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ORIGINAL PAPER

Overexpression of Arabidopsis *MYB96* confers drought resistance in *Camelina sativa* via cuticular wax accumulation

Saet Buyl Lee · Hyojin Kim · Ryeo Jin Kim · Mi Chung Suh

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Abstract
Key message **Camelina has been highlighted as an emerging oilseed crop. Transgenic Camelina plants overexpressing Arabidopsis *MYB96* exhibited drought resistance by activating expression of Camelina wax biosynthetic genes and accumulating wax load.**
Abstract Camelina (*Camelina sativa* L.) is an oilseed crop in the *Brassicaeae* family with potential to expand biofuel production to marginal land. The aerial portion of all land plants is covered with cuticular wax to protect them from desiccation. In this study, the Arabidopsis *MYB96* gene was overexpressed in Camelina under the control of the CaMV35S promoter. Transgenic Camelina plants overexpressing Arabidopsis *MYB96* exhibited normal growth and development and enhanced tolerance to drought. Deposition of epicuticular wax crystals and total wax loads increased significantly on the surfaces of transgenic leaves compared with that of non-transgenic plants. The levels of alkanes and primary alcohols prominently increased in transgenic Camelina plants relative to non-transgenic plants. Cuticular transpiration occurred more slowly in transgenic leaves than that in non-transgenic plants. Genome-wide identification of Camelina wax biosynthetic genes enabled us to determine that the expression levels of *CsKCS2, CsKCS6, CsKCR1-1, CsKCR1-2, CsECR*, and *CsMAH1* were approximately two to seven-fold higher in transgenic Camelina leaves than those in non-transgenic leaves. These results indicate that MYB96-mediated transcriptional regulation of wax biosynthetic genes is an approach applicable to generating drought-resistant transgenic crops. Transgenic Camelina plants with enhanced drought tolerance could be cultivated on marginal land to produce renewable biofuels and biomaterials.

Keywords *Camelina sativa* · Cuticular wax · Drought resistance · MYB96 · Transcription factor · Transformation

Abbreviations

VLCFAs	Very long-chain fatty acids
KCS	β-Ketoacyl-CoA synthase
KCR	β-Ketoacyl-CoA reductase
HCD	β-Hydroxyacyl-CoA dehydratase
ECR	Enoyl-CoA reductase
CER	Eceriferum
MAH1	Mid-chain alkane hydroxylase1
FAR3/CER4	Fatty acyl-CoA reductase
WSD1	Bifunctional wax synthase/acyl-CoA:diacylglycerol acyltransferase
GL1	Glossy1
WSL1	Wax crystal sparse leaf1
WDA1	Wax-deficient anther1
DWA1	Drought-induced wax accumulation
LACS	Long-chain acyl-CoA synthetase
TF	Transcription factors
SEM	Scanning electron microscopy
GC	Gas chromatography
RT-PCR	Reverse transcription-polymerase chain reaction
RB	Right border

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S. B. Lee · H. Kim · R. J. Kim · M. C. Suh (✉)
Department of Bioenergy Science and Technology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Korea
e-mail: mcsuh@chonnam.ac.kr

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E9-T	Pea rbcs-E9 terminator
BAR	Bialaphos-resistance gene
NOS-P	Nopaline synthase gene promoter
35S-P	Cauliflower mosaic virus 35S promoter
NOS-T	Nopaline synthase gene terminator
LB	Left border
NT	Non-transgenic plants

Introduction

Camelina [*Camelina sativa* (L.) Crantz], known as false flax or gold of pleasure, is an oilseed crop that belongs to the *Brassicaceae* (Cruciferous plants). Camelina seeds contain 30–40 % oil by dry weight and unsaturated fatty acids include approximately 90 % of the total seed oils; 35–40 % α-linolenic acid (18:3n-3), 20–25 % linoleic acid (18:2n-6), 15 % eicosanoic acid (20:1n-9), and 3 % erucic acid (22:1n-9) (Zubr 1997; Lu and Kang 2008). Camelina seed oil is useful for industrial applications such as the production of soaps, varnishes, cosmetics as well as food. Camelina has recently emerged as an oilseed crop to produce Camelina biofuel, because it can be grown on marginal land due to properties such as cold-stress tolerance and relatively low fertilizer requirements (Putnam et al. 1993; Zubr 1997). In addition, it has short life span (100–120 days) and can be easily transformed using the Agrobacterium-mediated floral dip method (Zubr 1997; Lu and Kang 2008; Hutcheon et al. 2010; Kang et al. 2011). Most recently Camelina transcriptome and genome data have become available (Nguyen et al. 2013; http://www.camelinagenome.org/; Kagale et al. 2014; http://www.camelinadb.ca/).

