Chapter 7
Cuticular Waxes and Its Application in Crop Improvement

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Abstract Cuticle and cuticular waxes form the first level of barrier between the land plants and their external environment. This hydrophobic layer protects the plant tissues from excessive non-stomatal water loss, controls exchange of gases and solutes, conferring tolerance to enormous abiotic and biotic challenges. The cuticular waxes synthesized in epidermal cells is a complex mixture of very long-chain fatty acids, their esters, and derivatives. Its biosynthesis, transport, and deposition involve multiple genes and are tightly coordinated by complex molecular networks, which in turn is regulated in response to various environmental factors. Past few decades of research evidences from model as well as from non-model systems greatly expanded our understanding and knowledge of the genes involved in cuticular wax biosynthesis and its regulation in plants. This chapter briefly summarizes on the significance of cuticular waxes, its biosynthesis, transport, and deposition. Further, focus has been given toward the transcription factors identified in wax biosynthesis, its positive and negative regulators, and the targeted manipulation of cuticular wax biosynthesis in *Arabidopsis* and different crop plants resulted in tolerance toward adverse conditions.

Keywords Cuticle · Waxes · Wax biosynthesis · Transcription factors · Abiotic and biotic stress tolerance

7.1 Introduction

Plant cuticle forms the first layer of resistance between all land plants and their surroundings. It performs multiple functions of which the most important is to restrict the non-stomatal water loss (Kerstiens 1996; Goodwin and Jenks 2005; Mamrutha et al. 2010; McFarlane et al. 2014). The cuticle mainly consists of

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cutin, lipid, intra-, and epi-cuticular waxes accumulated on the plant surface. The C16 and C18 oxygenated aliphatic monomers derived from fatty acids (FAs) and glycerol form the insoluble polymer cutin that can resist mechanical damage and act as structural support for cuticular waxes (Kolattukudy 1980; Pollard et al. 2008). The cuticular waxes are subdivided into intra- and epi-cuticular. These are generally complex mixtures of very long-chain (VLC) saturated FA derivatives (Borisjuk et al. 2014). The intra-cuticular waxes are mixture of amorphous lipids implanted in the cutin that links the cuticle with the cell wall matrix, and epi-cuticular waxes are the surface lipids forming various crystal like or smooth film structures (Jetter and Schaffer 2001; Kunst and Samuels 2003). Waxes are chemically complex mixtures of lipids consisting of very long-chain fatty acids (VLCFA), hydrocarbons, alkanes, alkenes, ketones, aldehydes, esters, primary alcohols, secondary alcohols, triterpenes, flavonoids, and sterols (Rashotte et al. 1997; Nawrath et al. 2013; Lee and Suh 2015a; Xue et al. 2017). The wax concentration and chemical composition highly vary among plant species, tissues, and developmental stages and contribute to wax crystal morphology, structure, and surface hydrophobicity (Mamrutha et al. 2010, 2017).

Cuticular waxes can play various roles in plant protection against stresses such as cold, salinity, drought, high temperature, ultraviolet (UV) radiations, and mechanical damage (Jenks et al. 1994; Long et al. 2003; Mamrutha et al. 2010; Sajeevan et al. 2017b), bacterial and fungal pathogens, and insects (Eigenbrode and Espelie 1995; Eigenbrode and Jetter 2002; Ziv et al. 2018; Zhang et al. 2019a, b, c; Wang et al. 2019; Kong et al. 2020). In addition to its protective roles, the cuticle is also involved in regulating the plant developmental processes (Ingram and Nawrath 2017). Both biotic and abiotic stresses can act as environmental clues and alter the concentration and composition of waxes. The *Arabidopsis thaliana* (*At*) plants under drought/dehydration stress showed altered cuticular wax biosynthesis and increased epi-cuticular wax deposition (Kosma et al. 2009; Yang et al. 2011). Similarly, drought stress-induced epi-cuticular wax deposition was reported in plants such as cotton, rose, peanut, and tree tobacco (Bondada et al. 1996; Jenks et al. 2001; Samdur et al. 2003; Cameron et al. 2006). A high correlation between improved drought tolerance and higher cuticular waxes was reported in oats (Bengtson et al. 1978), sorghum (Jordan et al. 1984), rice (Islam et al. 2009), alfalfa, and crested wheat grass (Jefferson et al. 1989). In biotic stress, cuticular waxes act as the plant's first physical barrier restricting pathogen entry. On the other hand, pathogens can exploit the cuticular waxes to initiate their pre-penetration and infection processes in regulating the plant–pathogen interactions (Skamnioti and Gurr 2007; Ju et al. 2017; Cui et al. 2019). With its diverse role in multiple abiotic and biotic stresses, cuticular waxes have gained increasing attention and considered to be an indispensable trait for crop improvement.

7.2 Cuticular Wax Biosynthesis in Plants

Through various forward and reverse genetics approaches in model plants like *A. thaliana* and tomato and crop plants such as rice and barley, a number of genes engaged in cuticular wax biosynthesis, transport, and deposition was identified and characterized. From the current knowledge, cuticular wax biosynthesis can be divided into three steps—a de novo synthesis of the C16 or C18 FAs followed by the extension to form VLCFAs. In the third step, the synthesis of various derivatives of VLCFAs such as aldehydes, alcohols, alkanes, ketones, esters, etc. via either the alcohol- or alkane-forming pathways. These VLCFA derivatives are further transported across plasma membrane and deposited as intra- and epi-cuticular waxes.

In short, the cuticular wax biosynthesis begins in endoplasmic reticulum (ER) by the addition of two carbons donated by malonyl CoA for the extension of C16 and C18 fatty acid (FA) precursors formed in plastid. This extension process is a sequential cycle that is facilitated by fatty acid elongase (FAE) complex results in the formation of VLCFAs consists of 20–36 carbons. The FA extension carries through a series of four consecutive reactions of condensation (β-ketoacyl-CoA synthase, KCS), reduction (β-ketoacyl-CoA reductase, KCR), dehydration (-β-hydroxyacyl-CoA dehydratase, HCD), and a second reduction (enoyl-CoA reductase, ECR), for each of two carbon atom extension, that are collectively called elongase (Ohlrogge et al. 1978). Mutation in one of the four extension enzymes (elongase) will result in pleiotropic effects and severe reduction in overall cuticular waxes, indicating the importance of FA extension is an important rate limiting step in cuticular wax synthesis (Beaudoin et al. 2009; Seo and Park 2011). These VLCFAs are further modified/processed to form a variety of cuticular wax components through two distinct pathways—decarbonylation pathway (alkane forming) and acyl reduction pathway (alcohol forming) (Li et al. 2008; Rowland et al. 2006; Rowland and Domergue 2012). In *Arabidopsis*, decarbonylation pathway is predominantly responsible for the production of major derivatives of cuticular waxes with chain length between 21 and 35C atoms such as aldehydes, alkanes, ketones, and secondary alcohols. On the other hand, acyl reduction pathway leads to the production of primary alcohols and wax esters (Bernard and Joubes 2013; Lee and Suh 2015a). A simplified schematic representation of plant cuticular wax biosynthesis pathways in ER is shown in Fig. 7.1.

