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Gossypol: phytoalexin of cotton

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Sesquiterpenoids are a class of 15-carbon secondary metabolites that play diverse roles in plant adaptation to environment. Cotton plants accumulate a large amount of sesquiterpene aldehydes (including gossypol) as phytoalexins against pathogens and herbivores. They are stored in pigment glands of aerial organs and in epidermal layers of roots. Several enzymes of gossypol biosynthesis pathway have been characterized, including 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) and farnesyl diphosphate synthase (FPS) that catalyze the formation of the precursor farnesyl diphosphate (FPP), (+)-δ-cadinene synthase (CDN) which is the first enzyme committed to gossypol biosynthesis, and the downstream enzymes of CYP706B1 and methyltransferase. Expressions of these genes are tightly regulated during cotton plants development and induced by jasmonate and fungi elicitors. The transcription factor GaWRKY1 has been shown to be involved in gossypol pathway regulation. Recent development of new genomic platforms and methods and releases of diploid and tetraploid cotton genome sequences will greatly facilitate the elucidation of gossypol biosynthetic pathway and its regulation.

cotton, secondary metabolism, gossypol, sesquiterpenoid

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INTRODUCTION

Plant produces a wealth of specialized compounds, or secondary metabolites, which are heavily involved in mediating plant interaction with biotic and abiotic factors and plant adaptation to the changing environments (Fang et al., 2011). Of these, terpenoids constitute the largest group and play diverse roles in plant growth and development, as well as protecting plants from herbivores and pathogens (Tholl, 2015). Many of the terpenoids are also of high value in their pharmacological activities or industrial applications, such as artemisinin (a sesquiterpene lactone) in the treatment of malaria (Paddon and Keasling, 2014) and taxol (a diterpene alkaloid) in the treatment of a number of types of cancer (Guchelaar et al., 1994). Cotton (*Gossypium*. spp.), one of the most important economic crops and the major source of natural fiber for textile industry, accumulates a large amount of sesquiterpenoids as phytoalexins, of which the most known is gossypol.

SESQUITERPENOIDS IN COTTON

Among the sesquiterpenes produced by plant, some are released together with other volatiles and contribute to the scent or as odor signals to attract insects (Muhlemann et al., 2014), whereas others are modified by enzymes such as

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P450 monooxygenase, alcohol reductase, methyltransferase and glycosyltransferase, and then stored (Chen et al., 2011). Cotton plants produce both volatile and non-volatile sesquiterpenoids. The former includes β-caryophyllene, α-humulene and guaia-1(10),11-diene (Yang et al., 2013), whereas the later comprises desoxyhemigossypol (dHG), hemigossypol (HG), gossypol (G), hemigossypolone (HGQ), heliocides H_1, H_2, H_3 and H_4 (Bell et al., 1978), as well as 6-methoxy and 6,6′-dimethoxy derivatives (Bell et al., 1975; Dowd and Pelitire, 2006; Stipanovic et al., 1975), which have the same core skeleton of (+)-δ-cadinene and share common upstream synthetic steps (Figure 1 A).

The gossypol molecular possesses a chiral axis due to the restricted rotation around the binaphthyl, leading to the two enantiomers of (+)-gossypol and (−)-gossypol (Veech et al., 1976). In *Gossypium* species, both (+)-gossypol and (−)-gossypol widely exist, but at different ratios. A previous analysis of the contents and ratios of (+)/(−)-gossypol in 28 wild species and three domesticated species showed that (−)-gossypol took a major part in *G. darwinii*, *G. sturtianum*, *G. areysianum*, *G. longicalyx*, *G. harknessii* and *G. costulatum*, whereas in *G. hirsutum*, *G. anomalum*, *G. mustelinum*, *G. gossypioides* and *G. capitisviridis*. (+)-gossypol is the dominant form, which could make up to 97% in *G. hirsutum* var. *mariegalante* (Stipanovic et al., 2005). Moreover, the content and ratio also vary in tissues. For example, in flowers and roots of *G. barbadense* and *G. hirsutum*, there are more (+)-gossypol, whereas in seeds, (−)-gossypol ratio is higher (Cass et al., 2004; Stipanovic et al., 2006b).