The aerial parts of plants are covered with cuticular wax, which is involved in controlling non-stomatal water loss and gas exchange, preventing the attachment of dust and pathogen spores on the leaf surface, and protecting plants from UV irradiation (Kunst and Samuels 2009). To date, cuticular wax biosynthetic pathway has been well investigated in a model plant, *Arabidopsis thaliana*. Cuticular wax consists of very long-chain fatty acids (VLCFAs, ≥20 carbons) and their derivatives such as aldehydes, alkanes, secondary alcohols, ketones, primary alcohols, and wax esters (Kunst and Samuels 2009). Cuticular wax biosynthesis occurs in epidermal cells. The C16–C18 fatty acids produced from plastids are further elongated up to C34 fatty acids by fatty acid elongase complex, which is comprised of four enzymes, β-ketoacyl-CoA synthase (KCS), β-ketoacyl-CoA reductase (KCR), β-hydroxyacyl-CoA dehydratase (HCD), and enoyl-CoA reductase (ECR) located in the endoplasmic reticulum (Haslam and Kunst 2013; Lee and Suh 2013). The elongated VLCFA-CoAs are hydrolyzed to free VLCFAs by an unknown thioesterase and subsequently transformed into various wax components through alkane- and alcohol-forming pathways (Kunst and Samuels 2009; Bernard and Joubès 2013; Lee and Suh 2013; Li-Beisson et al. 2013). In the alkane-forming pathway, VLCFAs are converted into alkanes by CER1, CER3, and cytochrome b5 complexes (Bernard et al. 2012). Mid-chain alkane hydroxylase 1 (MAH1), which belongs to cytochrome P450 family, catalyzes the hydroxylation of alkanes and subsequent oxidation of secondary alcohols for the synthesis of secondary alcohols and ketones, respectively (Greer et al. 2007). In the alcohol-forming pathway, fatty acyl-CoA reductase (FAR3/CER4) and bifunctional wax synthase/acyl-CoA:diacylglycerol acyltransferase (WSD1) are involved in the formation of primary alcohols by the reduction of VLCFAs and in esterification of primary alcohols and acyl-CoAs with 16 carbon atoms, respectively (Rowland et al. 2006; Li et al. 2008). In addition, several genes involved in cuticular wax biosynthesis have been identified from crops such as maize (*Zea mays*), rice (*Oryza sativa*), and tomato (*Lycopersicon esculentum*) by characterization of wax-deficient mutants. Maize glossy 1 (GL1) and GL8 have been identified as homologs of Arabidopsis CER1 and KCR1, respectively (Hansen et al. 1997; Xu et al. 1997). Rice GL1-2, WSL1 (wax crystal sparse leaf 1), GL1-1/WSL2, WDA1 (wax-deficient anther 1), and DWA1 (drought-induced wax accumulation) are known to be Arabidopsis CER1, KCS, CER3, CER1, and LACS (long-chain acyl-CoA synthetase) homologs, respectively (Jung et al. 2006; Yu et al. 2008; Islam et al. 2009; Mao et al. 2012; Zhu and Xiong 2013). Tomato *LeCER6* has been reported to have Arabidopsis KCS activity (Vogg et al. 2004; Leide et al. 2007).

When plants are exposed to drought stress, they exhibit various physiological and biochemical responses such as stomatal closure, reduced transpiration, reduction of photosynthetic efficiency, and synthesis of osmoprotectants and secondary metabolites (Zingaretti et al. 2013). It has been reported that the amount of cuticular wax increases by approximately twofold in drought-treated Arabidopsis and tree tobacco (*Nicotiana glauca*) compared with plants grown in well-watered conditions (Cameron et al. 2006; Kosma et al. 2009). Similarly, total wax loads from cotton (*Gossypium hirsutum*) and sesame (*Sesamum indicum*) leaves increased by approximately 70 and 30 % under water stress conditions, respectively (Bondada et al. 1996; Kim et al. 2007). More recently, it has been reported that overexpression of several genes encoding transcription factors that activate cuticular wax biosynthesis increases drought tolerance in transgenic plants. For examples,

overexpression of *WIN1/SHN1* gene encoding an AP2/EREBP family transcription factor increased total wax amounts by sixfold and confers drought resistance in transgenic Arabidopsis (Aharoni et al. 2004). Elevation of cuticular wax content and resistance to drought were observed in transgenic alfalfa (*Medicago sativa*) and Arabidopsis overexpressing *Medicago truncatula WXP1* and *WXP2* (Zhang et al. 2005, 2007). Overexpression of the *Brassica napus BnLAS* gene encoding a GRAS transcription factor in Arabidopsis increased drought tolerance by up-regulating several genes involved in wax synthesis and regulation (Yang et al. 2011b). Overexpression of abscisic acid- and drought-induced Arabidopsis MYB96 activates the expression of genes involved in wax biosynthesis and increases accumulation of cuticular waxes, which ultimately results in a drought-resistant phenotype in Arabidopsis (Seo et al. 2011). Transgenic rice (*Oryza sativa*) overexpressing *OsWR1* also showed enhanced expression of genes involved in wax biosynthesis, reduced water loss, and enhancement of drought tolerance (Wang et al. 2012).

