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Review Article

Research Advances on Cuticular Waxes Biosynthesis in Crops: A Review

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Abstract

Cuticular waxes are the hydrocarbon consisting of very long chain primary alcohols, aldehydes, fatty acids, alkane and esters. They are hydrophobic layer which protect aerial plant organs and help plant species for adaptation in different environments. Wax deposition and chemical composition vary considerably among crop species. Cuticular waxes play a significant role against major abiotic stresses in plants such as drought, high salinity and cold. So, it draws close attention to molecular processes of cuticular wax biosynthesis under stress factors. Here, we briefly summarized to the existing knowledge on the cuticular waxes properties, diversity, morphological changes in leaf surface wax crystals and amount and composition of cuticular waxes. We also provide information about wax biosynthesis genes in crops. Recently, due to progress of plant genome sequence, numerous genes involved in biosynthesis of cuticular waxes have been characterized both for model plant (Arabidopsis) and crops such as rapeseed (*Brassica napus*), *Camelina spp*, potato (*Solanum tuberosum*), eggplant (*S. macrocarpon*), tomato (*S. lycopersicum*), barley (*Hordeum vulgare*), rice (*Oryza sativa*), maize (*Zea mays*), wheat (*Triticum astivum*), broccoli (*B. olericea*), sesame (*Sesamum indicum*), tobacco (*Nicotiana tabacum*), cucumber (*Cucumis sativus*), cabbage (*B. oleracea*) etc. Basic compositions of cuticular wax are alcohols, branched alkanes, alkenes, aldehydes, fatty acids, esters, ketones, triterpenoids and sterols in crops. However, they vary from one crop species to the other. Cuticular wax biosynthesis is organ-specific and depends upon developmental stages of crops, and induced by environmental stimuli. The genetic factors also control wax biosynthesis and composition. However, cuticular wax also acts as a photoprotector layer during photosynthesis and protect from UV light radiation. It is also linked to gas exchange and plant development. In this review, we have summarized the cuticular wax amounts and contents in different organs, and genes to be involved in cuicular wax biosynthesis in several crops. This knowledge may be helpful in potential applications for selection of crop for agricultural sustainability.

Keywords: Crops; Cuticular wax; Environmental stimuli; Wax biosynthesis; Wax diversity

Introduction

In growth and development stages, plants have to face enormous environmental stresses like drought, salinity, cold, heat, UV light, high radiation, insect or fungal and pathogens. The cuticular waxes are a surface layer of the plant which provide defense against pests and pathogens (Wink, 1988; Holmes and Keiller, 2002; Bargel *et al.*, 2004; Yeats and Rose, 2013). The cuticle is composed of two distinct layers which chemically separate compounds including a lipophilic cutin polymer matrix and waxes (Holloway, 1982; Jeffree, 1996; Kunst *et al.*, 2005). Cuticular waxes are most important elements which prevent uncontrolled evaporation of water at the leaves surfaces (Jetter and Riederer, 2000; Knoche *et al.*, 2000). The cuticular wax is composed of very long-chain fatty acid compounds (VLCFAs; C20 to C34). These VLCFAs compounds consist of Branched alkanes, primary alcohols, alkenes, aldehydes, secondary alcohols, β- and OH-β-diketones, esters and often triterpenoids and flavonoids (Jetter *et al.*, 2006; Samuels *et al.*, 2008). The genetic and environmental factors influence on the deposition and composition of cuticular waxes (Bianchi, 1995; Post-Beittenmiller, 1996). Cuticular wax biosynthetic pathways have been studied extensively in Arabidopsis (Hannoufa *et al.*, 1993; McNevin *et al.*, 1993; Jenks *et al.*, 1995; Suh *et al.*, 2005; Kunst and Samuels, 2009; Nawrath *et al.*, 2013). Several cuticular waxes genes from Arabidopsis were identified such as *FATB*, *LCAS1*, *LACS2*, *LCAS4*, *ACC1*, *KCS1*, *KCS2/DAISY*, *KCS6/CER6/CUT1*, *KCS9*, *KCS20*, *KCR1*, *HCD/PAS2*, *ECR/ECR10*, *CER2*, *CER2-LIKE1*, *CER2-LIKE2*, *CER1*, *RST1*, *CYTB5-B*, *CYTB5-C*, *CYTB-D*, *CYTB5-E*, *MAH1*, *FAR3/CER4* and *WSD1* (Lee and Suh, 2015). Number of cuticular wax genes has already been identified in crops (Table 1). Transcription, mRNA and post-translational modification are controlled by these genes in waxy and waxless plants (Von Wettstein-Knowles, 1995; Pu *et al.*, 2013; Lee *et al.*, 2015). However, genetic mechanism related to deposition of cuticular waxes in crops is still elusive and subject to further investigations.

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Cuticular wax composition also depends on leaf color, insect-plant interaction and plant development. The quantity of plant cuticular wax largely depends upon environment conditions. Researchers have great interest to comprehend the detail genetic behavior of wax biosynthesis genes in crops. In this review, we summarized the amount and contents of cuticular waxes in the different crops and focused on recent progress about the molecular and biological function of genes engaged in biosynthesis of cuticular wax. We also briefly discussed the properties and diversities of cuticular waxes.