There is considerable natural variation in contents of gossypol and related sesquiterpenoids among tissues and developmental stages of *Gossypium* species, ranging from 0.4% to 2% (Cai et al., 2004). In most cultivars, pericarps and mature seeds have relatively higher contents of up to 2%, whereas leaves have moderate and young tissues, such as hypocotyls, have the lowest (0.05%) (Cai et al., 2004). Such contents are also variable with growing stages and environmental factors, e.g. young leaves usually have

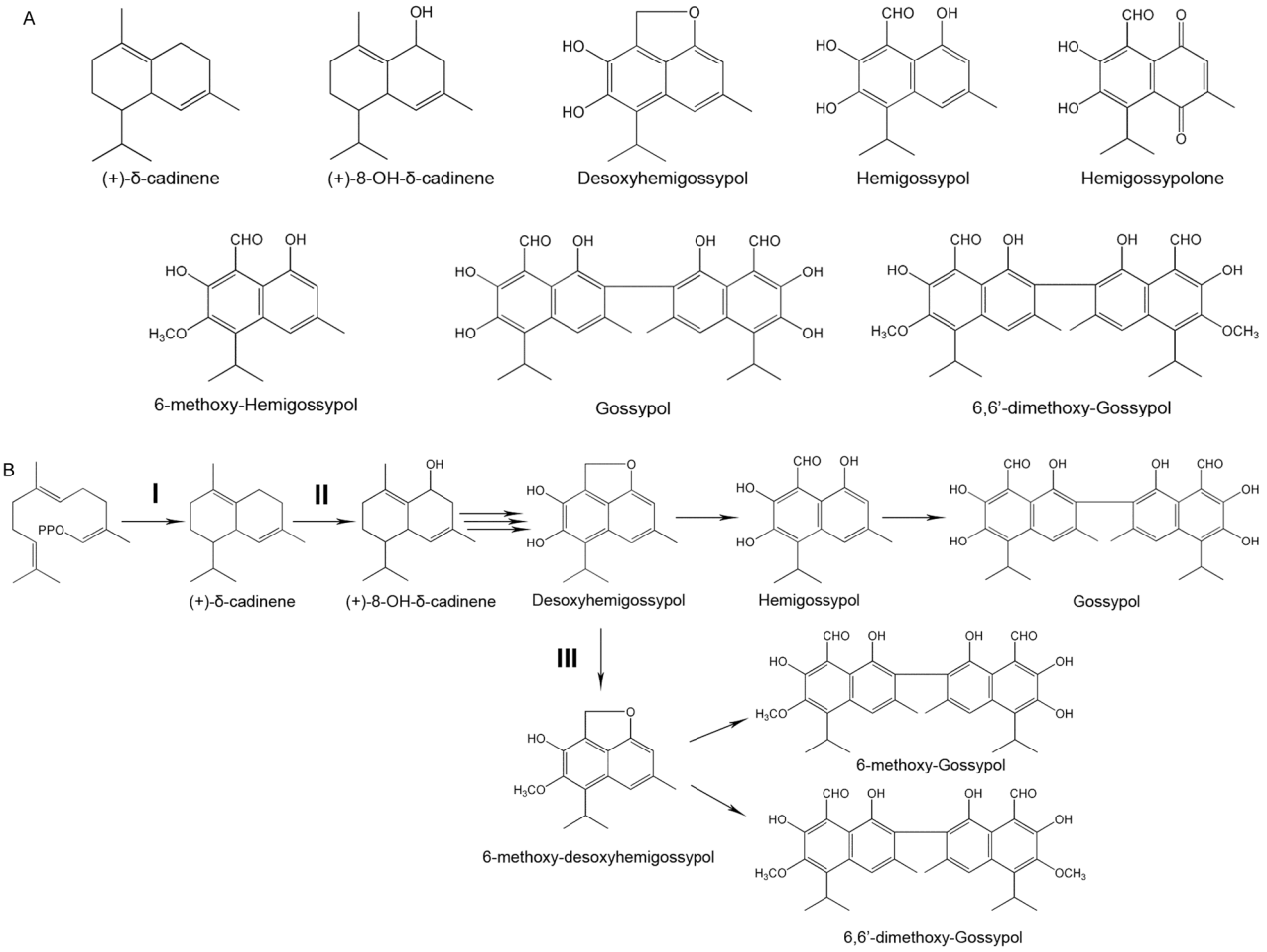

Figure 1 Structures and biosynthetic pathway of cadinene-type phytoalexins in cotton. A, Chemical structures of cadinene-type phytoalexins detected in cotton plants. B, Biosynthetic pathway of gossypol and derivates. The precursor farnesyl diphosphate (FPP) is cyclized into (+)-δ-cadinene by (+)-δ-cadinene synthase (I), and then hydroxylated into 8-hydroxy-(+)-δ-cadinene by CYP706B1 (II). After multiple hydroxylation and oxidation steps, it is converted to hemigossypol and then coupled into gossypol. The desoxyhemigossypol can also be converted to 6-methoxy-desoxyhemigossypol by desoxyhemigossypol-6-O-Methyltransferase (III) for the biosynthesis of 6-methoxy-gossypol and 6,6′-dimethoxy-gossypol.

higher levels of the sesquiterpenoids than old leaves; mechanical wounding, pathogen infection or herbivore insects ingestion can significantly increase the tissue phytoalexin levels (Heinstein, 1985).

GOSSYPOL IN PLANT-ENVIRONMENT INTERACTIONS

Insects like cotton bollworm (*Helicoverpa armigera*), aphids (*Aphis gossypii*) and lygus (*Lygus lucorum*), and pathogens of *Xanthomonas campestris*, *Aspergillus flavus* and *Verticillium dahliae*, cause fiber yield losses up to more than 30% (Chattannavar et al., 2010). Gossypol and derivates play important roles in defense against the pests and pathogens due to their cytotoxicity (Abraham et al., 1999; Liu et al., 1999b). For example, gossypol can inhibit *V. dahliae* spore germination at a concentration as low as 25 μmol L^{-1}, and inhibit