The MYB transcription factors belong to a large family having the MYB DNA-binding domain containing 52 amino acids (Stracke et al. 2001; Dubos et al. 2010). The MYB domain regulates the levels of transcripts by recognizing the MYB-binding sites, YAACKG or CNGTTR (Urao et al. 1993; Abe et al. 2003). The MYB TF family is classified into the 4R-MYB, 3R-MYB (R1R2R3-MYB), 1R-MYB or MYB-related, and R2R3-MYB classes by the number of repeated MYB domains. In particular, R2R3-MYB TF genes in Arabidopsis are involved in plant developmental processes including epidermal cell, trichome, and stomatal formation as well as various biotic and abiotic stresses including drought (Dubos et al. 2010). To date, five R2R3-MYB TF genes that regulate cuticle biosynthesis have been reported. MYB41 induced by desiccation and salt positively regulates the expression of genes involved in wax biosynthesis (Cominelli et al. 2008). MYB30 regulates the biosynthesis of VLCFAs, which are essential precursors for cuticular waxes and sphingolipids (Raffaele et al. 2008). MYB96 is the positive regulator of wax biosynthesis under drought condition (Seo et al. 2011). More recently, it was reported that MIXTA-like transcription factors MYB106 and MYB16 regulate cuticle development coordinately with WIN1/SHN1, which controls cutin biosynthesis and wax accumulation (Oshima et al. 2013).

In this study, Arabidopsis *MYB96* gene was overexpressed in Camelina under control of the CaMV35S promoter to develop transgenic Camelina plants with enhanced drought resistance. Accumulation of epicuticular crystals in transgenic and non-transgenic Camelina leaves was analyzed (SEM). Cuticular wax amounts and composition from leaves and stems of transgenic and non-transgenic Camelina plants were measured using gas chromatography (GC) and GC-mass spectrophotometry. The cuticular transpiration assay results suggested that drought tolerance in transgenic Camelina plants is closely related to increased levels of cuticular wax. Finally, quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis showed that heterologous overexpression of Arabidopsis *MYB96* activates expression of the Camelina wax biosynthetic genes *CsKCS2, CsKCS6, CsKCR1-1, CsKCR1-2, CsECR*, and *CsMAH1*. Taken together, these results provide the molecular and genetic tools to develop transgenic crops with improved drought tolerance using a transcription factor that controls cuticular wax biosynthesis.

Materials and methods

Plant material and growth condition

Camelina seeds [*Camelina sativa* (L.) Crantz, USDA GRIN seed accession PI 650140, "CAME" source population] were germinated on mixed soil (soil/vermiculite/perlite, 4:2:1, v/v/v). Camelina plants were grown in a culture room under long-day conditions (25 ± 3 °C, 16 h light/8 h dark cycle).

Camelina transformation and selection of Camelina transgenic plants

To introduce the Arabidopsis *MYB96* gene into Camelina plants, the 35S:*96-MYC* vector (Seo et al. 2011) was transformed into *Agrobacterium tumefaciens* (GV3101 strain) by the freeze–thaw method. Then, Camelina transformation was performed using the modified floral dipping method (Lu and Kang 2008; Liu et al. 2012). Agrobacterium including 35S:*96-MYC* was inoculated in YEP medium (containing 50 μg/mL of rifampicin and 25 μg/mL of kanamycin) and cultured at 30 °C overnight with shaking. The cultivated Agrobacterium was centrifuged at 190×*g* for 8 min and then resuspended in transformation solution (5 % sucrose and 0.05 % Silwet L-77) to a final concentration of $OD_{600} = 1.0$. After removing bloomed flowers and siliques from 5- to 6-week grown Camelina plants, the buds were submerged into a transformation solution containing Agrobacterium with 35S:*96-MYC*. Two minutes after submergence, the buds were wrapped in a plastic bag for 1 day to maintain humidity. T_1 transgenic seeds from transgenic Camelina plants were sowed on mixed soil and were treated with BASTA (0.03 % v/v, Bayer Crop Science) after 10 days twice in 2 weeks.

Herbicide-resistant transgenic Camelina plants were selected.

Genomic DNA isolation, RNA isolation, and PCR analysis

Genomic DNA was isolated from 10 transgenic Camelina plants using DNA extraction buffer (200 mM Tris–HCl, pH 7.5, 250 mM NaCl, 25 mM EDTA, and 0.5 % sodium dodecyl sulfate). To confirm successful introduction of the Arabidopsis *MYB96* gene, PCR was performed using the myc-pBA F1 and MYB96-NR1 primers shown in Table 1. Total RNAs were isolated from 3- to 6-week-old Camelina T_2 plants using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). The isolated total RNAs were reverse-transcribed with Gostrip Reverse Transcriptase (Promega, Madison, WI, USA) and used for RT-PCR and quantitative real-time RT-PCR. Quantitative real-time RT-PCR was executed using SYBR® FAST Universal 2× qPCR Master Mix (KAPA Biosystems, Wilmington, MA, USA) in a volume of 20 μL. The gene-specific primer set indicated in Table 1 and the Bio-Rad CFX96 Real-Time PCR system (Bio-Rad, Hercules, CA, USA) were used for quantitative real-time RT-PCR. Based on the fact that Camelina is known to have hexaploid genome (Kagale et al. 2014), the gene-specific primers designed from 3′-untranslated regions or the primers having mismatched sequences at the 3′-termini of primers were used (Kang et al. 2011). Only single products were amplified in each quantitative real-time RT-PCR.

