Leaf Cuticular Wax, a Trait for Multiple Stress Resistance in Crop Plants

Kunhikrishnan H. Dhanyalakshmi,

Raju Y. Soolanayakanahally, Tawhidur Rahman,

Karen K. Tanino and Karaba N. Nataraja

Abstract

Cuticular waxes form the primary interface between a plant and its external environment. The most important function of this hydrophobic interface is regulation of non-stomatal water loss, gas exchange and conferring resistance to a wide range of biotic as well as abiotic stresses. The biosynthesis, transport and deposition of the cuticular waxes are tightly coordinated by complex molecular networks, which are also often regulated in response to various developmental, biotic as well as abiotic cues. Evidences from model as well as non-model systems suggest that targeted manipulation of the molecular regulators of wax biosynthetic pathways could enhance plant resistance to multiple stresses as well as enhance the post-harvest quality of produce. Under the current scenario of varying climatic conditions, where plants often encounter multiple stress conditions, cuticular waxes is an appropriate trait to be considered for crop improvement programs, as any attempt to improve cuticular traits would be advantageous to the crop to enhance its adaptability to diverse adverse conditions. This chapter briefs on the significance of cuticular waxes in plants, its biosynthesis, transport and deposition, its implication on plant resistance to adverse conditions, and the current options in targeted manipulation of wax-traits for breeding new crop types.

Keywords: cuticular waxes, wax biosynthesis, biotic stress, abiotic stress, stress resistance

1. Introduction

In the current era of increasing uncertainties in crop production, emerging constraints and risks demand technical and technological advances in the agricultural sector, and integrative approaches, such as Climate Smart Agriculture (CSA), to address the interlinked challenges of food security and climate change. While maintaining food security is a major challenge for future, the possible solution is to enhance crop productivity along with nutritional security. However, this stance is remarkably limited by the different abiotic as well as biotic environments, where the crops grow and develop.

Drought, excess water (flooding), extremes of temperatures (cold, chilling, frost, and heat), salinity, high and/or low light, mineral deficiency, and toxicity are the common abiotic stresses for crop production. These stresses alter plant metabolism, growth, development, and in extreme cases cause the cessation of

vegetative and reproductive growth. Some of the abiotic stresses such as drought, high temperature and salinity can influence the occurrence and spread of biotic agents like pathogens, insects, and weeds [1]. In crops like tomato, cucurbits and rice, temperature is one of the most important deciding factors for the occurrence of bacterial diseases [2]. Temperature can also alter the incidence of vector-borne diseases by modifying spread of vectors.

But, in their natural environment, plants face combination of stresses, especially under the changing climate scenario. The effect of stresses would be more pronounced under combined (biotic and abiotic) stresses [3], while simultaneous occurrence of abiotic and biotic stresses are more destructive to crop production [4]. Hence, there exists a need now, to look for common traits that can contribute for plant adaptation to such multifarious stressful conditions and sustain crop productivity as well. In this scenario, it is desirable to have a single trait that can confer tolerance to multiple (abiotic and biotic) stresses. Cuticular waxes, a major component of plant cuticle covering all the aerial parts of the plants, can be considered as an important trait for combined stress resistance.

2. Cuticular waxes: a component of plant cuticle

The cuticle is a unique structure developed by land plants during the course of their evolution from an aquatic to a terrestrial lifestyle [5]. The primary role of this lipophilic layer, comprising cutin and cuticular waxes, was to limit non-stomatal water loss by functioning as a physical barrier between the plant surface and its external environment [6]. Development of a cuticular barrier is one of the major adaptive mechanisms for survival and growth of plants under water limiting terrestrial conditions [7]. As the primary barrier between the aerial surface of plants and the external environment, the cuticle also protect the plants from mechanical rupture or injury, toxic gases and ultra violet radiation [8–10]. The cuticle also has notable roles associated with growth and developmental processes like preventing epidermal fusion by establishing normal organ boundaries [11], and phytohormone homeostasis [12]. The cuticle and its components are known to play essential roles as signaling molecules for pathogens and for the plants themselves [13]. Another important role is in fruits, where it influences quality, defense and post-harvest shelf life [14]. In fruits, water retention [15] firmness [16] and its responses to physical and biotic stresses are also influenced by the cuticle [17].

The cuticle is composed of a covalently linked scaffold of cutin and a mixture of soluble cuticular lipids (SCL), called as waxes [10, 18]. Structurally, cutin is made of covalently cross linked C16 or C18 oxygenated fatty acids and glycerol, forming the most abundant structural component of the cuticle [19]. The waxes within the cuticle function as an actual barrier against the diffusion of water or solutes [20, 21]. The waxes occur in two layers; forming two distinct physical layers called intra- and epi-cuticular waxes [22]. The former is dispersed within the cutin polymer while the epi-cuticular wax is deposited on the outer surface as crystals or films [22, 23]. This outermost layer can be physically stripped off the surfaces using aqueous glue [23, 24]. These waxes are composed of a variety of organic solvent-soluble lipids; consisting of very-long-chain fatty acids (VLCFA) and their derivatives. The major composition of VLFCAs are alkanes, wax esters, branched alkanes, primary alcohols, alkenes, secondary alcohols, aldehydes ketones, and unsaturated fatty alcohols, as well as cyclic compounds including terpenoids and metabolites such as sterols and flavonoids [19, 25–27]. Wax composition varies with crop species and differs in their functions and responses to biotic and abiotic environments [10].

As per recent studies, intra-cuticular waxes form the primary transpirational barrier and the contribution of epi-cuticular waxes as a transpirational barrier

depends on the species-specific cuticle composition [28]. In species like *Tetrastigma voinierianum*, *Oreopanax guatemalensis*, *Monstera deliciosa*, and *Schefflera elegantissima*, intra-cuticular wax pre-dominantly act as a transpirational barrier while in *Citrus aurantium*, *Euonymus japonica*, *Clusia flava*, and *Garcinia spicata*, both intra- as well as epi-cuticular waxes had equal contribution as transpirational barriers [28]. A study from *Prunus* suggests that intra-cuticular waxes of the cuticle form the actual transpirational barrier [29] and not epi-cuticular waxes [30].

3. Ecological significance of cuticular waxes

The cuticular waxes confer diverse surface properties to plant parts, which actually play the key role in controlling non-stomatal water loss and gas exchange, and protection from external environment. Leaf cuticular wax amount and crystal morphology regulated post-harvest water loss from leaves [31]. Epi-cuticular wax films give glossy appearance to leaves and fruits, while wax crystals (β-diketones) conferred dull, glaucous appearance to leaves and stems [10]. The thickness [5] composition and properties of the waxes vary with crop species and are found to be induced under diverse stressful conditions [32]. These differences reflect their functions and responses to biotic and abiotic environments [10]. Importance of cuticular wax accumulation in plant resistance to both biotic as well as abiotic stress conditions is now well documented [12, 33, 34].

3.1 Abiotic stresses

One of the most important roles of the waxes is to protect the plant surfaces from excessive solar and ultraviolet (UV) radiations. Cuticular waxes scatter UV-B radiation [35] and was demonstrated in apple [36]. As per studies from model systems as well as crops, increased cuticular wax biosynthesis improves drought stress resistance [37]. In rice, wheat, barley and sorghum, grain yield under water limiting conditions have positive correlation with wax content [38–41] . Hence, in crop plants, higher cuticular wax content is a promising trait for stress resistance as well as yield under water limiting conditions [27]. In mulberry, increasing wax load is useful to manage post-harvest water losses [42]. In barley, cuticular wax components act as a barrier to water loss and contribute to salt stress resistance [43]. Heat stress resistance is also positively correlated with wax accumulation in bahia grass [44]. Under heat stress, the wax load in sorghum was correlated with its ability to maintain the canopy temperature cool, resulting in reduced water loss [45]. Similarly, pea varieties with thicker wax load also exhibited lower canopy temperature, thereby limiting water loss and associated heat stress [46].

Cuticular waxes play an important role in preventing non-stomatal water loss during drought and high temperature stress, as well as enabling frost avoidance. Such climatic stressors can induce a heavier wax load and change the chemical composition of waxes by accumulating longer aliphatic compounds on plant tissues [47]. Drought increases stiffness and quality of the plant cuticle under climate change [48]. Similarly, the leaf cuticular surface is the first barrier blocking destructive ice penetration into the leaf cells in freezing avoidance mechanisms [49]. Using a hydrophobic film, Wisniewski et al. [50] showed the importance of the epi-cuticular hydrophobicity enabling avoidance of freezing in sensitive plants. The critical nature of the cuticular layer in frost avoidance of corn is also clearly demonstrated [51]. Freezing avoidance is the only mechanism of frost resistance in sensitive plants. In fact, the first demonstration of a transgenic organism in agriculture was the alteration of the cell wall protein secondary structure on ice nucleating bacteria, *Pseudomonas syringae* and *Erwinia herbicola*, which then prevented ice nucleation across the cuticle and avoided leaf damage [52, 53]. In future, injury due to frost stress will be more, not

less under global warming [54]. Hence, a better understanding of stress-induced wax modification among crop plants holds promise to cope with climate change.

3.2 Biotic stresses

The cuticle and its components act as signaling molecules to favor fungal growth and development, and infections in plants [55, 56]. Surface waxes act as cues to activate fungal developmental processes like appressorium formation, pre-penetration processes, etc., in crop plants like avocado, wheat, rice, maize and peanut [13, 57–59]. However, the hydrophobic nature of the cuticle also renders it a barrier for bacterial as well as fungal pathogens [60], a desirable trait for disease resistance. Waxes are known to protect lotus from pathogen infection [61]. It repulses pathogen spores and atmospheric pollutants like acid rain and ozone [32]. Another role of waxes is in plant-insect interaction; to attract or to serve as a deterrent [62]. It prevents insect attachment to plant surface oviposition and feeding [63, 64] and hence confer tolerance to insects in crop plants [65, 66].

4. Molecular biology of cuticular wax biosynthesis and deposition

Studies in *Arabidopsis* and subsequently, barley, rice and tomato systems have significantly contributed for the elucidation of the complex regulatory pathways underlying the biosynthesis, transport and deposition of wax components on plant surfaces [26, 27, 67]. Cuticular wax biosynthesis predominantly occurs in epidermal cells. The biosynthetic pathway initiates exclusively in the outer membranes of the plastids of epidermal cells where C16 and C18 fatty acids are synthesized, exported to the cytosol as acyl-CoAs and then elongated up to C34 at the endoplasmic reticulum (ER); through a series of enzymatic reactions [19, 26]. The synthesized components are subsequently transported through the apoplastic pathway and deposited on the cuticle. The key steps involved [32] are summarized here.

4.1 Synthesis of malonyl-CoA

The *de novo* fatty acid biosynthesis initiates with the synthesis of malonyl-CoA. It is initiated with the transfer of a bicarbonate derived CO_2 molecule to the biotin moiety of a biotin carboxylate carrier protein (BCCP), that form N-1,2 carboxybiotin biotin carboxylate carrier protein-BCCP. The reaction is catalyzed by biotin carboxylase (BC). The CO_2 is further transferred to acetyl-CoA by carboxyltransferases (CT). Acetyl-CoA carboxylase (ACCase), a multifunctional enzyme system then catalyzes the formation of malonyl-CoA, from acetyl-CoA [32], which will be subsequently used for *de novo* fatty acid biosynthesis.

4.2 De novo fatty acid biosynthesis

De novo synthesis of acyl chain in the stroma of plastids is catalyzed by a series of enzymatic steps, which collectively forms fatty acid synthase complex (FAS). The series of reactions with the catalyzing enzymes are:

a. Condensation of malonyl-acyl carrier protein (manolyl-ACP) with acetyl-CoA to form 3-ketoacyl-ACP catalyzed by β-ketoacyl-ACP synthase (KAS III).

b. Reduction of 3-β-ketoacyl-ACP to 3-hydroxyacyl-ACP, catalyzed by 3-βketoacyl-ACP reductase.

c. Dehydration of 3-hydroxyacyl-ACP to *trans-*Δ^2*-*enoyl-ACP, catalyzed by β-hydroxy acyl ACP dehydratase.

d. Reduction of *trans-*Δ^2*-*enoyl ACP to Acyl-ACP by Enoyl ACP reductase.

