role in plant evolution including growth and development, flowering, fruit ripening, seed germination and senescence (Rouet-Mayer *et al*. 1992; Saravitz and Siedow 1995; Sung and Chiu 1995; Kausch and Handa 1997; Fukuchi-Mizutani *et al*. 2000; Ye *et al*. 2000). LOX gene expression is regulated by different hormones such as ABA (Melan *et al*. 1993), JA (Creelman and Mullet 1997), and also by different forms of stress, such as pathogen attack (Melan *et al*. 1993) and wounding (Porta *et al*. 1999).

LOXs have been detected during potato tuber development and several research groups have suggested that LOXs are involved in the control of potato tuberization (Bachem *et al*. 1996; Royo *et al*. 1996; Kolomiets *et al*. 2001). Potato LOXs are encoded by a large multigene family and several LOX cDNAs have been isolated from potato tubers, roots, and leaves (Geerts *et al*. 1994; Casey 1995; Kolomiets *et al*. 1996a, 1996b; Royo *et al*. 1996; Fidantsef and Bostock 1998). Royo *et al*. (1996) characterized three distinct classes of LOX genes in potato plants based on their deduced amino acid sequences and their patterns of expression. Lox1 genes were expressed mostly in tubers and roots and comprise enzymes with 9-LOX activity. Lox2 genes were expressed in leaves only and Lox3 genes were expressed in leaves and roots that produce 13-HPOT, the precursor of JA and related compounds. Accumulation of Lox1 class transcripts detected in the apical and subapical regions of newly formed tuber, specifically in vascular tissue of the perimedullary region, which is the site of the most active cell growth during tuber enlargement *in situ* hybridization (Kolomiets *et al*. 2001). LOX activity was suppressed in relation to reduced tuber yield, decreased average tuber size, and a disruption of tuber formation (Kolomiets *et al*. 2001). An inhibitor of LOX, naproxen and SHAM also declined LOX activity in potato plants (Kolomiets *et al*. 2001; Gao *et al*. 2003). It has been observed that all enzymes of the LA cascade, LOX, AOS, and AOC, differentially localize within chloroplasts, and are mainly found associated with thylakoid membranes (Farmaki *et al*. 2007).

In our study, the effect of temperature, which is one of

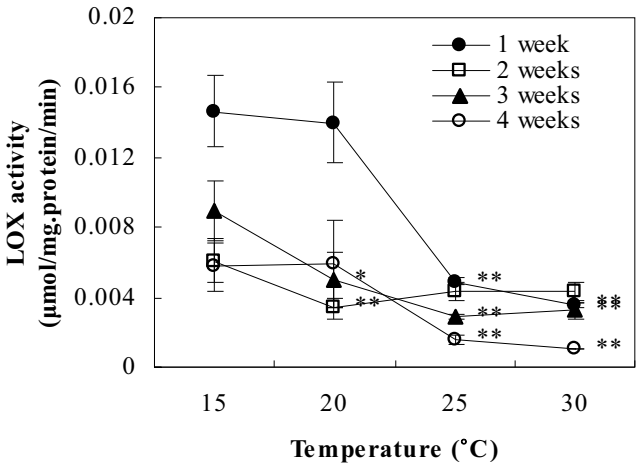

Fig. 5 The effect of growing temperature on LOX activity in potato leaves. Potato leaves were collected 1 (●), 2 (□), 3 (▲), and 4 (○) weeks after temperature treatments and determined at 30°C. Values represent the means of three independent measurements ±SE. * and ** indicate significant differences ($p < 0.05$ and $p < 0.01$) respect to the growing temperature of 15°C. Reprinted from **Nam KH, Minami C, Kong F, Matsuura H, Takahashi K, Yoshihara T** (2005) Relation between environmental factors and the LOX activities upon potato tuber formation and flower-bud formation in morning glory. *Plant Growth Regulation* **46**, 253-260, with kind permission of Springer Science and Business Media, ©2005.