Table 1 Oligonucleotide sequences used in this study

Name	Sequences (5′–3′)
myc-pBA F1	GGCGCGCCTGTACAGACGTCT
MYB96 NF1	CCCCGGGAATGGGAAGACCACCTTGC
MYB96 NR1	GGTCGACCTTTTTGAGCTTCTTCTTC
CsACT_F	ACAATTTCCCGCTCTGCTGTTGTG
CsACT_R	AGGGTTTCTCTCTTCCACATGCCA
CsKCS2-1_F1	GATTCACATCACATATCTTCAATCC
CsKCS2-1_R1	GCCAGTTTCTTTTAAACACAGCG
CsKCS6_F1	CGGAGAAGCCTTGAAGGCTAAC
CsKCS6_R1	CGAAGGCCTGCTTGAAGTCTG
CsKCR1-1_F2	GATCCGGAATAAGTGAACTCAGG
CsKCR1-1_R2	GGATATACAAAGATTCAGGAGCAT
CsKCR1-2_F1	GTGAAACGGATTAAAGAGGCG
CsKCR1-2_R1	ACGCTTCCTCGCTAGCAGAC
CsECR-3_F2	CCCTTGTACACACCGGTCG
CsECR-3_R2	GGTAGCCTCCAGACCCACTG
CsCER1_F1	CTATCATACCACACCGGCC
CsCER1_R1	GGTCCAAATCGGAGGGAAG
CsCER3-1_F1	CAAGTGACCAAATACAATGCCGCA
CsCER3-1_R1	CAAGTTGCGAGTCCTTCACCATC
CsMAH1-1_F1	GTCAACAAGGTTGGCGACTTTAAG
CsMAH1-1_R1	CACTTGTGGATGCTCTGCTTCAC
CsCER4_F2	CTGGTTAAGCATGTCTTCTAATAAGTTC
CsCER4_R2	CATTACAGAGATGCAAATTAATGCTG
CsWSD1-1_F1	GACGACCAGGGATGCAGAGG
CsWSD1-1_R1	GTCATCCGTATCACCTCCGC

Drought stress treatments

Drought stress was applied according to the method of Seo et al. (2009). Briefly, 18-day-old plants grown in mixed soil (approximately 300 g) were withheld from water for 2 weeks and then re-watered, and survival rates were calculated 3 days later.

Stomatal density analysis

Leaves of 5- to 6-week-old Camelina plants were incubated in methanol overnight to remove chlorophyll and subsequently placed in lactic acid. Stomatal densities of adaxial and abaxial leaves were determined under a Leica DM2500 microscope (Leica Microsystems, Wetzlar, Germany).

Cuticular transpiration and chlorophyll leaching assays

Leaves of 5- to 6-week-old Camelina plants grown in soil were used. Non-transgenic and transgenic Camelina plants were acclimated in dark for 5 h for the cuticular transpiration assay. The fourth or fifth leaves were excised from the stem and soaked in water for 1 h in the dark to equilibrate water contents. The leaves were weighted gravimetrically using a microbalance. Dark acclimated leaves (fourth or fifth leaves) were incubated on ice for 30 min and immersed in 30 mL of 80 % ethanol for 24 h for chlorophyll extraction assays. The amounts of extracted chlorophylls were measured at an absorbance of 647 and 664 nm according to method of Lolle et al. (1998). Data are expressed as percentage of the total chlorophyll contents after 24 h.

SEM analysis

Field-emission SEM (FE-SEM) was used to observe cuticular wax crystals. Hand cut segments of 6-week-old leaves from non-transgenic and transgenic Camelina plants overexpressing Arabidopsis *MYB96* were fixed with 1 % osmium tetroxide vapor for 24 h and air dried for 1 day. Segments were mounted on standard aluminum stubs and coated with platinum particles using a Hitachi E1030 sputter coater. The coated samples were viewed with a Hitachi S4700 FE-SEM.

