

RESEARCH ARTICLE

The secondary laticifer differentiation in rubber tree is induced by trichostatin A, an inhibitor of histone acetylation

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Abstract The secondary laticifer, a specific tissue in the secondary phloem of rubber tree, is differentiated from the vascular cambia. The number of the secondary laticifer in the trunk bark of rubber tree is positively correlated with rubber yield. Although jasmonates have been demonstrated to be crucial in the regulation of secondary laticifer differentiation, the mechanism for the jasmonate-induced secondary laticifer differentiation remains to be elucidated. By using an experimental morphological technique, the present study revealed that trichostatin A (TSA), an inhibitor of histone deacetylation, could induce the secondary laticifer differentiation in a concentration-dependent manner. The results suggest that histone acetylation is essential for the secondary laticifer differentiation in rubber tree.

Keywords *Hevea brasiliensis*, histone acetylation, laticifer differentiation, trichostatin, vascular cambia

effective than jasmonate acid and methyl jasmonate (MeJA) in inducing the secondary laticifer differentiation^[6]. The mechanical wounding-induced secondary laticifer differentiation is limited to the wounded area and depends on endogenous jasmonates which acts downstream of reactive oxygen species caused by dehydration of the wounded tissues^[7–9]. In addition, the induction of secondary laticifer differentiation induced by both the mechanical wounding and methyl jasmonate was influenced by season, which is associated with the content of endogenous cytokinin and the activity of vascular cambia^[10]. Here, we reported the effect of trichostatin A (TSA), an inhibitor of histone deacetylation^[11], on induction of the secondary laticifer differentiation, which gives a new clue for understanding the regulation of the secondary laticifer differentiation in rubber tree.

1 Introduction

Rubber tree (*Hevea brasiliensis*) is the main source of natural rubber in the world^[1]. The rubber biosynthesis occurs uniquely in laticifer, a specific tissue^[2,3]. The number of the secondary laticifer in the trunk bark of rubber tree is positively correlated to the rubber yield. The secondary laticifer is consist of numerous laticifer cells that are differentiated from the fusiform initials of vascular cambia^[4]. The secondary laticifer differentiation is induced by such factors as jasmonic acid (JA)^[4], mechanical wounding^[5] and coronatine (COR)^[6], an active jasmonate homolog^[6]. The coronatine is more

2 Materials and methods

2.1 Plant materials

Rubber tree clone CATAS7-33-97 budded on rootstocks were grown at the Experimental Station of the Rubber Research Institute (RRI) of the Chinese Academy of Tropical Agricultural Sciences (CATAS), Danzhou, Hainan Province, China. The plants were pruned each year and epicormic shoots grew from the latent buds on the pruned branches. Each epicormic shoot flushed five to six times a year and includes a series of foliage clusters which were classified by the length of stem. Therefore, each of these morphologically distinct growth increments represents a growth flush and is referred to as an extension unit (EU)^[4]. Under natural condition, none of the secondary laticifer appear in the stem of EU1 and EU2 (counted from the top of the shoot)^[4,5,10], which is convenient for distinguishing the induced secondary laticifer.

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2.2 Treatments

In June, when the epicormic shoots had produced more than two extension units, the epidermis and outer parts of cortex of $0.5\text{ cm} \times 1\text{ cm}$ area of stem were scratched with a razor blade at the site 1 cm below the lowest foliage leaf of EU1. The wounded surfaces were (1) directly exposed to air (mechanical wounding), (2) applied with sterile water or 5% dimethyl sulfoxide (DMSO) and wrapped immediately with parafilm, (3) applied with 0.07% methyl jasmonate and wrapped immediately with parafilm, (4) applied with a gradient concentration of coronatine and wrapped immediately with parafilm, and (5) applied with a gradient concentration of trichostatin A and wrapped immediately with parafilm. Coronatine was purchased from Sigma (St. Louis, MO, USA) and trichostatin A was purchased from Selleck (Houston, TX, USA). Samples were collected seven days after treatments. Five replicates were conducted for each treatment. To test the effect of trichostatin A on the secondary laticifer differentiation, the treatments applied were water (negative control) as well as coronatine, mechanical wounding and methyl jasmonate (positive control).