the critical requirements for potato tuber formation, on LOX activity was examined (Nam *et al*. 2005) (**Fig. 5**) using UV spectrophotometry (Gao *et al*. 2003). During the initial stages of tuberization, that is, one week after the 15°C temperature treatment, high levels of LOX activity were observed. These findings suggested that potato tuber induction is correlated with LOX activity and is also dependent on the growing temperatures. Another experiment in which the relationships between LOX and light or dark treatment in a typical SD plant, morning glory (*Pharbitis nil*), showed that LOX activity was greatly enhanced up to 30 min and then declined after switching from light to dark conditions. However, the activity did not vary on switching from dark to light conditions (Nam *et al*. 2005). This suggests that the appearance of flower buds in *P. nil* might be attributed to the activation of LOX which can be initiated by dark treatment. A report by Ye *et al*. (2000) using the *Arabidopsis thaliana* plant, suggested that LOX may mediate a photoperiodic signal in the transition from vegetative growth to bolting and reproductive growth.

13(*S*)-HPOT converted from LA by 13-LOX catalysis is metabolized by an AOS into an unstable allene oxide, which is cyclized by an AOC to *cis*-(+)-OPDA (9*S*,13*S*) carrying the enantiomeric structure of the naturally occurring JA (Feussner and Wasternack 2002). AOS enzymes are members of the cytochrome P450 enzyme family, subfamily CYP74 (Howe and Schilmiller 2002). AOS proteins have been observed in various plant organs (Gardner 1975; Blée and Joyard 1996; Caldelari and Farmer 1998; Laudert and Weiler 1998; Grechkin and Hamberg 2000) and cloned from several plant species (Maucher *et al*. 2000; Froehlich *et al*. 2001; Itoh *et al*. 2002). In potato plants, the activity of AOS was shown in stolons, roots and developing tubers (Hamberg 2000) and three cDNAs encoding AOS (*StAOS*1-3) were isolated (Stumpe *et al*. 2006). In western blotting analysis, AOS protein largely accumulated under inductive photoperiod conditions for flower-bud formation in *P. nil*, suggesting that AOS probably plays a role in flower-bud formation in *P. nil* (Kong *et al*. 2005a). AOC gene was cloned from tomato (Ziegler *et al*. 2000) and *A. thaliana* plants (Stenzel *et al*. 2003). Kong *et al*. (2005a) reported that AOC protein plays an essential role in the initial JA accumulation induced by theobroxide. To perform the conversion of the unstable allene oxide to the first cyclic precursor of JA, the association between AOC and AOS is required to be in close proximity (Farmaki *et al*. 2007). In our unreported study, the AOS/AOC branch to JA biosynthesis was stimulated in response to low temperature and resulted in high endogenous levels of both AOS and the AOC enzyme.

Quantitative and qualitative analysis of the products

In the LA cascade, 13-LOX derived products have been closely correlated with potato tuberization (Yoshihara *et al*. 1989; Koda *et al*. 1991; Pelacho and Mingo-Castel 1991; Castro *et al*. 2000; Kolomiets *et al*. 2001; Pruski *et al*. 2002; Sarkar *et al*. 2006). TA and TAG with strong tuber-inducing activities, where TAG might be more important, were isolated from potato leaves using the bioassay of a potato single-node stem segment culture (Koda and Okazawa 1988; Koda *et al*. 1988; Yoshihara *et al*. 1989). Similar to TA and TAG, JA and its methyl ester have also shown tuber-inducing activities in potato (Koda *et al*. 1991). TA and its glucoside (TAG) are structurally related to JA and when JA applied to potato leaves was further metabolized to TAG within 2 weeks (Yoshihara *et al*. 1996).