Cuticular wax analysis

Cuticular wax was analyzed using the method described by Seo et al. (2011). Camelina plants were used. Cuticular wax was extracted from 6-week-old Camelina leaves in chloroform for 30 s. *n*-Octacosane, docosanoic acid, and 1-tricosanol were added to the extraction solvent as internal standards (Sigma, St. Louis, MO, USA). Subsequently, the solvent was evaporated under nitrogen and dissolved in 100 μL of pyridine with 100 μL of bis-*N*,*N*-(trimethylsilyl)trifluoroacetamide (Sigma). The wax mixtures were heated at 90 °C for 30 min to convert to trimethylsilyl derivatives and re-dried under nitrogen. The dried wax mixtures were dissolved in heptane/toluene (1:1) for qualitative and quantitative composition analyses using GC–MS (GCMS-QP2010, Shimazu, Tokyo, Japan; column 60-m HP-5, 0.32-mm i.d., $df = 0.25$ mm, Agilent Technologies, Palo Alto, CA, USA) and GC. The analyte was injected at 220 °C, and the temperature was held for 4.5 min. The temperature was increased to 290 °C at 3 °C/min, maintained for 10 min, raised to 300 °C at 2 °C/min, and then maintained for 15 min at 300 °C. Data were statistically analyzed using Student's *t* test ($*P < 0.05$).

Accession numbers

Sequence data from this article can be found in the EMBL/GenBank data libraries under accession numbers *CsKCS2* (KJ461880), *CsKCS6* (KJ461881), *CsKCR1-1* (KJ461882), *CsKCR1-2* (KJ461883), *CsECR* (KJ461884), *CsCER1* (KJ461885), *CsCER3* (KJ461886), *CsMAH1* (KJ461887), *CsCER4* (KJ461888), and *CsWSD1* (KJ461889).

Results

Expression of Arabidopsis *MYB96* in Camelina

The binary vector 35S:96-MYC was constructed under the control of CaMV35S promoter to overexpress Arabidopsis *MYB96* in Camelina (Seo et al. 2011). It was introduced into Camelina plants using the *Agrobacterium*-mediated floral dip method (Lu and Kang 2008; Liu et al. 2012) (Fig. 1a). The harvested T_1 transgenic seeds were sown on soil, 10-day-old seedlings were sprayed using 0.03 % (v/v) BASTA solution (Bayer Crop Science, Seoul, Korea) twice in 2 weeks, and herbicide-resistant Camelina plants were selected (Fig. 1b). Stable integration of the Arabidopsis *MBY96* gene was confirmed from 10 T_2 independent transgenic plants by genomic DNA PCR analysis (Supplementary Fig. 1a). Quantitative real-time RT-PCR analysis showed that expression of Arabidopsis *MYB96* was approximately threefold to 33-fold higher in transgenic Camelina plants than that in non-transgenic Camelina plants (Fig. 1c, Supplementary Fig. 1b). Under normal growth conditions (16 h light/8 h/dark, 25 ± 3 °C), no growth retardation or abnormal development was observed in transgenic Camelina plants overexpressing Arabidopsis *MYB96* compared with that in non-transgenic Camelina plants (Supplementary Fig. 2).

Transgenic Camelina plants overexpressing Arabidopsis *MYB96* confer increased resistance to drought

In a previous report (Seo et al. 2009), both an Arabidopsis *MYB96* activation tagging line (*myb96-ox*) and overexpressing line (*35S:MYB96*) exhibited drought-stress resistance. To examine whether the transgenic Camelina plants overexpressing Arabidopsis *MYB96* are drought-tolerant, non-transgenic and three independent transgenic Camelina lines (T_3 generation) overexpressing Arabidopsis *MYB96* were exposed to a drought stress condition. As shown in Fig. 2, average survival rates were 42.6 % (±5 %, standard error) in non-transgenic Camelina plants and 84.8, 85.4, and 83.2 % (±5, 3, and 9 %, standard error) in *MYB96* OX-5, *MYB96* OX-6, and *MYB96* OX-7 transgenic Camelina plants overexpressing Arabidopsis *MYB96*, respectively.

Subsequently, the cuticular transpiration rate was measured after stomata were almost completely closed by dark treatment for 5 h. Water loss occurred more rapidly in non-transgenic Camelina leaves, but more slowly in transgenic Camelina leaves (Fig. 3a). Chlorophylls were extracted more slowly from transgenic Camelina leaves overexpressing Arabidopsis *MYB96* compared with those in non-transgenic leaves (Fig. 3b). These results indicate that overexpressing Arabidopsis MYB96 confers drought resistance to Camelina plants by altering cuticle permeability.

An increase of cuticular wax deposition in leaves of transgenic Camelina plant overexpressing Arabidopsis *MYB96*

In a previous report, Arabidopsis MYB96 acted an activator of cuticular wax biosynthesis. Increased cuticular wax load was observed in activation-tagged *myb96-1D* mutants overexpressing Arabidopsis *MYB96* (Seo et al. 2011). To examine whether heterologous expression of Arabidopsis *MYB96* in Camelina plays a similar role regulating cuticular wax biosynthesis, we investigated deposition of cuticular wax crystal on the surfaces of leaves in transgenic Camelina plants overexpressing Arabidopsis *MYB96* using the SEM. Cuticular wax crystals were clearly observed on the surfaces of leaves in transgenic Camelina