This complex also includes an acyl carrier protein (ACP), a cofactor component of FAS to which the growing acyl chain remains esterified. These sequential reactions result in a fully reduced acyl chain, extended by two carbons in each cycle [68] through the sequential round of condensation, reduction, dehydration and second-reduction steps [69]. Repetition of the cycle for six times generates palmitoyl-ACP (16:0-ACP), where the condensation reactions are catalyzed by KAS I. One final cycle reaction between palmitoyl-ACP and malonyl-ACP utilizes KAS II to generate stearoyl-ACP (18:0-ACP). These products are further processed by stearoyl-ACP desaturase (introduce double bonds), plastidial acyltransferases, and acyl-ACP thioesterases (hydrolases). The fatty acyl-ACP thioesterases (FATA and FATB) hydrolyzes the C16-C18 acyl-acyl carrier proteins to generate fatty acids, which are then exported out of the plastids to undergo modifications in the ER [69].

4.3 Elongation of fatty acids

The C16 and C18 compounds, hydrolyzed by acyl-ACP thiosterases are activated into C16- and C18-CoA by long chain acyl-CoA synthetases (LACSs) and exported to the ER. The C16 and C18 acyl-CoA then act as a substrate for fatty acid elongase (FAE) complex, localized on the ER, which adds two carbons successively to form VLCFAs with C26-C34 chains. FAE complex are heterotetramers of independently transcribed, monofunctional proteins. They operate a reiterative cycle of four reactions catalyzed by

i. β-Ketoacyl-CoA synthase (KCS) that catalyze the two carbon condensation to acyl-CoA.

ii. β-Ketoacyl-CoA reductase (KCR) that catalyze the reduction of β-ketoacyl-CoA.

iii. β-Hydroxyacyl-CoA dehydratase (HCD) that catalyze the dehydration of β-hydroxyacyl-CoA.

iv. Enoyl-CoA reductase (ECR) that reduces the enoyl-CoA ultimately leading to VLCFAs [69–71].

4.4 Wax biosynthetic pathways

The elongated products are further modified to produce wax components i.e., to primary alcohols, alkyl esters, aldehydes, alkanes, secondary alcohols, ketones and free fatty acids, via two pathways (i) acyl reduction pathway (generates primary alcohols and wax esters) and (ii) decarbonylation pathway (generates alkanes, aldehydes, secondary alcohols, and ketones).

i. Acyl-reduction pathway: fatty acyl-CoAs are converted into primary alcohols catalyzed by fatty acyl-CoA reductase (FAR) through an intermediate aldehyde [71]. A bi-functional wax synthase/acyl-CoA:diacylglycerol acyltransferase (WS/DGAT) enzyme, WSD1 condenses the generated fatty alcohols and C16:0 acyl-CoA into wax esters [26].

ii. Decarbonylation pathway: acyl-CoAs are reduced to aldehyde intermediate by FAR, which are subsequently decarbonylated into alkanes, catalyzed by aldehyde decarbonylase. Stereospecific hydroxylation of alkanes catalyzed by midchain alkane hydroxylase 1 (MDH1) give rise to secondary alcohols, and oxidation of these alcohols form corresponding ketone [32]. Additional hydroxylation and oxidation reactions lead to the esterification of secondary alcohols with fatty acids and formation of diols, hydroxyl ketones and diketones [32].

4.5 Transport and deposition of cuticular waxes

The wax components generated are then transferred from the ER to the plasma membrane (PM) through Golgi and trans-golgi network mediated vesicle trafficking or non-vesicular trafficking [72]. Further, adenosine triphosphate binding cassette (ABC) transporters in the plasma membrane (homodimers and heterodimers) export the wax components to the epidermal surface [73]. Lipid transfer proteins (LTPs) like glycosylphosphatidylinositol (GPI)-anchored LTPs (LTPGs), attached to the outer surface of the plasma membrane are also
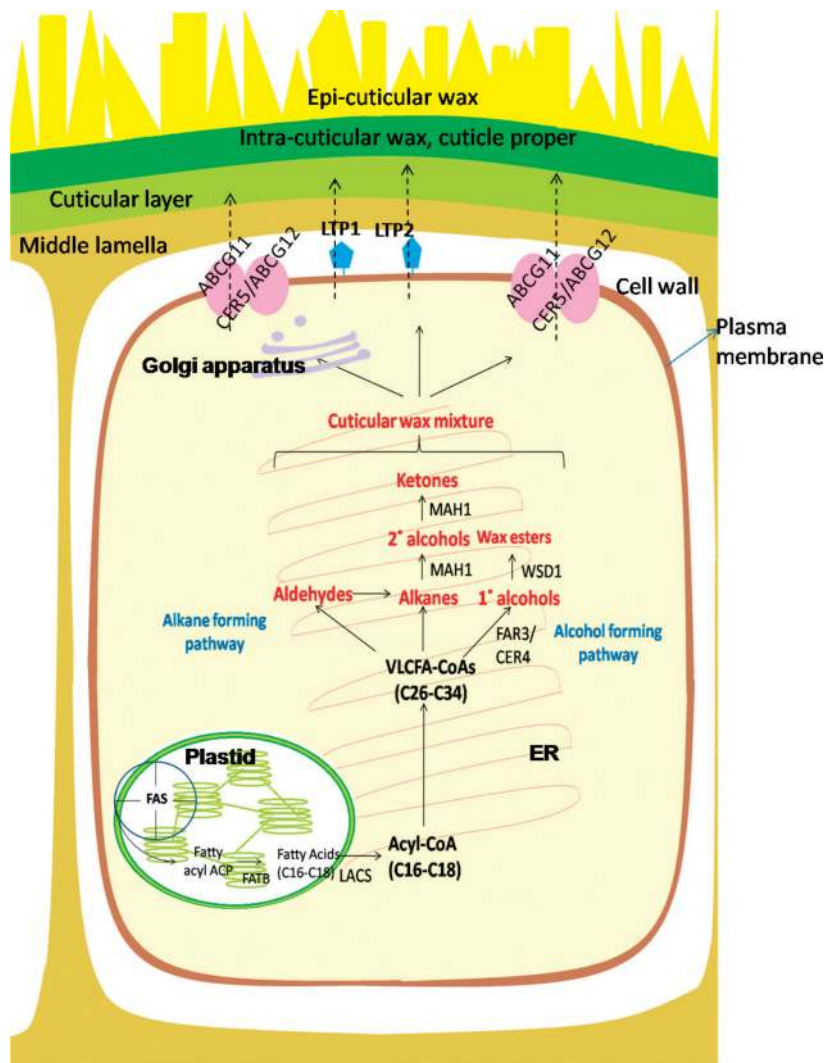

Figure 1.
Schematic representation of wax biosynthesis, transport and deposition in plants.

Gene	Protein type	Role	Reference
Cuticular wax biosynthesis			
ACC1	Acetyl CoA carboxylase	Synthesis of malonyl CoA substrates	[79]
FATB	Acyl acyl carrier protein thioesterase	Supply of saturated fatty acids for wax biosynthesis	[80]
CUT1 /CER6/KCS6	VLCFA condensing enzyme (β-ketoacyl-CoA synthase)	Regulation of VLCFA biosynthesis/elongation of 24C fatty acids	[81]
CER1/CER22	Aldehyde decarbonylase	VLC alkane biosynthesis	[82]
KCS1	β-ketoacyl-CoA synthase	Elongation of 24C fatty acids	[83]
KCS20; KCS2/DAISY	3-ketoacyl-coenzyme A synthase	Required for VLCFA elongation to C22	[84]
LACS1/CER8; LCAS2	Long chain acyl CoA synthetase	Synthetase activity for VLCFAs C20-C30	[85]
KCS9	3-ketoacyl-coenzyme A synthase	Elongation of C22-C24 fatty acids	[86]
WAX2/YRE/FLP1/ CER3	Aldehyde-generating acyl-CoA enzyme	Required for synthesis of aldehydes, alkanes, secondary alcohols, and ketones; biosynthesis of cuticular membrane	[76, 87]
CER10	Enoyl-CoA reductase	Biosynthesis of VLCFA	[88]
CER4/FAR3	Alcohol forming fatty acyl CoA reductase	Formation of C24:0 and C26:0 primary alcohols	[89]
CYP96A15 (cytochrome P450 enzyme)	Midchain alkane hydrolase	Formation of secondary alcohols and ketones (stem cuticular wax)	[78]
WSD1	Wax ester synthase/diacylglycerol acyltransferase	Wax ester biosynthesis	[90]
PASTICCINO2 (PAS2)	3-hydroxy-acyl-CoA dehydratase	VLCFA synthesis in association with CER10, an enoyl-CoA reductase	[91]
KCR1	β-Ketoacyl-CoA reductase	Required for VLCFA elongation	[70]
CER2	BAHD acyltransferase	Fatty acid elongation beyond C28	[92]
CER17 (ECERIFERUM1)	Acyl-CoA desaturase like 4	n-6 desaturation of very long chain acyl-CoAs	[93]
Transport and deposition			
AtWBC12/CER5	ATP binding cassette (ABC) transporter	Transport of cuticular waxes	[94]
LTPG1	Lipid transport protein	Cuticular wax export or accumulation	[74]
ABCG11/WBC11/ DESPERADO	ATP binding cassette (ABC) transporter	Secretion of surface waxes in interaction with CER5	[73, 95]
LTPG2	Lipid transport protein	Cuticular wax export or accumulation	[96]
GLN1, ECH		Vesicle trafficking	[72]

Table 1.
Key genes involved in wax biosynthesis, transport and deposition identified from the model system Arabidopsis.

directly or indirectly involved in wax export [74]. A brief representation of wax biosynthesis, transport and deposition with key genes, is presented in **Figure 1** (adapted from [19, 26, 27, 32, 69, 71]).

Early studies in barley mutants with little or no wax on aerial plant parts, called glossy or glaucous were termed as eceriferum (cer), where cera means wax and ferre means to bear [75]. Subsequently, the wax defective mutants in *Arabidopsis* with bright, shiny, or glossy stems or leaves were also termed as *eceriferum* (*cer*) [76]. The wax locus from maize and *Brassica napus* is termed as *glossy* [68]. With the help of forward genetic screens using wax defective mutants and reverse genetic approaches [77, 78], considerable progress has been achieved in understanding wax biosynthesis, transport and deposition. **Table 1** gives an overlook of the key genes involved in wax biosynthesis, transport and deposition identified from the model system *Arabidopsis*.

5. Regulation of cuticular wax biosynthesis

While the complex wax biosynthesis and transport pathways are well determined, the information on underlying regulatory mechanisms is still fragmentary. There is limited information that these processes and their candidate pathway genes are influenced by developmental factors. The cuticle development is an intrinsic part of cell developmental processes like organ development, cell partitioning, etc. [11]. PAS2, acy-CoA dehydratase, regulating the synthesis of VLCFA during wax biosynthesis in the epidermis is essential for proper cell proliferation during development [97]. Wax deposition is also known to occur in an organ-specific manner during its development and is influenced by diverse environmental conditions as well [17]. The available information on the exact developmental regulation of wax biosynthesis is however, limited. As per evidences from leek (*Allium porrum* L.), wax accumulation and elongation activities are highly induced within a defined and an identifiable region of leaf [98]. The expression of plastidial fatty acid synthase (FAS), FAEs that regulate elongation of long-chain fatty acids in the microsomal membranes and acyl ACP-thioesterases are probable targets of developmental regulation, depending upon the need to produce fatty acid precursor pools [98]. Some of the key genes involved in wax biosynthesis are also affected by defects in the organization of organelles, especially the ER. A mutation of PEX10 (peroxisome biogenesis factor 10) in *Arabidopsis*, which disrupted the ER network, in turn lead to mislocalization of CER4, CER1, SHN1 and WAX2, affecting cuticular wax biosynthesis [99].

There is increasing evidence to show that wax biosynthesis and its pathway genes are regulated at transcriptional, post-transcriptional and translational levels [26, 100]. A wide range of abiotic factors like light, water, temperature, salinity etc., influence wax biosynthesis and deposition. An increase in cuticular wax content is observed in bean, barley and cucumber on exposure to UV-B light [101]. In cotton, enhanced UV-B radiation specifically increased the epicuticular wax load on the adaxial surface of leaves [102]. There is an also an up-regulation of wax biosynthetic genes in salt tolerant rice genotypes under stress [103]. Although the underlying mechanisms have not been well explored in the above conditions, there is sufficient information on the influence of drought or moisture stress on wax biosynthesis in plants. A significant increase in wax load in *Arabidopsis* plants subjected to water stress is indicative of its regulation under drought [17]. In crops like rice, wheat, tobacco, alfalfa, peanut and cotton, etc., an increase in cuticular wax accumulation was observed under moisture stress condition [104]. Drought induced accumulation of wax biosynthesis is positively correlated with drought tolerance in crops like oat, rice, wheat and forage crops, etc. [104–107].

