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Role of Plant Fatty acid Elongase (3 keto acyl-CoA Synthase) gene in Cuticular Wax Biosynthesis.

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Review Article

ABSTRACT

Plant surfaces are ensheathed by cuticular wax, amorphous intra-cuticular embedded in cutin polymer and crystalloid epi-cuticular that imparts a whitish appearance, confers drought resistance by reducing stomatal transpiration and also protects from U.V Radiation, phytophagous insects etc. Very long chain fatty acids acts as precursors for cuticular wax bio-synthesis. Wax bio-synthesis begins with fatty acid synthesis in the plastid (*de novo* synthesis of C16 and C18) and elongation of fatty acids in endoplasmic reticulum (C20 – C34) by four distinct enzymes *3-ketoacyl-CoA synthase*, *3-ketoacyl-CoA reductase*, *3-hydroxacyl-CoA dehydratase, trans-2,3-enoyl-CoA reductase (KCS, KCR, HCD, ECR)*. The *KCS*, a fatty acid elongase, determines the chain length and substrate specificity of the condensation reaction, a rate limiting step and the subsequent elongated products alkanes, aldehydes, primary alcohols, secondary alcohols, ketones and wax esters. 21 *KCS* genes were annotated in *Arabidopsis thaliana* Genome of which some KCSs were identified involved in cuticle formation *(CER6) (CUT1), KCS1, KCS2, (DAISY), KCS2O and FDH)*.The current review will focus on the bio-chemical, genetic and molecular approaches on *KCS*s genes, predominantly *KCS1* in plants particularly useful in identifying and characterizing gene products involved in wax bio-synthesis, secretion and function for developing transgenic crops that combat various stresses.

INTRODUCTION

Cuticular wax is a special features developed by plants to seal the aerial organs and to tolerate various biotic and abiotic stresses.Epicuticular wax is the first line of defense to prevent non-stomatal harmful water loss [forming a continuous hydrophobic barrier obstructing water loss from plant organs].[3]Other functions in plant are protection from Ultraviolet [UV] light by reflection [24,45], restrict the attachment and growth of insects [38] increased plant resistance against pathogens like bacteria and fungi[6]and reduces water deposition on the surface of the plant thus reducing retention of dust, pollen and air pollutants [26] and plays a role in plant–insect [38] and plant–pathogen interactions [6]. Reduction in the amount of epicuticular wax on plant surface showed increased rates of transpiration. Brushing waxes off the excised leaves significantly increased the rate of water loss [15].Similarly, leaves of rice [*Oryza sativa*], dipped for two seconds in chloroform to remove epicuticular waxes, and exhibited more than a two fold increase in cuticular conductance to water vapor compared to control leaves [39]. Leaf glaucousness [waxinees] is a characteristic referred to as a plant adaptation to drought [22]. Cuticular wax is a general term for a complex mixture of hydrophobic compounds including homologous series of very long chain aliphatic lipids [fatty acids, alcohols, aldehydes, alkanes, ketones and wax esters] which are all derived from VLCFAs by the fatty acid elongase mechanism.

Mechanism of Fatty Acid Elongation

The Fatty Acid Elongase mechanism involves four successive reactions catalyzed by four distinct enzymes [*KCS, KCR, HCD* and *ECR*] organized in an endoplasmic reticulum [ER]-associated complex, the acyl-CoA elongases.

The first step of fatty acid elongation is the condensation by the *KCS* [or condensing enzyme] of a long chain acyl-CoA with a malonyl-Co A. The 3-ketoacyl-CoA is then reduced by a *KCR* to 3-hydroxyacyl-CoA. It is then *HCD* to form a trans-2, 3-enoyl-CoA. The final elongation step is the reduction by the *ECR* which yields a two carbon acyl Co A. Considering the condensing activity; several genes have been identified as encoding the *KCS*. The first one, *FAE1*, was isolated from the *Arabidopsis thaliana* mutant *fae1* and characterized as the seed-specific *KCS* gene responsible for the synthesis of VLCFA present in storage lipids [19]. In the *Arabidopsis* genome, 21 *FAE1*-like genes are present [7]. Only eight of them have been demonstrated to encode proteins able to catalyze VLCFA production [4,40,54]. These *KCS* enzymes have different substrate specificities suggesting that multiple fatty acid elongases exist in plants. Three *KCS*s have been shown to be involved in the formation of the cuticle in *Arabidopsis*: *CER6* [9,18,36], *KCS1* [53] and *FDH* [42, 59].Complete loss of function of *CER6* or *KCS1* leads to the accumulation of wax derivatives with 24 carbons suggesting that these two *KCS*s are involved in the biosynthesis of C26 and longer VLCFAs for the production of epicuticular waxes. In addition, loss of function of *FDH [FIDDLEHEAD]* results in ectopic organ fusions suggesting a role of this *KCS* in organ development. Another *KCS* named *HIC* has been described as a negative regulator of stomatal development that responds to CO2 concentration [14]. The 3D structure of the *KCS* proteins using the model generated for the *KCS1* protein, analyzed the subcellular localization in tobacco cells by confocal microscopy of 7 *KCS* proteins as well as that of the two reductases of the acyl-CoA elongase complex, *KCR* and *ECR.* Twenty-one *KCS* genes have been previously identified in the *Arabidopsis* genome [7].