Properties of Cuticular Wax

Plant response and adaptation to abiotic and biotic stresses: Plant transpiration depends on two factors. Basically plant transpiration take place through stomata and a non-stomatal component is also there. Bernard and Joubès (2013) reported that there is significance correlation between the lipid cuticle layer and transpiration which was first proof about the role of cuticle for non-stomatal water loss (Stiles, 1994). Stomata remains close during water stress or night time, and provide space for significant cuticule transpiration. Several plant studies such as on tobacco and sesame also showed that wax biosynthesis was increased during water stress, and played an important role in preventing the cuticuler desiccation (Cameron *et al*., 2006; Kim *et al*., 2007). In Arabidopsis, water and osmotic stresses increased wax deposition that in turn were associated with a resistance to water stress, and cuticle formation could be a part of mechanism to acquire tolerance to water stress (Kosma *et al.*, 2009). Amount of wax and resistance to water flow depends on the cuticle biosynthesis enzymes (Aharoni *et al*., 2004; Bourdenx *et al*., 2011; Seo *et al*., 2011). The regulation of cuticle permeability mainly depends on the wax deposition mechanisms during water stress. Several studies have reported that increase in cuticle permeability reduces wax load and vice versa (Chen *et al*., 2003; Zhang *et al*., 2005; Kosma *et al*., 2009; Lü *et al*., 2009, 2012; Bourdenx *et al*., 2011; Seo *et al*., 2011). Higher cuticle permeability depends on increased amount of cutin and waxes depositions.

Cuticular waxes need for plant development: Beside their major contribution to stress tolerance, cuticular waxes are active players in the growth and developmental processes in plant. Kurdyukov *et al.* (2006) reported that organ fusion phenotypes were frequently associated with severe imperfections in either cuticular wax or cutin biosynthesis, as noticed in, *bodyguard, Wddlehead, cer3*/*wax2* and numerous other mutants. Other studies on Arabidopsis also indicated that *lacs1lacs2* double-mutant plants demonstrated pleiotropic phenotypes such as organ fusion, unusual flower development and decreased seed set (Weng *et al.*, 2010). The wax-deficient *cer1* mutant of Arabidopsis had a conditional male-sterile phenotype that reduced pollen viability contributed to the low seed yield (Aarts *et al*., 1995). These studies indicated that a deficiency in cuticular waxes biosynthesis or cutin synthesis has a effects on cuticular barrier, water movement, defend against drought stress and protect organ fusion. Arabidopsis mutant's analyses help us to understand of changes in cuticular waxes amount and composition during different developmental stages and add conception to the action of cuticular waxes in plant physiology.

Diversity of Cuticular Wax

Despite our relatively advanced understanding of wax compound structure and biosynthesis in Arabidopsis, crucial questions remain unanswered about how chemical composition determines the physical properties of the cuticular wax mixture. Before addressing these questions, a thorough understanding of the major dimensions of cuticular wax diversity is needed, in particular, the diversity in the chemical structures and diversity in the waxes covering on different biological organs. It will move us closer to a fundamental understanding of the relationships between structure and function in the plant cuticular wax diversity.

Structural diversities of wax compound: Structural diversity may be evident from the aliphatic tail (e.g., number of unsaturations, aliphatic branches, TCN, etc) or functional groups (e.g., number of functional groups, positions on the aliphatic tail, oxidation state, etc) in the wax molecules. Some of those compounds were found in large amount in different plants and crops, indicating that they play a vital role in the properties of cuticular waxes and alter cuticular wax mixture. Moreover, their biosynthesis mechanisms of converting branched wax precursors into branched wax compounds are still not clear. It is needed to characterize branched wax compounds biosynthesis pathway to remove uncertainty of model species. Fatty acids, primary alcohols, alkenes, wax esters, aldehydes and branched alkanes are major cuticular wax components. However, wax profiles in different plant organs also revealed that some plants accumulate secondary functional groups with major cuticular wax compounds (Gunthardt-Goerg, 1986; Wen *et al.*, 2006). Ketones, ketoalcohols, and ketoaldehydes found on the surfaces of the fern *Osmunda re-alias* (Jetter and Riederer, 2000), while β-diketones are present on the surface of wheat and barley (Tulloch and Weenink, 1969; Jackson, 1971; Han-Avivi *et al.*, 2016; Schneider *et al.*, 2016; Huang *et al.*, 2017). It was proved that the true diversity of cuticular waxes presents in different plants. Thus, our knowledge about branched wax compound and biosynthesis is still nascent stage.

Biological variability in wax coverage and composition: Aerial plant organs are covered with waxes throughout their developmental stages. The quantity and composition of wax in plants depend upon the stresses in an age-dependent manner. Indeed, previous studies had reported that wax amount and compositions vary between plant surfaces of

Table 1: Genes known to be involved in cuticular wax biosynthesis in crop species