The transcript levels of several genes involved in wax biosynthetic pathways are regulated in response to abiotic stresses. FAR5, a fatty acyl CoA reductase, in wheat responsible for accumulation of long chain primary alcohols of C26:0, C28:0 and C30:0 are regulated by drought, ABA and cold [108]. The transcripts of KCS2/DAISY, a 3-ketoacyl-coenzyme A synthase required for the elongation of VLCFA are up regulated under water deficit conditions [84]. Osmotic stress induces the expression of CER1, that regulates alkane biosynthesis; while the over expression of CER1 increased susceptibility to bacterial and fungal pathogens [109]. Hypoxia is also known to affect total wax loads on *Arabidopsis*. The expression of KCS, KCR1, ECR/CER10 and PAS2, components of fatty acid elongase complex in *Arabidopsis* stem and leaves is affected which in turn affects the production of VLCFA precursors of wax biosynthesis. The wax synthesis genes like MAH1, CER3, CER4, WSD1, etc., and several genes associated with wax and lipid transport are also affected by hypoxia [110]. There is also indication on the regulation of wax biosynthesis in response to cold. Acteyl-CoA carboxylase plays the essential role for cold acclimation in *Arabidopsis*. In sensitive to freezing3 (*sfr3*) mutants, with a missense mutation in ACC1, the long chain components of leaf cuticular wax were reduced and there was inhibition on the wax deposition on inflorescence stem, which rendered the plants sensitive to cold stress [111]. Wax biosynthesis is also reported to be regulated in response to carbon dioxide (CO_2) concentration. This is mediated by HIC (High Carbon Dioxide), a gene encoding a 3-keto acyl coenzyme A synthase (KCS)-an enzyme involved in the synthesis of very-long-chain fatty acids that influences stomatal development in *Arabidopsis* [112].

With the identification of several transcription factors (TFs), transcriptional regulatory mechanisms are considered to be a major contributor for the wax biosynthesis [113]. WIN1/SHN1 (WAX INDUCER 1/SHINE1) is a TF from AP2/EREBP family initially reported to regulate cuticular wax and then cutin biosynthesis by regulating the expression of *CER1*, *KCS1*, *CER2*, *LACS2*, *GPAT4*, *CYP86A4*, *CYP86A7* and *HTH*-like genes [114]. SHN1 overexpression increased drought tolerance in *Arabidopsis* [115]. Wax synthesis regulatory gene 1 (WR1) from rice [116] and SHN1 from wheat [117], both homologs of WIN1/SHN1 from *Arabidopsis* also reduced water loss and improved drought tolerance. Transcriptional repression by diurnally controlled DEWAX2 is another important for regulator of wax biosynthesis in *Arabidopsis*. Compared to wild type, the total wax loads in *dewax2*, were increased by 12 and 16% respectively in rosette and cauline leaves [118, 119]. Another candidate from AP2/ERF TF family, WRINKLED4 (WRI4) positively regulates wax biosynthesis in stems. *wri4* mutants expressed 28% reduction of total wax loads in stems, although siliques and leaves were unaffected. Hence WRI4 act as a transcriptional activator to regulate the expression of LACS1, KCR1, PAS2, ECR and WSD1, to maintain the levels of 29C long alkanes, ketones and secondary alcohols in stems [113]. MYB94, regulate the expression of wax biosynthetic genes like WSD1, KCS2/DAISY, CER2, FAR3 and ECR to activate cuticular wax biosynthesis and is up regulated by drought and ABA. This also conferred tolerance to drought stress in *Arabidopsis* and *Camelina* [120]. MYB96, an ABA responsive TF also regulates wax biosynthesis under drought [121]. In *Camelina*, MYB96 activated the expression of wax biosynthetic genes KCS2, KCS6, KCR1-1, KCR1-2, ECR, and MAH1 which resulted in high levels of alkanes and primary alcohols and improved drought tolerance [120]. MYB96 acts as a component of plant disease resistance, through salicylic acid mediated signaling [122]. Both MYB94 and MYB96 share a common region containing MYB consensus motifs in the promoter of their target wax biosynthetic genes [123]. Hence MYB94 and MYB96 have an additive role on plant cuticular wax biosynthesis and under drought and ABA conditions.

In addition, to transcriptional regulation, wax biosynthesis is regulated by other events. Expression of CER3/WAX2/YRE, an aldehyde-generating acyl-CoA enzyme
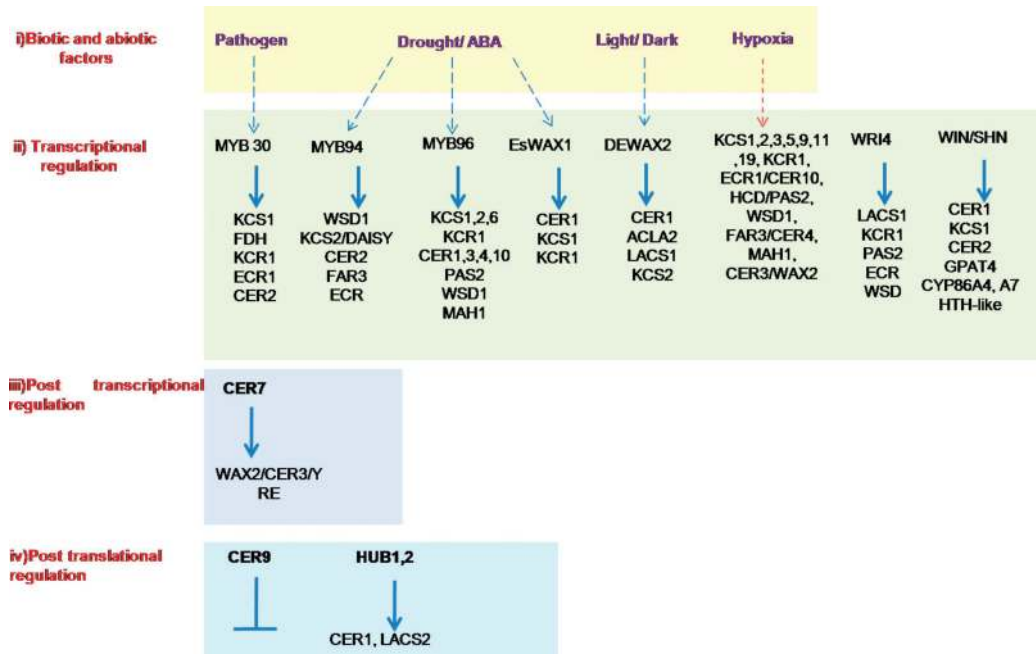
Figure 2.
Brief representation of the key regulatory events in wax biosynthesis and their targets.

in the wax biosynthetic pathway is regulated by CER7, a core RNA processing and degrading exosomal subunit. CER7 regulates WAX2 transcript levels by degrading a specific mRNA species encoding its negative regulator [124]. Many of such regulators have been identified from model systems as well as crop species and a brief overview of the key regulatory events and their targets has been presented in **Figure 2**.

6. Cuticular wax trait in imparting stress resistance

Under field conditions, crops encounter multiple biotic and/or abiotic stresses simultaneously at different stages of developments. Cuticular waxes have a direct role in multiple stress tolerance in crops [109]. In cucumber, wax biosynthesis has been shown to have key roles in influencing the plant responses to biotic as well as abiotic stresses [125]. In sorghum, genes regulating leaf waxes have critical role in regulating tolerance to drought and heat stress [45]. Considering the relevance of cuticular waxes under diverse biotic as well as abiotic stressful conditions, as discussed above and under combined stress conditions, it can be an ideal trait to tackle multiple stresses in crop plants.

6.1 Biotic stresses

6.1.1 Pathogens

Being the outermost layer of plant cuticle, the epi-cuticular wax can serve as a first line of physical defense against pathogens and herbivores. However, increasing thickness and hydrophobicity of the cuticle through over-deposition of the wax may not necessarily increase the resistance of the plant against biotic stresses. The composition and structure of wax in the cuticle can constitute the source of signals for the foreign invaders and for the plants themselves. Thus, the roles of cuticular wax could be multifunctional and can vary not only for various plant species but

also for different kinds of pathogens. Functional study of the *DEWAX* gene, a negative regulator of wax biosynthesis in *Arabidopsis*, is a good example of this complexity. The *dewax* mutant line in *Arabidopsis*, with increased epicuticular wax and decreased cuticular permeability, showed susceptibility to the fungal pathogen *Botrytis cinerea*, but resistance to the bacterial pathogen *Pseudomonas syringae* [126]. Moreover, *DEWAX* overexpressing lines in *Arabidopsis* and *Camelina* showed inverse defense modulations to *B. cinerea* and *P. syringae* as compared to *dewax* mutant in *Arabidopsis* [126].

Wax and cutin components in the plant cuticle could function in pattern- and effector-triggered immunity (PTI and ETI) and could serve to generate local and systemic acquired resistance against numerous pathogens [127]. During plant-pathogen interaction, the plant cuticle can be affected by enzymes synthesized and secreted by the pathogens. Many fungal pathogens synthesize and secrete hydrolytic enzymes (for example, cutinases, esterases and lipases) at the early stage of infection that directly target the cuticle [128–131]. *Fusarium oxysporum* secretes cutinases that degrade cutin layers in the cuticle and generates cutin monomers that support fungal adherence to the host plant and facilitate the initiation of infection [128]. Hexadecanediol, a cutin component in rice can facilitate spore germination and differentiation for pathogenic fungi *Magnaporthe grisea* and *B. cinerea* [55]. Presence of a very-long-chain C_{26} aldehyde (a wax component) was important for the barley powdery mildew fungus (*Blumeria graminis*) to initiate infection in host plant species. Germination and appressorial differentiation of *B. graminis* were strongly prohibited in aldehyde free *glossy11* mutant in corn. Spraying of *n*-hexacosanal (C_{26}-aldehyde) or wax preparation from wild-type corn can restore the conidial formation and differentiation [59].

Plant can also recognize the attachment of pathogens and activate defense responses against them, in which pathogen-infection generated plant products, such as cutin monomers or cell wall oligosaccharides, can act as signaling molecules [132]. Defense responses in plants are often manifested as alternations of the cuticle. *Colletotrichum acutatum* infection in citrus resulted in increased lipid synthesis in the epidermal cell and increased deposition of those lipids in cuticle, the process eventually changes the structure of the cuticle [133]. Cuticular biosynthesis was also found to be up-regulated in tomato fruit following infection by fungal pathogen *C. gloeosporioides* [134].

Cuticular permeability plays a vital role in almost all plant-pathogen interactions. A more permeable cuticle can lead to either resistance or susceptibility to pathogens. Elevated deposition of cuticular wax as well as the presence of hydrophobic wax components (e.g., very-long-chain alkanes or ketones) can make a cuticle less permeable. Mutation or overexpression of genes that diminish biosynthesis of various wax components can generate the opposite effect. There are number of wax-deficient mutant and transgenic lines in *Arabidopsis* and other plant species with diminished cuticular permeability showed resistance to the fungal pathogen *B. cinerea* [34, 127]. However, the phenomenon is not true for all wax deficient plant lines. Wax and cutin deficient *acp4* and *gl1* mutants in *Arabidopsis* displayed increased sensitivity to *B. cinerea* [135, 136]. Mutations in SHINE transcription factors in other studies also showed alteration in cuticular wax accumulation, and susceptibility to *B. cinerea* infection [137, 138].

6.1.2 Insects and herbivores

Epicuticular wax also plays important roles in plant interaction with insects and herbivores. Flowering plants have evolved with cuticular wax of various forms, sizes and structures that are either enabling the attachment and movement of

pollinating insects, or reducing the attachment of herbivorous insects and pests on the plant surfaces. Reducing the attachments of herbivores on plant surfaces is a part of a plant defense strategy against herbivores.