2.3 Light microscopy

To eliminate tannin-like substances, which can be mistaken for the rubber inclusion in laticifer, bark samples were fixed in 80% ethanol for 24 h at room temperature, treated with iodine and bromine in glacial acetic acid, and embedded in paraffin after dehydration. Sections ($12\text{ }\mu\text{m}$ in thickness) were cut with a microtome and stained with fast green. Laticifer in sections could be recognized since rubber in the laticifer was in brown color due to the iodine-bromine treatment. Sections of the bark samples were made for examination under a light microscope (Leica DMLB, Leica Microsystems Wetzlar GmbH, Wetzlar, Germany) and the phloem tissues in bark cross sections were measured.

2.4 Statistics

Data collection from five sections of independent shoots was referred to Hao and Wu^[4]. Significant deviation analysis was performed by Duncan's test ($P < 0.05$).

3 Results

There was no effect of water on induction of the secondary laticifer differentiation 7 d after treatment (Fig. 1a; Table 1). But one line of the secondary laticifer was induced in the secondary phloem of the stem by mechanical wounding (Fig. 1b) or 0.07% methyl jasmonate (Fig. 1c). Although no secondary laticifer was detected in the stem treated with $100\text{ nmol}\cdot\text{L}^{-1}$ coronatine

(Fig. 1d), few of the secondary laticifer cells (0.092 lines) appeared near the vascular cambia in the secondary phloem when the coronatine concentration increased to $1\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ for 7 d (Fig. 1e). The number of the secondary laticifer cells increased noticeably (0.234 lines) when the concentration increased to $2.5\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ (Fig. 1f; Table 1). The secondary laticifer cells exhibit as a continuous line in the secondary phloem of stem bark applied with $10\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ coronatine (Fig. 1g). There was no significant difference in the number of secondary laticifers between the treatments of 10 and $20\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ coronatine (Fig. 1h; Table 1).

Under the conditions when all the mechanical wounding, jasmonates and coronatine could induce the secondary laticifer differentiation, we found the trichostatin A (TSA), a histone deacetylation inhibitor, had also effect on inducing the secondary laticifer differentiation. Similar to the coronatine effect, TSA induced the secondary laticifer differentiation in a concentration-dependent manner. As the solvent of TSA, 5% dimethyl sulfoxide had no effect on inducing the secondary laticifer differentiation (Fig. 2a). Same was for $1\text{ nmol}\cdot\text{L}^{-1}$ trichostatin A (Fig. 2b). There appeared several laticifer cells upon treatment with $10\text{ nmol}\cdot\text{L}^{-1}$ TSA (Fig. 2c), although they were somewhat difficult to be detected. When TSA concentration was increased to $100\text{ nmol}\cdot\text{L}^{-1}$, the number of the secondary laticifer cells increased obviously (Fig. 2d; Table 1). Even more the secondary laticifer cells were detected upon treatment with $1\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ TSA (Fig. 2e; Table 1). However, there was no difference in the number of the secondary laticifers between treatments of $1\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ and $10\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ trichostatin A (Fig. 2f; Table 1).

4 Discussion

The effect of linolenic acid and mechanical wounding on inducing the secondary laticifer differentiation may be ascribed to the increased level of endogenous jasmonates^[7]. Although the jasmonic acid and methyl jasmonate are not the active form, (+)-7-iso-jasmonoyl-L-isoleucine^[8], they are effective in inducing the secondary laticifer differentiation^[4,10]. The coronatine could structurally and functionally mimic the active form of jasmonates^[6]. It bonds to COI1, the receptor of active jasmonate, and activates jasmonate signaling^[12]. With respect to the applied concentration, coronatine is more effective than jasmonic acid^[9,11,13–15] and methyl jasmonate^[16] while less effective than TSA in inducing the secondary laticifer differentiation.