Mechanism	Species	Organs	Protein family name	Abbreviation	Reference
		Seedling leaf	Arabidopsis *KCS6* homolog	*GL4*	Avato *et al.*, 1987; Liu *et al.*, 2009
		Seedling leaf	Arabidopsis *KCR* homolog	*GL8a*	Xu *et al.*, 1997; Dietrich *et al.*, 2005
	Maize	Seedling leaf	Arabidopsis *KCR* homolog	*GL8b*	Dietrich *et al.*, 2005
		Seedling leaf	Arabidopsis *CER3* homolog	*GL1*	Hansen *et al.*, 1997; Sturaro *et al.*, 2005
		Seedling leaf	Arabidopsis *CER2* homolog	*GL2*	Lemieux, 1996; Velasco *et al.*, 2002
		Leaf	KCS	*WSL1*	Yu *et al.*, 2008
		Shoot	KCS	*ONI1*	Ito *et al.*, 2011
		Anther	Arabidopsis *CER1* homolog	*WDA1*	Jung *et al.*, 2006
		Leaf	Arabidopsis *CER1* homolog	*OsGL1-6*	Zhou *et al.*, 2013
		Leaf	Arabidopsis *CER3*, maize *GL1* homolog	*OsGL1-2*	Islam *et al.*, 2009
		Leaf	Arabidopsis *CER3* homolog	*OsGL1-1/WSL2*	Qin *et al.*, 2011; Mao *et al.*, 2012
		Leaf	AMP-binding domin contained	*DWA1*	Zhu and Xiong, 2013
		Leaf	Arabidopsis *CER3* homolog/ Maize *GL1* homolog	*OsGL1-3*	Zhou *et al.*, 2015
		Leaf	A homolog of the *MBOAT* transferase family	*OsWS1*	Xia *et al.*, 2015
	Rice	Leaf	$NAD^+/NADP^+$-dependent sterol dehydrogenase	*OsHSD1*	Zhang*et al.*, 2016
		Leaf	*KCR*	*WSL3*	Gan *et al.*, 2016
		Leaf	*KCS*	*WSL3*	Hong-bing *et al.*, 2017
		Leaf	Arabidopsis *CER6* homolog	*WSL4*	Wang *et al.*, 2017
		Leaf	*CER6-like KCS*	*LeCER6*	Vogg *et al.*, 2004;
	Tomato	Fruit	B-Amyrin synthesis	*SITTS1*	Wang *et al.*, 2011
Cuticular wax biosynthesis		Fruit	Oxidosqualene cyclase	*SITTS2*	Wang *et al.*, 2011
		Leaf	Unknown	*BnaA.GL*	Pu *et al.*, 2013
	B. napus	Leaf	lipid transfer proteins	*BraLTP1*	Liu *et al.*, 2014
	Brassica rapa	Leaf	Arabidopsis *CER2* homolog	*BrWax1*	Zhang *et al.*, 2013a
		Leaf	Arabidopsis *KCS2* homolog	*CsKCS2*	
		Leaf	Arabidopsis *KCS6* homolog	*CsKCS6*	
		Leaf	Arabidopsis *KCR 1* homolog	*CsKCR1-1*	Lee *et al.*, 2014
	C. sativa	Leaf	Arabidopsis *KCR 1* homolog	*CsKCR1-2*	
		Leaf	Arabidopsis *ECR* homolog	*CsECR*	
		Leaf	Arabidopsis *KCS2* homolog	*CsMAH1*	
	Cucumber	Leaf	Arabidopsis *WAX2* homolog	*CsWAX2*	Wang *et al.*, 2015a
		Leaf	Arabidopsis *CER1* homolog	*CsCER1*	Wang *et al.*, 2015b
		leaf	lipid transfer proteins	*BoLTP2*	
		Leaf	Arabidopsis *CER3* homolog	*BoCER3*	
		Leaf	Arabidopsis *KCS1* homolog	*BoKCS1*	
	Cabbage	Leaf	Arabidopsis KCR1homolog	*BoKCR1*	Laila *et al.*, 2017
		Leaf	Arabidopsis *LACS1* homolog	*BoLACS1*	
		Leaf	alkane hydroxylase *CYP96A15*	*BoMAH1*	
		Leaf	Arabidopsis *CER4* homolog	*BoFAR3*	
		Leaf	Arabidopsis *WSD1*-like family	*BoWSD1*	
		Leaf	Arabidopsis *CER4-6* homolog	*W1W2*	Zhang *et al.*, 2013b
		Leaf	*CER1* and *CER3* homologs	*W3*	Zhang *et al.*, 2015
		Leaf	Arabidopsis *CER4* homolog	*TaFAR1*	Wang *et al.*, 2015b
	Wheat	Leaf	Arabidopis *CER4* homolog	*TaFAR5*	Wang *et al.*, 2015c
		Leaf	Arabidopsis *CER4* homolog	*TaFAR2, TaFAR3, TaFAR4,*	Wang *et al.*, 2016
		Spike	miRNA (MIRNA)	*W1-COE and / or W2-COE*	Huang *et al.*, 2017
		Leaf	Arabidopsis *CER4* homolog	*Ae.tFAR1,Ae.tFAR2, Ae.tFAR3,Ae.tFAR4, Ae.tFAR6*	Wang *et al.*, 2017
		Leaf	Arabidopsis *CER4* homolog	*TaFAR6,TaFAR7, TaFAR8*	Chai *et al.*, 2018
	Barley	Spike	PKS (DMP), Hydrolase (DMH),CYP450 (DMC)	*Cer-cqu*	Hen-Avivi *et al.*, 2016; Schneider *et al.*, 2016
		Leaf	Protein phosphatase 2C family protein	*Cer-b*	Zhou *et al.*, 2017

different ages (Atkin and Hamilton, 1950; Gülz *et al.*, 1992; Viougeas *et al.*, 1995). Largely, the understanding of cuticular wax deposition on plant surfaces needs further studies (Suh *et al.*, 2005). Plant surface development depends on cuticular wax deposition at different environmental conditions. It is needed to investigate the dimensions of biological variability and its relationship with the developmental biology of the plants. Thus, Cuticular waxes display structural diversity in their aliphatic tails and functional group (s) and biological variability depending on surfaces of different species, surfaces of different plant organs, and organ surfaces at different ages.

Cuticular Wax Morphology

The wax morphology was examined on both the adaxial and abaxial sides of leaf by scanning electron microscopy (SEM) to achieve insight into epicuticular wax crystals (Fig. 1). Leaf blades were collected from D genome (*Aegilops tauschii*) at three plant development stages (seedling, heading and filling stages). Two forms of wax crystals: platelets and tubules (A–L Figs) were found in the wheat leaf. Barthlott (1998) classified the epicuticular waxes. Some platelets shape wax crystals were joined to their adjacent crystals making a dense network. The length of platelet shaped wax crystals was between 0.3 and 0.7 μm and height between 0.3 and 0.5 μm. The platelet shaped wax crystals had irregular margins, and were present at different angles with respect to each other (Fig. 1 A–L). So, the wax morphology changed during their development stages. It is concluded that as the plant ages, the cuticular wax morphology changes on the leaf surface (Wang *et al.*, 2015a).