Most plant body surfaces are covered with a two-dimensional (2D) epicuticular wax film of various thicknesses. In many species, wax film is protrudes with three-dimensional (3D) wax crystals. Wax crystals can generate various shapes as revealed by electron microscopic analysis, such as rodlets, threads, platelets and tubules [61]. The complexity of these various shapes originates from the molecular self-assembly of various wax components, in which morphology of those crystals is also cor-related with the presence of specific chemical components in the wax [139, 140]. Many experimental studies and reports from various plants species (for example, from genera *Eucalyptus*, *Pisum*, *Brassica*) have shown that 3D wax crystals have protective functions against insects, in general, including the herbivorous insects [141]. Studies with *Eucalyptus* species in canopy found that glaucous juvenile leaves containing high quantities of wax crystals were less prone to herbivorous infestation as compared to the glossy adult leaves [142]. Feeding rates of flea beetles, *Phyllotreta cruciferae*, on low-wax glossy (*eceriferum, cer*) *Brassica napus* mutant lines were much higher as compared to the wild-type *B. napus* [143]. Cuticular surfaces with wax crystals also interferes with the attachment, locomotion and foraging behavior of predatory insects and parasitoids [65, 144]. *Pisum sativum* lines with higher prevalence of crystalline epicuticular wax (CEW) were found more favorable for four predatory coccinellid species to attach, move and consume more aphids as compared to the *P. sativum* mutant line with reduced CEW [145]. Flowering stems with high CEW of numerous other plant species (for example, species under the genera *Salix*, *Hypenia*, *Eriope*) often generate slippery surfaces that prevent the movement of nectar robbers, ants and other plant pests [141, 146].

Several hypotheses have been proposed and tested on the mechanisms of wax crystal inhibition of insect attachment inhibition: (i) roughness hypothesis; (ii) contamination hypothesis; (iii) fluid absorption hypothesis [141]. Wax crystals, in general, generate a micro-rough surface on the cuticle that may prevent adhesive pads of the insects to stick, preventing them to successfully attach to the plant sur-face [144, 147, 148]. Contamination hypothesis proposed that detached wax crystals of the cuticular surface of some plants can adhere to the insect attachment organs (e.g., adhesive pads), contaminate those, and as such subsequent insect attachment becomes challenging and unsuccessful [147–149]. Adhesive pads of many insects secret fluids, which can also enhance wax crystal contamination to attachment organs. Fluid secretion from the adhesive pads are supposed to help insects to pursue successful attachment to the plants. However, there is evidence certain plant species have crystalline wax coverage that can absorb the fluids secreted by the adhesive pads and prevent the insects to successfully attach to the cuticle [150, 151].

The study of cuticular wax involvement in biotic stress resistance is complex with a multitude of organisms spanning insects to disease. The story is still not clear and field situations in which interactions between organisms and abiotic stresses and the role of cuticular wax needs to be evaluated. Nevertheless, certain consistencies are evident in that permeability of the cuticular layer appears to be important in patho-gen invasion and wax crystals play an important role in insect intervention by the cuticular layer. These areas of research merit further investigation.

6.2 Abiotic stresses

As mentioned above, abiotic stresses such as drought, extremes of tempera-tures, salinity, etc., cause significant losses in crop productivity. Since most of the

stresses occur simultaneously, crop breeders are looking for traits contributing for multiple stress resistance. From this context, cuticular wax can serve as ideal trait. Drought stress, a major abiotic stresses in tropical regions, influences the biosynthesis and composition of cuticular wax in crops [27]. The importance of cuticular wax in desiccation tolerance is evident that, compared to gymnosperms and angiosperms, many early extant plants such as ferns, and horsetails are more sensitive to dehydration [152]. In crops like pea, cuticular wax load increases when subjected to drought stress [46]. In rice, *gl1*-1/wsl2 and *gl1-2* loss-of-function mutants with reduced wax load exhibited sensitivity to drought compared to the wild type plants [104, 153]. Drought stress is known to increase the wax content and alter composition of cuticular wax in many plants such as pea [46], *Arabidopsis* [17, 115], tobacco [154], alfalfa [155]. Significant correlations between the wax content and yield, drought tolerance and water-use efficiency have been reported in different crops such as sorghum [38], barley [156], rice [41], and wheat [157, 158]. These reports demonstrate that less wax or non-waxy crops/genotypes are sensitive to desiccation with poor drought-tolerance compared to the crops having more cuticular wax [105]. The existing evidences suggests cuticular wax is responsible for reducing non-stomatal transpiration by increasing cuticular resistance [43]. The cuticular waxes also have roles in imparting resistance to salinity stress, mainly by regulating residual transpiration. A significant negative correlation observed between residual transpiration and total wax content, reports residual transpiration could be a fundamental mechanism by which plants optimize water-use efficiency under salinity stress [43]. As discussed above, wax accumulation also correlated with high temperature resistance in plants [44]. Leaf surface waxes help to maintain cooler canopy in sorghum under heat stress [45]. The cuticular waxes can further help in protecting plants from high light stress [101]. The cuticular wax has a role in protecting plants from excessive ultraviolet (UV) light and there are reports indicating that elevated UV-B radiation can affect plant cuticular wax formation [101, 159, 160]. Based on the existing information, as mentioned above, cuticular wax, can be treated as the first protective layer and an important trait contributing for both biotic and abiotic stresses.

7. Attempts by crop biologists to manipulate cuticle traits

7.1 Breeding

Identification of genomic regions contributing wax traits is crucial in manipulating wax characteristics using breeding approaches. In rice, quantitative trait loci (QTL) linked to the leaf epi-cuticular layer was identified corresponding to EM15_10-ME8_4-R1394A-G2132 region on chromosome 8 [161]. In sorghum, a crop with the ability to produce profuse amounts of EW, B̲LO̲O̲M̲-C̲UTICLE (BLMC) locus from chromosome 10, was identified to account for profuse wax production. BLMC region corresponds to approximately 153,000 bp with three co-segregating markers and an acyl CoA oxidase with seven other putative candidates. BLMC mutation affected C28-C30 free fatty acid fractions and hence cuticle properties in culm and leaves, disrupted EW production and increased plant death rating in field at anthesis [162]. With the genetic analysis of F2 population from HUAYOU2 (P1 X M36), BoWax1 locus (*Brassica oleraceae* Wax 1) is identified to be controlling glossy green trait in cabbage, due to a deletion mutation of two nucleotides in the cDNA of Bol013612 of HUAYOU2. BoWax1 locus maps to chromosome CO1 [163]. The wax biosynthetic pathway genes identified in pearl millet were co-located to the QTL

controlling biomass production under early drought stress and stay green traits [164]. Targeted breeding using the modern molecular breeding for this trait would be useful.

7.2 Transgenic

With the elucidation of wax biosynthetic pathways and identification of key regulators, attempts were made in crop plants to engineer cuticle properties and to enhance stress tolerance traits. One of the early reports in engineering wax traits and thereby improved stress tolerance was from *Medicago sativa* (alfalfa), a forage legume. WXP1, a transcriptional regulator from *Medicago truncatula*, upregulated by drought, cold and ABA, was over expressed in alfalfa, which significantly increased the leaf cuticular wax load, mainly contributed by the C30 primary alcohol. The transgenic plants exhibited enhanced tolerance to drought and rapid recovery under rehydration [155]. Over expression of SlSHN1, a close homolog of the WIN/SHN gene from *Arabidopsis*, in tomato using constitutive CaMV 35S promoter improved drought tolerance, with higher cuticular wax deposition on leaf epidermal tissue. The transgenic plants displayed delayed wilting, improved water status and reduced water status [165]. MYB96, a transcriptional regulator over-expressed in *Camelina*, an emerging biofuel crop, which generated plants with enhanced drought tolerance. The expression levels of CsKCS2, CsKCS6, CsKCR1-1, CsKCR1-2, CsECR, and CsMAH1 were highly upregulated in the transgenic plants which resulted in a significant increase in the deposition of epicuticular waxes and total wax loads. This gives an option to cultivate the crop on marginal lands to produce renewable biofuels and bioresource [120]. It was further demonstrated that ectopic expression of DEWAX, a negative regulator of cuticular wax biosynthesis increased tolerance to *Botrytis cinerea* in *Camelina* [126]. A study from groundnut by over-expressing the KCS1 gene from a drought tolerant genotype improved cuticular was load and drought tolerance in a susceptible genotype [166]. Likewise, several of such regulators have been identified from model systems as well as crop species and used for engineering crop plants to enhance stress tolerance.

8. Options for manipulation of wax traits for individual and/combined stress tolerance

In crop plants, due to the nature of combined stressors interactions, the stress effect is not always additive [3]. While working with glossy mutants of *Zea mays* (*gl4*), an enhanced colonization of bacteria, was observed leading to more leaf blight pathogen growth compared to the wild type [167]. The thin cuticle provided leaf blight pathogen, an easy access to nutrient and water in *gl4* mutant indicating that cuticular wax thickness is a useful trait to identify plants' resistance to combined stressors. Additionally, wax layer structure and composition are equally important in conferring defense mechanisms. As rightly pointed in Ref. [1], such combined studies allow us to understand the shared and specific effects of biotic and abiotic stressors.

Wild relatives and landraces have long been recognized as a source of genes for breeding major field and horticulture crops. During domestication of wheat, tomato, rice, soybean and corn, yield was the focus trait. This in turn narrowed the genetic diversity for other biotic and abiotic stressors [168]. For example, during domestication of modern wheat, due to a phenotyping bottleneck a largely

overlooked drought trait in wheat breeding program is glaucousness [169]. Such beneficial allelic variants lost in cuticle related traits can be introgressed back by crossing an elite line with its wild relatives. Apart from genetic diversity, a mutation population (EMS or gamma irradiation) provides an alternative avenue to target crop improvement via selection of cuticle-associated trait variations [170]. In fleshy tomatoes, a mutant line underlying for *delayed fruit deterioration* (DFD), is characterized for minimal transpirational water-loss and enhance post-harvest shelf life [171]. A recent alternative for trait manipulation is CRISPR-Cas9 system which is a precise gene-editing technology. This new method accelerates the evaluation of beneficial cuticle-associated alleles in different genetic backgrounds [172]. In similar lines, small RNA based transgenic strategy is also emerging as a molecule of choice to deal with combined biotic and abiotic resistance in crops [173].

9. Conclusion

There is sufficient evidence to argue that cuticle and cuticular waxes are involved in the regulation of multiple biotic and abiotic interactions. The cuticular wax can be treated as an important trait contributing for multiple stress resistance. Concerted efforts have been made to elucidate the synthesis and deposition of cuticular waxes in plants. Further analysis of the key regulatory steps involved in the formation of cuticular waxes, and also the role played by diverse types of wax components and structures in stress response is needed. This information could be incorporated in crop improvement programs (via marker assisted selection for wax genes). Since there are promising options emerging to analyze the cuticular wax trait using modern synchrotron technology [174] as well as now widely recognized techniques to observe ice propagation in real time across the cuticle [175] crop breeders have the potential to improve their efficiency of selection based on these traits. Recent progress in genomics can substantially help major field and horticulture crops to buffer the impacts of climate change. In addition, new genome-editing technologies will provide interesting tools to characterize and engineer waxes in crops. Unraveling key regulators and network partners of surface wax synthesis would aid in targeted manipulation of the trait using modern biotechnological applications. There are options to analyze the cuticular wax trait using modern non-destructive approaches. Crop breeders can use these tools to improve their efficiency of selection for the trait, and effectively pyramid the trait in elite genotypes to combat combined stresses.