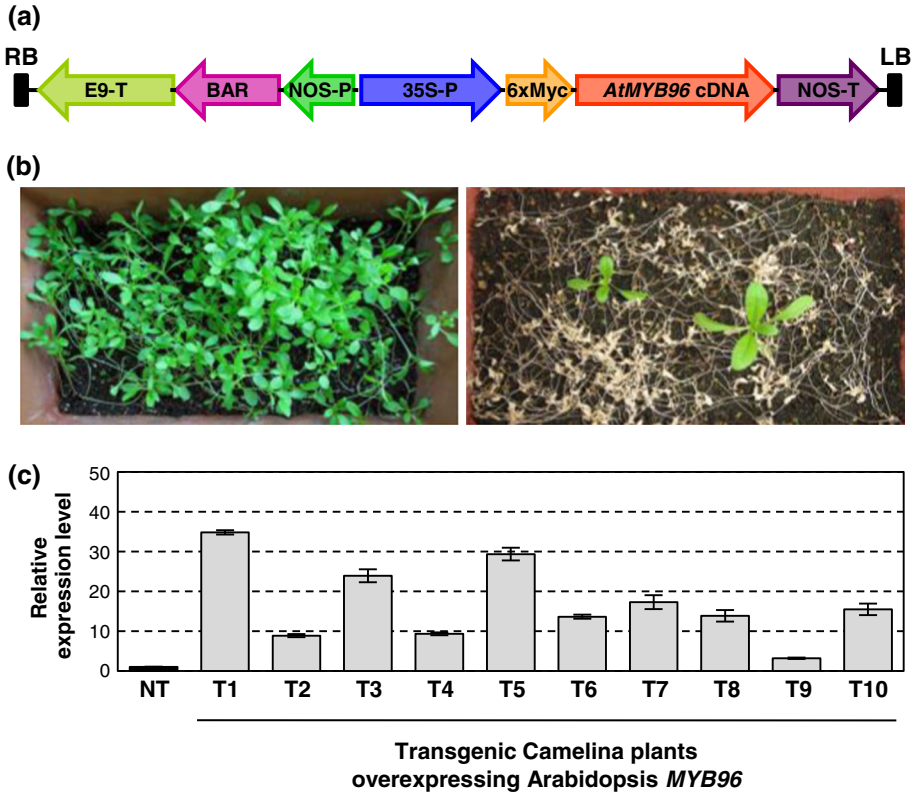

Fig. 1 Generation of transgenic Camelina plants overexpressing Arabidopsis *MYB96*. **a** Schematic diagram of the 35S:*96-MYC* binary vector construct containing the *BAR* gene used for plant transformation. *RB* right border, *E9-T* pea rbcs-E9 terminator, *BAR* bialaphos-resistance gene, *NOS-P* nopaline synthase gene (NOS) promoter, *35S-P* cauliflower mosaic virus 35S promoter, *NOS-T* NOS terminator, *LB* left border. **b** Selection of transgenic Camelina plants overexpressing Arabidopsis *MYB96*. The 10-day-old plants on the left were sprayed with a 0.03 % (v/v) Basta solution. Basta was applied twice for 2 weeks until all leaf surfaces were wet. Herbicide-resistant transgenic Camelina plants on the right were selected 2 weeks after the Basta application. **c** Expression level of Arabidopsis *MYB96* transcripts in transgenic Camelina plants (T_2) overexpressing Arabidopsis *MYB96*. Total RNA was extracted from 3-week-old plants. The isolated RNAs were subjected to quantitative real-time RT-PCR. *CsACT* was used to determine the quantity and quality of the cDNAs (Hutcheon et al. 2010). Each value is the mean of triplicate experiments. *Bars* indicate standard errors. *NT* non-transgenic plants

plants overexpressing Arabidopsis *MYB96*, unlike non-transgenic plants (Fig. 4).

Based on the SEM analysis results, the composition and amount of cuticular waxes were analyzed in the leaves of non-transgenic and transgenic Camelina plants overexpressing Arabidopsis *MYB96* by gas chromatography/mass spectrometry (GC/MS) and GC. The alkanes (about 30 %) and primary alcohols (about 58 %) were predominantly present in cuticular wax of Camelina leaves. Minor fatty acid content (about 12 %) was also detected (Fig. 5a). Total wax loads increased by 22–61 % in transgenic Camelina plants overexpressing Arabidopsis *MYB96*, compared with those in non-transgenic plants (Fig. 5a). The levels of almost all cuticular wax components were increased in transgenic Camelina plants overexpressing Arabidopsis *MYB96*, compared with those in non-transgenic plants, and increased levels of alkanes and primary alcohols were prominent (Fig. 5b). These results indicate that heterologous expression of Arabidopsis *MYB96* in Camelina plays a role as a positive regulator of cuticular wax accumulation. Thus, increased cuticular wax loads confer drought resistance in transgenic Camelina plants overexpressing Arabidopsis *MYB96*.