Table: KCS Genes in Arabidopsis

S.NO	GENE NAME	GENE NAME IN LITERATUIRE	ACCESSION NUMBER	FUNCTION
1	KCS1	KCS1	At1g01120	Involved in critical fatty acid elongation process
2	KCS2	KCS2	At1g04220	Involved in bio-synthesis of VLCFAs
3	KCS3	KCS3	At1g07720	Involved in bio-synthesis of VLCFAs
4	KCS4	KCS4	At1g19440	Involved in bio-synthesis of VLCFAs
5	KCS5	KCS5, CER60	At1g25450	Involved in bio-synthesis of VLCFAs
6	KCS6	KCS6,CER6, CUT1	At1g68530	Involved in bio-synthesis of VLCFAs
7	KCS7	KCS7	At1g71160	Involved in bio-synthesis of VLCFAs
8	KCS8	KCS8	At2g15090	Involved in bio-synthesis of VLCFAs
9	KCS9	KCS9	At2g16280	Involved in bio-synthesis of VLCFAs
10	KCS10	KCS10, FDH	At2g26250	Involved in the synthesis of long chain lipids found in cuticle
11	KCS11	KCS11	At2g26640	Involved in bio-synthesis of VLCFAs
12	KCS12	KCS12	At2g28630	Involved in bio-synthesis of VLCFAs
13	KCS13	KCS13, HIC	At2g46720	Involved in bio-synthesis of VLCFAs
14	KCS14	KCS14	At3g10280	Involved in bio-synthesis of VLCFAs
15	KCS15	KCS15	At3g52160	Involved in bio-synthesis of VLCFAs
16	KCS16	KCS16	At4g34250	Involved in bio-synthesis of VLCFAs
17	KCS17	KCS17	At4g34510	Involved in bio-synthesis of VLCFAs
18	KCS18	KCS18, KCS2	At4g34520	Involved in bio-synthesis of VLCFAs
19	KCS19	KCS19, FAE1	At5g04530	Involved in bio-synthesis of VLCFAs
20	KCS20	KCS20	At5g43760	Involved in bio-synthesis of VLCFAs
21	KCS21	KCS21	At5g49070	Involved in bio-synthesis of VLCFAs

Adopted from Je´roˆme Joube`s et al., [21], Blacklock and Jaworski 1 [4] ; 2, Costaglioli et al., [7] ; 3, Efremova et al., [8] ;4, Fiebig et al. [9] ;5, Ghanevati and Jaworski ., [12] 6, Ghanevati and Jaworski [13]; 7, Gray et al., [14]; 8, Hooker et al., [18]; 9, James and Dooner [20]; 10, James et al., [19]; 11, Kunst et al., [29]; 12, Kunst and Clemens [28]; 13, Lolle et al., [34] ; 14, Millar and Kunst [37]; 15, Millar et al., [36]; 16, Paul et al., [40]; 17, Pruitt et al., [42]; 18, Rossak et al., [46]; 19, Stasolla et al., [49]; 20, Todd et al., [53]; 21, Trenkamp et al., [54].

As *KCS1* is the most expressive among all the *KCS* genes [21] the attention is given mainly for this gene which is involved in the crucial fatty acid elongase, a rate limiting step [5,53,59].

3-KETO ACYL CO-A SYNTHASE 1

KCS 1 is a condensing enzyme involved in the critical fatty acid elongase process. [ACESSION NO: #AT1G01120]. A cDNA encoding *KCS* was identified in *Arabidopsis thaliana*. The recombinant protein was expressed in yeast and its function as a fatty acid elongase condensing enzyme confirmed. The analysis of T-DNA tagged kcs-1 mutant demonstrated the in vivo involvement of *KCS-1* in very long chain fatty acid and wax bio-synthesis in vegetative tissues. It elongates C18:0-Co A through C26:0-Co A and is more active towards saturated fatty acids. Molecular weight of polypeptide is 58.0 kDa [from nucleotide sequence] [21] .