7.3 Transporters of Cuticle Precursors

The cutin and wax precursors synthesized in ER are transported across the plasma membrane, cell wall, and the emerging cuticular membrane. To date, most of the steps involved in wax biosynthesis are well understood, but the mechanism of transport is poorly known. A close group of half transporters ABCG, an ATP binding cassette, are shown to be involved in the transport of both wax and cutin

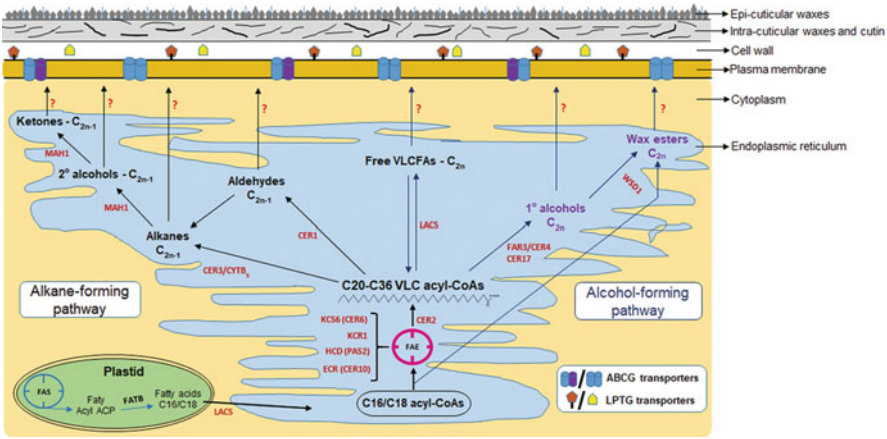

Fig. 7.1 A simplified representation of biosynthesis of cuticular waxes in endoplasmic reticulum (ER). In the biosynthesis of cuticular waxes, fatty acid elongation (FAE) complex catalyzed four sequential consecutive reactions—condensation by KCS6, reduction by KCR1, dehydration by HCD, and a second reduction by ECR for the formation of VLCFAs. These elongated VLCFAs are then modify into different wax derivatives via alkane- and alcohol-forming pathways. Further, wax derivatives were transported to the plasma membrane (PM) by a fully unknown mechanism then to the extracellular matrix by ABC transporters and delivered to the cuticle by yet to be discovered machinery possibly implicating LTPG1 (CER—ECERIFERUM; LACS1—Long-Chain Acyl CoA Synthase1; MAH1—Mid chain Alkane Hydroxylase1; WSD1—Wax Synthase/Diacylglycerol acyltransferase1). The cuticular wax biosynthesis model is adapted from Bernard and Joubes (2013), Yeats and Rose (2008), and Lewandowska et al. (2020)

derivatives across the plasma membrane (Do et al. 2018). The *Arabidopsis* genome consists of four ABCG transporters—ABCG11, ABCG12, ABCG13, and an uncharacterized ABCG15 (Pighin et al. 2004; Bird et al. 2007; Panikashvili et al. 2011). The ABCG11 is a homodimer likely to export cutin precursors (Bird et al. 2007; Elejalde-Palmett et al. 2021) and ABCG11 and ABCG12/CER5 need to form heterodimer for wax secretion (Bird et al. 2007; McFarlane et al. 2010). The ABCG13, third half transporter, was reported to be involved in the cutin deposition in *Arabidopsis* flowers (Panikashvili et al. 2011).

A full transporter ABCG32 identified from *A. thaliana*, *Hordeum spontaneum,* and *Oryza sativa* is involved in cutin deposition (Bessire et al. 2011; Chen et al. 2011). More recently, another ABC transporter from rice (*OsABCG9*), an ortholog of *AtABCG11*, has been reported that specifically transport wax but not cutin (Nguyen et al. 2018). Despite having well-documented evidences to show the involvement of different ABC transporters in trafficking cuticular lipids, there is lack of evidences to demonstrate the substrate specificity in vitro. Till date, all the ABC transporters identified from different systems are members of the ABCG subfamily that are involved in the transport of lipids and hydrophobic compounds (Moitra et al. 2011). Studies have shown that ABC transporter mutants resulted in lipid embodiments intracellularly. This further supports the direct involvement of

ABC transporters in cuticular lipid transport (Pighin et al. 2004; Bird et al. 2007; Bessire et al. 2011).

Glycosyl phosphatidyl inositol (GPI)-anchored lipid transfer proteins (LTPs), LTPG1 and LTPG2, are plasma membrane bound that are involved in the transport of wax derivatives (Debono et al. 2009; Lee et al. 2009a; Kim et al. 2012). These LTPs are a unique class of soluble proteins that can bind to a variety of lipid substrates (Yeats and Rose 2008). It is proposed that the apoplastic LTPs are involved in the trafficking of wax derivatives, although genetic or biochemical evidences are clearly lacking (Yeats and Rose 2008). Recently, *Arabidopsis* mutant analysis demonstrated the involvement of gnom like1-1 (GNL1) and echidna (ECH)-dependent endo-membrane vesicle transport of waxes to plasma membrane-localized ATP-binding cassette transporters (McFarlane et al. 2014).

7.4 Transcriptional Regulation in Biosynthesis of Cuticular Waxes

Efforts to elucidate the biosynthesis of cuticular wax pathway and its players were mainly identified through mutants and concentrated with the model plant, *Arabidopsis*. A large number of genes involved in the biosynthesis of cuticular waxes has been identified, isolated, and characterized (Jetter et al. 2006; Jetter and Kunst 2008; Samuels et al. 2008). In *Arabidopsis*, more than 190 genes have been identified to be involved in the biosynthesis of cuticular waxes, its transport, or deposition (Li-Beisson et al. 2013). Among these, CER1, CER2, CER6/CUT1, KCS1, IDDLEHEAD (FDH), and WAX2 from *Arabidopsis*, GL1 and GL8 from maize encode wax synthesis and transport related enzymes (Aarts et al. 1995; Hansen et al. 1997; Todd et al. 1999; Fiebig et al. 2000; Chen et al. 2003; Zhang et al. 2005). A summary of genes identified from the model plant *Arabidopsis* that are involved in cuticular wax biosynthesis, transport, or deposition is detailed in Table 7.1.