Changes in epigenetic modification are involved in regulating the active state of tumor suppressor genes and proto-oncogenes^[17]. Therefore, tumor is considered to be a kind of cellular epigenetic disease^[18–20]. Such histone deacetylation inhibitors as *O*-benzylhydroxylamine

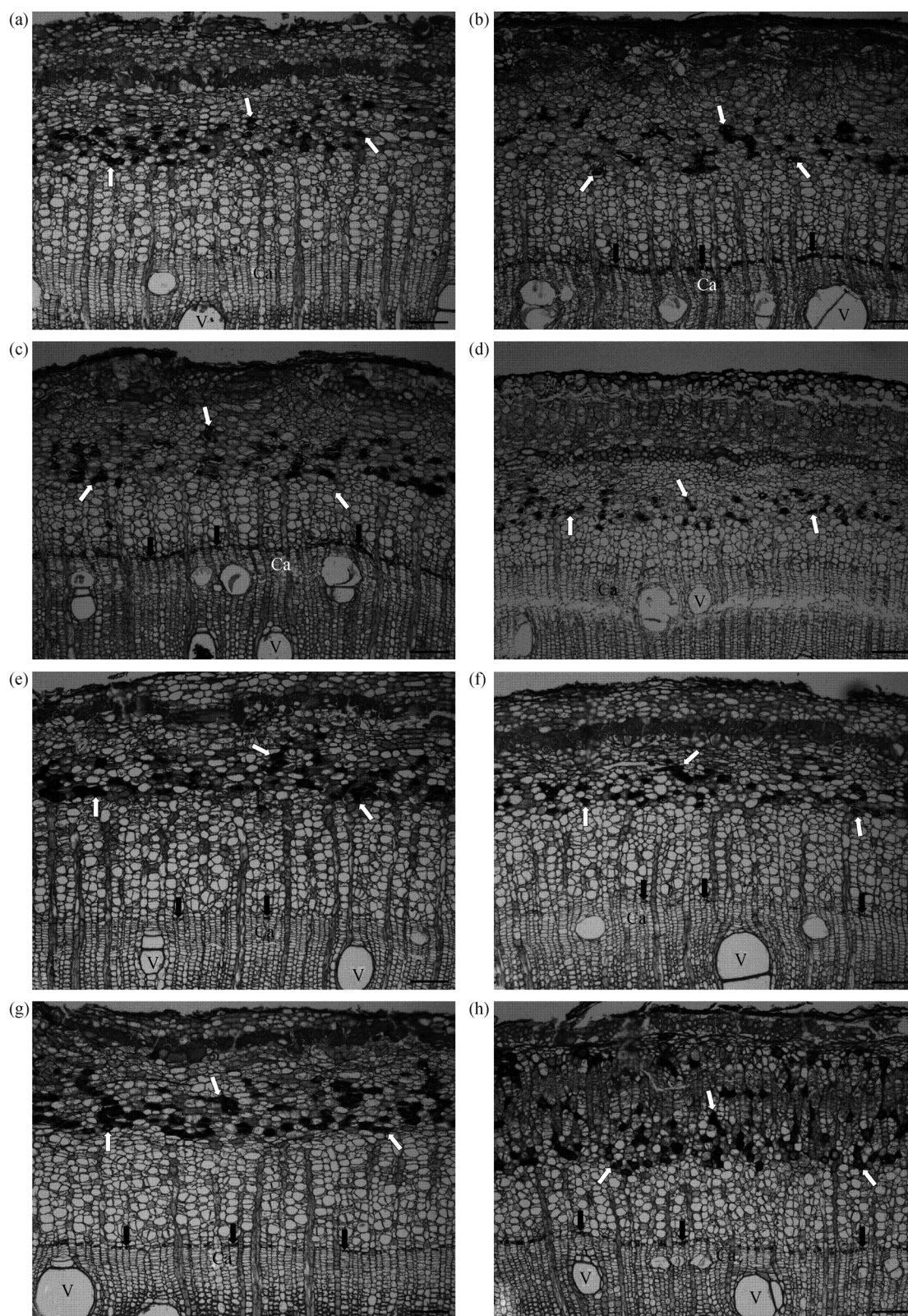


Fig. 1 Light microscopic photos of the bark cross sections. The stem of epicormic shoots was respectively treated with water (a), mechanic wounding (b), 0.07% methyl jasmonate (c) and coronatine at different concentrations (d–h). White arrow, the primary laticifers; black arrow, the secondary laticifers; Ca, cambium; ST, sieve tube; V, vessel. Scale bar = 100 μm.