Enzymatic Reaction of *KCS1*

A long chain 2, 3, 4-saturated fatty acyl co A + malonyl co A ↔ a long chain 3 oxalo acyl co A + CO2 + Co enzyme A. The structure of *KCS1* shows that out of 8 sub classes [α, β, γ, δ, ζ, ε, η, θ] *KCS1* belongs to the sub class δ [showing similarity to *KCS13, KCS14*]. By using the ARAMEMNON plant membrane protein database server [http://aramemnon.botanik.unikoeln. de/index.ep] [Schwacke R] [48], identified *KCS1* anchored to the membrane with two trans -membrane domains [residues 44-66 & 85-103]. Proposed model of *KCS1* is triangular shape with prominent arm made of 2 parallel α helices [A1 and A2] linked to the protein by a complex network of coils. The size, shape and position of this arm make it suitable for anchoring to the membrane. The body of the protein shows α-β-α-α [4 layered] structure. Amino acid sequence of *KCS* is CYS[258] HIS [454] ASN[458] of which "Active Cystine residue" is the acyl acceptor in the first step of condensation and creates electronic environment resembling in acyl chain elongation reaction. A fourthresidue that is a serine at position 317 has been demonstrated to be crucial for the activity of the *FAE1* enzyme in *Brassica napus* [25]. Volume and spatial organization of *KCS1*, putative binding pocket is compatible with docking of malonyl co A and acyl chain alpha carbons. The chromosomal locations of *KCS1* genes in relation to the segmental duplication history were analyzed and found that *KCS1, KCS13* of δ subclass have evolved from an older segmental duplication with a good statistical significance [5]. Localization of GFP-*KCS* fusion protein in Endoplasmic reticulum modeling shows accessible putative phosphorylation sites, predictions have been made using at high stringency and mapped on the models to check accessibility.

The functions of *KCS1* were, it is involved in the formation of the cuticle, [53] involved in the bio-synthesis of C26 and long VLCFAs [very long chain fatty acid synthesis] for the production of epi-cuticular waxes, fatty acid biosynthesis pathway, fatty acid elongase activity, lipid biosynthesis process, transferase activity [transfer of acyl groups other than aminoacyl group] Very long chain fatty acid metabolic process. Activity of *KCS1* in-vitro condensation assay of isolated [HIS] 6*KCS* gene expression is ubiquitous in roots, stem and epidermis [4].

KCS1 is the most expressive gene for different abiotic stresses among all the members of *KCS* family [*KCS1 to KCS21*] duly supported by the evidences from bio-chemical, genomic and molecular approaches in various plants.

Contribution from Bio-Chemical, Genomic and Molecular Approaches:

Wax Bio-synthesis pathways exemplification has been possible due to the development of complex techniques like gas chromatography and mass spectrometry that allowed for the identification of wax components and exquisite genetic and bio-chemical studies using radio labeled tracers. As there is limited knowledge on enzymes involved in wax bio-synthesis regarding the association of these enzymes with membranes, insufficient amount of epidermal tissue for enzyme purification, enzyme activity assay substrates being insoluble in aqueous buffers are not commercially available. Genetic approaches alone were initially not successful in revealing the identity of loci involved in wax production. By studying wax biosynthesis in *Arabidopsis* twenty-two mutants were identified, called the *eceriferum* mutants [*cer*], displaying varying degrees of waxlessness *cer1- cer10* are very glossy and *cer 11- cer22* are less so, with altered epicuticular wax patterns compared to the wild type [27]. Homologous mutations to *eceriferum* were also found in other plants, e.g. in barley, where the locus is also termed *eceriferum*. Barley is the most extensively studied species, with 85 identified *cer* loci. In maize and *Brassica napus* the mutants are named *glossy*, referring to the leaves' glossy surface. Scanning electron microscopy revealed that the epi-cuticular wax crystals on these mutants are absent or exhibit particular morphological changes. Chemical analyses demonstrated that mutants have a lower wax load or altered chemical composition. Thus, biochemical functions of the mutated gene products are difficult to predict from the alterations in wax compositions from the visual screens but only mutants with significant decreases in epicuticular crystalloid accumulation would be isolated in the screen with lesions in regulatory genes, or genes affecting epidermal development. Mutants affecting individual biosynthetic enzymes would be difficult to find due to their more subtle biochemical phenotypes. Interestingly the *KCS* genes, that take part in the control of VLCFA biosynthesis, show different tissue specific expression patterns by both molecular and histo-chemical tests. The varying expression levels of *KCS* isoforms in different tissues suggest the specific roles of these enzymes [23]. The examination of the *eceriferum* mutants also resulted in the discovery of the importance of the operation of the *KCS* gene family. Since the expression of these genes is essential for the production of VLCFAs, their inhibition results in the block of wax biosynthesis [23] Yephremov et al [59]. Made a list of the candidate genes involved in wax biosynthesis, suggesting that the synthesized molecules by the elongase complexes are commonly modified, and therefore their proper constitution is crucial for the possibilities of later modifications.