Acknowledgements

Author details

Kunhikrishnan H. Dhanyalakshmi[1], Raju Y. Soolanayakanahally[2],
Tawhidur Rahman[3], Karen K. Tanino[3] and Karaba N. Nataraja[1*]

1 Department of Crop Physiology, University of Agricultural Sciences, GKVK,
Bengaluru, India

2 Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada,
Saskatoon, SK, Canada

3 Department of Plant Sciences, College of Agriculture and Bioresources,
University of Saskatchewan, Saskatoon, SK, Canada

*Address all correspondence to: nataraja_karaba@yahoo.com

IntechOpen

References

[1] Pandey P, Ramegowda V, Senthil-Kumar M. Shared and unique responses of plants to multiple individual stresses and stress combinations: Physiological and molecular mechanisms. Frontiers in Plant Science. 2015;**6**:723. DOI: 10.3389/fpls.2015.00723

[2] Kudela V. Potential impact of climate change on geographic distribution of plant pathogenic bacteria in Central Europe. Plant Protection Science. 2009;**45**:27-32. DOI: 10.17221/2832-PPS

[3] Atkinson NJ, Urwin PE. The interaction of plant biotic and abiotic stresses: From genes to the field. Journal of Experimental Botany. 2012;**63**:3523-3543. DOI: 10.1093/jxb/ers100

[4] Ramegowda V, Senthil-Kumar M. The interactive effects of simultaneous biotic and abiotic stresses on plants: Mechanistic understanding from drought and pathogen combination. Journal of Plant Physiology. 2015;**176**:47-54. DOI: 10.1016/j.jplph.2014.11.008

[5] Riederer M, Schreiber L. Protecting against water loss: Analysis of the barrier properties of plant cuticles. Journal of Experimental Botany. 2001;**52**:2023-2032. DOI: 10.1093/jexbot/52.363.2023

[6] Kerstiens G. Cuticular water permeability and its physiological significance. Journal of Experimental Botany. 1996;**47**:1813-1832. DOI: 10.1093/jxb/47.12.1813

[7] Kenrick P, Crane PR. The origin and early evolution of plants on land. Nature. 1997;**389**:33. DOI: 10.1038/37918

[8] Reina-Pinto JJ, Yephremov A. Surface lipids and plant defenses. Plant Physiology and Biochemistry. 2009;**47**:540-549. DOI: 10.1016/j.plaphy.2009.01.004

[9] Sieber P, Schorderet M, Ryser U, Buchala A, Kolattukudy P, Metraux JP, et al. Transgenic *Arabidopsis* plants expressing a fungal cutinase show alterations in the structure and properties of the cuticle and postgenital organ fusions. The Plant Cell. 2000;**12**:721-738. DOI: 10.1105/tpc.12.5.721

[10] Yeats TH, Rose JKC. The formation and function of plant cuticles. Plant Physiology. 2013;**163**:5-20. DOI: 10.1104/pp.113.222737

[11] Bellec Y, Harrar Y, Butaeye C, Darnet S, Bellini C, Faure JD. Pasticcino2 is a protein tyrosine phosphatase-like involved in cell proliferation and differentiation in *Arabidopsis*. The Plant Journal. 2002;**32**:713-722. DOI: 10.1046/j.1365-313X.2002.01456.x

[12] Wang Z-Y, Xiong L, Li W, Zhu J-K, Zhu J. The plant cuticle is required for osmotic stress regulation of abscisic acid biosynthesis and osmotic stress tolerance in *Arabidopsis*. The Plant Cell. 2011;**23**:1971-1984. DOI: 10.1105/tpc.110.081943

[13] Podila GK, Rogers LM, Kolattukudy PE. Chemical signals from avocado surface wax trigger germination and appressorium formation in *Colletotrichum gloeosporioides*. Plant Physiology. 1993;**103**:267-272. DOI: 10.1104/pp.103.1.267

[14] Martin LBB, Rose JKC. There's more than one way to skin a fruit: Formation and functions of fruit cuticles. Journal of Experimental Botany. 2014;**65**:4639-4651. DOI: 10.1093/jxb/eru301

[15] Kosma DK, Parsons EP, Isaacson T, Lu S, Rose JK, Jenks MA. Fruit cuticle lipid composition during development in tomato ripening mutants. Physiologia Plantarum. 2010;**139**:107-17. DOI: 10.1111/j.1399-3054.2009.01342.x

[16] Isabel L, Burcu B, Goulao Luis F. The fruit cuticle as a modulator of postharvest quality. Postharvest Biology and Technology. 2014;**87**:103-112. DOI: 10.1016/j.postharvbio.2013.08.012

[17] Isaacson T, Kosma DK, Matas AJ, Buda GJ, He Y, Yu B, et al. Cutin deficiency in the tomato fruit cuticle consistently affects resistance to microbial infection and biomechanical properties, but not transpirational water loss. The Plant Journal. 2009;**60**:363-77. DOI: 10.1111/j.1365-313X.2009.03969.x

[18] Baker EA. Chemistry and morphology of plant epicuticular waxes. In: Linnean Society Symposium Series. 1982

[19] Lee SB, Suh MC. Advances in the understanding of cuticular waxes in *Arabidopsis thaliana* and crop species. Plant Cell Reports. 2015;**34**:557-572. DOI: 10.1007/s00299-015-1772-2

[20] Schonherr J. Resistance of plant surfaces to water loss: Transport properties of cutin, suberin and associated lipids. In: Physiological Plant Ecology II. Berlin, Heidelberg: Springer; 1982. pp. 153-179

[21] Schönherr J, Riederer M. Foliar penetration and accumulation of organic chemicals in plant cuticles. In: Reviews of Environmental Contamination and Toxicology. New York, NY: Springer; 1989. pp. 1-70

[22] Jeffree CE. The fine structure of the plant cuticle. Biology of the Plant Cuticle. 2006;**23**:11-125. DOI: 10.1002/9780470988718.ch2

[23] Buschhaus C, Jetter R. Composition differences between epicuticular and intracuticular wax substructures: How do plants seal their epidermal surfaces? Journal of Experimental Botany. 2011;**62**:841-853. DOI: 10.1093/jxb/erq366

[24] Jetter R, Schäffer S. Chemical composition of the *Prunus laurocerasus*

leaf surface. Dynamic changes of the epicuticular wax film during leaf development. Plant Physiology. 2001;**126**:1725-1737. DOI: 10.1104/pp.126.4.1725

[25] Jetter R, Kunst L, Samuels AL. Composition of plant cuticular waxes. Biology of the Plant Cuticle. 2008;**23**:145-181. DOI: 10.1002/9781119312994.apr0232

[26] Lee SB, Suh MC. Recent advances in cuticular wax biosynthesis and its regulation in *Arabidopsis*. Molecular Plant. 2013;**6**:246-249. DOI: 10.1093/mp/sss159

[27] Xue D, Zhang X, Lu X, Chen G, Chen Z-H. Molecular and evolutionary mechanisms of cuticular wax for plant drought tolerance. Frontiers in Plant Science. 2017;**8**:621. DOI: 10.3389/fpls.2017.00621

[28] Jetter R, Riederer M. Localization of the transpiration barrier in the epi-and intracuticular waxes of eight plant species: Water transport resistances are associated with fatty acyl rather than alicyclic components. Plant Physiology. 2016;**170**:921-934. DOI: 10.1104/pp.15.01699

[29] Zeisler-Diehl V, Müller Y, Schreiber L. Epicuticular wax on leaf cuticles does not establish the transpiration barrier, which is essentially formed by intracuticular wax. Journal of Plant Physiology. 2018;**227**:66-74. DOI: 10.1016/j.jplph.2018.03.018

[30] Zeisler V, Schreiber L. Epicuticular wax on cherry laurel (*Prunus laurocerasus*) leaves does not constitute the cuticular transpiration barrier. Planta. 2016;**243**:65-81. DOI: 10.1007/s00425-015-2397-y

[31] Mamrutha HM, Mogili T, Lakshmi KJ, Rama N, Kosma D, Kumar MU, et al. Leaf cuticular wax amount and crystal morphology regulate post-harvest

water loss in mulberry (*Morus* species). Plant Physiology and Biochemistry. 2010;**48**:690-696. DOI: 10.1016/j.plaphy.2010.04.007

[32] Shepherd T, Griffiths DW. The effects of stress on plant cuticular waxes. New Phytologist. 2006;**171**:469-499. DOI: 10.1111/j.1469-8137.2006.01826.x

[33] Seo PJ, Park C-M. Cuticular wax biosynthesis as a way of inducing drought resistance. Plant Signaling & Behavior. 2011;**6**:1043-1045. DOI: 10.4161/psb.6.7.15606

[34] Serrano M, Coluccia F, Torres M, L'Haridon F, Metraux JP. The cuticle and plant defense to pathogens. Frontiers in Plant Science. 2014;**5**:1-8. DOI: 10.3389/fpls.2014.00274

[35] Krauss P, Markstädter C, Riederer M. Attenuation of UV radiation by plant cuticles from woody species. Plant, Cell and Environment. 1997;**20**:1079-1085. DOI: 10.1111/j.1365-3040.1997.tb00684.x

[36] Solovchenko A, Merzlyak M. Optical properties and contribution of cuticle to UV protection in plants: Experiments with apple fruit. Photochemical & Photobiological Sciences. 2003;**2**:861-866. DOI: 10.1039/B302478D

[37] Zhu L, Guo J, Zhu J, Zhou C. Enhanced expression of EsWAX1 improves drought tolerance with increased accumulation of cuticular wax and ascorbic acid in transgenic *Arabidopsis*. Plant Physiology and Biochemistry. 2014;**75**:24-35. DOI: 10.1016/j.plaphy.2013.11.028

[38] Jordan WR, Shouse PJ, Blum A, Miller FR, Monk RL. Environmental physiology of sorghum. II. Epicuticular wax load and cuticular transpiration. Crop Science. 1984;**24**:1168-1173. DOI: 10.2135/cropsci1984.0011183X002400060038x

[39] Monneveux P, Reynolds MP, González-Santoyo H, Peña RJ, Mayr L, Zapata F. Relationships between grain yield, flag leaf morphology, carbon isotope discrimination and ash content in irrigated wheat. Journal of Agronomy and Crop Science. 2004;**190**:395-401. DOI: 10.1111/j.1439-037X.2004.00116.x

[40] Gonzalez A, Ayerbe L. Effect of terminal water stress on leaf epicuticular wax load, residual transpiration and grain yield in barley. Euphytica. 2010;**172**:341-349. DOI: 10.1007/s10681-009-0027-0

[41] Zhu X, Xiong L. Putative megaenzyme DWA1 plays essential roles in drought resistance by regulating stress-induced wax deposition in rice. Proceedings of the National Academy of Sciences. 2013;**110**:17790-17795. DOI: 10.1073/pnas.1316412110

[42] Sajeevan RS, Nataraja KN, Shivashankara KS, Pallavi N, Gurumurthy DS, Shivanna MB. Expression of Arabidopsis SHN1 in Indian mulberry (*Morus indica* L.) increases leaf surface wax content and reduces post-harvest water loss. Frontiers in Plant Science. 2017;**8**:418. DOI: 10.3389/fpls.2017.00418

[43] Hasanuzzaman M, Davies NW, Shabala L, Zhou M, Brodribb TJ, Shabala S. Residual transpiration as a component of salinity stress tolerance mechanism: A case study for barley. BMC Plant Biology. 2017;**17**:107. DOI: 10.1186/s12870-017-1054-y

[44] Tischler CR, Burson BL. Evaluating different bahiagrass cytotypes for heat tolerance and leaf epicuticular wax content. Euphytica. 1995;**84**:229-235. DOI: 10.1007/BF01681815

[45] Awika HO, Hays DB, Mullet JE, Rooney WL, Weers BD. QTL mapping and loci dissection for leaf epicuticular wax load and canopy temperature depression and their association with

QTL for staygreen in Sorghum bicolor under stress. Euphytica. 2017;**213**:207. DOI: 10.1007/s10681-017-1990-5

[46] Sanchez FJ, Manzanares M, de Andres EF, Tenorio JL, Ayerbe L. Residual transpiration rate, epicuticular wax load and leaf colour of pea plants in drought conditions. Influence on harvest index and canopy temperature. European Journal of Agronomy. 2001;**15**:57-70. DOI: 10.1016/ S1161-0301(01)00094-6

[47] Heredia-Guerrero JA, Guzman-Puyol S, Benítez JJ, Athanassiou A, Heredia A, Domínguez E. Plant cuticle under global change: Biophysical implications. Global Change Biology. 2018;**24**:2749-51. DOI: 10.1111/gcb.14276

[48] Domínguez E, Heredia-Guerrero JA, Heredia A. The biophysical design of plant cuticles: An overview. New Phytologist. 2011;**189**:938-949. DOI: 10.1111/j.1469-8137.2010.03553.x

[49] Wisniewski M, Fuller M. Ice nucleation and deep supercooling in plants: New insights using infrared thermography BT-cold-adapted organisms: Ecology, physiology, enzymology and molecular biology. In: Margesin R, Schinner F, editors. Berlin, Heidelberg: Springer; 1999. pp. 105-118. DOI: 10.1007/978-3-662-06285-2_6