Cuticular Waxes in Crops

Amounts and contents of cuticular waxes in crop: It is very well known that cuticular waxes deposition varies across crops and from organ to organ (Barthlott *et al.*, 1998; Kosma *et al.*, 2010; Buschhaus and Jetter, 2011; Bernard and Joubès, 2013). Cuticular wax contents were measured in different organs of Arabidopsis, rapeseed (*Brassica napus*), *Camelina* spp, potato (*S. tuberosum*), eggplant (*S. macrocarpon*), tomato (*S. lycopersicum*), barley (*Hordeum vulgare*), maize (*Zea mays*), rice (*Oryza sativa*), wheat (*Triticum astivum*), broccoli (*B. olericea*), sesame (*Sesamum indicum*), tobacco (*Nicotianatabacum*), cucumber (*Cucumis sativus*) and cabbage (*B. oleracea*) (Table 2). Previous studies have shown that in Arabidopsis ecotype Columbia-0, the wax was 0.7–1.5, 13–24, 13 μg/cm^2 in leaves, stems and siliques, while it was 23-82 and 36–170 μg/g in flowers and seed coat, respectively. Alkane contributed up to 50% of total wax loads and represented the most dominant wax compound in all organs of Arabidopsis. In stems, silique walls, flowers, and seed coats, secondary alcohols and ketones were present but their very low amounts was noticed on leaf (Jenks *et al.*, 1995; Bernard and Joubès, 2013; Lee and Shu, 2015).

In rapeseed, cuticular wax amount in leaves is considerably higher than Arabidopsis leaves, while other wax compounds were similar (Pu *et al.*, 2013). In rapeseed breeding line 6-3476, the amount of wax in leaves (687–2255 μg/g) was similar to flower of Arabidopsis (2382 μg/g) (Tassone *et al.*, 2016). In *camelina sativum* var Celina, the amount of wax in leaves, stem, flower and seed coat was 6.24, 164, 264, 0.24 μg/cm^2, respectively. Wax esters (74%) in leaf and triterpenoids/sterols (53%) in stems were observed, but the primary alcohols (65%) were detected in seed coats (Razeq *et al.*, 2014).

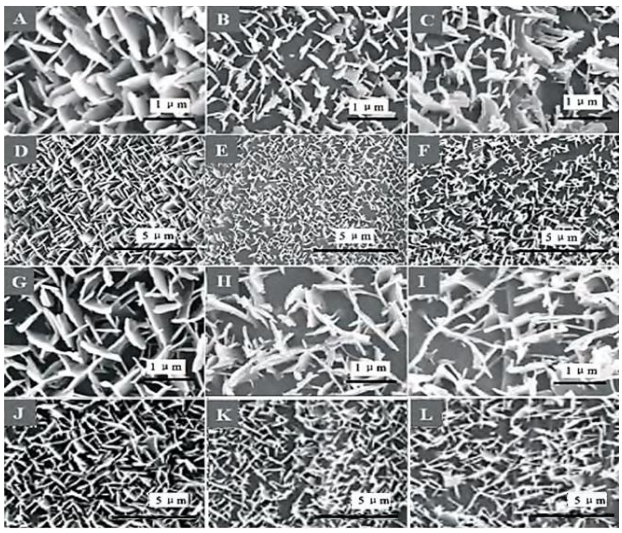

Fig. 1: Epicuticular wax crystals patterns on the adaxial and abaxial leaf surfaces of the wheat D genome (*A. tauschii*) detected by SEM at three stages of plant development. A, D are the adaxial surface of leaves during seedling stage; B, E are the adaxial surface of leaves during heading stage; C, F are the adaxial surface of leaves during filling stage; G, J are the abaxial of leaves during seedling stage; H, K are the abaxial surface of leaves during seedling stage; I, L are the abaxial surface of leaves during Filling stage. The micrographs are at a resolution of 10,000× and 30,000×, and the bars indicate 1 μm and 0.5 μm, respectively

Furthermore, in leaf of *Camelina MYB96* transgenic line, the amount of wax was 2.9 μg/cm^2 while in the leaf of Robinson variety it was 0.72 μg/cm^2 (Tomasi *et al.*, 2017). In another report, the wax compositions of cultivar *C. sativum* var Celina was similar to that reported by Razeq *et al.* (2014). Furthermore, the amount and composition in leaves of *C. sativa* 1.37 μg/cm^2, *C. rumelica* 2.01 μg/cm^2, *C. hispida* 0.85 μg/cm^2 and *C. microcarpa* 0.84 μg/cm^2 were reported but total wax loads were lower than younger leaves. In all Camelina species, the primary alcohols and alkanes were dominant components followed by wax esters, fatty acids and aldehydes. Interestingly, *C. sativa* var MYB96 synthesized higher levels of primary alcohols. It indicates that MYB96 might be an effective gene involved in the primary alcohols biosynthesis (Tomasi *et al.*, 2017). In potato, the amount of wax in the leaves was 5 μg/cm^2 in Perkoz, 6 μg/cm^2 in Aster and Maryna, and 7 μg/cm^2 in Ibis. Alkanes were dominant compound and primary alcohols were second major class in all potato varieties (Szafranek and Synak, 2006). In Gboma eggplant plant, the wax in cultivar UVPP was 2.7 μg/cm^2 and in Urafiki it was 2.3 μg/cm^2 were observed in leaves. Alkanes, primary alcohols, fatty acids, sterols, and triterpenols were also found. Alkanes (47–56%) were the dominant component in cuticular wax in Gboma Eggplant. Sterols content were observed much higher than triterpenes, consisting of 19 and 32% of the total waxes in Urafiki and UVPP cultivars, respectively (Halinski *et al.*, 2012).