Table 1 Statistics of the secondary laticifer cells induced by different factors

Representative photo in Fig. 1			Representative photo in Fig. 2		
No.	Treatment	Number of laticifer cells	No.	Treatment	Number of laticifer cells
a	Water	0 ^d	a	5% DMSO	0 ^d
b	Wounding	1.005±0.005 ^a	b	1 nmol·L ⁻¹ TSA	0 ^d
c	0.07% MeJA	0.988±0.005 ^a	c	10 nmol·L ⁻¹ TSA	0.032±0.003 ^c
d	100 nmol·L ⁻¹ COR	0 ^d	d	100 nmol·L ⁻¹ TSA	0.32±0.006 ^b
e	1 μmol·L ⁻¹ COR	0.092±0.002 ^c	e	1 μmol·L ⁻¹ TSA	0.983±0.005 ^a
f	2.5 μmol·L ⁻¹ COR	0.234±0.006 ^b	f	10 μmol·L ⁻¹ TSA	0.999±0.005 ^a
g	10 μmol·L ⁻¹ COR	0.996±0.006 ^a			
h	20 μmol·L ⁻¹ COR	0.995±0.005 ^a			

Note: Data are means±SE of the number of laticifer cells in five sections from independent shoots. Letters within the “No.” column stand for different figures while letters within the “Number of laticifer cells” column indicate statistically significant differences ($P < 0.05$).