growth of *Rhizopusv nigricans* at 100 μmol L^{-1} (Turco et al., 2007). Hemigossypolone shows inhibitory activities against *A. flavus* (Mellon et al., 2011), and the effects of decreasing the larval weights and moth eclosion rates on cotton bollworm and beet armyworm (*Spodoptera exigua*) have also been reported (Kong et al., 2010; Stipanovic et al., 2006a, 2008). Enantiomers of gossypol have different performances of these activities. Generally, (−)-gossypol is more active than (+)-gossypol in pathogen resistance, but both are equivalent toward insects. The (−)-gossypol was found to be four times more active than (+)-gossypol in its inhibitory effect over the initial growth of *A. flavus* (Mellon et al., 2011), while both enantiomers could significantly lengthen days-to-pupation and decrease pupal weight of *H. virescens* and *Helicoverpa zea* (Stipanovic et al., 2008; Stipanovic et al., 2006b). Moreover, some artificially modified gossypol derivates were found to have anti-microbe, anti-cancer and anti-virus activities in recent years (Przybylski et al., 2009; Yin et al., 2011). Of these, the aromatic gossypol Schiff bases were found to exhibit high inhibitory activities toward tobacco mosaic virus (TMV), providing promising candidates for plant virus control in the field (Li et al., 2014).

During the long term of plant-insect co-evolution, insects have also developed diverse approaches to locate host plants for feeding (Wu and Baldwin, 2010). As the major insect pest of cotton, cotton bollworm (*H. armigera*) is able to live on cotton plants to complete its life cycle. To deal with gossypol, it has developed strong detoxification system that involves the P450 monooxygenase CYP6AE14 (or GIP for gossypol induced P450). When suppressed by plant mediated insect RNA interference (RNAi) approach, decreased gossypol tolerance of bollworm was observed (Mao et al., 2007), and transgenic cotton plants expressing the double stranded RNA against GIP (dsGIP) acquired enhanced resistance to cotton bollworms (Mao et al., 2011).