[50] Wisniewski M, Glenn DM, Fuller MP. Use of a hydrophobic particle film as a barrier to extrinsic ice nucleation in tomato plants. Journal of the American Society for Horticultural Science. 2002;**127**:358-364. DOI: 10.21273/ JASHS.127.3.358

[51] Hamilton K. Identification of Ultrastructural and Biochemical Markers of Frost Avoidance in the Cuticular Layer of Corn. Canada: University of Saskatchewan; 2017

[52] Lindow SE, Arny DC, Upper CD. Erwinia herbicola: A bacterial

ice nucleus active in increasing frost injury to corn. Phytopathology. 1978;**68**:523-527

[53] Lindow SE. The role of bacterial ICE nucleation in frost injury to plants. Annual Review of Phytopathology. 1983;**21**:363-384. DOI: 10.1146/annurev. py.21.090183.002051

[54] Storey K, Tanino K, editors. Nature at Risk: Temperature Adaptation and Climate Change. Wallingford, UK: CABI Press; 2011

[55] Gilbert RD, Johnson AM, Dean RA. Chemical signals responsible for appressorium formation in the rice blast fungus *Magnaporthe grisea*. Physiological and Molecular Plant Pathology. 1996;**48**:335-346. DOI: 10.1006/pmpp.1996.0027

[56] Ahmed A, Crawford T, Gould S, Ha YS, Hollrah M, Noor-E-Ain F, et al. Synthesis of (R)-and (S)-10, 16-dihydroxyhexadecanoic acid: Cutin stereochemistry and fungal activation. Phytochemistry. 2003;**63**:47-52. DOI: 10.1016/S0031-9422(03)00003-7

[57] Reisige K, Gorzelanny C, Daniels U, Moerschbacher BM. The C28 aldehyde octacosanal is a morphogenetically active component involved in host plant recognition and infection structure differentiation in the wheat stem rust fungus. Physiological and Molecular Plant Pathology. 2006;**68**:33-40. DOI: 10.1016/j.pmpp.2006.05.006

[58] Hegde Y, Kolattukudy PE. Cuticular waxes relieve self-inhibition of germination and appressorium formation by the conidia of *Magnaporthe grisea*. Physiological and Molecular Plant Pathology. 1997;**51**: 75-84. DOI: 10.1006/pmpp.1997.0105

[59] Hansjakob A, Riederer M, Hildebrandt U. Wax matters: Absence of very-long-chain aldehydes from the leaf cuticular wax of the glossy11 mutant of

maize compromises the prepenetration processes of *Blumeria graminis*. Plant Pathology. 2011;**60**:1151-1161. DOI: 10.1111/j.1365-3059.2011.02467.x

[60] Martin JT. Role of cuticle in the defense against plant disease. Annual Review of Phytopathology. 1964;**2**:81-100. DOI: 10.1146/annurev.py.02.090164.000501

[61] Barthlott W, Neinhuis C. Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta. 1997;**202**:1-8. DOI: 10.1007/s004250050096

[62] Eigenbrode SD, Espelie KE. Effects of plant epicuticular lipids on insect herbivores. Annual Review of Entomology. 1995;**40**:171-194. DOI: 10.1146/annurev.en.40.010195.001131

[63] White C, Eigenbrode SD. Effects of surface wax variation in *Pisum sativum* on herbivorous and entomophagous insects in the field. Environmental Entomology. 2000;**29**:773-780. DOI: 10.1603/0046-225X-29.4.773

[64] Hariprasad K V, van Emden HF. Mechanisms of partial plant resistance to diamondback moth (*Plutella xylostella*) in brassicas. International Journal of Pest Management. 2010;**56**:15-22. DOI: 10.1080/09670870902980834

[65] Eigenbrode SD, Jetter R. Attachment to plant surface waxes by an insect predator. Integrative and Comparative Biology. 2002;**42**:1091-1099. DOI: 10.1093/icb/42.6.1091

[66] Wójcicka A. Surface waxes as a plant defense barrier towards grain aphid. Acta Biologica Cracoviensia s Botanica. 2015;**57**:95-103. DOI: 10.1515/abcsb-2015-0012

[67] Yeats TH, Buda GJ, Wang Z, Chehanovsky N, Moyle LC, Jetter R, et al. The fruit cuticles of wild tomato species exhibit architectural and chemical diversity, providing a new model for studying the evolution of cuticle function. The Plant Journal. 2012;**69**:655-666. DOI: 10.1111/j.1365-313X.2011.04820.x

[68] Kunst L, Samuels AL. Biosynthesis and secretion of plant cuticular wax. Progress in Lipid Research. 2003;**42**:51-80. DOI: 10.1016/S0163-7827(02)00045-0

[69] Post-Beittenmiller D. Biochemistry and molecular biology of wax production in plants. Annual Review of Plant Physiology and Plant Molecular Biology. 1996;**47**:405-430. DOI: 10.1146/annurev.arplant.47.1.405

[70] Beaudoin F, Wu X, Li F, Haslam RP, Markham JE, Zheng H, et al. Functional characterization of the *Arabidopsis* β-Ketoacyl-Coenzyme A reductase candidates of the fatty acid elongase. Plant Physiology. 2009;**150**:1174 LP-1191. DOI: 10.1104/pp.109.137497

[71] Kunst L, Samuels L. Plant cuticles shine: Advances in wax biosynthesis and export. Current Opinion in Plant Biology. 2009;**12**:721-727. DOI: 10.1016/j.pbi.2009.09.009

[72] McFarlane HE, Watanabe Y, Yang W, Huang Y, Ohlrogge J, Samuels AL. Golgi- and Trans-Golgi Network-mediated vesicle trafficking is required for wax secretion from epidermal cells. Plant Physiology. 2014;**164**:1250 LP-1260. DOI: 10.1104/pp.113.234583

[73] Bird D, Beisson F, Brigham A, Shin J, Greer S, Jetter R, et al. Characterization of *Arabidopsis* ABCG11/WBC11, an ATP binding cassette (ABC) transporter that is required for cuticular lipid secretion. The Plant Journal. 2007;**52**:485-498. DOI: 10.1111/j.1365-313X.2007.03252.x

[74] Debono A, Yeats TH, Rose JKC, Bird D, Jetter R, Kunst L, et al. *Arabidopsis* LTPG is a glycosylphosphatidylinositol-anchored

lipid transfer protein required for export of lipids to the plant surface. The Plant Cell. 2009;**21**:1230-1238. DOI: 10.1105/tpc.108.064451

[75] Lundqvist U, von Wettstein D. Induction of eceriferum mutants in barley by ionizing radiations and chemical mutagens. Hereditas. 1962;**48**:342-362. DOI: 10.1111/j.1601-5223.1962.tb01818.x

[76] Kurata T, Kawabata-Awai C, Sakuradani E, Shimizu S, Okada K, Wada T. The YORE-YORE gene regulates multiple aspects of epidermal cell differentiation in *Arabidopsis*. The Plant Journal. 2003;**36**:55-66. DOI: 10.1046/j.1365-313X.2003.01854.x

[77] Jenks MA, Tuttle HA, Eigenbrode SD, Feldmann KA. Leaf epicuticular waxes of the eceriferum mutants in *Arabidopsis*. Plant Physiology. 1995;**108**:369-377. DOI: 10.1104/pp.108.1.369

[78] Greer S, Wen M, Bird D, Wu X, Samuels L, Kunst L, et al. The Cytochrome P450 Enzyme CYP96A15 is the midchain alkane hydroxylase responsible for formation of secondary alcohols and ketones in stem cuticular wax of *Arabidopsis*. Plant Physiology. 2007;**145**:653 LP-667. DOI: 10.1104/pp.107.107300

[79] Lu S, Zhao H, Parsons EP, Xu C, Kosma DK, Xu X, et al. The glossyhead allele of ACC reveals a principal role for multidomain Acetyl-Coenzyme A Carboxylase in the biosynthesis of cuticular waxes by *Arabidopsis*. Plant Physiology. 2011;**157**:1079-1092. DOI: 10.1104/pp.111.185132

[80] Bonaventure G, Salas JJ, Pollard MR, Ohlrogge JB. Disruption of the FATB gene in *Arabidopsis* demonstrates an essential role of saturated fatty acids in plant growth. The Plant Cell.

2003;**15**:1020-1033. DOI: 10.1105/tpc.008946

[81] Fiebig A, Mayfield JA, Miley NL, Chau S, Fischer RL, Preuss D. Alterations in CER6, a gene identical to CUT1, differentially affect long-chain lipid content on the surface of pollen and stems. The Plant Cell. 2000;**12**:2001-2008. DOI: 10.1105/tpc.12.10.2001

[82] Sakuradani E, Zhao L, Haslam TM, Kunst L. The CER22 gene required for the synthesis of cuticular wax alkanes in *Arabidopsis thaliana* is allelic to CER1. Planta. 2013;**237**:731-738. DOI: 10.1007/s00425-012-1791-y

[83] Todd J, Post-Beittenmiller D, Jaworski JG. KCS1 encodes a fatty acid elongase 3-ketoacyl-CoA synthase affecting wax biosynthesis in *Arabidopsisthaliana*. The Plant Journal. 1999;**17**:119-130. DOI: 10.1046/j.1365-313X.1999.00352.x

[84] Lee S-B, Jung S-J, Go Y-S, Kim H-U, Kim J-K, Cho H-J, et al. Two *Arabidopsis* 3-ketoacyl   CoA synthase genes, KCS20 and KCS2/DAISY, are functionally redundant in cuticular wax and root suberin biosynthesis, but differentially controlled by osmotic stress. The Plant Journal. 2009;**60**:462-475. DOI: 10.1111/j.1365-313X.2009.03973.x

[85] Lü S, Song T, Kosma DK, Parsons EP, Rowland O, Jenks MA. *Arabidopsis* CER8 encodes LONG-CHAIN ACYL-COA SYNTHETASE 1 (LACS1) that has overlapping functions with LACS2 in plant wax and cutin synthesis. The Plant Journal. 2009;**59**:553-564. DOI: 10.1111/j.1365-313X.2009.03892.x

[86] Kim J, Jung JH, Lee SB, Go YS, Kim HJ, Cahoon R, et al. *Arabidopsis* 3-Ketoacyl-Coenzyme A Synthase9 is involved in the synthesis of tetracosanoic acids as precursors of cuticular waxes, suberins,

sphingolipids, and phospholipids. Plant Physiology. 2013;**162**:567-580. DOI: 10.1104/pp.112.210450

[87] Chen X, Goodwin SM, Boroff VL, Liu X, Jenks MA. Cloning and characterization of the WAX2 gene of *Arabidopsis* involved in cuticle membrane and wax production. The Plant Cell. 2003;**15**:1170-1185. DOI: 10.1105/tpc.010926

[88] Zheng H, Rowland O, Kunst L. Disruptions of the *Arabidopsis* Enoyl-CoA reductase gene reveal an essential role for very-long-chain fatty acid synthesis in cell expansion during plant morphogenesis. The Plant Cell. 2005;**17**:1467-1481. DOI: 10.1105/tpc.104.030155

[89] Rowland O, Zheng H, Hepworth SR, Lam P, Jetter R, Kunst L. CER4 encodes an alcohol-forming fatty acyl-coenzyme A reductase involved in cuticular wax production in *Arabidopsis*. Plant Physiology. 2006;**142**:866-877. DOI: 10.1104/pp.106.086785

[90] Li F, Wu X, Lam P, Bird D, Zheng H, Samuels L, et al. Identification of the wax ester Synthase/Acyl-Coenzyme A:Diacylglycerol Acyltransferase WSD1 required for stem wax ester biosynthesis in *Arabidopsis*. Plant Physiology. 2008;**148**:97-107. DOI: 10.1104/pp.108.123471

[91] Bach L, Michaelson L V, Haslam R, Bellec Y, Gissot L, Marion J, et al. The very-long-chain hydroxy fatty acyl-CoA dehydratase PASTICCINO2 is essential and limiting for plant development. Proceedings of the National Academy of Sciences. 2008;**105**:14727-14731. DOI: 10.1073/pnas.0805089105