The key regulators involved in the biosynthesis of waxes and cuticular components deposition are transcription factors (TFs). Different families of TFs belong to ethylene-responsive factors (ERFs), myeloblastosis family (MYB), and homeodomain-leucine zipper class IV (HD-Zip IV) factors identified as regulators of wax biosynthesis, of which ERFs gained more importance (Aharoni et al. 2004; Seo et al. 2011). Overexpression of these TFs lead to changes in wax biosynthesis, its accumulation, and changes in the chemical composition (Broun et al. 2004). It has also been demonstrated that overexpression of these TFs often resulted in increased stress tolerance (Broun et al. 2004; Javelle et al. 2010; Seo and Park 2011). However, despite their obvious positive effects on plant protection, it was also demonstrated that the ectopic expression could negatively affect plant growth, yield, and decreased stress tolerance (Aharoni et al. 2004; Zhang et al. 2005). A summary of TFs identified that play a role in the biosynthesis of cuticular waxes, targeted genes,

Table 7.1 Genes identified in the biosynthesis of cuticular waxes and its transport from the model plant *Arabidopsis thaliana*

Sl. No.	Gene abbreviation	Protein family name	Role	Function	Reference
1.	*KCS1*	β-Ketoacyl-coenzyme A synthase	Extension of 24C FA	Biosynthesis	Todd et al. (1999)
2.	*CUT1/CER6/ KCS6*	β-Ketoacyl-coenzyme A synthase	Regulation of VLCFA biosynthesis/24C FA extension	Biosynthesis	Fiebig et al. (2000), Hooker et al. (2002)
3.	*FATB*	Fatty acyl-ACP thioesterase B	Providing saturated FAs for wax biosynthesis	Biosynthesis	Bonaventure et al. (2003)
4.	*CER10/ECR/ ECR10*	Trans-2,3-Enoyl-coenzyme A reductase	VLCFA biosynthesis	Biosynthesis	Zheng et al. (2005)
5.	*PASTICCINO2 (PAS2)/HCD*	β-Hydroxyacyl-coenzyme A dehydratase	VLCFA synthesis in association with CER10, an enoyl-CoA reductase	Biosynthesis	Bach et al. (2008)
6.	*KCR1*	β-Ketoacyl-coenzyme A reductase	VLCFA extension	Biosynthesis	Beaudoin et al. (2009)
7.	*KCS20*; *KCS2/ DAISY*	β-Ketoacyl-coenzyme A synthase	VLCFA extension to C22	Biosynthesis	Franke et al. (2009), Lee et al. (2009b)
8.	*LACS1/CER8*; *LCAS2*; *LCAS4*	Long-chain acyl CoA synthetase	Synthetase activity for VLCFAs C20-C30	Biosynthesis	Lü et al. (2009), Jessen et al. (2011), Weng et al. (2010)
9.	*ACC1*	Acetyl-coenzyme A carboxylase	Malonyl CoA substrate synthesis	Biosynthesis	Lü et al. (2011)
10.	*CER2*; *CER2-Like1/2*	BAHD acyltransferase	FA extension beyond C28	Biosynthesis	Haslam et al. (2012, 2015), Pascal et al. (2013)
11.	*KCS9*	β-Ketoacyl-coenzyme A synthase	Extension of C22-C24 FAs	Biosynthesis	Kim et al. (2013)
12.	*CER17 (ECERIFERUM1)*	Acyl-CoA desaturase like 4	n-6 desaturation of VLC acyl-CoAs	Biosynthesis	Yang et al. (2017)

(continued)

Table 7.1 (continued)

Sl. No.	Gene abbreviation	Protein family name	Role	Function	Reference
13.	*WAX2/YRE/FLP1/ CER3*	Aldehyde-generating acyl-CoA enzyme	Synthesis of aldehydes, alkanes, 2o-alcohols, and ketones; cuticular membrane biosynthesis	Biosynthesis—Alkane-forming pathway	Chen et al. (2003), Rowland et al. (2007), Bernard et al. (2012)
14.	*CER1/CER22*	Aldehyde decarbonylase	Biosynthesis of VLC alkane	Biosynthesis—Alkane-forming pathway	Bourdenx et al. (2011), Bernard et al. (2012), Sakuradani et al. (2013)
15.	*RST1—RESURRECTION1*	Aldo/keto reductase/ cytochrome C/G-protein-coupled receptor family 1	May act in reduction of acyl-CoAs to aldehydes	Biosynthesis—Alkane-forming pathway	Chen et al. (2005)
16.	*CYTB5-B/C/D/E*	Cytochrome B5	Redox-dependent synthesis of VLC alkanes	Biosynthesis—Alkane-forming pathway	Bernard et al. (2012)
17.	*CYP96A15* (cytochrome P450 enzyme)/*MAH1*	Midchain alkane hydrolase 1	Formation of 2o-alcohols and ketones	Biosynthesis—Alkane-forming pathway	Greer et al. (2007)
18.	*CER4/FAR3*	FA CoA reductase	Formation of C24:0 and C26: 0 1o-alcohols	Biosynthesis—Alcohol-forming pathway	Rowland et al. (2006)
19.	*WSD1*	Wax ester synthase/ diacylglycerol acyltransferase	Wax ester biosynthesis	Biosynthesis—Alcohol-forming pathway	Li et al. (2008)
20.	*ABCG12/CER5*	ATP-binding cassette (ABC) transporter	Cuticular waxes transport	Transport	Pighin et al. (2004)
21.	*ABCG11/WBC11/ DESPERADO*	ATP-binding cassette (ABC) transporter	Secretion of surface waxes in interaction with CER5	Transport	Bird et al. (2007), Luo et al. (2007), Panikashvili et al. (2011)

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Table 7.1 (continued)

Sl. No.	Gene abbreviation	Protein family name	Role	Function	Reference
22.	*GLN1; ECH*	Vesicle trafficking	Vesicle trafficking	Transport	McFarlane et al. (2014)
23.	*LTPG1; LTPG2*	GPI-anchored lipid transfer protein (LTPG)	Export or accumulation of cuticular waxes	Transport/ deposition	DeBono et al. (2009), Lee et al. (2009a). Kim et al. (2012)

and cuticular composition affected identified through overexpression or down-regulation is detailed in Table 7.2.