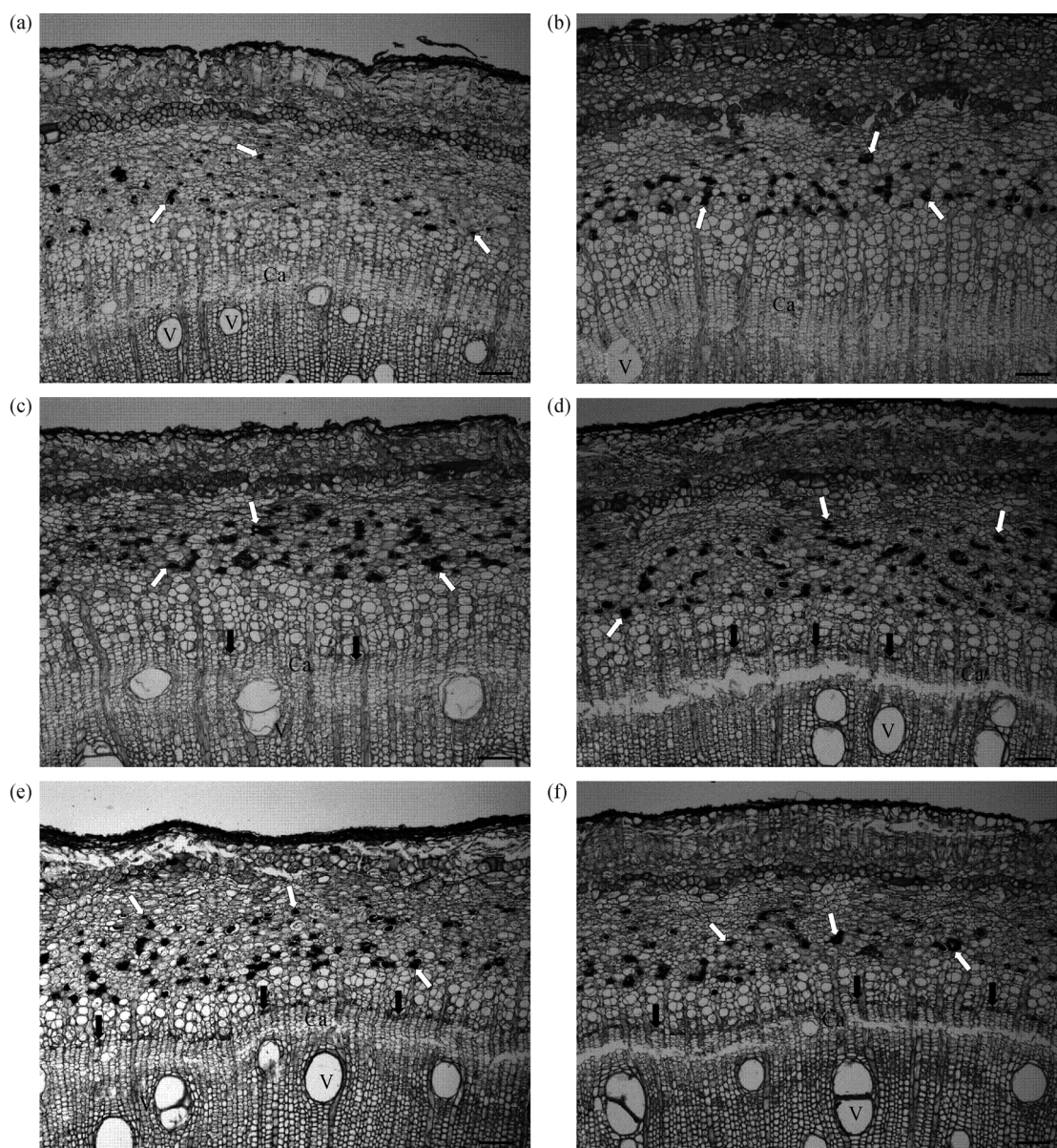


Fig. 2 Light microscopic photos of the bark cross sections. The stem of epicormic shoots was respectively treated with 5% dimethyl sulfoxide (a) and TSA at different concentrations (b–f). White arrow, the primary laticifers; black arrow, the secondary laticifers; Ca, cambium; ST, sieve tube; V, vessel. Scale bar = 100 μm.

hydrochloride (trichostatin A), benzhydroxamic acid derivative (SAHA), cyclic depsipeptide, benzamide derivatives (MS-275 and CI-994) and fatty acid (valproic acid and phenylbutyric acid) can inhibit the cell proliferation or activate cell apoptosis in the case of hepatocellular carcinoma^[21], esophageal squamous cell carcinoma^[22], colon mucosa cancer^[23]. So, the histone deacetylation inhibitor (HDACi)^[24,25] as well as DNA methyltransferase inhibitors (DNMTs)^[26] may serve as a new way of “epigenetic therapy.” In the present study, TSA is effective in inducing the secondary laticifer differentiation in rubber tree, suggesting that inhibition of cell proliferation may result in activation of cell differentiation.

Post-translational modifications of histones are involved in modulating dynamic changes in chromatin structure and gene activity^[27]. Histone acetylation is generally associated with transcription activation, whereas histone deacetylation results in transcription repression^[12,15]. The dynamic changes in deacetylation and acetylation of histones are modulated by histone deacetylase (HDA) and histone acetyltransferase^[28]. By modulating the histone acetylation or directly interacting with target proteins, the HDAs have pleiotropic effects on regulating plant growth and development^[16–19], abiotic stress responses^[18,20,26] and photomorphogenesis^[24]. The TSA-induced secondary laticifer differentiation suggests that HDA(s) may negatively regulate the jasmonate signaling. It is well known that Jasmonate ZIM-domain(JAZ) proteins act as repressors of jasmonate signaling^[29]. Available data show that JAZ proteins (JAZ1, JAZ3, JAZ9) recruit HDA6 as a corepressor to modulate histone acetylation and inhibit jasmonate signaling^[30]. In addition, the HDA6 also interacts with COI1 in *Arabidopsis*^[31]. On the other hand, the HDA6 seems to be required for the expression of jasmonate responsive genes in *Arabidopsis* HDA6 mutant, *axe1-5*, and HDA6 RNA-interfering plants^[25].

5 Conclusions

Histone acetylation may be associated with jasmonate signaling in regulating the secondary laticifer differentiation. Elucidating their relationship will deepen our knowledge about the secondary laticifer differentiation in rubber tree.