[92] Haslam TM, Mañas-Fernández A, Zhao L, Kunst L. *Arabidopsis* ECERIFERUM2 is a component of the fatty acid elongation machinery required for fatty acid extension to exceptional lengths. Plant Physiology. 2012;**160**:1164 -1174. DOI: 10.1104/pp.112.201640

[93] Yang X, Zhao H, Kosma DK, Tomasi P, Dyer JM, Li R, et al. The acyl desaturase CER17 is involved in producing wax unsaturated primary alcohols and cutin monomers. Plant Physiology. 2017;**173**:1109-1124. DOI: 10.1104/pp.16.01956

[94] Pighin JA, Zheng H, Balakshin LJ, Goodman IP, Western TL, Jetter R, et al. Plant cuticular lipid export requires an ABC transporter. Science. 2004;**306**:702-704. DOI: 10.1126/science

[95] Luo B, Xue X-Y, Hu W-L, Wang L-J, Chen X-Y. An ABC transporter gene of Arabidopsis thaliana, AtWBC11, is involved in cuticle development and prevention of organ fusion. Plant and Cell Physiology. 2007;**48**:1790-802. DOI: 10.1093/pcp/pcm152

[96] Kim H, Lee SB, Kim HJ, Min MK, Hwang I, Suh MC. Characterization of Glycosylphosphatidylinositol-Anchored Lipid Transfer Protein 2 (LTPG2) and overlapping function between LTPG/LTPG1 and LTPG2 in cuticular wax export or accumulation in *Arabidopsis thaliana*. Plant and Cell Physiology. 2012;**53**:1391-1403. DOI: 10.1093/pcp/pcs083

[97] Nobusawa T, Okushima Y, Nagata N, Kojima M, Sakakibara H, Umeda M. Synthesis of very-long-chain fatty acids in the epidermis controls plant organ growth by restricting cell proliferation. PLoS Biology. 2013;**11**:1-14. DOI: 10.1371/journal.pbio.1001531

[98] Rhee Y, Hlousek-Radojcic A, Ponsamuel J, Liu D, Post-Beittenmiller D. Epicuticular wax accumulation and fatty acid elongation activities are induced during leaf development of leeks. Plant Physiology. 1998;**116**:901 LP-911. DOI: 10.1104/pp.116.3.901

[99] Kamigaki A, Kondo M, Mano S, Hayashi M, Nishimura M. Suppression of peroxisome biogenesis factor 10 reduces cuticular wax accumulation by disrupting the er network in *Arabidopsis thaliana*. Plant and Cell Physiology. 2009;**50**:2034-2046. DOI: 10.1093/pcp/pcp152

[100] Mamrutha HM, Nataraja KN, Rama N, Kosma DK, Mogili T, Lakshmi KJ, et al. Leaf surface wax composition of genetically diverse mulberry (*Morus* sp.) genotypes and its close association with expression of genes involved in wax metabolism. Current Science. 2017;**112**:759-766. DOI: 10.18520/cs/v112/i04/759-766

[101] Steinmüller D, Tevini M. Action of ultraviolet radiation (UV-B) upon cuticular waxes in some crop plants. Planta. 1985;**164**:557-564. DOI: 10.1007/BF00395975

[102] Kakani VG, Reddy KR, Zhao D, Sailaja K. Field crop responses to ultraviolet-B radiation: A review. Agricultural and Forest Meteorology. 2003;**120**:191-218. DOI: 10.1016/j.agrformet.2003.08.015

[103] Shankar R, Bhattacharjee A, Jain M. Transcriptome analysis in different rice cultivars provides novel insights into desiccation and salinity stress responses. Scientific Reports. 2016;**6**:23719. DOI: 10.1038/srep23719

[104] Islam MA, Du H, Ning J, Ye H, Xiong L. Characterization of glossy1-homologous genes in rice involved in leaf wax accumulation and drought resistance. Plant Molecular Biology. 2009;**70**:443-56. DOI: 10.1007/s11103-009-9483-0

[105] Guo J, Xu W, Yu X, Shen H, Li H, Cheng D, et al. Cuticular wax accumulation is associated with drought tolerance in wheat near-isogenic lines. Frontiers in Plant Science. 2016;**7**:1809. DOI: 10.3389/fpls.2016.01809

[106] Bengtson C, Larsson S, Liljenberg C. Effects of water stress on cuticular transpiration rate and amount and composition of epicuticular wax in seedlings of six oat varieties. Physiologia Plantarum. 1978;**44**:319-324. DOI: 10.1111/j.1399-3054.1978.tb01630.x

[107] Saneoka H, Ogata S. Relationship between water use efficiency and cuticular wax deposition in warm season forage crops grown under water deficit conditions. Soil Science and Plant Nutrition. 1987;**33**:439-448. DOI: 10.1080/00380768.1987.10557590

[108] Wang Y, Wang M, Sun Y, Wang Y, Li T, Chai G, et al. FAR5, a fatty acyl-coenzyme A reductase, is involved in primary alcohol biosynthesis of the leaf blade cuticular wax in wheat (*Triticum aestivum* L.). Journal of Experimental Botany. 2015;**66**:1165-78. DOI: 10.1093/jxb/eru457

[109] Bourdenx B, Bernard A, Domergue F, Pascal S, Léger A, Roby D, et al. Overexpression of *Arabidopsis* ECERIFERUM1Promotes wax very-long-chain alkane biosynthesis and influences plant response to biotic and abiotic stresses. Plant Physiology. 2011;**156**:29-45. DOI: 10.1104/pp.111.172320

[110] Kim H, Choi D, Suh MC. Cuticle ultrastructure, cuticular lipid composition, and gene expression in hypoxia-stressed *Arabidopsis* stems and leaves. Plant Cell Reports. 2017;**36**:815-827. DOI: 10.1007/s00299-017-2112-5

[111] Amid A, Lytovchenko A, Fernie AR, Warren G, Thorlby GJ. The sensitive to freezing3 mutation of *Arabidopsis thaliana* is a cold-sensitive allele of homomeric acetyl-CoA carboxylase that results in cold-induced cuticle deficiencies. Journal of Experimental Botany. 2012;**63**:5289-5299. DOI: 10.1093/jxb/ers191

[112] Gray JE, Holroyd GH, van der Lee FM, Bahrami AR, Sijmons PC,

Woodward FI, et al. The HIC signalling pathway links CO_2 perception to stomatal development. Nature. 2000;**408**:713. DOI: 10.1038/35047071

[113] Park CS, Go YS, Suh MC. Cuticular wax biosynthesis is positively regulated by WRINKLED4, an AP2/ERF-type transcription factor, in *Arabidopsis* stems. The Plant Journal. 2016;**88**: 257-270. DOI: 10.1111/tpj.13248

[114] Broun P, Poindexter P, Osborne E, Jiang C-Z, Riechmann JL. WIN1, a transcriptional activator of epidermal wax accumulation in *Arabidopsis*. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:4706-4711. DOI: 10.1073/pnas.0305574101

[115] Aharoni A, Dixit S, Jetter R, Thoenes E, van Arkel G, Pereira A. The SHINE Clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in *Arabidopsis*. The Plant Cell. 2004;**16**:2463-2480. DOI: 10.1105/tpc.104.022897

[116] Wang Y, Wan L, Zhang L, Zhang Z, Zhang H, Quan R, et al. An ethylene response factor OsWR1 responsive to drought stress transcriptionally activates wax synthesis related genes and increases wax production in rice. Plant Molecular Biology. 2012;**78**: 275-288. DOI: 10.1007/s11103-011-9861-2

[117] Bi H, Shi J, Kovalchuk N, Luang S, Bazanova N, Chirkova L, et al. Overexpression of the TaSHN1 transcription factor in bread wheat leads to leaf surface modifications, improved drought tolerance, and no yield penalty under controlled growth conditions. Plant, Cell and Environment. 2018;**41**:2549-2566. DOI: 10.1111/pce.13339

[118] Go YS, Kim H, Kim HJ, Suh MC. Arabidopsis cuticular wax biosynthesis is negatively regulated by the DEWAX gene encoding an AP2/ERF-Type transcription factor. The Plant Cell. 2014;**26**:1666-1680. DOI: 10.1105/tpc.114.123307

[119] Kim H, Go YS, Suh MC. DEWAX2 transcription factor negatively regulates cuticular wax biosynthesis in *Arabidopsis* leaves. Plant and Cell Physiology. 2018;**59**:966-977. DOI: 10.1093/pcp/pcy033

[120] Lee SB, Kim H, Kim RJ, Suh MC. Overexpression of *Arabidopsis* MYB96 confers drought resistance in Camelina sativa via cuticular wax accumulation. Plant Cell Reports. 2014;**33**:1535-1546. DOI: 10.1007/s00299-014-1636-1

[121] Seo PJ, Lee SB, Suh MC, Park M-J, Go YS, Park C-M. The MYB96 transcription factor regulates cuticular wax biosynthesis under drought conditions in *Arabidopsis*. The Plant Cell. 2011;**23**:1138-1152. DOI: 10.1105/tpc.111.083485

[122] Seo PJ, Park CM. MYB96-mediated abscisic acid signals induce pathogen resistance response by promoting salicylic acid biosynthesis in *Arabidopsis*. New Phytologist. 2010;**186**:471-483. DOI: 10.1111/j.1469-8137.2010.03183.x

[123] Lee SB, Kim HU, Suh MC. MYB94 and MYB96 additively activate cuticular wax biosynthesis in *Arabidopsis*. Plant and Cell Physiology. 2016;**57**:2300-2311. DOI: 10.1093/pcp/pcw147

[124] Hooker TS, Lam P, Zheng H, Kunst L. A core subunit of the RNA-processing/degrading exosome specifically influences cuticular wax biosynthesis in *Arabidopsis*. The Plant Cell. 2007;**19**:904-913. DOI: 10.1105/tpc.106.049304

[125] Wang W, Liu X, Gai X, Ren J, Liu X, Cai Y, et al. *Cucumis sativus* L. WAX2 plays a pivotal role in wax biosynthesis, influencing pollen fertility and plant

biotic and abiotic stress responses. Plant and Cell Physiology. 2015;**56**:1339-1354. DOI: 10.1093/pcp/pcv052

[126] Ju S, Go YS, Choi HJ, Park JM, Suh MC. DEWAX transcription factor is involved in resistance to *Botrytis cinerea* in *Arabidopsis thaliana* and *Camelina sativa*. Frontiers in Plant Science. 2017;**8**:1210. DOI: 10.3389/fpls.2017.01210

[127] Ziv C, Zhao Z, Gao YG, Xia Y. Multifunctional roles of plant cuticle during plant-pathogen interactions. Frontiers in Plant Science. 2018;**9**:1088. DOI: 10.3389/fpls.2018.01088

[128] Woloshuk CP, Kolattukudy PE. Mechanism by which contact with plant cuticle triggers cutinase gene expression in the spores of *Fusarium solani* f. sp. pisi. Proceedings of the National Academy of Sciences. 1986;**83**: 1704-1708. DOI: 10.1073/pnas.83.6.1704

[129] Leroch M, Kleber A, Silva E, Coenen T, Koppenhöfer D, Shmaryahu A, et al. Transcriptome profiling of Botrytis cinerea conidial germination reveals upregulation of infection-related genes during the prepenetration stage. Eukaryotic Cell. 2013;**12**:614-626. DOI: 10.1128/EC.00295-12

[130] Garrido SM, Kitamoto N, Watanabe A, Shintani T, Gomi K. Functional analysis of FarA transcription factor in the regulation of the genes encoding lipolytic enzymes and hydrophobic surface binding protein for the degradation of biodegradable plastics in *Aspergillus oryzae*. Journal of Bioscience and Bioengineering. 2012;**113**:549-555. DOI: 10.1016/j.jbiosc.2011.12.014

[131] Wang B, Liang X, Gleason ML, Zhang R, Sun G. Genome sequence of the ectophytic fungus Ramichloridium luteum reveals unique evolutionary adaptations to plant surface niche. BMC Genomics. 2017;**18**:729. DOI: 10.1186/s12864-017-4118-3

[132] Malinovsky FG, Fangel JU, Willats WGT. The role of the cell wall in plant immunity. Frontiers in Plant Science. 2014:**5**:178. DOI: 10.3389/fpls.2014.00178