7.4.1 APETALA2/Ethylene Responsive Factor

The APETALA2/Ethylene Responsive Factor (AP2/ERF) superfamily is known to be one of the largest plant-specific families of TF involved in diverse plant physiological processes (Licausi et al. 2013). These TFs can regulate the gene expression transcriptionally and posttranslationally at different stages of plant growth and development, hormone signaling, and in response to various abiotic and biotic stresses (Elliott et al. 1996; Xu et al. 2011; Licausi et al. 2013). The AP2/ERF proteins were first identified from *Arabidopsis*, typically consists of a highly conserved AP2 domain of 40–70 amino acids in length (Jofuku et al. 1994). Based on the number of AP2 and other DNA binding domains, they are categorized into four different subfamilies—AP2, ERF, DREB (Dehydration Responsive Element Binding), and RAV (related to ABI3/VP1) (Mizoi et al. 2012). Members of AP2 subfamily consist of two AP2/ERF domains (Sakuma et al. 2002). The ERFs and DREB subfamilies contain single AP2 domain that usually binds to an ethylene responsive (AGCCGCC) cis-element designated as GCC-box (Ecker 1995; Eini et al. 2013). However, the RAV subfamily proteins are characterized by the presence of two different DNA binding domains, AP2/ERF and B3 (Kagaya et al. 1999).

The first TF identified and reported to be involved in cuticular wax biosynthesis was WAX INDUCER1/SHINE1 (WIN1/SHN1) from *Arabidopsis* simultaneously by two independent research groups designated as WAX INDUCER1 (WIN1) and SHINE1 (SHN1) (Aharoni et al. 2004; Broun et al. 2004). WIN1/SHN1 belonging to the subfamily of AP2/ERF TFs is a member of a clade of three close homolog proteins (SHN2 and SHN3) in *Arabidopsis* genome belonging to group V or B6 (Sakuma et al. 2002; Nakano et al. 2006). All three SHN clade genes exist with a single intron in nature, and no splice variants are reported. The *AtWIN1/SHN1* share 55% and 71% protein sequence homology with *AtSHN2* and *AtSHN3*, respectively (Aharoni et al. 2004). All three SHN proteins contain three conserved domains/

Table 7.2 A list of TFs identified in biosynthesis of cuticular waxes, their target genes, and the chemical components

Sl. No.	Plant	TF name	TF type	Target genes	Affected chemical components	Reference
1.	*Arabidopsis thaliana*	WIN1/ SHN1	AP2-EREBP	CYP86A7, CYP86A4, Lipase-like, HTH-like GPDHc1 NLM2, GPAT4, CER1, KCS1, CER2, FAE1	C30/34 FA, C28/30 aldehyde, C27/C29/C33 alkane, C16:0 and C18:1 ω-HFAs, C31 and C29 alkanes	Aharoni et al. (2004), Broun et al. (2004), Kannangara et al. (2007)
2.	*Medicago truncatula*	WXP1	ERF	FSE-like: MtTC79579, MtTC80406, MtTC87247, LCR-like: TC81689, TC84740, WAX2-like: TC82822, KAR (GL8-like)	C30/C28 primary alcohols, C29-C33 alkanes, C18:1 DSA, C22/C24 FAs, C22/C23/C30 aldehydes, cholesterol, sitosterol	Zhang et al. (2005)
3.	*Medicago truncatula*	WXP2	ERF	–	C28/C32/ C18/C22 FAs, C32/C28 aldehyde C30/C34/C32 primary alcohols, C31/C23/ C25/C27 alkane, cholesterol, sitosterol	Zhang et al. (2007)
4.	*Arabidopsis thaliana*	MYB41	R2R3-type MYB	WIN1/SHN1 LACS2, ATT1, LTPs, GDSL-lipases, hydrolase α/β-fold family, AtEXP5	–	Cominelli et al. (2008)
5.	*Hordeum vulgare*	Nud	ERF	–	–	Taketa et al. (2008)
6.	*Solanum lycopersicum*	MYB12	R2R3-type MYB	21 Phenylpropanoid/ flavonoidrelated transcripts, 8 genes related to FA metabolism	Mostly metabolites associated with a phenylpropanoid pathway	Adato et al. (2009)

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Table 7.2 (continued)

Sl. No.	Plant	TF name	TF type	Target genes	Affected chemical components	Reference
7.	*Zea mays*	OCL1	HD-Zip IV	nsLTP, CYP78A6-like, ABC transporter, SEC14	C25 alkane, C24, C26, and C28 alcohols, C48 ester, C28 and C30 aldehydes	Javelle et al. (2010)
8.	*Arabidopsis thaliana*	SHN2/3	AP2-EREBP	–	–	Aharoni et al. (2004), Shi et al. (2011)
9.	*Arabidopsis thaliana*	MYB96	R2R3-type MYB	RD22, some GH3 genes, KCR1, SER1, KCS1, KCS2, KCS6, PAS2, CER3, ESR, WBC11 and other ABC transporters, LTP	–	Seo et al. (2009, 2011)
10.	*Oryza sativa*	CFL1	WW domain	WIN1/SHN1, BDG, FDH	–	Wu et al. (2011)
11.	*Arabidopsis thaliana*	HDG1	HD-Zip IV	–	–	Wu et al. (2011)
12.	*Eucalyptus gunnii*	CBF1a/b	DREB/CBF	–	–	Navarro et al. (2011)
13.	*Arabidopsis thaliana*	WRI1/3/4	AP2/EREBP	PKp2, MAT, KASI, KASIII, ENR, FATA, G3PDH, ROD1, BCCP2	C16:0/C18:0/C18:1/C18:2 Data for triple wri1wri3 wri4 mutants: DSAs, C16:0/C18:1/C18:2 ω-OH and C16 10,16OH DCAs	Cernac and Benning (2004), Masaki et al. (2005), To et al. (2012)
14.	*Arabidopsis thaliana*	NFXL2	NFXL	BDG1, SHN1, SHN2, SHN3	–	Lisso et al. (2012)
15.	*Arabidopsis thaliana*	ANL2	HD-Zip IV	–	C16:0, C18:0, C18:1, C18:2, and ω-OH DCAs, C27, C31, and C33 alkanes	Nadakuduti et al. (2012)
16.	*Oryza sativa*	WR1	ERF	OsKCS2, OsLACS1,	C16/C20/C24/C26/	Wang et al. (2012)

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Table 7.2 (continued)

