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Mechanical wounding-induced laticifer differentiation in rubber tree: An indicative role of dehydration, hydrogen peroxide, and jasmonates

Wei-Min Tian*, Shu-Guang Yang, Min-Jing Shi, Shi-Xin Zhang, Ji-Lin Wu

Ministry of Agriculture Key Laboratory of Biology and Genetic Resources of Rubber Tree/State Key Laboratory Breeding Base of Cultivation and Physiology for Tropical Crops, Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, Hainan, 571737, PR China

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ABSTRACT

The secondary laticifer in the secondary phloem of rubber tree are a specific tissue differentiating from vascular cambia. The number of the secondary laticifers is closely related to the rubber productivity of *Hevea*. Factors involved in the mechanical wounding-induced laticifer differentiation were analyzed by using paraffin section, gas chromatography–mass spectrometry (GC–MS), and Northern-blot techniques. Dehydration of the wounded bark tissues triggered a burst of hydrogen peroxide, abscisic acid, and jasmonates and up-regulated the expression of *HbAOSa*, which was associated with the secondary laticifer differentiation strictly limited to the wounded area. Application of exogenous hydrogen peroxide, methyl jasmonate, and polyethylene glycol 6000 (PEG6000) could induce the secondary laticifer differentiation, respectively. Moreover, 6-Benzylaminopurine, a synthetic cytokinin, enhanced the methyl jasmonate-induced secondary laticifer differentiation. However, the dehydration-induced secondary laticifer differentiation was inhibited by exogenous abscisic acid. Diphenyleneiodonium chloride (DPI), a specific inhibitor of NADPH oxidase, was effective in inhibiting the accumulation of hydrogen peroxide as well as of jasmonates upon dehydration. It blocked the dehydration-induced but not the methyl jasmonate-induced secondary laticifer differentiation. The results suggested a stress signal pathway mediating the wound-induced secondary laticifer differentiation in rubber tree.

Introduction

Reactive oxygen species (ROS) are produced in plant cells primarily as a by-product of aerobic metabolism (Cho et al., 2009). ROS comprise hydrogen peroxide (H_2O_2), superoxide anion ($O^{2.-}$), hydroxyl radical ($HO^{\cdot}$), and singlet oxygen ($^1O_2$), and arise from such processes as photosynthetic electron transport, fatty acid β-oxidation and respiration in the mitochondria, chloroplasts, and peroxisomes (Asada, 2006; Rhoads et al., 2006; Miller et al., 2008). The level of ROS inside the cell is critically controlled by the relevant protective mechanisms using compartmentalized isozymes of catalase, superoxide dismutase, or peroxidase (Bolwell and Wojtaszek, 1997). Under stress conditions, such as mechanical wounding, herbivore or pathogen attack, a so-called oxidative burst—a rapid rise in ROS occurs (Sagi et al., 2004; Cho et al., 2009). In mammals, a membrane-associated NADPH oxidase is responsible for the respiratory oxidative burst in phagocytes (Cho et al., 2009). This multi-component enzyme uses electrons derived from intracellular NADPH to generate superoxide anion, which subsequently dismutates to more stable H_2O_2 derivative (Bokoch and Knaus, 2003). In plants, the respective enzymes for oxidative burst caused by pathogen attack or mechanical wounding are primarily ascribed to the plasma membrane-located NADPH oxidases, homologs of the mammalian gp^{91phox} respiratory burst NADPH oxidase subunit (Orozco-Cardenas et al., 2001; Torres et al., 2002; Sagi et al., 2004). ROS generated by plasma membrane-located NADPH oxidases also play an important role in regulating plant growth and development, including the growth of root hair and pollen tube (Foreman et al., 2003; Potocky et al., 2007), stomata closure (Kwak et al., 2003; Cho et al., 2009; Srivastava et al., 2009), lignification in differentiating xylem vessels (Barceló, 2005), seed after-ripening (Muller et al., 2009), and seed dormancy (Liu et al., 2010). In addition, exogenous H_2O_2 has a positive effect on the cotton fiber initiation (Zhang et al., 2010), and the H_2O_2 generated from extracellular peroxidases and CuZn-superoxide dismutase (SOD) is required for seed germination in *Pisum sativum* (Kranner et al., 2010) and for the development and lignification of the secondary walls of tracheary elements in the *Zinnia* cell-culture system (Karlsson et al., 2005). To our knowledge, there is no evidence to show the involvement of H_2O_2 in the regulation of specific tissue differentiation within vascular system.

* Corresponding author. Tel.: +86 898 23300309.
E-mail address: wmtian@163.com (W.-M. Tian).

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Jasmonate signaling plays a pivotal role in regulating responses of plants to wounding (Turner et al., 2002; Wasternack et al., 2006; Koo and Howe, 2009) and activating secondary metabolism (Blechert et al., 1995; Memelink et al., 2001; Devoto et al., 2005; Chen et al., 2006). It is also involved in the regulation of plant growth and development, including growth inhibition (Yan et al., 2007; Zhang and Turner, 2008), pollen maturation (Turner et al., 2002), stamen filament growth (Cheng et al., 2009), glandular trichome development (Li et al., 2004), tuberization, bulb formation, determination of plant structure, and thigmomorphogenesis (Koda, 1997). Lots of what we currently know about jasmonate signaling comes from studies on Arabidopsis and tomato (Turner et al., 2002; Chini et al., 2009; Fonseca et al., 2009; Yan et al., 2009). Little is known, however, about the role of jasmonates in regulating cell differentiation as well as the jasmonate signaling in woody plants, although available data show that both exogenous jasmonic acid and linolenic acid are effective in inducing the secondary laticifer differentiation in rubber tree (*Hevea brasiliensis* Muell. Arg.) (Hao and Wu, 2000), and the traumatic resin duct development are enhanced by exogenous methyl jasmonate in Norway spruce (*Picea abies* L. Karst) (Martin et al., 2002). The effect of methyl jasmonate on traumatic resin duct formation is mediated by ethylene (Hudgins and Franceschi, 2004).

