Journal of Plant Physiology **165** (2008) 233—238

Available online at www.sciencedirect.com

ScienceDirect

JOURNAL OF PLANT PHYSIOLOGY

www.elsevier.de/jplph

SHORT COMMUNICATION

# Temperature regulates tuber-inducing lipoxygenase-derived metabolites in potato (*Solanum tuberosum*)

**Kyong-Hee Nam, Fanjiang Kong, Hideyuki Matsuura, Kosaku Takahashi, Kensuke Nabeta, Teruhiko Yoshihara***

*Laboratory of Bio-organic Chemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kitaku, Sapporo 060-8589, Japan*

Received 19 January 2007; received in revised form 30 April 2007; accepted 30 April 2007

**KEYWORDS**
Hydroperoxy-linolenic acid;
Jasmonic acid;
Temperature;
Tuberonic acid;
Tuberonic acid glucoside

## Summary

Temperature is one of the major environmental factors affecting potato tuberization. It has been suggested that lipoxygenase (LOX) mediates between temperature and tuber induction. In this study, the contents of the LOX-derived metabolites hydroperoxylinolenic acid (HPOT), jasmonic acid (JA), tuberonic acid (TA) and tuberonic acid glucoside (TAG) were analyzed in leaves of potatoes growing at different temperatures. At low, tuber-inducing temperature, endogenous levels of JA, TA and TAG rise, indicating their crucial role in tuber induction. The concentration of 13(S)-HPOT seems not to be directly affected by temperature. Instead, the molecule has only a short half-life in leaves and is readily metabolized.


## Introduction

In 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 (Taylor et al., 1992). Temperature is one of the major environmental factors affecting potato tuberization. Cool temperatures are very favorable for tuber induction, while high temperatures exert negative influences (Ewing and Struik, 1992). It has been proposed that partitioning of assimilates is balanced

*Abbreviations:* AOC, allene oxide cyclase; AOS, allene oxide synthase; GC–SIM–MS, gas chromatography–selected ion monitoring–mass spectrometry; HPOT, hydroperoxylinolenic acid; JA, jasmonic acid; LD, long day; LOX, lipoxygenase; Me-JA, methyl jasmonate; SD, short day; TA, tuberonic acid; TAG, tuberonic acid glucoside

*Corresponding author. Present address: Asahikawa University, Women's Junior College, Japan.
Tel.: +81 11 706 2505; /fax: +81 11 706 2505.
*E-mail address:* yosihara@chem.agr.hokudai.ac.jp (T. Yoshihara).

0176-1617/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2007.04.003

by temperature, as the amount partitioned to tubers is decreased, while the amount to other parts of the plant is increased at high temperatures (Ewing and Struik, 1992). Besides temperature and other specific environmental cues, growth hormones and several other endogenous factors are also involved in the control of tuberization in potato (Xu et al., 1998; Jackson, 1999).

Many studies have reported that potato tuberization is closely correlated with metabolites of the linolenic acid cascade such as jasmonic acid (JA), methyl jasmonate (Me-JA) and tuberonic acid glucoside (TAG) (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). In plants, linoleic and linolenic acids are initially oxygenated to form 9(*S*)-hydroperoxylinolenic acid (HPOT) or 13(*S*)-HPOT by lipoxygenase (LOX) and then are 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 $\beta$-oxidative enzymes (Siedow, 1991). Next, JA is metabolized to tuberonic acid (TA) and finally converted into TAG, which has been identified as the endogenous tuber-inducing substance of potato (Yoshihara et al., 1989). Thus, it is very certain that 13-LOX-derived products are closely related to potato tuberization.

Theobroxide, isolated from the culture filtrate of the fungus *Lasiodiplodia theobromae*, has also been identified as a natural tuber-inducing compound in potato (Nakamori et al., 1994). It induces tuberization and flower bud formation in potato and morning glory (*Pharbitis nil*), respectively, under non-inductive photoperiods (Yoshihara et al., 2000). Moreover, theobroxide-treated potato and morning glory plants have strongly increased levels of endogenous JA and TA (Yang et al., 2004; Gao et al., 2005; Kong et al., 2005). However, salicylhydroxamic acid, a JA biosynthesis inhibitor, suppresses the inductive effect of theobroxide on potato tuber formation and reduces the endogenous contents of JA and TA (Gao et al., 2003). Theobroxide stimulates the activity of LOX, the key enzyme in JA biosynthesis in potato and morning glory plants (Gao et al., 2005; Kong et al., 2005).

In our previous report, the relationship between potato production and LOX activity was investigated as a function of growing temperature (Nam et al., 2005). The highest LOX activity was measured and the earliest tubers were induced under the low-temperature conditions of 15 °C, suggesting that potato tuber induction is correlated with LOX activity, which depends upon the growing temperature. Since LOX catalyzes the reaction from linolenic acid to HPOT in the linolenic acid cascade, it is very probable that the amounts of LOX-derived metabolites also depend on growing temperature. In a continuation of our previous study, in this report we investigated the effects of LOX-derived metabolites on potato tuber induction in relation to growing temperatures.

## Materials and methods

### Plant materials

Potatoes (*S. tuberosum* L.) were planted as previously described (Nam et al., 2005). The potato cultivar used in this study was Irish Cobbler. Stored tubers were germinated in complete darkness at 25 °C for 4 weeks, and then a cylinder (2-cm diameter × 3-cm long) with a single sprout for each tuber was obtained using a cork borer. The cylinders were kept in darkness for 2 days, until one layer of dry periderm had formed on the surface of the cylinders. Cylinders were planted in pots filled with a mixture of peatmoss and perlite (2:1, v/v) and grown under LD (18-h light/6-h dark) conditions in growth chambers (NK System, Biotron NC 350, Japan) equipped with 20 strip fluorescent lamps to provide white light (150 $\mu$mol m$^{-2}$ s$^{-1}$), at a temperature of 25 °C and relative humidity of 60% until temperature treatments. Plants were given tap water every 2 days and were fertilized with liquid Hyponex (500 ×, Hyponex Japan Co. Ltd) twice a week.

### Temperature treatments

Temperature treatments were carried out as previously described (Nam et al., 2005). Temperature treatments were applied over 2 weeks after plants had been grown at 25 °C under LD (18-h light/6-h dark) conditions. Potato plants were transferred to growth chambers controlled to temperatures of 15, 20, 25 and 30 °C under SD (10-h light/14-h dark) conditions.

### Determination of endogenous 9(*S*)- and 13(*S*)-hydroperoxylinolenic acids

The purification and quantification of HPOT were performed essentially as described by Göbel et al. (2002) with some modifications. About 1 g of frozen leaf tissue was added to 10 mL of extraction buffer (hexane/2-propanol, 3:2). (6Z,9Z,11E,13S)-3-Hydroxy-6,9,11-octadecatrienoic acid ($\gamma$-13-HOT) was added as an internal standard. The extract was centrifuged at 4500*g* at 4 °C for 10 min. The clear upper phase was collected and the pellet was extracted three times with 3 mL each of extraction medium. To the combined organic phases, a 6.7% (w/v) solution of potassium sulfate was added to a volume of 47 mL. After vigorous shaking, the upper organic layer was removed. The upper layer containing the oxylipin