Sl. No.	Plant	TF name	TF type	Target genes	Affected chemical components	Reference
				OsCER3, OsCUT1, OsFDH1/2, OsKCS1, OsLACS1–2, OsCER1/2, OsFAE1-L	C30/C32 FAs, C32/C22 alcohols, C25/C27/ C29/C31 alkanes, C48 ester	
17.	*Oryza sativa*	WR2/3/4	ERF	–	–	Wang et al. (2012)
18.	*Solanum lycopersicum*	CD2	HD-Zip IV	–	Trans-CA, di OH C16:0 and ω-OH C18:1 Ph. C16:0, C20:0, and C22:0 DCAs, C27, C29, C30, and C31 alkanes CC18:0 FA	Nadakuduti et al. (2012)
19.	*Solanum lycopersicum*	SHN3	AP2-EREBP	SlGL2, SlMIXTA, SlCYP77A1, SlCYP86A8, SlCYP86A69	BA, trans-CA, C16:0 and C16–9/10-H DFAs, C16–9/10, 16Di, C16-ω, C18:1–2 HFAs	Mintz-Oron et al. (2008), Shi et al. (2013)
20.	*Arabidopsis thaliana*	MYB106/ NOK	R2R3-type MYB	WIN1/SHN1, FDH, LACS2, CYP84A4, CYP77A6, Atg04570, KCS1, CER1, CER2, LCR, LACS2	–	Gilding and Marks (2010), Oshima et al. (2013)
21.	*Arabidopsis thaliana*	MYB16	R2R3-type MYB	WIN1/SHN1, FDH, LACS2, CYP84A4, CYP77A6, Atg04570, KCS1, CER1, CER2, LCR, LACS2	–	Oshima et al. (2013)
22.	*Eucalyptus grandis*	SHN1/2	ERF	–	–	Marques et al. (2013)
23.	*Solanum lycopersicum*	SHN1	AP2-EREBP	GDSL, Enoyl-CoA reductase,	–	Al-Abdallat et al. (2014)

(continued)

Table 7.2 (continued)

Sl. No.	Plant	TF name	TF type	Target genes	Affected chemical components	Reference
				acyl-CoA synthase, Fiddle-head, HOT-HEAD-like		
24.	*Eutrema salsugineum*	WAX1	R2R3-type MYB	*CER1*, *KCS2*, *KCR1*, *VTC1*, *GLDH*, *MIOX4*	–	Zhu et al. (2014)
25.	*Arabidopsis thaliana*	MYB94, MYB96	R2R3-type MYB	*KCR1*, KCS1, K*CS2/DAISY*, *KCS6*, *CER2*, *CER1*, *CER3*, *WSD1*	–	Lee and Suh (2015b), Lee et al. (2016)
26.	*Arabidopsis thaliana*	WRI4	AP2/ EREBP	LACS1, KCR1, PAS2, ECR, WSD1, PKP1, PKP2, BCCP2, ENR1, PDH- E1a	C24 and C28 FAs, C27 alkanes, and C24, C26, C28 primary alcohols, C29 ketones	Park et al. (2016)
27.	*Hordeum vulgare*	WIN1	AP2/ EREBP	KAS2, CYP86A2, CYP89A2, LACS2	–	Kumar et al. (2016)
28.	*Arabidopsis thaliana*	DEWAX2	AP2/ ERF	CER1, ACLA2, LACS1, LACS2, KCS12	C29 and C31 alkanes, C28 primary alcohols, C29 ketone	Kim et al. (2018)
29.	*Brassica napus*	WIN1/ SHN1	AP2/ EREBP	BCCP1, GPAT9, LPAT5, DGAT2 *LACS2*, *KCS1*, *KCR1*, *CER1*	C29-alkanes, C31-alkanes, C28-alcohol, C29-alcohol	Liu et al. (2019)
30.	*Jatropha curcas*	WRKY	WRKY	Cab40, Lhcb5, Rca1, WIN1	FA, fatty alcohols, carboxylic acid, alkene, terpene, triterpenoid, aldehyde	More et al. (2019)
31.	*Cucurbita moschata*	WIN1	AP2/ ERF	CER1, CER1-1, CER4, KCS1, ABC	Ester (C20: C22, C20: C24, C20: C26, C20: C28, C20: C24), alkanes	Zhang et al. (2019a)

(continued)

Table 7.2 (continued)

Sl. No.	Plant	TF name	TF type	Target genes	Affected chemical components	Reference
					(C29 and C31)	
32.	*Malus domestica*	SHINE2	AP2/ EREBP	MYB30, MYB96, LACS2, CER1, CER3, CER6, KCS1, WIN1, DEWAX, SHINE3	Alkanes, alcohols, aldehydes, FAs	Zhang et al. (2019b)
33.	*Malus domestica*	MYB30	R2R3-type MYB	*WRI1*, *WIN1*, *ACBP1*, *LACS2*, *SHINE2*, *SHINE3*, *KCS1*	C29 alkanes, C31 alcohols, C29 aldehydes, C16 FAs, C29 ketones, and C29 and C30 esters	Zhang et al. (2019c)
34.	*Eustoma grandiflorum*	*MIXTA-like 1*	R2R3-type MYB	*CER3*, *CER6*, *CER10*, *KCS1*, *KCR1*, *CYP77A6*, *WIN1*	–	Wang et al. (2020)
35.	*Arabidopsis thaliana*	RAP2.4	AP2/ DREB	KCS2, CER1	VLC-alkane, C27, C29, C31, C33	Yang et al. (2020)

motifs, AP2 domain at N-terminal, a middle, and C-terminal conserved motifs (Nakano et al. 2006).

The overexpression of WIN1/SHN1 showed increased accumulation of cutin/leaf epi-cuticular waxes and resulted in improved dehydration tolerance of transgenic *Arabidopsis* and downregulation leads to decreased cutin content in the outer parts of the plant (Aharoni et al. 2004; Broun et al. 2004; Kannangara et al. 2007). The WIN1/SHN1 overexpression also reflected in altering the structure of leaf epidermis and stomatal index, trichrome number, and branching (Aharoni et al. 2004). Therefore, it is possible that WIN1/SHN1 and other AP2 domain superfamily members not only involved in cuticle formation but also function in other metabolic pathways. Shi et al. (2011) showed that WIN1/SHN1 TF plays an important role in the metabolism of cell wall. The constitutive overexpression of WIN1/SHN1 TF leads to an upregulation of three downstream genes (*CER1*, *CER2*, and *KCS1*) that were initially identified and known to be involved in the biosynthesis of epidermal waxes (Broun et al. 2004). Transgenic *Arabidopsis* plants expressing WIN1/SHN1 resulted in altering the expression of 12 genes, of which 11 were upregulated and one with an unknown function strongly downregulated (Kannangara et al. 2007). Even though

WIN1/SHN1 overexpression altered cuticular wax load and resulted in improved stress tolerance, evidences for the direct activation of downstream genes by WIN1/SHN1 are still lacking. In the last few years, WIN/SHN-related members were identified and characterized from different crops like soybean, cotton, barley, wheat, etc. Overexpression resulted in altered cuticle properties and imparts tolerance to multiple abiotic stresses (Xu et al. 2016; Bi et al. 2018; Djemal et al. 2018; Djemal and Khoudi 2015, 2021).