Very-long-chain fatty acids [VLCFAs] are important functional components of various lipid classes, including cuticular lipids in the higher plant epidermis and lipid-derived second messengers. *FAE1,* the seed-specific *β-ketoacyl-CoA synthase* [*KCS*] catalyzes the first rate-limiting step in VLCFA biosynthesis. Misexpression of *FAE1* changes the VLCFAs in different classes of lipids. In *Arabidopsis*, mutations in the FATTY ACID ELONGATION [*FAE1*] gene result in reduced levels of very long chain fatty acids in seed [20, 30, 33]. The genes encoding *FAE*1 and its homologs have been cloned from *Arabidopsis* [19], *Jojoba* [31] and *Brassica napus* [2]. Ghanevati M et al.,[12]

performed site directed mutagenesis experiments on the *Arabidopsis FAE1* decipher the importance of Cys and His as residues in condensing enzyme catalysis, shown that Cys-223 and four His residues are essential for the enzyme activity. Extensive characterization of *FAE1*and the related *KCS1* has confirmed that these genes encode condensing enzymes [12,46,53]. The *FAE1*- like gene family appears to be unique to plants.

In Garden nasturtium [*Tropaeolum majus*] seeds the fatty acid elongase involved in producing significant amounts of erucic acid [22:1] [70%–75%] of total fatty acid [41], and accumulates trierucin as the predominant triacylglycerol [TAG] in its seed oil [51]. Using a degenerate primers approach, a cDNA of a putative embryo *FAE* was obtained, containing a 1,512-bp open reading frame encoding a protein of 504 amino acids. A genomic clone of the nasturtium *FAE* gene was isolated and sequence analyses indicated the absence of introns, restricted to the embryo[from Northern hybridization], encoded by a small multigene family [from Southern hybridization]. The cDNA into two different heterologous chromosomal backgrounds [*Arabidopsis* and tobacco [*Nicotiana tabacum*]] under the control of a seed-specific [napin] promoter and the tandem 35S promoter for functional confirmation. Seed-specific expression resulted in an 8-fold increase in erucic acid proportions in *Arabidopsis* seed oil, while constitutive expression in transgenic tobacco tissue resulted in increased proportions of VLCFAs indicate that the nasturtium *FAE* gene encodes a condensing enzyme involved in the biosynthesis of VLCFAs, utilizing monounsaturated and saturated acyl substrates. With its strong and unique preference for elongating 20:1-CoA, *FAE* gene product is used for directing or engineering increased synthesis of erucic acid.

In Jojoba manipulation of the microsomal fatty acyl-Co A elongation pathway converts high erucic acid rapeseed oil rich in VLCFAs [used as an industrial feedstock] into canola [edible oil] devoid of VLCFAs significant for agriculture. The cDNA was cloned from developing *Jojoba* embryos [involved in microsomal fatty acid elongation] which is homologous to the *Arabidopsis[FAEI]* gene encoding KCS.The characterization and transformation of low erucic acid rapeseed with the *Jojoba* cDNArestored *KCS* activity for developing embryos and altered the transgenic seed oil composition to contain high levels ofVLCFAs [35].

In *Jojoba* a gene encoding the *KCS* was isolated from developing seeds of *Lunaria annua,* Brassicaceae [rich in nervonic acid] complimented the canola [*Brassica napus*].Mutation of this gene knocked out the production of very long chain fatty acids in the seed oil. The influence of the *Jojoba KCS* substrate preferences was evident from the appearance of up to 5% nervonic acid in the transgenic oil nervonic acid present in jojoba oil but found at very low levels in rapeseed oil [31]. Expression of the *Lunaria KCS* in transgenic *Brassica* resulted in rapeseed oils with greater than 25% nervonic acid.