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Compliance with ethics guidelines Shixin Zhang, Shaohua Wu, and Weimin Tian declare that they have no conflict of interest or financial conflicts to disclose.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Lau N S, Makita Y, Kawashima M, Taylor T D, Kondo S, Othman A S, Shu-Chien A C, Matsui M. The rubber tree genome shows expansion of gene family associated with rubber biosynthesis. *Scientific Reports*, 2016, **6**: 28594
2. Gomez J. Anatomy of hevea and its influence on latex production. *Malaysian Rubber Research & Development Board Monography*, 1982
3. Dooner H, Kermicle J. Displaced and tandem duplications in the long arm of chromosome 10 in maize. *Genetics*, 1976, **82**(2): 309–322
4. Hao B Z, Wu J L. Laticifer differentiation in *Hevea brasiliensis*: induction by exogenous jasmonic acid and linolenic acid. *Annals of Botany*, 2000, **85**(1): 37–43
5. Tian W M, Shi M J, Yu F Y, Wu J L, Hao B Z, Cui K M. Localized effects of mechanical wounding and exogenous jasmonic acid on the induction of secondary laticifer differentiation in relation to the distribution of jasmonic acid in *Hevea brasiliensis*. *Acta Botanica Sinica*, 2003, **45**(11): 1366–1372
6. Ichihara A, Shiraishi K, Sato H, Sakamura S, Nishiyama K, Sakai R, Furusaka A, Matsumoto T. The structure of coronatine. *Journal of the American Chemical Society*, 1977, **99**(2): 636–637
7. Tian W M, Yang S G, Shi M J, Zhang S X, Wu J L. Mechanical wounding-induced laticifer differentiation in rubber tree: an indicative role of dehydration, hydrogen peroxide, and jasmonates. *Journal of Plant Physiology*, 2015, **182**: 95–103
8. Fonseca S, Chico J M, Solano R. The jasmonate pathway: the ligand, the receptor and the core signalling module. *Current Opinion in Plant Biology*, 2009, **12**(5): 539–547
9. Benedetti C E, Xie D, Turner J G. COI1-dependent expression of an *Arabidopsis* vegetative storage protein in flowers and siliques and in response to coronatine or methyl jasmonate. *Plant Physiology*, 1995, **109**(2): 567–572
10. Zhang S X, Tian W M. Cross talk between cytokinin and jasmonates in regulating the secondary laticifer differentiation in rubber tree (*Hevea brasiliensis* Muell. Arg.). *Journal of Rubber Research*, 2015, **18**(1): 38–48
11. Wasternack C, Xie D. The genuine ligand of a jasmonic acid receptor: improved analysis of jasmonates is now required. *Plant Signaling & Behavior*, 2010, **5**(4): 337–340
12. Katsir L, Schilmiller A L, Staswick P E, He S Y, Howe G A. COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proceedings of the National Academy of Sciences of the United States of America*, 2008, **105**(19): 7100–7105
13. Fonseca S, Chini A, Hamberg M, Adie B, Porzel A, Kramell R, Miersch O, Wasternack C, Solano R. (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. *Nature Chemical Biology*, 2009, **5**(5): 344–350
14. Wasternack C, Hause B. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *Annals of Botany*. *Annals of Botany*, 2013, **111**(6): 1021–1058
15. Geng X, Jin L, Shimada M, Kim M G, Mackey D. The phytotoxin coronatine is a multifunctional component of the virulence armament of *Pseudomonas syringae*. *Planta*, 2014, **240**(6): 1149–