7.4.2 Homologous of WIN/SHN

In rice, four homologous of *Arabidopsis* WIN/SHN were identified and designated as Wax Synthesis Regulatory genes 1–4 (*OsWR1–4*) (Wang et al. 2012). Sequence homology studies showed the *OsWR1* protein sequence is closely related to *AtWIN1/SHN1* protein. Transgenic rice plants overexpressing *OsWR1* resulted in decreased cuticle permeability, in contrast to the results exhibited in *AtWIN1/SHN1* overexpression studies by Aharoni et al. (2004), but an enhancement in drought tolerance has been reported in both the cases. The decreased cuticle permeability of *OsWR1* overexpression was due to alterations in long-chain FAs and alkanes. In addition, it was demonstrated that *OsWR1* could interact with wax-related genes, *OsLACS2* and *OsFAE1-L*, by direct binding to GCC and DRE elements present in the promoter region. The *OsWR1* overexpression in rice resulted in more than two-fold upregulation of 12 wax biosynthesis-related genes and four cutin biosynthesis genes. The overexpression also showed an increased expression of non-cuticle biosynthesis genes involved in membrane stabilization and reactive oxygen species (ROS) scavenging such as late embryogenesis abundant protein (LEA3), ascorbate peroxidase (APX1), superoxide dismutase (SOD), and catalase (Cat A and Cat B) that could independently contribute to improved drought tolerance. On the other hand, silencing of *OsWR1* by RNAi resulted in significant downregulation of many of those genes (Table 7.2) and partial silencing resulted in decreased transcript levels of Cat A and Cat B (Wang et al. 2012).

7.4.3 Negative Regulators of WIN/SHN

Two negative regulators, NUCLEAR FACTOR X LIKE2 (NFXL2) and SPINDLY (SPY) of *WIN/SHN* genes, were reported. The *Arabidopsis* NFXL2 mutant analysis showed difference in the composition of cutin, reduced stomatal aperture, and an increase in drought tolerance by regulating the expression of all three SHN genes (Lisso et al. 2012). Further analysis revealed that *NFXL2* gene could act as a negative regulator for WIN1/SHN1 and several others by directly interacting with the gene promoter region. Thus, NFXL2 protein modulated the cuticle components biosynthesis through a direct repression of *WIN1/SHN* gene (Lisso et al. 2012).

7.4.4 WAX PRODUCTION1 (WXP1)

WXP1, an AP2 domain containing TF from *Medicago truncatula* (*Mt*), is evidently distinct from other AP2/ERF TF family genes. The *MtWXP1* identified to be a homolog to ERFs has 53% amino acid sequence identity with RAP2.4. The WXP1 transcript was highly upregulated by abscisic acid (ABA) treatment in both shoot and root of seedlings, and upregulation was observed only in shoot under cold and drought stress. Under cold stress conditions, the upregulation was very rapid and could be detected within 30 minutes (Zhang et al. 2005). The constitutive overexpression of WXP1 in alfalfa resulted an increase in total leaf wax load to nearly 40% and enhanced drought stress tolerance (Zhang et al. 2005). The gas chromatography-mass spectrometry (GCMS) analysis of transgenic plants leaves showed a significant difference in multiple wax derivatives such as higher content of C30 alcohol moieties (25–35%) and elevated levels of other wax components (Table 7.2). The increase in wax content resulted in reduced water loss by decreasing water permeability, lower chlorophyll leaching, and showed better tolerance to drought stress. The WXP1 overexpression resulted in the upregulation of three FAE-like and two LACERATA (LCR, encoding cytochrome P450 monooxygenases) wax biosynthesis pathway genes (Zhang et al. 2005).

The constitutive overexpression of *MtWXP1* and its paralog WXP2 significantly enhanced the deposition of cuticular waxes due to the accumulation of specific wax components and their chain length distributions on leaves of *Arabidopsis* (Zhang et al. 2007). The WXP1 and WXP2 transgenic lines showed higher levels of n-alkanes. The primary alcohol levels were increased in WXP1 plants but showed an opposite trend in WXP2 as compared to their wild type plants (Table 7.2). The WXP1 plants did not show any changes in the cuticle permeability while WXP2 resulted in decreased levels. Surprisingly, detached leaves of WXP1 and WXP2 transgenic plants retained better water content and showed significantly enhanced survival under drought stress conditions. Under the low-temperature stress, WXP1 transgenic plants showed an improved tolerance while WXP2 was susceptible as compared to control plants. The *Arabidopsis* plants constitutively expressing WXP1 did not exhibit any negative effects on plant growth and development; however, slower plant growth was observed in WXP2 overexpression (Zhang et al. 2007).

7.4.5 WRINKLED and CBF TFs

The WRINKLED1 (*WRI1*) gene contain two AP2 domain was identified from *Arabidopsis* through the mutant analysis. The mature seeds of *wri1* mutants showed a wrinkled appearance and decreased content of water-insoluble oils (Cernac and Benning 2004). The overexpression of WRI1 resulted in 10–20% increased seed oil content without reducing the seed number (Cernac and Benning 2004). The WRI3 and WRI4 homologs of WRI1 were identified to be involved in gene expression of

the synthesis of acyl chain and glycerol backbones that are main precursors of different lipid biosynthetic pathways (To et al. 2012). On the other hand, significant downregulation of most glycolytic and late FA biosynthetic genes were observed in *wri* triple mutants. The C-repeat binding factor genes (*CBF1a* and *1b*) associated with drought and cold tolerance were found to be involved in the regulation of deposition of cuticular waxes in *Eucalyptus gunii*, an Australian drought- and cold-tolerant tree species (Navarro et al. 2011). The eucalyptus transgenic plants exhibited a high accumulation of anthocyanins, decrease in the stomatal density, reduced growth, better water retention capacity with reduced leaf area, and increase in leaf thickness and leaf cell size as compared to the control plants. Also, transgenic plant leaves showed a higher density of oil glands, and amount of cuticular waxes were significantly higher (Navarro et al. 2011). Overexpression of *CBF4 TF* gene in grape vine resulted in enhanced freezing tolerance and decreased electrolyte leakage due to freezing. The mRNA expression profiling of transgenic line showed the expression of CBF4 targets the lipid metabolism, epi-cuticular wax formation, and cell wall structure-related genes (Tillett et al. 2012). So far, these are the only reports showing the involvement of *CBF* genes on cuticle wax deposition (Table 7.2).