More than detoxification, insects can even utilize one phytoalexin to enhance their resistance to other toxins. The cotton bollworm larvae that fed on gossypol-containing glanded cotton leaves were significantly more tolerant to the insecticides of cyhalothin and monocrotophos than those fed on gossypol-free glandless cotton leaves, although such tolerance could not be accumulated or inherited (Kong et al., 2010). In this quickly induced pesticide resistance, multiple gossypol-inducible P450s were found contribute to the δ-methrin tolerance, and knocking down of the P450s by plant-mediated gene silencing approach rendered the larvae more sensitive to the insecticide (Schuler, 2012). The CYP6AE14, which is related to gossypol detoxification, was found to have epoxidation activity towards aldrin, further demonstrating that insect pests can utilize secondary metabolites from their major host plants to elaborate defense systems against other toxic compounds (Tao et al., 2012). Additionally, gossypol is also reported to increase the magnitude and dominance of fitness costs of Bt toxin in pink bollworm (*Pectinophora gossypiella*) (Williams et al., 2011).

BIOSYNTHESIS OF CADINENE-TYPE SESQUITERPENOIDS

Till now, little is known about the biosynthesis of cadinene-type sesquiterpenoids in cotton. As a compound derived from sesquiterpene, gossypol is synthesized in plant cells via the mevalonic acid pathway (MVA pathway). The common precursor farnesyl diphosphate (FPP) is cyclized into (+)-δ-cadinene and then hydroxylated into 8-hydroxy-(+)-δ-cadinene. After multiple hydroxylation and oxidation steps, it is converted to hemigossypol and then coupled into gossypol. Several enzymes involved in this process have been isolated and characterized, including 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) (Joost et al., 1995), farnesyl diphosphate synthase (FPS) (Liu et al., 1999a), (+)-δ-cadinene synthase (CDN) (Benedict et al., 1995; Chen et al., 1995; Meng et al., 1999; Tan et al., 2000), a P450 monooxygenase (CYP706B1) (Luo et al., 2001; Wang et al., 2003), P450 reductase (Yang et al., 2010), as well as a methyltransferase (Liu et al., 1999b). Recently, evidences suggest that the CYP82D109 (P450) is potentially a new gene involved in this pathway (Wagner et al., 2015) (Figure 1 B).

3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR)

In plant cells, the enzyme HMGR catalyzes the reduction of 3-hydroxy-3-methylglutaryl coenzyme A, which is a rate-limiting step of the cytosolic MVA pathway for the biosynthesis of isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) (Kirby and Keasling, 2009). In *Arabidopsis* (Enjuto et al., 1994) and rice (Kim et al., 2004) genomes, there are two or more *HMGR* genes, and the cotton genome also contains multiple HMGRs as revealed by EST data mining and genome searching

(www.phytozome.net). Since gossypol is the major sesquiterpene aldehyde of cotton, it is not surprising that expression of *HMGR* genes can be strongly induced by pathogen inoculation (Joost et al., 1995). Moreover, when stimulated with fungal elicitor, *G. barbadense* exhibited a more rapid induction of *HMGR* gene expression and enzyme activity than *G. hirsutum*, consistent with the fact that *G. babadense* is more resistant to pathogens among these two cultivated allotetraploid cotton species (Joost et al., 1995).

Farnesyl diphosphate synthase (FPS)

FPS catalyzes the coupling of IPP and DMAPP into FPP, which can then be converted by terpene synthases into structurally diverse sesquiterpenes or by squalene synthases for triterpene metabolism. In *G. arboreum*, the FPS is a protein consisting of 342 amino acid residues, which shares high sequence identities with those from *Arabidopsis* (78.9%), *Artemisia annua* (80.7%) and maize (71.6%). Similar to *HMGR*, both the transcript and the protein accumulation can be strongly induced by fungal elicitor, implying its important role in cotton plants defense (Liu et al., 1999a).

(+)-δ-cadinene synthase (CDN)

Early studies revealed that, when inoculated with *X. campestris*, cotton seedlings accumulate a large amount of sesquiterpenes, including δ-cadinene, ε-cadinene and α-muurolene, among which δ-cadinene takes a major portion (Davis and Essenberg, 1995). Moreover, the total proteins isolated from cotton cotyledons treated with pathogen elicitors was able to catalyze the conversion of FPP into (+)-δ-cadinene at a high rate, indicating that (+)-δ-cadinene is a critical precursor or intermediate for phytoalexin biosynthesis (Benedict et al., 1995; Davila-Huerta et al., 1995; Davis and Essenberg, 1995). Later characterization of multiple (+)-δ-cadinene synthase (CDN) proteins with different sequences revealed that it is encoded by a family includes at least *CDN-A*, *CDN-B*, *CDN-C* and *CDN-D* subfamilies (Benedict et al., 1995; Benedict et al., 2001; Chen et al., 1995; Davis et al., 1996; Davis and Essenberg, 1995; Meng et al., 1999), and each has variable expression pattern in different tissues (Benedict et al., 2004; Meng et al., 1999; Tan et al., 2000) and can be induced by pathogens (Davila-Huerta et al., 1995; Davis et al., 1996; Liu et al., 1999a) (Figure 1B).