Table 2: Cuticular wax amounts and contents in different organs in crops

Species	Organs	Total Loads	Components (relatives %)													Reference
			Fatty acids	Aldehydes	Alkanes	Primary alcohols	Sec. alcohols	Ketons	Wax esters	Iso-alkanes	Anteiso-alkanes	Alkenols	Alkenes	Triterpenoids & sterols	Ferulate Esters/ Phenol	
	Leaf	0.7–1.5[m]	1	14	73	8	0	0	4	0	0	0	0	0	0	Bernard and Joubès, 2013; Li-Beisson *et al*., 2013
Arabidopsis	Stem[a]	13–24[m]	1	7	44	12	9	22	5	0	0	0	0	0	0	Bernard and Joubès, 2013; Li-Beisson *et al.*, 2013
	Silique[a]	13[m]		1	4	50	14	9	22	0	0	0	0	0	0	Kim *et al.*, 2012
	Flower[a]	2382[n]	0	2	62	5	15	14	2	0	0	0	0	0	0	Bernard and Joubès, 2013
	Seed coat[a]	36–170[n]	0	9	51	13	18	9	0	0	0	0	0	0	0	Li *et al.* 2007; Bernard and Joubès, 2013
	Leaf	29.4[m]	1	4	55	2	10	27	2	0	0	0	0	0	0	Pu *et al.*, 2013
Rapeseed	Leaf[b]	687–2255[n]	0	0	0	0	0		0	0	0	0	0	0	0	Tassone *et al.*, 2016
	Leaf[c]	6.2[m]	3	0	3	20	0	0	74	0	0	0	0	0	0	
C. sativa	Steam[c]	16[m]	6	0	28	13	0	0	0	0	0	0	0	53	0	Razeq *et al.*, 2014
	Flower[c]	264[n]	14	0	64	1	0	0	0	0	0	0	0	21	0	
	Seed coat[c]	0.2[m]	6	0	29	65	0	0	0	0	0	0	0	0	0	
C. sativa	Leaf[d]	2.9 [m]	16	2	35	42	0	0	3	0	0	0	0	0	0	
C. sativa	Leaf[e]	0.72[m]	9	0	9	49	0	0	17	0	0	0	0	0	0	
C. sativa	Leaf[c]	0.83m	7	0	17	50	0	0	25	0	0	0	0	0		Tomasi *et al.*, 2017
C sativum	leaf[c]	1.37m	12	1	22	43	0	0	14	0	0	0	0	0	0	
C. *rumelica*	Leaf	2.01[m]	6	1	35	40	0	0	17	0	0	0	0	0	0	
C. hispida	Leaf	0.85[m]	7	0	20	27	0	0	40	0	0	0	0	0	0	
C. microcarpa	Leaf	0.84[m]	4	0	45	44	0	0	0	0	0	0	0	0	0	
Potato	Leaf [f]	6[m]	9	0	61	12	1	1	3	0	0	0	0	2	0	
	Leaf [g]	7[m]	9	0	65	10	0	1	5	0	0	0	0	1	0	Szafranek and Synak, 2006
	Leaf [h]	6[m]	10	0	61	11	1	2	2	0	0	0	0	1	0	
	Leaf [i]	5[m]	5	0	61	7	1	2	2	0	0	0	0	1	0	
Egg Plant	Leaf[j]	2.7[m]	3	0	47	16	0	0	0	0	0	0	5	19	0	Halinski *et al.*, 2012
	Leaf[k]	2.3[m]	5	0	56	18	0	0	0	0	0	0	5	32	0	
	Leaf[l]	6.7[m]	0	0	74	3	0	0	0	18	0	0	0	5	0	Wang *et al.*, 2011
	Anther[j]	2155[n]	21	0	33	0	0	0	0	33	6	0	0	7	0	Smirnova *et al*., 2013
	Fruit[m]	8.4[m]	7	4	37	6	0	0	0	5	1	4	16	21	0	Isaacson *et al.*, 2009
	Fruit[n]	10.2[m]	3	2	49	6	0	0	0	2	0	5	9	24	0	Kosma *et al.*, 2010
Tomato	Leaf [L1]	2094[n]	2	0	72	1	0	0	0	0	0	0	0	10	0	
	Leaf[L2]	2118[n]	1	0	75	1	0	0	0	0	0	0	0	7	0	
	Leaf[L3]	1686[n]	1	0	73	1	0	0	0	0	0	0	0	12	0	
	Leaf[L4]	1131[n]	4	0	73	2	0	0	0	0	0	0	0	7	0	
	Leaf[L5]	1019[n]	3	0	77	1	0	0	0	0	0	0	0	7	0	
	Leaf[Pen1]	2351[n]	3	0	38	1	0	0	0	0	0	0	0	2	0	
	Leaf [Pen2]	3933[n]	4	0	53	2	0	0	0	0	0	0	0	7	0	Halinski *et al.*, 2015
	Leaf [Pen3]	3738[n]	11	0	35	3	0	0	0	0	0	0	0	19	0	
	Leaf [Pen4]	3971[n]	1	0	71	1	0	0	0	0	0	0	0	1	0	
	Leaf [Pen5]	3116[n]	45	0	34	0	0	0	0	0	0	0	0	11	0	
	Leaf[LxPen]	2657[n]	1	0	62	1	0	0	0	0	0	0	0	4	0	
	Leaf[Pim1]	1342[n]	1	0	71	2	0	0	0	0	0	0	0	5	0	
	Leaf [LxPim]	1069[n]	2	0	71	1	0	0	0	0	0	0	0	5	0	
	Leaf[o]	14.7[m]	9	4	1	75	0	0	11	0	0	0	0	0	0	Von Wettstein-Knowles, 1971; Avato *et al.*, 1982
Barley	Leaf[p]	14.6m	2	1	1	6	0	59	0	0	0	0	0	0	0	Yu *et al.*, 2008