7.4.6 Myeloblastosis Family (MYB)

To date, many MYB TFs have been shown to be involved in the complex network that control cuticle biosynthesis, cell-wall modification, and cuticle deposition in the model plant *Arabidopsis*. A R2R3-type MYB TF in *Arabidopsis*, *MYB41* is reported to be involved in the cuticle biosynthesis and wax transport regulation (Cominelli et al. 2008). *AtMYB41* interacts with mitogen-activated protein kinase 6 (MPK6), a member of protein kinases family interacts with a number of signaling pathways involved in plant development and responses to stress (Hoang et al. 2012). It was demonstrated that *AtMYB41* can physically interact with MPK6 and get phosphorylated at residue Ser251, which can enhance MYB41 DNA binding capacity to the *LTP* gene promoter. This was further proved by wild type *AtMYB41* gene overexpression that showed improved tolerance to high salinity while overexpression of a mutated MYB41 (Serine 251 to alanine) resulted in decreased tolerance to salt stress (Hoang et al. 2012).

A R2R3-type MYB protein, MYB96, identified as a stress-responsive TF modulates the responses of drought stress by combining the auxin and ABA signals (Seo et al. 2009). The *Arabidopsis* mutant plants overexpressing MYB96 suppressed the lateral root growth but were resistant to drought stress, while the knockout mutants were highly sensitive to drought stress (Seo et al. 2009). The microarray results showed upregulation of a large group of genes encoding the wax biosynthetic enzymes by MYB96, specifically those of VLCFA condensing enzymes (Seo et al. 2011; Table 7.2). Most of the target genes of MYB96 were also upregulated under drought stress and ABA due to the presence of MYB-responsive cis-element "TAACTA/G" in their promoter. The transgenic *AtMYB96* plants showed increased

epi-cuticular wax crystal deposition in leaves but reduced in stem and showed a slight change in the color of leaves. Also, these plants were significantly shorter with no characteristic "shiny" phenotype; however, no changes in epidermal development was observed (Seo et al. 2011). The *myb96* loss of function mutant was susceptible to drought stress due to the alteration in cuticular wax biosynthesis (Guo et al. 2013). A closely related *MYB94 TF* gene can effectively replace MYB96 in cuticular wax biosynthesis (Lee et al. 2016). The MYB94 and MYB96 TFs are closely related and can additively function in the biosynthesis of waxes under drought stress and well-watered conditions via an ABA-dependent pathway (Lee et al. 2016).

The role of *AtMYB96* in frost tolerance and response to biotic stresses were also reported (Guo et al. 2013; Seo and Park 2011). The *LTP3* gene overexpression resulted in increased freezing tolerance without showing an effect on CBF expression or their target cold regulated (COR) genes. The MYB96 directly binds to the *LTP3* gene promoter results in positive regulation of LTP3 expression results in enhanced freezing tolerance, consistent with MYB96 overexpressing transgenic plants (Guo et al. 2013). An inhibitor of the rust germ tube differentation1 (*irg1*) mutant showed complete loss of epi-cuticular wax crystals in the abaxial surface and consequent reduction in the surface hydrophobicity that conferred non-host resistance to biotrophic fungal pathogens. The abaxial leaf surface wax composition analysis of *irg1* mutant showed 90% reduction in primary alcohols (C30) and alkanes (C29 and C31) were increased compared to control (Table 7.2). It is proposed that IRG1 may be a direct or indirect regulator of *MtMYB96* transcription; however, there is no evidence to claim that IRG1 could regulate the cuticular wax biosynthesis-related genes directly or is performed only through MYB96.

MIXTA, an MYB-related TF, has been identified with the role in cuticular wax biosynthesis and epidermal cell shape formation. The *Arabidopsis* and *Torenia fournieri* MYB106 and MYB16, MIXTA-like TF genes can regulate the development of cuticle that coordinate with TF WIN1/SHN1 (Oshima et al. 2013; Table 7.2). The downregulation of *MYB106* and *MYB16 TF* genes resulted in cuticle deficiencies of flowering organs, organ adhesion, and decreased epi-cuticular wax crystals. Microarray results showed MYB106 and WIN1/SHN1 TFs regulate similar set of genes (Oshima et al. 2013; Table 7.2). Among these, the genes involved in the accumulation of waxes such as FDH, KCS1, and CER2 and cutin biosynthesis genes such as LACERATA and LONG-CHAIN ACYL COA SYNTHETASE2 were identified. The overexpression of MYB16 in *Arabidopsis* resulted in the accumulation of waxy substances on leaves, and both MYB106 and MYB16 downregulation by RNAi leads to reduced expression of cuticular wax biosynthesis genes *LACERATA* and *ECERIFERUM1* with severe permeable cuticle phenotype (Oshima and Mitsuda 2013; Oshima et al. 2013).

7.4.7 Homeodomain-Leucine Zipper Class IV Factors

The homeodomain leucine zipper IV (HD-Zip IV) TFs are predominantly expressed in epidermal cells with epidermis-related functions have been identified from number of plant systems (Javelle et al. 2011; Chew et al. 2013). Maize (*Zea Mays*) Outer Cell Layer 1 (*ZmOCL1*) gene is a member of HD-ZIP IV comes under the subclass of HD-ZIP homeodomain proteins, was detected in protoderm, floral organs, and developing leaves (Ingram et al. 1999, 2000). The transgenic maize plants overexpressing *ZmOCL1* gene had less effect on phenotype as compared to its control, but the transcriptome analysis revealed expression of many genes involved in the metabolism of lipids and its transport (Javelle et al. 2010). Some of the genes identified are carboxylesterase, type 2 LTP, phosphatidylinositol transport protein, three ABC transporters, and FA reductase (Table 7.2). The FA reductases responsible for the long-chain primary alcohol synthesis from FA precursors were closely related to CER4 protein in *Arabidopsis* (Rowland et al. 2006). The transgenic plants of *ZmOCL1* did not show significant changes in the wax layer structure or size as compared to the wild-type plants. However, wax chemical component analysis showed a significant increase in C32 alcohol content and decrease in C32 aldehydes in the young leaves of *ZmOCL1* transgenic. Few of the independent transgenic lines showed significant two- to threefold increase in C44 to C48 wax esters as compared to the control (Javelle et al. 2010).

7.4.8 Curly Flag Leaf1, a Negative Regulator of HD-Zip IV

The Curly Flag Leaf1 (*CFL1*) gene, a WW domain encoding protein, was reported as a negative regulator of cuticle development (Wu et al. 2011). The overexpression of *OsCFL1* and *AtCFL1* in transgenic *Arabidopsis* plants resulted in an organ fusion phenotype with decreased levels of epi-cuticular waxes and defective cuticles. Yeast two hybrid assay provided evidences for direct interaction of *AtCFL1* with HDG1, a HD-Zip IV protein (Wu et al. 2011). The *HDG1* gene suppression resulted in a defective cuticle phenotype in transgenic *Arabidopsis*, similar to that of the CFL1 overexpressing plants. The *AtCFL1* overexpression and HDG1 downregulation in transgenic *Arabidopsis* resulted in the downregulation of FIDDLEHEAD (FDH) and BODYGUARD (BDG), two cuticle biosynthesis-associated genes. The BDG encodes a member of the α/β-hydrolase fold protein superfamily and FDH is also known as KCS10 (Kurdyukov et al. 2006; Wellesen et al. 2001; Yephremov et al. 1999). It was demonstrated that HDG1 could function as a positive regulator by directly binding to the L1 boxes in the promoters of *BDG* and *FDH* genes. The HDG1 function is negatively regulated by CFL1, thereby affecting the cuticle development (Wu et al. 2011; Table 7.2).