A distinctive characteristic of woody plants is their sustainable secondary growth via the production of secondary vascular elements by vascular cambia (Matte Risopatron et al., 2010). The investigation of both primary and secondary vascular development has made good progress in recent years (Aloni, 2013; Lucas et al., 2013; Duval et al., 2014). A bark girdling system suitable for investigating plant vascular development and regeneration has been built up (Chen et al., 2014). The secondary laticifer cells in the secondary phloem of rubber tree are differentiated from the fusiform initials of vascular cambia (Hao and Wu, 2000). An alternative mechanical wounding system without bark girdling is more suitable for investigating the secondary laticifer cell differentiation in rubber tree, because the different fusiform initials of the existent vascular cambia differentiate into secondary laticifer cells in a synchronous manner and the wound-induced laticifer cells can be easily detected by histochemical staining (Wu et al., 2002; Tian et al., 2003). In the present study, the physiological cues mediating the wound-induced secondary laticifer differentiation were analyzed by using this experimental system.

Material and methods

Plant materials

Plants of rubber tree clone CATAS7-33-97 budded on rootstocks were grown on the Experimental Farm of Chinese Academy of Tropical Agricultural Sciences (CATAS) on Hainan Island. The plants were pruned each year and epicormic shoots grew from the latent buds on the pruned trunks. The epicormic shoot flushes five to six times a year and therefore consists of a series of foliage clusters, separated by lengths of leafless stem (Hao and Wu, 2000). Each of these morphologically distinct growth increments represents a growth flush, and is referred to as an extension unit (EU) (Hao and Wu, 2000). Although the primary laticifer is present in the leaves and bark of every EU, the secondary laticifer does not emerge in the stem of EU1–2 (counted from the top of the shoot) (Hao and Wu, 2000), which is convenient to distinguish the induced secondary laticifers in these parts. In the present study, the stem of EU1 of one-year-old epicormic shoots is treated as follows.

Experimental design

For the secondary laticifer differentiation induced by dehydration, the epidermis, and outer parts of cortex of 0.2 cm × 1 cm area of stem were scratched with a razor blade at the site 1 cm below the lowest foliage leaf of EU1. The wounded surfaces of each of five epicormic shoots were (1) directly exposed to air, (2) wrapped immediately with parafilm membrane, and (3) applied with PEG6000 powder, and then wrapped immediately with parafilm membrane, respectively. Samples (including parts of xylem) were collected by peeling with a razor blade 10 days after treatments and fixed in 80% ethanol for paraffin section. To see if the dehydration-induced secondary laticifers could be detected earlier, samples were also collected five days after treatment.

For the effect of dehydration on the level of endogenous H_2O_2, ABA, JA, and AOS expression, two batches of epicormic shoots were chosen for treatment of either direct exposure of the wounded surface to the air or being wrapped immediately with parafilm membrane after mechanical wounding, respectively. The wounded area was of 8 cm × 1 cm instead of 0.2 cm × 1 cm so as to collect enough samples for these purposes. Each batch consisted of five epicormic shoots for every time interval as indicated in corresponding figures.

For the effect of exogenous H_2O_2, ABA, methyl jasmonate, and 6-Benzylaminopurine (BAP) on secondary laticifer differentiation, 0.2 M H_2O_2, 0.53% ABA, 2 mM methyl jasmonate, 0.9% 6-Benzylaminopurine (BAP), and as control, 0.1 M PBS buffer were applied on the wounded surfaces immediately after mechanical wounding and wrapped with parafilm membrane, respectively. Five epicormic shoots were chosen for each treatment. Samples (including parts of xylem) were collected 10 days after treatment by peeling with a razor blade and fixed in 80% ethanol for paraffin section.

For the inhibition of ABA and DPI on secondary laticifer differentiation, we developed an experimental system which was suitable for pharmacological analysis of the inhibiting effect of chemicals on laticifer differentiation. In this experimental system, the wounded area was immediately applied with chemicals and wrapped with parafilm membrane for 2 h, then exposed to the air for 2 h, and followed by chemical application and wrapping again until sampling. With the experimental system, 0.53% ABA, 0.4 mM DPI, and as control, 0.1 M PBS were respectively applied to the wounded sites of each of five epicormic shoots. Samples (including parts of xylem) were collected 10 days after treatment by peeling with a razor blade and fixed in 80% ethanol for paraffin section.

Light microscopy

To eliminate tannin-like substances which may be mistaken for the rubber inclusions in laticifer cells, samples were fixed in 80% ethanol for 24 h at room temperature, and then treated with iodine and bromine in glacial acetic acid (Shi and Hu, 1965), and embedded in paraffin after dehydration. Sections (thickness 20 μm) were cut with a microtome and stained with fast green (0.5 g in 100 ml of 95% ethanol). The laticifer cells in sections could be traced without any staining on the basis of rubber inclusions in brown color under a Leica DMLB microscope (Leica, Wetzlar, Germany). Staining with fast green was done just to strengthen the image contrast.

Preparation of total RNA

Bark samples were collected and stored at −80 °C until they were used to extract total RNA. Samples were ground to a fine powder in liquid nitrogen using a mortar and pestle. Then, 1.5 g powder was mixed with 15 mL extraction buffer (133 mM Trisbase, 66 mM EDTA, 666 mM NaCl, 13.3 mM dithiothreitol (DTT), 2.7% (w/v)