[133] Marques JPR, Amorim L, Spósito MB, Appezzato-da-Glória B. Ultrastructural changes in the epidermis of petals of the sweet orange infected by *Colletotrichum acutatum*. Protoplasma. 2016;**253**:1233-1242. DOI: 10.1007/s00709-015-0877-3

[134] Alkan N, Fortes AM. Insights into molecular and metabolic events associated with fruit response to post-harvest fungal pathogens. Frontiers in Plant Science. 2015;**6**:889. DOI: 10.3389/fpls.2015.00889

[135] Xia Y, Gao Q-M, Yu K, Lapchyk L, Navarre D, Hildebrand D, et al. An intact cuticle in distal tissues is essential for the induction of systemic acquired resistance in plants. Cell Host and Microbe. 2009;**5**:151-165. DOI: 10.1016/j.chom.2009.01.001

[136] Xia Y, Yu K, Navarre D, Seebold K, Kachroo A, Kachroo P. The glabra1 mutation affects cuticle formation and plant responses to microbes. Plant Physiology. 2010;**154**:833-846. DOI: 10.1104/pp.110.161646

[137] Sela D, Buxdorf K, Shi JX, Feldmesser E, Schreiber L, Aharoni A, et al. Overexpression of AtSHN1/WIN1 provokes unique defense responses. PLoS One. 2013;**8**:e70146. DOI: 10.1371/journal.pone.0070146

[138] Buxdorf K, Rubinsky G, Barda O, Burdman S, Aharoni A, Levy M. The transcription factor SlSHINE3 modulates defense responses in tomato plants. Plant Molecular Biology. 2014;**84**:37-47. DOI: 10.1007/s11103-013-0117-1

[139] Koch K, Ensikat H-J. The hydrophobic coatings of plant surfaces:

Epicuticular wax crystals and their morphologies, crystallinity and molecular self-assembly. Micron. 2008;**39**:759-772. DOI: 10.1016/j.micron.2007.11.010

[140] Bargel H, Koch K, Cerman Z, Neinhuis C. Evans Review No. 3: Structurefunction relationships of the plant cuticle and cuticular waxes a smart material? Functional Plant Biology. 2006;**33**:893-910. DOI: 10.1071/FP06139

[141] Gorb E V, Gorb SN. Anti-adhesive effects of plant wax coverage on insect attachment. Journal of Experimental Botany. 2017;**68**:5323-5337. DOI: 10.1093/jxb/erx271

[142] Brennan EB, Weinbaum SA. Effect of epicuticular wax on adhesion of psyllids to glaucous juvenile and glossy adult leaves of *Eucalyptus globulus* Labillardière. Australian Journal of Entomology. 2001;**40**:270-277. DOI: 10.1046/j.1440-6055.2001.00229.x

[143] Bodnaryk RP. Leaf epicuticular wax, an antixenotic factor in *Brassicaceae* that affects the rate and pattern of feeding of flea beetles, *Phyllotreta cruciferae* (Goeze). Canadian Journal of Plant Science. 1992;**72**:1295-1303. DOI: 10.4141/cjps92-163

[144] Eigenbrode SD. The effects of plant epicuticular waxy blooms on attachment and effectiveness of predatory insects. Arthropod Structure and Development. 2004;**33**:91-102. DOI: 10.1016/j.asd.2003.11.004

[145] Eigenbrode SD, White C, Rohde M, Simon CJ. Behavior and effectiveness of adult *Hippodamia convergens* (Coleoptera: Coccinellidae) as a predator of *Acyrthosiphon pisum* (Homoptera: Aphididae) on a wax mutant of *Pisum sativum*. Environmental Entomology. 1998;**27**:902-909. DOI: 10.1093/ee/27.4.902

[146] Gorb E, Gorb S. How a lack of choice can force ants to climb up waxy plant stems. Arthropod-Plant Interactions. 2011;**5**:297-306. DOI: 10.1007/s11829-011-9143-6

[147] Gorb E, Haas K, Henrich A, Enders S, Barbakadze N, Gorb S. Composite structure of the crystalline epicuticular wax layer of the slippery zone in the pitchers of the carnivorous plant *Nepenthes alata* and its effect on insect attachment. Journal of Experimental Biology. 2005;**208**:4651-4662. DOI: 10.1242/jeb.01939

[148] Gorb E, Böhm S, Jacky N, Maier L-P, Dening K, Pechook S, et al. Insect attachment on crystalline bioinspired wax surfaces formed by alkanes of varying chain lengths. Beilstein Journal of Nanotechnology. 2014;**5**:1031-1041. DOI: 10.3762/bjnano.5.116

[149] Borodich FM, Gorb E V, Gorb SN. Fracture behaviour of plant epicuticular wax crystals and its role in preventing insect attachment: A theoretical approach. Applied Physics A. 2010;**100**:63-71. DOI: 10.1007/s00339-010-5794-x

[150] Dirks J-H, Clemente CJ, Federle W. Insect tricks: Two-phasic foot pad secretion prevents slipping. Journal of the Royal Society, Interface. 2010;**7**:587-593. DOI: 10.1098/rsif.2009.0308

[151] Gorb E V, Hofmann P, Filippov AE, Gorb SN. Oil adsorption ability of three-dimensional epicuticular wax coverages in plants. Scientific Reports. 2017;**7**:45483. DOI: 10.1038/srep45483

[152] Edwards D, Abbot GD, Raven JA. Cuticles of early land plants: A palaeoecophysiological evaluation. In: Kersteins G, editor. Plant Cuticles: An Integrated Functional Approach. Oxford: Bios; 1996. pp. 1-31

[153] Qin B-X, Tang D, Huang J, Li M, Wu X-R, Lu L-L, et al. Rice OsGL1-1 is

involved in leaf cuticular wax and cuticle membrane. Molecular Plant. 2011;**4**: 985-995. DOI: 10.1093/mp/ssr028

[154] Cameron KD, Teece MA, Smart LB. Increased accumulation of cuticular wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco. Plant Physiology. 2006;**140**:176-183. DOI: 10.1104/pp.105.069724

[155] Zhang J-Y, Broeckling CD, Blancaflor EB, Sledge MK, Sumner LW, Wang Z-Y. Overexpression of WXP1, a putative *Medicago truncatula* AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (*Medicago sativa*). The Plant Journal. 2005;**42**:689-707. DOI: 10.1111/j.1365-313X.2005.02405.x

[156] Febrero A, Fernández S, Molina-Cano JL, Araus JL. Yield, carbon isotope discrimination, canopy reflectance and cuticular conductance of barley isolines of differing glaucousness. Journal of Experimental Botany. 1998;**49**: 1575-1581. DOI: 10.1093/jxb/49.326.1575

[157] Richards RA, Rawson HM, Johnson DA. Glaucousness in wheat: Its development and effect on water-use efficiency, gas exchange and photosynthetic tissue temperatures. Functional Plant Biology. 1986;**13**: 465-473. DOI: 10.1071/PP9860465

[158] Johnson DA, Richards RA, Turner NC. Yield, water relations, gas exchange, and surface reflectances of near-isogenic wheat lines differing in glaucousness. Crop Science. 1983;**23**:318-325. DOI: 10.2135/cropsci1983.0011183X002300020033x

[159] Jansen MAK, Gaba V, Greenberg BM. Higher plants and UV-B radiation: Balancing damage, repair and acclimation. Trends in Plant Science. 1998;**3**:131-135. DOI: 10.1016/S1360-1385(98)01215-1

[160] Fukuda S, Satoh A, Kasahara H, Matsuyama H, Takeuchi Y. Effects of ultraviolet-B irradiation on the cuticular wax of cucumber (*Cucumis sativus*) cotyledons. Journal of Plant Research. 2008;**121**:179-189. DOI: 10.1007/s10265-007-0143-7

[161] Srinivasan S, Gomez SM, Kumar SS, Ganesh SK, Biji KR, Senthil A, et al. QTLs linked to leaf epicuticular wax, physio-morphological and plant production traits under drought stress in rice (*Oryza sativa* L.). Plant Growth Regulation. 2008;**56**:245-256. DOI: 10.1007/s10725-008-9304-5

[162] Burow GB, Franks CD, Acosta-Martinez V, Xin Z. Molecular mapping and characterization of BLMC, a locus for profuse wax (bloom) and enhanced cuticular features of Sorghum (*Sorghum bicolor* (L.) Moench.). Theoretical and Applied Genetics. 2009;**118**:423-431. DOI: 10.1007/s00122-008-0908-y

[163] Liu D, Dong X, Liu Z, Tang J, Zhuang M, Zhang Y, et al. Fine mapping and candidate gene identification for wax biosynthesis locus, BoWax1 in *Brassica oleracea* L. var. capitata. Frontiers in Plant Science. 2018:**9**:309. DOI: 10.3389/fpls.2018.00309

[164] Debieu M, Sine B, Passot S, Grondin A, Akata E, Gangashetty P, et al. Response to early drought stress and identification of QTLs controlling biomass production under drought in pearl millet. PLoS One. 2018;**13**:e0201635. DOI: 10.1371/journal.pone.0201635

[165] Al-Abdallat AM, Al-Debei HS, Ayad JY, Hasan S. Over-expression of SlSHN1 gene improves drought tolerance by increasing cuticular wax accumulation in tomato. International Journal of Molecular Sciences. 2014;**15**:19499-19515. DOI: 10.3390/ijms151119499

[166] Lokesh U, Venkatesh B, Kiranmai K, Nareshkumar A, Amarnathareddy V, Rao GL, et al. Overexpression of

ß-Ketoacyl Co-A Synthase1 gene improves tolerance of drought susceptible groundnut (*Arachis hypogaea* L.) cultivar K-6 by increased leaf epicuticular wax accumulation. Frontiers in Plant Science. 2019;**9**:1869. DOI: 10.3389/fpls.2018.01869

[167] Marcell LM, Beattie GA. Effect of leaf surface waxes on leaf colonization by*Pantoea agglomerans* and *Clavibacter michiganensis*. Molecular Plant-Microbe Interactions. 2002;**15**:1236-1244. DOI: 10.1094/MPMI.2002.15.12.1236

[168] Tanksley SD, McCouch SR. Seed banks and molecular maps: Unlocking genetic potential from the wild. Science. 1997;**277**:1063-1066. DOI: 10.1126/science.277.5329.1063

[169] Hen-Avivi S, Savin O, Racovita RC, Lee W-S, Adamski NM, Malitsky S, et al. A Metabolic gene cluster in the wheat W1 and the barley Cer-cqu loci determines β-Diketone biosynthesis and glaucousness. The Plant Cell. 2016;**28**:1440-1460. DOI: 10.1105/tpc.16.00197

[170] Oladosu Y, Rafii MY, Abdullah N, Hussin G, Ramli A, Rahim HA, et al. Principle and application of plant mutagenesis in crop improvement: A review. Biotechnology and Biotechnological Equipment. 2016;**30**:1-16. DOI: 10.1080/13102818.2015.1087333

[171] Saladié M, Matas AJ, Isaacson T, Jenks MA, Goodwin SM, Niklas KJ, et al. A reevaluation of the key factors that influence tomato fruit softening and integrity. Plant Physiology. 2007;**144**:1012-1028. DOI: 10.1104/pp.107.097477

[172] Cermak T, Curtin SJ, Gil-Humanes J, Cegan R, Kono TJY, Konecna E, et al. A multipurpose toolkit to enable advanced genome engineering in plants. The Plant Cell. 2017;**29**:1196-1217. DOI: 10.1105/tpc.16.00922

[173] Sajeevan RS, Parvathi MS, Nataraja KN. Leaf wax trait in crops for drought and biotic stress tolerance: Regulators of epicuticular wax synthesis and role of small RNAs. Indian Journal of Plant Physiology. 2017;**22**:434-447. DOI: 10.1007/s40502-017-0333-9

[174] Willick IR, Lahlali R, Vijayan P, Muir D, Karunakaran C, Tanino KK. Wheat flag leaf epicuticular wax morphology and composition in response to moderate drought stress are revealed by SEM, FTIR-ATR and synchrotron X-ray spectroscopy. Physiologia Plantarum. 2018;**162**:316-332. DOI: 10.1111/ppl.12637

[175] Wisniewski M, Lindow SE, Ashworth EN. Observations of ice nucleation and propagation in plants using infrared video thermography. Plant Physiology. 1997;**113**:327-334. DOI: 10.1104/pp.113.2.327