Compared to most sesquiterpene synthases with complex product spectrum (Yang et al., 2013), the CDNs produce single product of (+)-δ-cadinene. X-ray crystal structures of unliganded CDN and those with Mg^{2+} ions and substrate analogues revealed unusual features. Like other terpenoid synthases, CDN contains a characteristic aspartate-rich DDTYD motif on helix D that interacts with Mg^{2+} ions. However, it does not contain the "NSE/DTE" motif on helix H that specifically chelates Mg^{2+} ion. Instead, it interacts with Mg^{2+} ion with a second aspartate-rich motif of DDVAE, which is more similar to the FPS (Gennadios et al., 2009). Moreover, the G helix was found to play a very important role in (+)-δ-cadinene formation, and mutations at G helix changed its specific activity into higher selectivity to germacrene D-4-ol *in vivo* (Yoshikuni et al., 2006).

P450 monooxygenase and P450 reductase

The multiple hydroxyls in gossypol structure imply that more than one P450 monooxygenase are likely involved in modification steps of (+)-δ-cadinene backbone. The first characterized P450 is CYP706B1, which was shown to catalyze the hydroxylation of (+)-δ-cadinene at 8-position (Luo et al., 2001) (Figure 1B). Moreover, when the (^{3}H)(+)-δ-cadinene and its 8-hydroxy derivative (^{3}H)(+)-8-hydroxy-δ-cadinene were infiltrated into cotyledons of cotton plants, this specific radioactivity was detected in hemigossypol, supporting the (+)-8-hydroxy-δ-cadinene as intermediate for gossypol biosynthesis (Wang et al., 2003).

Recently, the P450 monooxygenase CYP82D109 was found to be involved in gossypol biosynthesis. It was differentially expressed in gossypol-containing and gossypol-free cotton plants, and, when silenced by RNAi, a 90% reduction of hemigossypolone and heliocides levels and a 70% reduction of gossypol level in the terminal leaves were observed. The accumulation of δ-cadinen-2-ol, as well as induction of lacinilene C pathway, suggest that these pathways are inter-connected (Wagner et al., 2015). However, enzymatic activity of this P450 remains unknown.

The catalytic activity of P450 monooxygenase requires the co-factor P450 reductase that utilizes NADPH to provide electron to the catalytic center of P450 monooxygenase. Like other plants, such as *Arabidopsis*, cotton also has two P450 monooxygenase reductases, one is expressed constitutively and the other can be induced rapidly by environmental stimuli, such as fungi elicitor or mechanical wounding (Yang et al., 2010).

Peroxidase, laccase and dirigent protein

It is still unclear how 8-hydroxy-(+)-δ-cadinene is converted into desoxyhemigossypol. However, desoxyhemigossypol can be decomposed rapidly in solution to hemigossypol, which can be retarded by the reducing agents like ascorbic acid or the enzyme catalase, and be increased by superoxide dismutase (Stipanovic et al., 1992), indicating that this is may be a natural oxidative reaction. The peroxidase and laccase present in cotton embryo extracts can catalyze the bimolecular coupling of hemigossypol into gossypol, and this reaction is dependent on the addition of H_2O_2 and can be inhibited by sodium azide. This *in vitro* phenolic coupling resulted in 53% (+)-gossypol and 47% (−)-gossypol which is in close agreement to the 49% (+)-gossypol and 51% (−)-gossypol ratio found in cotton seeds (Benedict et al., 2006; Veech et al., 1976).