JA has been involved in various morphogenic events including bulb formation and tuberization (Koda 1997). Formation of plant storage organs such as tubers and bulbs are controlled by photoperiod. In onion (*Allium cepa* L.) plants, bulb formation occurs in leaf blades in response to the stimulus of LD photoperiods, whereas tuber formation in potato plants was initiated in stolon tips by SD stimulus. In the potato, tuberization begins with cessation of stolon elongation followed by a swelling at the sub-apical region brought about by radial cell expansion (Booth 1963; Cutter 1978; Koda and Okazawa 1983b; Peterson *et al*. 1985; Xu *et al*. 1998b). Mita and Shibaoka (1983) reported that bulb formation of onion plants, which is caused by the lateral expansion of leaf sheath cells, was accompanied by the disruption of cortical microtubules in the cells. Later, JA and MeJA were found to disrupt cortical microtubules in suspension cultures of tobacco BY-2 cells and potato cells (Abe *et al*. 1990; Matsuki *et al*. 1992). In potato plants, the cessation of stolon elongation and cell expansion in the sub-apical meristem region was induced in response to JA (Takahashi *et al*. 1994) and once JA has induced cell expansion of a potato tuber, reorientation of the cortical microtubules occurred (Shibaoka 1991; Koda 1997). Abdala *et al*. (2000) have reported that endogenous levels of JA increased in roots between swollen stolon and tuber set and these organs may facilitate the action of JA on the orientation of microtubules during cell expansion in stolons. In the recent experiment on the effect of JA on histology, exogenously applied JA resulted in the enlargement of meristems, the increase in cell expansion, the reduction in the length of leaf primordia and the early differentiation of vascular tissue facilitating the movement of substances to the stolon tip (Cenzano *et al*. 2003). It has also been suggested that subapical meristem of the stolon might start to swell when the concentration of TAG reaches a sufficiently high level to induce tuberization (Yoshihara *et al*. 1996).

Based on our findings (Nam *et al*. 2005), it is very likely that increased LOX activity results in an increase in the total amount of cascade products. To understand the role of temperature in the LA cascade in detail, the contents of 9(*S*)-HPOT and 13(*S*)-HPOT in potato leaves were determined (Nam *et al*. 2008) (**Fig. 6**) by reverse phase-HPLC (Göbel *et al*. 2002). LOX catalyzes both pathways to 9(*S*)-HPOT and 13(*S*)-HPOT in the LA cascade. In this regard, a selective catalysis of LOX toward either 9(*S*)-HPOT or 13(*S*)-HPOT depending on temperature is difficult to be considered. Therefore, when LOX activity is enhanced at a given temperature, both reactions to 9(*S*)-HPOT and 13(*S*)-HPOT will be enhanced equally. However, only the 9(*S*)-HPOT level was enhanced under experimental conditions. This discrepancy can be explained by means of the differences in reaction rates. Since neither 9(*S*)-HPOT nor 13(*S*)-HPOT is a final product in the LA cascade, they are trans-