- 1165
16. Zhang S, Liu S, Tian W. Effect of vascular cambia activity on secondary laticifer differentiation induced by mechanical wounding in rubber tree (*Hevea brasiliensis* Muell. Arg.). *Chinese Journal of Tropical Crops*, 2011, **32**(6): 1037–1041 (in Chinese)
17. Kinzler K W, Vogelstein B. Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature*, 1997, **386**(6627): 761–763
18. Bartlett T E, Chindera K, McDermott J, Breeze C E, Cooke W R, Jones A, Reisel D, Karegodar S T, Arora R, Beck S, Menon U, Dubeau L, Widschwendter M. Epigenetic reprogramming of fallopian tube fimbriae in BRCA mutation carriers defines early ovarian cancer evolution. *Nature Communications*, 2016, **7**: 11620
19. Peitzsch C, Cojoc M, Hein L, Kurth I, Mabert K, Trautmann F, Klink B, Schrock E, Wirth M P, Krause M, Stakhovsky E A, Telegeev G D, Novotny V, Toma M, Muders M, Baretton G B, Frame F M, Maitland N J, Baumann M, Dubrovskaya A. An epigenetic reprogramming strategy to resensitize radioresistant prostate cancer cells. *Cancer Research*, 2016, **76**(9): 2637–2651
20. Suva M L, Riggi N, Bernstein B E. Epigenetic reprogramming in cancer. *Science*, 2013, **339**(6127): 1567–1570
21. Buurman R, Sandbothe M, Schlegelberger B, Skawran B. HDAC inhibition activates the apoptosome via Apaf1 upregulation in hepatocellular carcinoma. *European Journal of Medical Research*, 2016, **21**(1): 26
22. Heim C, Binder E B. Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics. *Experimental Neurology*, 2012, **233**(1): 102–111
23. Wilson A J, Byun D S, Popova N, Murray L B, L'Italien K, Sowa Y, Arango D, Velcich A, Augenlicht L H, Mariadason J M. Histone deacetylase 3 (HDAC3) and other class I HDACs regulate colon cell maturation and p21 expression and are deregulated in human colon cancer. *Journal of Biological Chemistry*, 2006, **281**(19): 13548–13558
24. Chistiakov D A, Myasoedova V A, Orekhov A N, Bobryshev Y V. Epigenetically active drugs inhibiting DNA methylation and histone deacetylation. *Current Pharmaceutical Design*, 2016, **22**(999): 1
25. Wu K, Zhang L, Zhou C, Yu C W, Chaikam V. HDA6 is required for jasmonate response, senescence and flowering in *Arabidopsis*. *Journal of Experimental Botany*, 2008, **59**(2): 225–234
26. Hrgovic I, Doll M, Kleemann J, Wang X F, Zoeller N, Pinter A, Kippenberger S, Kaufmann R, Meissner M. The histone deacetylase inhibitor trichostatin A decreases lymphangiogenesis by inducing apoptosis and cell cycle arrest via p21-dependent pathways. *BMC Cancer*, 2016, **16**(1): 763
27. Almeida V R, Vieira I A, Buendia M, Brunetto A T, Gregianin L J, Brunetto A L, Klamt F, de Farias C B, Abujamra A L, Lopez P L, Roesler R. Combined treatments with a retinoid receptor agonist and epigenetic modulators in human neuroblastoma cells. *Molecular Neurobiology*, 2016: 1–10
28. Jenuwein T, Allis C D. Translating the histone code. *Science*, 2001, **293**(5532): 1074–1080
29. Wager A, Browse J. Social Network: JAZ protein interactions expand our knowledge of jasmonate signaling. *Frontiers in Plant Science*, 2012, **3**: 41
30. Zhu Z, An F, Feng Y, Li P, Xue L, A M, Jiang Z, Kim J M, To T K, Li W, Zhang X, Yu Q, Dong Z, Chen W Q, Seki M, Zhou J M, Guo H, A M, Jiang Z, Kim J M, To T K, Li W, Zhang X, Yu Q, Dong Z, Chen W Q, Seki M, Zhou J M, Guo H. Derepression of ethylene-stabilized transcription factors (EIN3/EIL1) mediates jasmonate and ethylene signaling synergy in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, 2011, **108**(30): 12539–12544
31. Devoto A, Nieto-Rostro M, Xie D, Ellis C, Harmston R, Patrick E, Davis J, Sherratt L, Coleman M, Turner J G. COI1 links jasmonate signalling and fertility to the SCF ubiquitin-ligase complex in *Arabidopsis*. *Plant Journal*, 2002, **32**(4): 457–466