However, as introduced earlier the ratio of (+)/(−)-gossypol varies greatly among species and cultivars, e.g. in

G. hirsutum var. mariegalante, the (+)-gossypol accounts for about 98% (Stipanovic et al., 2005). In plants, a family of dirigent proteins plays a key role in determining the stereo specificity. For example, the dirigent protein from *Forsythia intermedia* can capture the E-coniferyl alcohol and stereo-selectively couple to (+)-pinoresinol (Davin et al., 1997). In cotton, a dirigent protein was reported to control the stereo-selective dimerization of hemigossypol in the laccase-catalyzed reaction. Although the dirigent protein itself does not exhibit catalytic activity, the (+)-gossypol ratio increased (up to 80%) with the concentration of this protein (Liu et al., 2008).

S-Adenosyl-L-Methionine: Desoxyhemigossypol-6-O-Methyltransferase

In some cultivars, gossypol can be methylated into 6-methoxy-gossypol and/or 6,6′-dimethoxy-gossypol (Bell et al., 1975; Stipanovic et al., 1975). The desoxyhemigossypol-6-O-Methyltransferase (dHG-6-OMT) from cotton stele tissue infected with *V. dahliae* was able to specifically methylate desoxyhemigossypol at 6-position, but not hemigossypol or gossypol, producing desoxyhemigossypol-6-methyl-ether (Liu et al., 1999b) (Figure 1B).

REGULATION OF GOSSYPOL BIOSYNTHESIS

In aerial tissues of cotton plants, gossypol and related sesquiterpenoids are largely accumulated in pigment glands in high concentrations ranging from 100 to 400 μm, whereas in roots, they are deposited in epidermal layers (Bolek et al., 2010). Different tissues contain varied spectra of compounds. Generally, in the roots and seeds gossypol is predominant, whereas in the glands of foliage diversified cadiene-type sesquiterpenoids are present, including desoxyhemigossypol (dHG), hemigossypol (HG), gossypol (G), hemigossypolone (HGQ), as well as heliocides H_1, H_2, H_3 and H_4 (Bell et al., 1975).

Biosynthesis of gossypol is tightly regulated spatially and temporally. At different developmental stages, the sesquiterpene aldehyde content and the related gene expression level vary greatly. In seedlings of *G. arboreum*, *CDN-C* and *CDN-A* transcripts were detected in both roots and cotyledons immediately after germination, and sesquiterpene cyclase activities were found to be high. However, both transcripts and enzyme activities then dropped quickly to an undetectable level in cotyledons but increased in roots during the following days of growth. These suggest that the *CDN* transcripts, proteins and gossypol, too, in cotyledons are not produced *de novo* but derived from a source that was formed previously, possibly accumulated during ovule development (Tan et al., 2000).

Cotton seed contains a high concentration of gossypol. During ovule (embryo) development, biosynthesis of gossypol is tightly regulated: before 15 DPA (day post-anthesis), gossypol is undetectable; from 15–20 DPA, gossypol begin to be synthesized and then accumulated rapidly in later stages, and after 30 DPA the pigment glands containing mostly gossypol can be viewed clearly. Consistent with this, transcripts of *CDN-C* and *CYP706B1* are also undetectable before 15 DPA, then start to accumulate at about 20 DPA and peak at about 40 DPA (Meng et al., 1999).

As a phytoalexin, gossypol can be strongly induced to accumulate by pathogen infection and insect attack. For example, contents of gossypol and hemigossypol in *G. arboreum* suspension cultures increased 8-fold after 72 h of co-culturing with *V. dahliae* elicitor (Heinstein, 1985), which is possibly mediated by a family of glycoproteins (Davis et al., 1998). Similarly, inoculation of bacteria on cotton cotyledons also induced the formation of cadinene-type sesquiterpenes (Davila-Huerta et al., 1995). Such inducement is faster in the resistant cotton cultivars of *G. barbadense* than in the susceptible cotton cultivars of *G. hirsutum* (Bianchini et al., 1999), and seems to be related to the jasmonate signaling, as jasmonate (MeJA) also induces gossypol accumulation and the gene expression (Luo et al., 2001; Xu et al., 2004). Consistently, expressions of biosynthesis pathway genes, including the *HMGR*, *FPS*, *CDNs* and as *CYP706B1*, have all been reported to exhibit inducible expression patterns upon stimulations (Joost et al., 1995; Liu et al., 1999a).