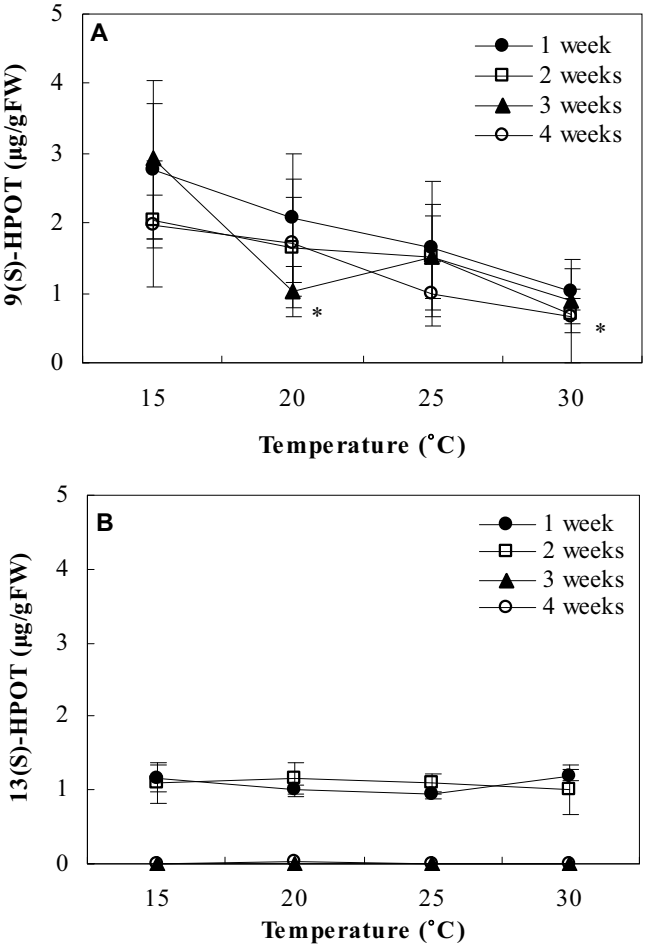

Fig. 6 Endogenous levels of 9(*S*)-HPOT (A) and 13(*S*)-HPOT (B) in potato leaves after 1-4 weeks' temperature treatment of 2-week-old plants. Values represent the means of three independent measurements ±SE. * indicates significant differences ($p < 0.05$) respect to the growing temperature of 15°C, according to the Bonferoni test. 9(*S*)- and 13(*S*)-HPOT = 9(*S*)- and 13(*S*)-hydroperoxy linolenic acids. Reprinted from **Nam KH, Kong F, Matsuura H, Takahashi K, Nabeta K, Yoshihara T** (2008) Temperature regulates tuber-inducing lipoxygenase-derived metabolites in potato (*Solanum tuberosum*). *Journal of Plant Physiology* **165**, 233-238, with kind permission of Elsevier Ltd., ©2008.