Transcript levels of Camelina cuticular wax biosynthetic genes were up-regulated in transgenic Camelina plants

Arabidopsis *MYB96* activates the expression of genes involved in the cuticular wax biosynthesis (Seo et al. 2011). To determine whether heterologous expression of Arabidopsis *MYB96* affects the expression of cuticular wax biosynthetic genes in transgenic Camelina, the transcript levels of putative Camelina wax biosynthetic genes were examined. First, the putative wax biosynthetic genes were retrieved from a Camelina genome sequencing database through BLAST searches. The BLAST search was conducted using genomic DNA or amino acid sequences

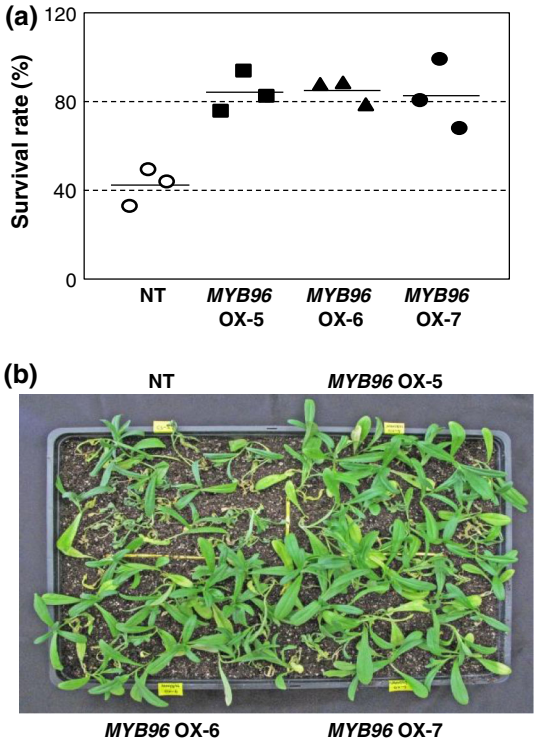

Fig. 2 Transgenic Camelina plants overexpressing Arabidopsis *MYB96* exhibit increased drought resistance. Eighteen-day-old plants were exposed to dehydration by withholding water for 2 weeks. Dehydrated plants were re-watered, and survival rates were calculated. Survival rates from individual transgenic lines (T_3) and non-transgenic plants (NT) are shown in **a**. Each point indicates the mean of an individual experiment. The mean of three experiments is marked by *black lines*. **b** Recovered plants after re-watering are presented in the image (NT, *MYB96* OX-5, *MYB96* OX-6, and *MYB96* OX-7)

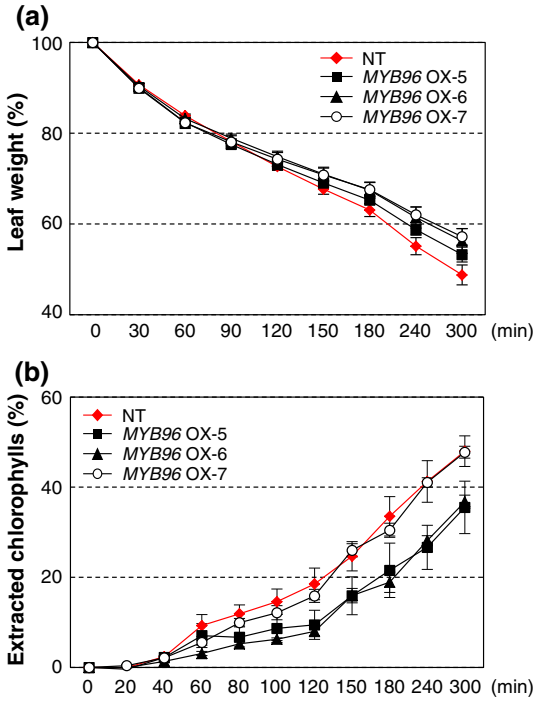

Fig. 3 Cuticle permeability in leaves of transgenic Camelina plants (T_3) overexpressing Arabidopsis *MYB96*. **a** Water loss assays. Five- to six-week-old plants were acclimated to the dark for 5 h. The fourth or fifth leave was excised from the stem and soaked in water for 1 h in the dark to equilibrate water contents. The leaves were weighed gravimetrically using a microbalance. **b** Chlorophyll extraction assays. *Dark* acclimated leaves were immersed in 80 % ethanol for 24 h. Data are expressed as the percentage of total chlorophyll contents after 24 h. *Bars* indicate standard errors. Each value is the mean of three independent measurements

obtained from the Arabidopsis database (http://www.arabidopsis.org/). Consequently, nine genes, which might be involved in cuticular wax biosynthesis in Camelina, were isolated and named *CsKCS2, CsKCS6, CsKCR1, CsECR, CsCER1, CsCER3, CsMAH1, CsCER4,* and *CsWSD1* (Table 2).