Table 2: Continued

Table 2: Continued

	Leaf [q]	7.57[m]	18	37	2	32	0	0	11	0	0	0	0	0	0	
	Blade															Mao *et al.*,
	Leaf Sheath[q]	5.8[m]	14	32	3	36	0	0	16	0	0	0	0	0	0	2012
Rice	Leaf Blade[r]	8.4[m]	34	31	7	24	0	0	4	0	0	0	0	0	0	
	Leaf Sheath[r]	4.5[m]	43	24	9	23	0	0	0	0	0	0	0	0	0	
	Leaf Blade[s]	6.2[m]	58	0	19	22	0	0	0	0	0	0	0	0	0	Wang *et al.*, 2017
	Leaf	8.2[m]	0	25	4	69	0	0	2	0	0	0	0	0	0	
Maize[t]	Blade															Javelle *et al.*,
	Leaf Sheath	4.5[m]	0	42	14	39	0	5	0	0	0	0	0	0	0	2010
	Leaf [1]	3.4 [m]	6	3	14	71	0	3	2	0	0	0	0	0	0	
	Leaf [2]	3.6 m	4	3	10	79	0	1	2	0	0	0	0	0	0	
	Leaf [3]	3.9 [m]	5	4	15	68	0	6	3	0	0	0	0	0	0	
	Leaf [4]	3.6 [m]	5	3	11	68	0	12	2	0	0	0	0	0	0	
	Spike [1]	4.5 [m]	13	1	40	18	0	37	0	0	0	0	0	0	0	Wang *et al.*,
Wheat	Spike [2]	5.8 [m]	11	1	31	30	0	27	0	0	0	0	0	0	0	2015a
	Spike [3]	7.7 [m]	10	1	19	7	0	63	0	0	0	0	0	0	0	
	Spike [4]	6.6 [m]	10	1	31	11	0	47	0	0	0	0	0	0	0	
	Leaf[5]		1	0	34	1	0	63	1	0	0	0	0	0	0	Zhang *et al.*, 2015
	Leaf[6]	16[m]	1	3	9	0	0	14	9	0	0	55	0	0	0	
	Peduncle [7]	4.9[m]	1	1	7	0	0	81	2	0	0	2	0	0	0	Racovita *et al.*, 2016
	Seedling leaf[7]	5.4m	1	5	2	84	0	0	1	0	0	0	0	0	0	
	Flag leaf[7]	8.4m	2	9	6	77	0	1	1	0	0	0	0	0	0	Wang *et al.*,
	Leaf sheaths[7]	3.5m	2	4	15	57	0	15	3	0	0	0	0	0	0	2017
	Peduncles[7]	2.9m	3	13	37	23	0	36	2	0	0	0	0	0	0	
	Glumes[7]	1.0m	2	7	10	30	0	10	2	0	0	0	0	0	0	
	Anthers[7]	0.3m	54	1	27	3	0	27	1	0	0	0	0	0	0	
Broccoli	Leaf18	1929 n	13	20	39	3	5	19	0	0	0	0	0	0	0	Lee *et al.*,
	Leaf19	3733 n	15	13	40	3	7	22	0	0	0	0	0	0	0	2015
Sesame	Leaf10	7.69 m	0	11	68	0	0	0	0	0	0	0	0	0	0	Kim *et al.*, 2007
Tobacco	Leaf[11]	13.9m	7.8	0	74	7	0	0	0	0	0	0	0	0	0	Cameron *et al.*, 2006
Cucumber	Friut12	1.3m	21	22	46	0	3	0	0	2	0	0	2	0	3	Wang *et al.*,
	Stem12	1.6m	6	8	82	0	10	0	0	1	0	0	1	0	3	2015a
	Leaf12	1.8m	8	15	62	0	8	0	0	5	0	0	0	0	2	
Cabbage	Leaf13	0	0	6	34	6	14	31	0	0	0	0	0	0	0	Laila *et al.*, 2017

Note: number 0 indicates that trace or undetectable amounts were audited. [a]ecotype col-0, [b]breeding line 6-3476, [c]cultivar *Cemelina sativum* var celina, [d] *C. sativum* var *MYB96*, [e]*C. sativum* var robinson, [f]potato cultivar aster, [g]potato cultivar ibis, [h]potato cultivar maryna, [i]potato cultivar perkoz, [j]egg plant cultivar uvpp, [k]Gboma egg plant cultivar urafiki, [l]cultivar micro tom, [m]cultivar tomato m82, [n]cultivar tomato ailsa craig, [l1] tomato cultivar vf-36, [l2]tomato cultivar wild (ecuador), [l3]tomato cultivar wild (ecuador), [l4]tomato cultivar nagcarlang, [l5] tomato cultivar wild (usa),[pen1]tomato cultivar wild (peru), [pen2]tomato cultivar wild (peru), [pen3] tomato cultivar wild (peru), [pen4]tomato cultivar wild (peru), [pen5]tomato cultivar wild (peru), [lxpen]tomato cultivar hybrid, [pim1]tomato cultivar wild (ecuador), [lxpim]tomato cultivar hybrid, [o]barley cultivar bonus, [p]barley cultivar bowman, [q]rice cultivar nipponbare, [r]rice cultivar japonica, [s]maize inbred line a188, [1]wheat cultivar a14, [2]wheat cultivar jing 2001, [3]wheat cultivar fanmai 5,[4]wheat cultivar shanken 99, [5]wheat cultiver bob white,[6]wheat cultivar bethlehem,[7]wild grass *Ae. tauschii*. [8]broccoli cultivar mc 117,[9]broccoli cultivar mc91, [10]seasame cultivar various, [11]tobacco cultivar graha, [12]cucumber cultivar wild type, [13]cabbage cultivar *b. oleracea* , [m] unit = μg/cm^2, [n]unit = μg/g

In tomato cultivar Micro-Tom, amount and composition of cuticular waxes in leaf and anther were 6.7 μg/cm^2 and 2155 μg/g, respectively, and the most abundant components were alkanes (74%) in leaves (Wang *et al.*, 2011). Tomato cultivar M82 and cultivar Ailsa Craig in fruits contained 8.4 μg/cm^2 and 10.2 μg/cm^2 wax total load, respectively while alkanes and alkenes were the dominant wax component (Isaacson *et al.*, 2009; Kosma *et al.*, 2010). However, the most dominant waxes components of these varieties were alkanes ranged from 34 to 71% (Halinski *et al.*, 2015). In tomato leaves, fruits and anthers, branched alkanes, alkenes, and cyclic compounds were detected (Isaacson *et al.*, 2009; Wang *et al.*, 2011; Smirnova *et al.*, 2013; Halinski *et al.*, 2015). This finding was in the agreement with the other researchers that cuticular waxes in tomato leaf were consisted by hydrocarbons (Zygadlo *et al.*, 1994; Smith *et al.*, 1996; Vogg *et al.*, 2004). Primary alcohols, aldehydes and fatty acid were found to be principal components of cuticular wax, where alkanes occupied less than 15% of the total wax loads in leaves of barley, rice and maize (Von Wettstein-Knowles, 1971; Avato *et al.*, 1982; Javelle *et al.*, 2010; 2011; Mao *et al.*,

2012) whereas ketones were the most abundant component in leaves of barley cultivar Bowman (Yu *et al.*, 2008).