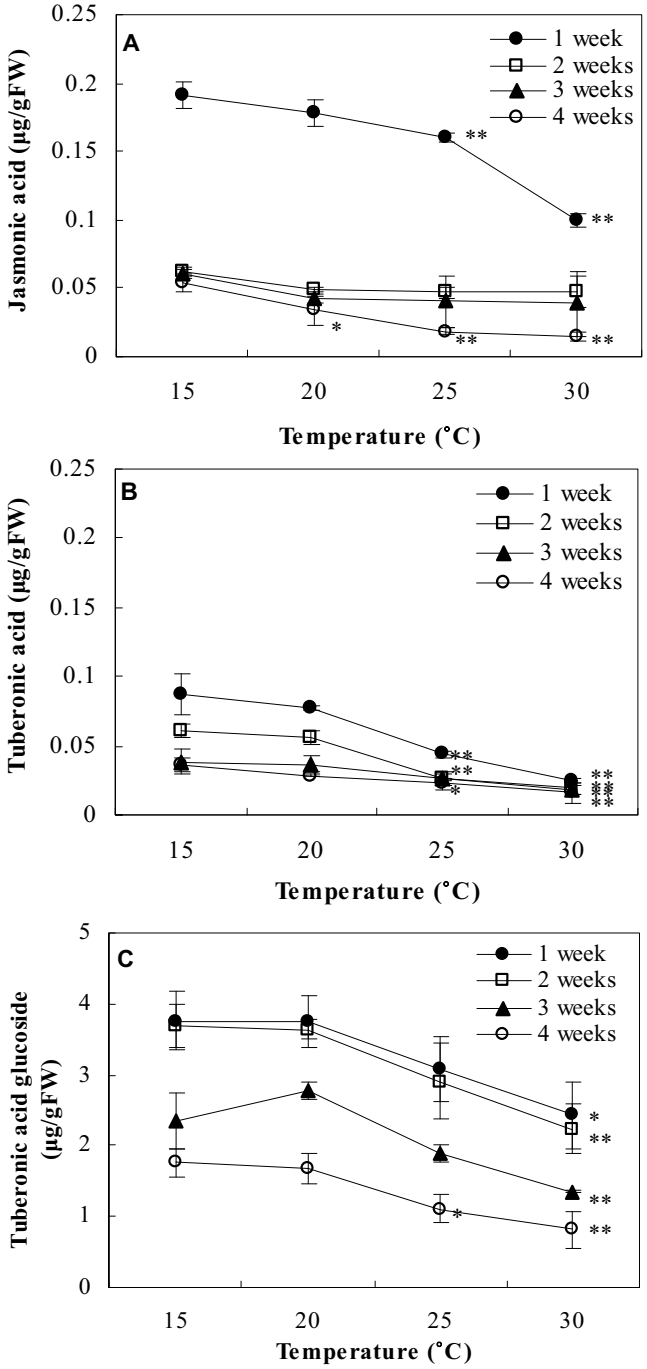

Fig. 7 Endogenous levels of jasmonic acid (A), tuberonic acid (B) and tuberonic acid glucoside (C) in potato leaves after 1-4 weeks' temperature treatment of 2-week-old plants. Values represent the means of three independent measurements ±SE. * and ** indicate significant differences ($p < 0.05$ and $p < 0.01$) with respect to the growing temperature of 15°C, according to the Bonferoni test. Reprinted from **Nam KH, Kong F, Matsuura H, Takahashi K, Nabeta K, Yoshihara T** (2008) Temperature regulates tuber-inducing lipoxygenase-derived metabolites in potato (*Solanum tuberosum*). *Journal of Plant Physiology* **165**, 233-238, with kind permission of Elsevier Ltd., ©2008.

formed via successive reactions to the next metabolites in the pathways. Under the proposed explanation, the high accumulation of 9(S)-HPOT implies that the successive reactions occur very slowly. On the other hand, the low and constant levels of 13(S)-HPOT suggest that the further reactions to JA, TA and TAG are very facile.

In addition, the endogenous contents of JA, TA and TAG were analyzed (Nam *et al*. 2008) by GC-SIM-MS (Matsuura *et al*. 2002). The highest JA content appeared at a growing temperature of 15°C and decreased as the growing temperature increased (**Fig. 7A**); this result was consistent with LOX measurements. Since low temperatures (15°C) were favorable for tuber induction, the relevance between JA and tuber induction is apparent to some extent. Also, for all temperatures studied, a relatively high JA content was observed one week after the temperature treatment, but declined sharply after two weeks. On the other hand, slightly high temperatures (20 and 25°C) are suitable for potato tuber growth. This observation that the content of JA declined as temperature increased and as the potato tuber developed suggests less association between JA and tuber growth. In the LA cascade, JA is metabolized to TA and finally into TAG (Siedow 1991). TAG has been suggested as a main endogenous tuber-inducing substance of potato (Koda *et al*. 1988; Yoshihara *et al*. 1989). As expected from the LA cascade, the endogenous levels of both TA and TAG showed a similar dependence on growing temperature to that of JA (**Fig. 7B** and **7C**). In particular, TAG was extraordinarily elevated at low temperature and the amounts of TAG were about 20-40 times larger than those of JA and TA in all cases. Because TAG is produced in leaves and is transferred to the stolon as a main tuber-inducing substance (Yoshihara *et al*. 1996), a high amount of TAG under low temperature conditions, demonstrates that TAG is the most important potato tuber-inducing substance. In summary, it is proposed that the increase in LOX, which is activated by low temperature, results in large amounts of endogenous JA, TA and TAG, which play a crucial role in potato tuber induction.