In rapeseed [*Brassica napus*], the two elongation steps from oleoyl-CoA [C18:1-CoA] to erucic acid are each controlled by alleles at two loci, E1 and E2 tightly linked to the *FAE1* gene loci. Advances in the biochemistry of the seed elongases and the cloning of the loci controlling the erucic acid content, the nature of the mutations that characterize the agriculturally important LEAR trait remain obscure. So the comparison of the component elongase activities in rapeseed varieties that vary in erucic acid content showed that an absence of *KCS* is associated with variation in the sequence of the *Bn-FAE1* gene. Low erucic acid rapeseed [LEAR] a near absence of VLCFAs in the seed oil lack L-ketoacyl-CoA synthase activity, acyl-CoA and ATP-dependent elongation activities in microsomes. High erucic acid rapeseed [HEAR] having high molecular mass acyl-CoA elongase complex in solubilised microsomes [from Size exclusion chromatography]. Transcripts for the *Bn-FAE1* genes were detected in LEAR embryos, immunoblots using antisera raised against the *L-ketoacyl-CoA synthase* indicated an absence of this protein. Comparison of the deduced amino acid sequences of immature embryo cDNAs reveals that LEAR alleles of *Bn-FAE1* encode variant *L- ketoacyl-CoA synthase* proteins. So, the coding region of LEAR was overexpressed for the development of transgenic seeds with significant increase in erucic acid.[52] To develop low level oferucic acid in rapeseeds by intron-spliced hairpin RNA, an inverted repeat unit of a partial *BnFAE1.1*gene interrupted by a spliceable intron cloned into pCAMBIA3301, and a seed-specific [Napin] promoter used to control the transcription of the transgene. Four transgenic plants harboring a single copy of transgene were generated. Expression of endogenous *BnFAE1.1* gene in developing T3 seeds was significantly reduced. In mature T3 seeds, erucic acid was decreased by 60.8 to 99.1% compared with wild type seeds, and accounted for 0.36 to 15.56% of total fatty acids. The level of eicosenoic acid was also greatly decreased. The expression of endogenous *BnFAE1.1* was efficiently silenced by the designed RNAi silencer, causing a significant down-regulation in the level of erucic acid. Therefore, the RNAi-mediated post-transcriptional silencing of *FAE1* gene to reduce oleic acid in rapeseeds was an efficient method to breed some new *Brassica napus* lines.

In Cotton several genes encoding putative *KCSs* were significantly up-regulated during early cotton fiber development. *GhCER6* cDNA containing an open reading frame of 1479 bp, encoding a protein of 492 amino acid residues homologous to the *Arabidopsis* condensing enzyme *CER6*, was isolated and cloned. In situ hybridization results demonstrated that *GhCER6* mRNA was detected only in the elongating wild-type cotton fiber cells.When *GhCER6* was transformed to the *Saccharomyces cerevisiae* elo3 deletion mutation strain, deficient in the production of 26-carbon fatty acids displayed a very slow growth phenotype, the mutant cells were found to divide similarly compared with those of the wild-type cells, heterologous expression of *GhCER6* restored the viability of the *Sacchromyces cerevisiae* haploid elo2 and elo3 double-deletion strain. Matrix-assisted laser desorption/ionization

time-of-flight mass spectrometry analysis showed that *GhCER6* was enzymatically active since the *yeast* elo2 and elo3 double-deletion mutant expressing the cotton gene produced verylong- chain fatty acids that are essential for cell growth suggesting that *GhCER6* encodes a functional *KCS* [60].

In wheat *TaCer6* was firstly cloned by rapid amplification of cDNA ends [RACE] and identified as a tissue-specific gene. To determine if environmental factors such as drought and low temperature induce *TaCer6* transcription, examination of the effects of these factors on *TaCer6* in two wheat cultivars was done, demonstrating that light was essential for *TaCer6* transcription, salt stress inhibited *TaCer6* expression, application of salicylic acid enhanced *TaCer6* transcripts accumulation. Polyethylene glycol [PEG 6000] and abscisic acid increased the expression of *TaCer6* more in the drought and cold tolerant cultivar Jinmai47 than in non-tolerant cultivar Shi4185. Low temperature increased *TaCer6* transcription in Jinmai47 while decreased it in Shi4185 *Cer6* encoding *KCS* catalyzes biosynthesis of VLCFA, which play an important role in holding water from astomatous dissipating, cell elongation, or fertility [32, 36, 43]. In *Arabidopsis Cer6* mutant, wax load was reduced to 6 - 7 % of wild-type levels and most of pollens were sterile [36].In tomato *LeCer6* mutant with leaf and fruit deficient in *n*-alkanes and aldehydes with chain lengths beyond C30, cuticular water loss was about four times larger than in wild tomato [32, 56]. Application of lignoceric acid [C24:0] in ovule culture medium to cotton lintless mutant *GhCer6* could overcome the inhibition of fiber elongation [43, 44]. The isolation and expression of wheat*Cer6*-like *KCS* cDNA, which was named as *TaCer6* [EU159697] [58].