In wheat, primary alcohols were the major components in leaves cuticular wax followed by alkanes, esters, aldehydes and fatty acids. Ketones deposition variations were observed in wheat leaves. In wheat leaves, ketones were found in trace amount the cuticular wax. However, in spike, a traceable amount of ketones was identified (Wang *et al.*, 2015a, 2017). In addition, there were large differences in the amounts of ketones among different wheat varieties (Wang *et al.*, 2015a; Zhang *et al.*, 2015). Wheat cultivar Bethlehem revealed wax coverage of 16 μg/ cm^2 in leaf and 49 μg/cm^2 in peduncles (Zhang *et al.*, 2015). Furthermore, in wheat cultivar Bethlehem, alkanols were 55% of total wax loads in leaf and β-diketone and hydroxy-β-diketones collectively comprised 81% of the total wax loads in peduncle. This happened due to discrepancy in the regulation of the acyl-reduction and β-diketone biosynthetic pathways in the two examined organs (Racovita *et al.*, 2016).

In broccoli cultivars MC117 and MC91, fatty acids (13 and 15%, respectively), aldehydes (20 and 13%, respectively), alkanes (38 and 39%, respectively) and ketones (19 and 21%, respectively) were found in the total wax loads in leaf (Lee *et al.*, 2015). In sesame, major components of waxes in leaves were alkanes (68% of total wax) and aldehydes (11% of total wax) (Kim *et al.*, 2007). In tobacco, the primary component of cuticular wax was alkanes, which constituted 75% of the total wax load fully expanded leaves while fatty acids and alcohols occupied smaller proportion of total wax loads (Cameron *et al.*, 2006). In cucumber, alkenes, primary alcohols, branched alkanes, phenols, esters, and aldehydes, phenols were major compounds in the cuticular waxes (Wang *et al.*, 2015a). In cabbage leaf, alkane contributed 34% followed by ketones 31% of total wax loads (Laila *et al.*, 2017).

In spite of a vast variation in cuticular wax loads and contents depending upon crops and organs, the prime reasons that responsible for these variations are not identified. Nevertheless, the present information may be used as a genetic source for determination of new genes associated with cuticular wax biosynthsis.

Biosynthesis of cuticular wax genes in crops: In crops, the genes responsible for biosynthesis of cuticular wax are not well characterized such as in Arabidopsis, though, recently notable advances have been made in this respect (Table 1). Identification and functional expression of the glossy (*gl*) mutants provided an opportunity to understand about wax biosynthesis in maize. Mutants of *GL4* and *GL8* are homolog of *AtKCS6* and *AtKCR*, respectively. They displayed a spectacular decrease in the amount of alkanes, alcohols and aldehydes in the leaves of wild type seedling (Avato *et al.*, 1987; Dietrich *et al.*, 2005; Liu *et al.*, 2009). In another study on maize, it was found that *gl1* and *gl2* mutants which were homolog to *AtCER3* and *AtCER2*, respectively, declined wax deposition in seedling leaves, especially the aldehydes levels significantly decreased or could not be identified. Contrarily, the *gl1gl2* double mutant enhanced the levels of wax esters (Bianchi *et al.*, 1979; Lemieux, 1996; Hansen *et al.*, 1997; Velasco *et al.*, 2002; Sturaro *et al.*, 2005). It was also found that *GL13*, an ABC transporter, was associated with cuticular wax deposition (Li *et al.*, 2013).

In rice, *crystal-spares leaf1* (*wsl1*) mutant, is a *KCS* gene which has a lesion of *WSL1* gene, catalyzed the creation of C20–C24 VLCFA precursors of leaf waxes. It reduced growth, fertility, leaf fusion and increased drought sensitivity due to wax-deficiency. This is indicated that *WSL1* might be involved in the deposition of other lipids associated with growth and development of the plant (Yu *et al.*, 2008). *ONION1 (ONI1)* is another homolog to rice *KCS* protein. It is accountable for synthesis of C20 and C22 saturated VLCFAs, which are important for development of shoot (Ito *et al.*, 2011). From functional expression of wax-*deficient anther1* ***(wda1)*** mutant, it was observed that a *WDA1* protein, which is homolog to *AtCER1*, was responsible for synthesis of VLC alkenes and alkanes in pollen and anthers (Jung *et al.*, 2006). Functional characterization of *OsGL1-6*, which is also homolog to *AtCER1*, reported that it is needed for wax biosynthesis on leaf blades. It was also found that reduced expression of the *OsGL1-6* gene was linked with notable decreases in total wax loads and enhanced drought sensitivity (Zhou *et al.*, 2013). Characterization of rice mutants, *gl1-2* and *gl1-1/wsl2*, revealed that these mutants reduced overall cuticular wax loads and increased sensitivity to drought stress (Islam *et al.*, 2009; Qin *et al.*, 2011; Mao *et al.*, 2012). Over expression of *Drought-Induced Wax Accumulation 1* (*DWA1*) held an AMP-binding domain, which increased VLCFA synthesis and enhanced drought resistance (Zhu and Xiong, 2013). *OsGL1-3* is homologous to maize *GL1* and *Arabidopsis WAX2/YRE/CER3/FLP*, which significantly increased biosynthesis of cuticular wax and enhanced tolerance to water stress (Zhou *et al.*, 2015). *OsWS1* belongs to the membrane-bound O-acyl transferase gene family, and is associated with wax biosynthesis in rice (Xia *et al.*, 2015). *OsHSD1* belongs to the short-chain dehydrogenase reductase family, which enhanced VLCFAs biosynthesis and soluble fatty acids in the leaves of the *oshsd1* mutant (Zhang *et al.*, 2016). *WSL3* encodes *KCR* in rice, which contributes to VLCFA biosynthesis and wax depositions in leaf. On the other hand in rice mutant, *wax crystal-sparse leaf 3* (*wsl3*) gene reduced epicuticular wax crystals and wax composition on the leaf surface (Gan *et al.*, 2016). *ORF4* is homologous to the *KCS6* family of *KCS*, which is similar to *WSL3* gene of rice and regulates cuticular wax formation (Hong-bing *et al.*, 2017). *WSL4* encodes a *KCS*, a homolog of *AtCER6*, which increased the cuticular wax load in rice leaves (Wang *et al.*, 2017).