REGULATION OF POTATO TUBER FORMATION BY CHEMICALS

Theobroxide (**Fig. 3, 6**), an epoxy cyclohexene compound isolated from the culture filtrate of the fungus *L. theobromae* (Nakamori *et al*. 1994), is purportedly involved in the regulation of various plant development processes. In order to explore the exact functions of theobroxide, various physiological and biochemical studies have been carried out systematically, in particular, in relation to the following three subjects; potato tuberization; *P. nil* flower bud formation; and stem elongation in *P. nil* and spinach (*Spinacia oleracea*).

Using a single segment *in vitro* bioassay, theobroxide was demonstrated to have potato microtuber inducing activity at a concentration of 5×10^{-6} M and it was almost identical to that of (±) JA (Nakamori *et al*. 1994). Furthermore, application of theobroxide (10^{-3} M in 100 ppm Tween 20 solution) onto the leaflet surface of potato plants induced tubers under non-inducing photoperiod conditions and enhanced total number and total fresh weight of tubers compared to that of controls without theobroxide (Yoshihara *et al*. 2000). In a study on the effect of different concentrations (10^{-5}, 10^{-4}, 10^{-3}, and 2×10^{-3} M) of theobroxide on the induction of potato microtuber formation, as the concentration of theobroxide was elevated, the tuberization ratio also increased (Yang *et al*. 2004). The total fresh weight of microtubers in 2×10^{-3} M theobroxide medium was about five times that of the microtubers grown in control medium. The treatment of SHAM, a JA biosynthesis inhibitor, suppressed the inductive effects of both theobroxide and SD photoperiod in potato tuber formation (Gao *et al*. 2003). In additional treatments of the theobroxide in the culture medium containing JA increased the tuberization ratio and fresh weight of microtubers more than the theobroxide and JA treatments alone (Gao *et al*. 2005). The yield of tubers of theobroxide-treated potato plants in the field was 20, 20 and 10% higher than that of untreated plants for cvs. 'Irish Cobbler', 'Kitaakari', and 'May Queen', respectively (unpublished data). Moreover, our unpublished study demonstrated that under suitable temperatures for potato tuberization, tuber inductions were enhanced by theobroxide, especially at low temperatures. In addition, high concentrations of theobroxide induced tubers even under non-inductive temperature conditions, that is 30°C, but it seems that excess amount of theobroxide only plays a neutral or even negative role in potato tuber induction.

In contrast to [2-^{14}C] (±) JA (Yoshihara *et al*. 1996), neither metabolism nor transportation occurred in an experiment involving the application of [3,6-^{3}H] (±) theobroxide to potato plants (unpublished data). It was concluded that theobroxide is not a single trigger for the tuber formation and might stimulate the biosynthesis of a common plant growth regulator. A number of studies showed a close relationship between theobroxide and JA in potato tuberization. Tuber induction of the potato, induced by theobroxide, is correlated with the stimulation of JA and TA syntheses and enhances the activity of LOX, a key enzyme for JA biosynthesis (Gao *et al*. 2003). Endogenous levels of JA reached a peak at a day 3 after theobroxide treatment, whereas no significant increase up to a day 28 was shown in control plants. Endogenous levels of TA in theobroxide-treated potato plants were almost the same up to one week as compared to the levels in non-treated plants, but a sharp increase of TA level was observed 2 weeks after theobroxide treatment. The activity of LOX after 60 min in theobroxide-treated plants was two times higher than in control plants. However, a JA biosynthesis inhibitor, SHAM, suppressed the inductive effect of theobroxide on potato tuberization and resulted in a reduction of the activity of LOX and the endogenous contents of JA and TA (Gao *et al*. 2003). In tissues obtained from *in vitro* cultures of potato single-node segments treated with theobroxide, endogenous JA was observed in both segments and microtubers, whereas TA was only detected in segments (Yang *et al*. 2004). Also, in both old and newly formed potato tissues, theobroxide increased the endogenous levels of JA and the activity of LOX (Gao *et al*. 2005). Simulative effect on LOX activity by theobroxide treatment was not stronger following an application of JA application. In addition, histological observation of the sections of potato stolons cultured *in vitro* showed that theobroxide may play a role in the swelling of microtubers formed *in vitro* in a similar manner as that of JA, suggesting that theobroxide may be trigger of JA production (Gao *et al*. 2005).