Till now only limited numbers of transcription factors have been reported to play roles in gossypol biosynthesis regulation. The W-box binding WRKY transcription factor family is related to plant defense (Eulgem and Somssich, 2007). In *Arabidopsis* there are more than 70 WRKY family proteins (Dong et al., 2003), which share the conserved 60-amino acid WRKYGQK box and a zinc finger domain (Zhang et al., 2014). The *G. arboreum* WRKY protein GaWRKY1 shows temporal and spatial expression pattern comparable to that of *CDN-A*, and is able to bind to the W-box in promoter of *CDN-A* and activate its expression, suggesting its role in the regulation of sesquiterpenoid biosynthesis (Xu et al., 2004).

GENETIC ENGINEERING OF GOSSYPOL TRAITS

Gossypol is toxic to human and animals (Dao et al., 2000; Shelley et al., 1999). Recent reports also showed its anti-cancer, antiviral, spermicidal and antimicrobial activities (Dao et al., 2000; Kim et al., 1984; Yildirim-Aksoy et al., 2004), and of which the (−)-gossypol seems to be more active than the (+)-gossypol (Blackstaffe et al., 1997; Shelley et al., 1999; Yildirim-Aksoy et al., 2004). Thus the breeding goal towards gossypol traits is expected to increase or maintain the phytoalexins in most organs of cotton plants for high resistance to pests and pathogens, meanwhile to eliminate these compounds in seeds to reduce the usage cost for the high quality edible oil and protein.

Most cotton plants have visible pigment glands on surface of aerial part, and are called “glanded cotton”, whereas

those deficient are called "glandless cotton", which are largely gossypol-free. Two genes, Gl_2 and Gl_3, are known to control the gland and gossypol traits, and their interactions form complex dominant or recessive gland trait (Dong et al., 2007). In China, the dominant glandless mutant "*Hai1*" (*G. barbadense*) was handed out by Cotton Research Institute of Chinese Academy of Agriculture Sciences in 1986 (Dong et al., 2007). Compared to the glanded cultivars, the glandless cotton cultivars have similar agronomic traits of fiber (Yuan et al., 2000), but, importantly, their seeds are gossypol-free and can be directly used to feed animals (Yuan et al., 2000). However, due to their increased susceptibility to diseases and pests, the glanded cultivars are now only cultivated at small scales.

Some wild cotton species, such as *Gossypium bickii* (2*n*=26, G genome), have the special trait of low gossypol in seed but possessing glands on the vegetative plant parts (Fryxell, 1965). In the hybrids derived from the cross of such wild species and *G. hirsutum*, individuals successfully introgressed of the low-gossypol seed trait can be selected (Zhu et al., 2005). Transgenic technology has also been employed to produce new valuable germplasms. By seed-specific suppression of the *CDN* expression, plants of ultra-low gossypol cottonseed (ULGCS) were developed (Sunilkumar et al., 2006), and field trial results confirmed the stability and specificity of the ULGCS trait, suggesting that this RNAi-based product has the potential to be commercially viable (Palle et al., 2013).

PERSPECTIVE: GOSSYPOL AT GENOMIC ERA

In the past years, great achievements have been made in sequencing the diploid (*G. raimondii* and *G. arboreum*) (Paterson et al., 2012; Wang et al., 2012) and tetraploid *G. hirsutum* and *G. barbadense* genomes (Cao, 2015; Liu et al., 2015; Zhang et al., 2015). These will greatly facilitate resolving the biosynthesis pathway and regulation mechanism of cadinene-type phytoalexins. However, one challenge of elucidating biosynthesis pathway of secondary metabolites is limited information of intermediates. Plant metabolomics studies metabolites at high throughput based on the mass spectrometry (MS) and nuclear magnetic resonance (NMR) technologies (Sumner et al., 2015), and has accelerated the elucidation of a variety of plant natural product biosynthetic pathways, such as saponins biosynthesis in *Glycyrrhiza uralensis* (Seki et al., 2008, 2011). When combined with purification and concentration technologies like high pressure liquid chromatography (HPLC) and solid phase extraction (SPE), the elucidation of cotton sesquiterpenoids biosynthesis pathway will greatly be promoted. Moreover, as one of the oldest natural allopolyploid crops, the cotton genome encounters multiple divergence and duplication events since the appearance of ancestor (Paterson et al., 2012). When and how these specialized metabolites are evolved, and what happened to the biosynthesis and regulatory genes in *Gossypium* genomes await further elucidation.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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