In tomato fruit cuticle, the *lecer6* mutant belongs to *CER6-like KCS* (*LeCER6*), decreased accumulation of alkanes and aldehydes but on the other hand, amounts of

triterpenoid increased in the total wax loads (Vogg *et al.*, 2004). In tomato fruits, the over expression of *SlTTS1* and *SlTTS2* enhanced biosynthesis of terpenoid (Wang *et al.*, 2011). However, alkane is the dominant wax component in the tomato wax but genes/proteins have not yet been identified for the biosynthesis of alkanes. In *B. napus*, glossy mutant *BnaA.GL* was characterized, which reduced wax biosynthesis and increased sensitivity to drought stress (Pu *et al.*, 2013). *BraLTP1* belongs to non-specific lipid transfer proteins (*nsLTPs*), which decreased wax deposition in leaves (Liu *et al.*, 2014). In *B. rapa*, the *BrWax1* gene is found on linkage group A01, which is involved in cuticular wax biosynthesis in leaves (Zhang *et al.*, 2013a). In *Camelina sativa*, few wax biosynthesis genes have been detected recently but their characterization is not complete yet (Lee *et al.*, 2014). In Cucumber, *CsWAX2* is homolog of *AtWAX2*, which performs fundamental functions in wax biosynthesis (Wang *et al.*, 2015a) and *CsCER1*, a homolog of *AtCER1*, played an important role in wax VLC alkanes biosynthesis (Wang *et al.*, 2015b). In Cabbage, *BoLTP2* gene is a non-specific *lipid-transfer protein1*, involved in the transformation of ketones to lipid which reduced wax deposition. *BoCER3* gene is homologous to *AtCER3* protein, which is related to wax deposition through converting aldehydes to alkanes. *BoKCS1* and *BoKCR1* are homologs to *AtKCS* and *AtKCR1* protein, respectively, which decreased acyl-CoAs synthesis and eventually influence total wax loads. Likewise, expressions of *BoLACS1* influenced wax depositions. Besides, the *BoMAH1* gene engaged in synthesis of secondary alcohols and ketones (Laila *et al.*, 2017).

In wheat, *W1W2* is a homolog of Arabidopsis *CER4-6* proteins. It produced hydroxyl-β-diketones, which enhanced drought tolerance through reducing cuticle permeability (Zhang *et al.*, 2013b). *W3*, a homolog of Arabidopsis *CER1* and *CER3*, is essential for β-diketone biosynthesis but suppresses its hydroxylation (Zhang *et al.*, 2015). *TaFAR1*, a homolog of Arabidopsis *CER4*, is an active acyl-CoA reductase. It produced primary alcohols, and as a result augmented total wax loads on wheat leaf blades (Wang *et al.*, 2015b). *TaFAR5*, a homolog to Arabidopsis *CER4*, is an alcohol-forming fatty acyl-coenzyme A reductase (*FAR*), which contributes significantly to produce primary alcohols in wheat leaf blade (Wang *et al.*, 2015c). *TaFAR2*, *TaFAR3*, and *TaFAR4* genes are a homolog of Arabidopsis *CER4* protein, which produced primary alcohols in cuticular wax (Wang *et al.*, 2016). From characterization of the *Iw* genes, it was found that *Iw* genes regulatory mechanism control *W-COE* expression and β-diketone formation (Huang *et al.*, 2017). In, Dgenome (*Ae. Tauschii*), *Ae.tFAR1*, *Ae.tFAR2*, *Ae.tFAR3*, *Ae.tFAR4*, and *Ae.tFAR6* are homolog to *AtCER4*, which principally accountable for deposition of primary alcohols (Wang *et al.*, 2017). In barley, the *Cer-cqu* gene cluster is involved in β-diketons biosynthesis which consists of several proteins families including type-III polyketide synthases, hydrolases, and cytochrome P450s (Hen-Avivi *et al.*, 2016; Schneider*et al.*, 2016). The barley *eceriferum-b.2* (*cer-b.2*) mutant produces β-diketons, which makes glossy leaf sheaths and deficient in the cuticular wax component, 14, 16-hentriacontanedione (Zhou *et al.*, 2017).

Conclusion

This review has revealed that wax compositional differences exist among different crops even organ to organ. High levels of structural diversities associates with cuticular wax deposition in crops are largely influenced by different genes. Several genes have been already identified, which are related to cuticular wax biosynthesis. Cuticular wax components are produced by two different complex pathways due to the influence of biotic and abiotic stress, which allow adaptive mechanism at the time of crop-environment interactions. Moreover, specific single wax components yet remain unknown during development and growth stages of different crops. Very few studies have focused on primary alcohols and ketons in wheat and barley, but most of the factors are still unidentified. Additionally, genome sequencing technologies have been progressed enormously, allowed identifying new race of cuticular waxes biosynthesis genes in crops. Knowledge on the wax biosynthesis mechanisms in different crops will be helpful to breed new crops cultivars better tolerant to environmental stresses.

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