In contrast to tuberization, flowering of potato plants is promoted under LD photoperiods (Turner and Ewing 1988). In experiments on the metabolism and transportation of JA in potato plants grown under different photoperiods, high accumulations of TAG were detected in tubers and flower-buds (Yoshihara *et al*. 1996). These results suggested that a common mechanism may be applicable to both tuberization and flower formation. Application of theobroxide stimulated flower-bud formation in potato plants grown under LD photoperiods (Yoshihara *et al*. 2000). Furthermore, theobroxide treatment of the leaf surfaces of *P. nil* plants induced flower-bud formation under non-inductive LD conditions and enhanced the number of flowers of seedling under inductive SD conditions (Yoshihara *et al*. 2000). Flower-bud formation in *P. nil* plants was suppressed by night-break and cotyledon-removal (Ogawa and King 1980; Vince-Prue and Gressel 1985), but this inhibitory effect was reversed by treatment with theobroxide (Gao *et al*. 2006). Besides, flower formation in LD rosette plants, such as spinach, was inhibited by application of theobroxide (Kong *et al*. 2006).

Theobroxide-induced flower bud formation in *P. nil* caused the increase of the endogenous levels of JA (Yang *et al*. 2004). Kong *et al*. (2005a) reported that theobroxide treatment resulted in high accumulations of JA under both SD and LD conditions and increased accumulation of LOX, AOS, and AOC proteins. Immunoblotting analysis of protein levels demonstrated a biphasic activation of AOC protein; the first and second activation of which were displayed at 30 min and 6 h, respectively after application of theobroxide. While LOX and AOS proteins are activated by theobroxide after the activation of AOC protein, suggesting that AOC is essential for theobroxide-induced JA biosynthesis in *P. nil*. Additionally, AOS protein, which is closely related to biosynthesis of a flowering inducing factor, 9,10-α-ketol octadecadienoic acid, accumulated markedly under SD conditions and by treatment of theobroxide, indicating that AOS probably plays a role in flower-bud formation in *P. nil*. On the other hand, the endogenous GA_1 and GA_3 contents in *P. nil* treated with theobroxide were relatively low, suggesting that GAs may be negatively involved in theobroxide-induced flower bud formation of *P. nil* (Gao *et al*. 2006).

Finally, theobroxide is associated with the inhibition of stem elongation in spinach and *P. nil* plants. Applied theobroxide suppressed stem elongation in *P. nil* under both SD and LD conditions and treatment of SHAM and GA_3 partially restored the inhibitory effect of theobroxide on stem elongation (Kong *et al*. 2005b). Stem length of seedlings exposed to night break and cotyledon removal in *P. nil* was shortened by supplemental applications of theobroxide (Gao *et al*. 2006). Stem elongation of spinach plants was declined by treatment of theobroxide under inductive LD conditions, but was reversed by the application of GA_3 (Kong *et al*. 2006).

LOX activity and endogenous JA levels were significantly enhanced, while endogenous GA_1 levels were decreased by theobroxide sprayed under both SD and LD conditions in *P. nil*. Therefore, stem elongation in *P. nil* may be caused by the balance between JA and GA biosynthesis (Kong *et al*. 2005b). Exogenous application of SHAM and GA_3 reversed the inhibition of stem elongation by theobroxide treatment controlling the endogenous JA level and LOX activity. It was also reported that the inhibitory effect of stem elongation in *P. nil* may be achieved through affecting endogenous contents of GA_{1+3} (Gao *et al*. 2005). In

spinach plants, the endogenous level of JA was unchanged and endogenous level of GA_1 was reduced by exogenous application of theobroxide under inductive LD conditions, suggesting that the suppression of stem length by theobroxide was likely due to a reduction of GA_1 biosynthesis (Kong *et al*. 2006).