In intact tomato [*Lycopersicon esculentum*] fruits are used, due to their astomatous surface, as a novel integrative approach to investigate composition, function and relationship of wax amounts and compositions of tomato were manipulated before measuring unbiased cuticular transpiration. First, successive mechanical and extractive wax removal steps allowed the selective modification of epi cuticular wax layer consisting of very long chain aliphatics and intra cuticular wax layers containing large quantities of triterpenoids. Second, applying reverse genetic techniques, a loss of function mutation with a transposon insertion in a very long chain fatty acid elongase *b- ketoacyl Co A synthase* [11].

Apathogen related tomato line called *dfd* [delayed fruit deterioration] described as a cutin-overexpressing cultivar, shown that it displays a higher level of water retention than the control cultivar, 'Ailsa Craig'. This effect is possibly due to the altered composition and greater amount of cuticular components in dfd tomatoes [47].

In apple [*Malus domestica* Borkh.] wax production is an important aspect of fruit development. To develop a fresh, shiny apple the analysis of expression levels of genes based on gene expression data from *Arabidopsis thaliana*, some genes in apple were assigned for analysis, EST sequences of apple used to design specific primers for RT-PCR experiments, by isolating intact RNA from different apple tissues and performing RT-PCR reaction in obtaining expression patterns using actin primer for the detection of intron containing genomic DNA, and tubulin primers as internal controls in RT-PCR experiments found tissue specific differences between the expression patterns of candidate genes, showing that *KCS1* and *KCS4* are overexpressed in the skin tissue, having skin-specific expression in apple fruit that are potentially involved in wax-biosynthesis [1].

ABA regulates the expression of many genes, including those encoding oil-body proteins in microspore-derived and zygotic embryos of *Brassica napus* [16, 17, 55, 57] . MYB96 transcription factor, the abscisic acid [ABA]-inducible plays a role in drought resistance, also regulates cuticular wax biosynthesis by binding directly to the promoters of genes encoding fatty acid elongating enzymes, like *KCS1, KCS2, KCS6* and *KCR1* that constitute a rate-limiting step in cuticular wax biosynthesis, downstream events are also activated and cuticular waxes are accumulated to a high level in the leaves and stems of the *myb96-1D* mutant. These observations indicate that the MYB96 transcription factor serves as a molecular web that incorporates drought stress signals to promote fatty acid elongation in Cuticular wax biosynthesis. In *myb96-1D* mutant *KCS, KCR, ECR* and *3-hydroxyacyl Co A dehydratase [PAS2]* are upregulated, *CER3 [ECERIFERUM 3], CYP96A15/MAH1 [CYTOCHROME P450 96 A1]* involved in decarbonylation pathway and *CER4* and Wax *ester synthase/diacylglycerol acyl transferase [WSD1]* functions in acyl reduction pathway. ABC transporter that exports cutin monomers and waxes, are also up regulated in the mutant, indicating that MYB96 regulates both cuticular wax biosynthesis and transport [50].

CONCLUSION

The emphasis to the plant cuticular wax is a reflection of its significant contribution to plant biology. Wax exterior to plant is the result of the metabolic activity of epidermis, its examination has contributed valuable information about the chemical diversity of wax composition within a single species and amongst taxa. The pioneering contributions of Joubes [23], Millar [36, 37], Kunst [28, 29] and others have shown us the basic wax biochemical pathways. The isolation of mutants in *Arabidopsis* and other plant species, combined with biochemical analyses of mutant phenotypes, resulted in identification of gene products involved in wax production, and cloning of a number of wax biosynthetic genes. Not all genes involved in the biochemistry, regulation and transport of wax to the plant surface have been identified by genetic approaches. The challenge now is to take the genomic tools

available to find genes whose functions are essential, redundant, or more subtle, like *KCS1* and investigate their specific contributions to cuticular wax production during the life cycle of the plant, develop transgenic crops for various stress tolerances.

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