7.5 Cuticular Wax, a Multifunctional Trait

Plant cuticle and cuticular waxes play multifunctional role in crop protection and survival against various abiotic and biotic stresses like transpiration water loss, drought, high light intensity, salinity, invading pathogens, and insect herbivores (Lewandowska et al. 2020). It is well documented and demonstrated that drought stress induces wax production (Aharoni et al. 2004; Zhang et al. 2005; Cameron et al. 2006). Significant correlations were observed between the content of waxes, yield, water use efficiency (WUE), and drought tolerance in crops like rice, wheat, barley, and sorghum (Jordan et al. 1984; Richards et al. 1986; Febrero et al. 1998; Zhu and Xiong 2013). These evidences point toward the fact that as the wax content decreases, the crop plants will become more sensitive in general to desiccation and drought stress compared to more waxy ones (Guo et al. 2016). The role of cuticular waxes in imparting salinity stress tolerance is through controlling the residual transpiration, which is negatively correlated with wax content (Hasanuzzaman et al. 2017). Higher leaf surface wax containing genotypes generally have a cooler canopy temperature that helps to resist high temperature or heat stress (Awika et al. 2017). Similarly, higher cuticular waxes can protect from high light conditions such as excessive ultraviolet (UV) radiations, indicating these stresses can affect and alter the plant cuticular waxes (Fukuda et al. 2008; Xue et al. 2017; Lewandowska et al. 2020).

Infection with plant pathogens can also result in increased epi-cuticular wax load and change the cuticular properties. Infections with fungal pathogens *Colletotrichum gloeosporioides* and *C. acutatum* in tomato and citrus plants resulted in increased cuticular wax biosynthesis, deposition, and changes the cuticular structure (Alkan and Fortes 2015; Marques et al. 2016). The increase in epi-cuticular wax load and changes in chemical composition may not always necessarily result in plant resistance against biotic stresses. The epi-cuticular waxes can play divergent roles in different plants and for different pathogens. This was demonstrated through the functional studies of the *Arabidopsis DEWAX* gene, a negative regulator of wax biosynthesis. The *Arabidopsis dewax* mutant lines showed an increased epi-cuticular wax were susceptible to *Botrytis cinerea* and resistant to *Pseudomonas syringae*, fungal and bacterial pathogens, respectively (Ju et al. 2017). Overexpression of DEWAX in *Arabidopsis* and *Camelina* showed inverse defense regulation to *Botrytis* and *Pseudomonas* (Ju et al. 2017).

7.6 Attempts to Manipulate Cuticular Trait

Attempts have been made to improve crop plants by targeting the wax biosynthesis pathway and altering the cuticular properties by conventional and modern breeding as well as through transgenic approaches. The prerequisite for crop improvement through breeding or transgenic approaches is to have the prior knowledge about the

genomic region/s and gene/s contributing for wax traits. This has been achieved to an extent through the loss- and gain-of-function mutants in either model or crop species. Over the domestication process of major crops like wheat, rice, corn, barley, soybean, and tomato, focus was on yield traits and the yield targeted breeding over generations resulted in reduced genetic diversity for other biotic and abiotic stressors in commercial varieties. A good source to regain the lost genetic diversity is to incorporate the wild relatives and landraces of crops plants in the breeding program. Multiple quantitative trait loci (QTL) regions involved in the biosynthesis of epi-cuticular waxes and its transport have been reported from multiple crops like rice, sorghum, cabbage, and pearl millet and can be used for marker-assisted breeding (MAS) programs (Srinivasan et al. 2008; Burow et al. 2009; Liu et al. 2018).

Considerable amount of work has been carried out in *Arabidopsis* to identify and characterize the functional and regulatory genes involved in cuticular wax biosynthesis (Aharoni et al. 2004; Kannangara et al. 2007; Seo et al. 2009; Shi et al. 2011; Yang et al. 2020). Many of these genes or its homologs identified from crop plants have been used for targeted engineering of wax biosynthetic pathway in crop plants that resulted in altered cuticle properties and showed multiple stress tolerance (Zhang et al. 2005, 2019a, b, c; Adato et al. 2009; Shi et al. 2013; Kumar et al. 2016; Sajeevan et al. 2017a; Liu et al. 2019; More et al. 2019; Wang et al. 2020). It has also been shown that ectopic expression of *Arabidopsis* or its homologs overexpression in biofuel crop, *Camelina sativa* and tree species like *Morus* and *Malus* resulted in altered total wax load, composition, structure, and contributed to drought tolerance (Lee et al. 2014; Sajeevan et al. 2017a; Zhang et al. 2019b, c). Alterations in the wax biosynthesis pathway is hampered due to the lack of clear knowledge in cuticular wax load, its chemical composition, and structural characteristics required to improve specific crops, and also to what extent these factors needs to be species- or tissue-specific.

7.7 Conclusion

Cuticle is a natural film covering the outer parts of the plant that consists of lipid polyesters covered and embedded with waxes that protect the tissues from multiple abiotic and biotic stresses. During the land plants evolution from aquatic to a more desiccating terrestrial environment, plants evolve to synthesize cuticular waxes as a fundamental morphological and physiological adaptation. There is a high level of compositional and structural differences exist in cuticular waxes among different crop plants and organs. These cuticular waxes are largely produced by two complex pathways controlled by the expression of different genes/enzymes in turn influenced by multiple environmental stresses. Although past decade advancement in genome sequencing technologies and through various forward and reverse genetics approaches allowed us to elucidate and understand the complex gene regulatory network involved in biosynthesis, transport, and deposition of cuticular waxes in

model as well as different crop plants, to an extent. We still have long way to go towards fully understanding the regulatory mechanisms controlling the cuticular wax biosynthesis, compositional and structural differences, transport, and deposition in response to various stressors. In addition, a limited understanding of the role of plant cuticle components as signaling molecules that promote resistance or susceptibility to biotic stresses needs to be further investigated. Unraveling these mechanisms would aid in targeted manipulation of the trait using modern biotechnological applications for the development of crop cultivars with improved health thereby promoting sustainable agriculture.

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