CONCLUDING REMARKS

The potato tuber is a specialized storage organ formed by the differentiation of the underground stolon and tuberization of potato is very much affected by the interaction between environmental, biochemical, and genetic factors. Based on an early grafting experiment (Gregory *et al*. 1956), it was proposed that a tuber-inducing substance is produced initially in leaves by environmental signals and transported into the stolons where the initiation of tuber development is carried out. Under SD conditions and cool temperatures, potato plants produce tubers, whereas they do not form tubers under LD conditions or high temperatures (Ewing and Struik 1992). Other factors such as plant hormones and several endogenous regulators have also been involved in potato tuberization (Xu *et al*. 1998a; Jackson 1999). Using the bioassay of a potato single-node stem segment culture, TAG has been identified in potato leaves as a tuber-inducing signal substance (Koda and Okazawa 1988; Koda *et al*. 1988; Yoshihara *et al*. 1989). TA which is the aglycone of TAG and JA exhibited similar activities on tuber induction to TAG (Yoshihara *et al*. 1989; Koda *et al*. 1991). In addition, several compounds isolated from the culture filtrates of the fungus *L. theobromae* showed strong microtuber inducing activity (Nakamori *et al*. 1994; Matsuura *et al*. 1998; Yang *et al*. 2000a, 2000b; Li *et al*. 2005). The generation of TAG, an endogenous tuber-inducing substance, is associated with a LA cascade. TAG is derived from JA, which can be synthesized from 13(*S*)-HPOT catalyzed by LOX as an initial enzyme in LA cascade (Siedow 1991). In the present review, the effects of LOX activity and LOX-derived metabolites on potato tuber induction in relation to growing temperature were presented.

A low temperature (15°C) was favorable for tuber induction of potato, while a relatively high temperature (20 and 25°C) was adaptable for tuber growth (Nam *et al*. 2005). LOX activity appeared at high level at the initial stage of potato tuberization and was stimulated by low growing temperatures of 15°C (Nam *et al*. 2005). This suggests that potato tuber induction is correlated with LOX activity depending on growing temperature. The enhanced LOX activity at a given temperature enhances both reactions of LA to 9(*S*)-HPOT and 13(*S*)-HPOT. Because neither 9(*S*)-HPOT nor 13(*S*)-HPOT is a final product in the LA cascade, they are transformed via a series of reactions to the next metabolites in each pathway. At this stage, the high level of 9(*S*)-HPOT may imply that the successive reactions are very slow and the low and constant level of 13(*S*)-HPOT suggests that the next reactions to JA, TA and TAG are very facile. As expected, the high endogenous levels of JA at low temperature were consistent with that of LOX protein (Nam *et al*. 2008). Also the endogenous levels of both TA and TAG showed a similar dependence on growing temperature, compared to that of JA (Nam *et al*. 2008). Therefore, it is proposed that the increase in LOX, which is activated by low temperature, results in large amounts of endogenous JA, TA and TAG, which have a crucial role in potato tuber induction.

Theobroxide, isolated from the culture filtrate of the fungus *L. theobromae*, has been proposed as a natural tuber-inducing compound in potato plants. Exogenously applied theobroxide strongly induced potato tuberization *in vitro* and *in vivo* under non-inductive photoperiod conditions (Nakamori *et al*. 1994; Yoshihara *et al*. 2000) and stimulated the activity of LOX and endogenous levels of JA and TA (Gao *et al*. 2003, 2005). Furthermore, theobroxide promoted the potato tuberization at low temperature, which is suitable for tuber induction, but it did not support the potato tuber growth step (unpublished data). Tubers were induced even under unsuitable temperatures, that is, 30°C by the treatments of theobroxide at higher concentration (unpublished data).

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