To measure the transcript levels of cuticular wax biosynthetic genes in transgenic Camelina overexpressing Arabidopsis *MYB96*, gene-specific primers were designed as described previously (Kang et al. 2011, Table 1). Total RNA was isolated from non-transgenic and transgenic Camelina plants, and quantitative real-time RT-PCR reactions were conducted with a gene-specific primer set. As shown in Fig. 6, the transcript levels of *CsKCS2, CsKCS6, CsKCR1-1, CsKCR1-2, CsECR*, and *CsMAH1* were upregulated by approximately 2.2-, 2.5-, 1.9-, 6.7-, 2.6-, and 3.7-fold in transgenic Camelina plants relative to that in non-transgenic plants, respectively. However, no significant changes in the transcript levels of *CsCER1, CsCER3, CsCER4,* or *CsWSD1* were observed in

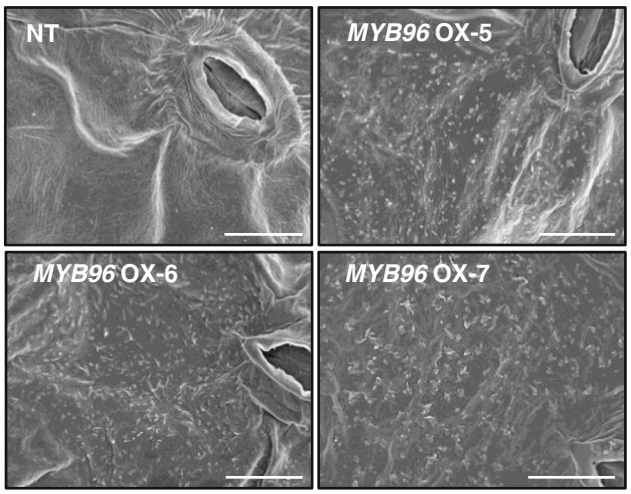

Fig. 4 Cuticular wax crystals increased on the surfaces of leaves in transgenic Camelina plants overexpressing Arabidopsis *MYB96*. Cuticular wax crystals were observed on the leaf surfaces of 6-week-old non-transgenic plants and transgenic Camelina plants (T_3) overexpressing Arabidopsis *MYB96* by scanning electron microscopy (SEM). *Bar* = 10 μm

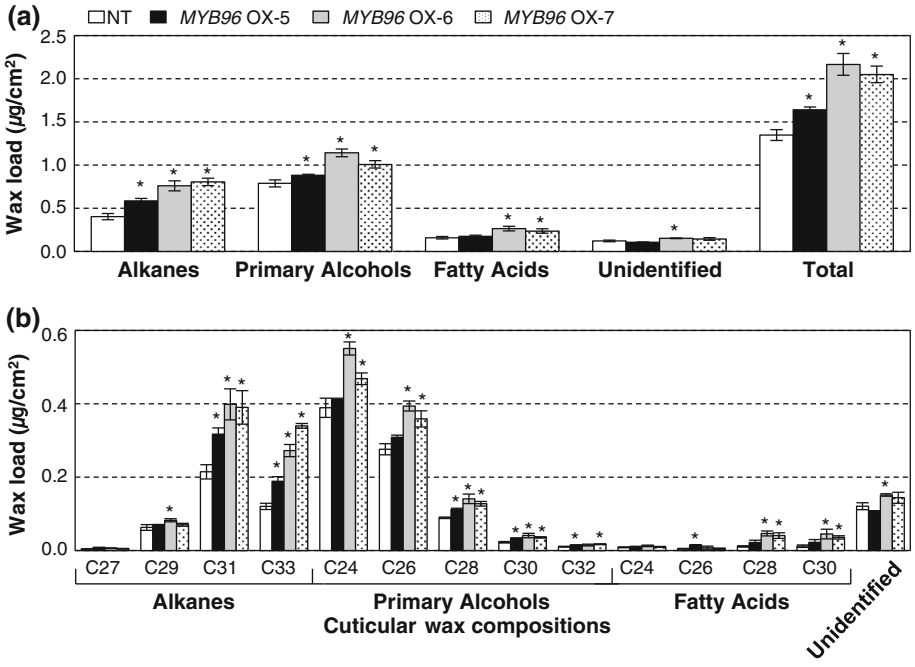

Fig. 5 Cuticular wax deposition was altered in leaves of transgenic Camelina plant overexpressing Arabidopsis *MYB96*. **a**, **b** The amount and composition of cuticular wax in non-transgenic plants and transgenic Camelina plants (T_3) overexpressing Arabidopsis *MYB96*. The 14th–18th leaves from the bottom part of 6-week-old Camelina stems were used for cuticular wax analysis. Each value is the mean of three independent measurements ± standard error. Data were statistically analyzed using Student's *t* test. *Asterisks* denote statistical differences with respect to the non-transgenic plants (*$P < 0.05$)

Table 2 Putative wax biosynthetic genes in *Camelina sativa*

Arabidopsis thaliana gene ID	Protein	Length (a.a)	GenBank accession number	Protein	Length (a.a)	Identity (%)
AT1G04220	KCS2	528	KJ461880	CsKCS2	510	88
AT1G68530	KCS6	497	KJ461881	CsKCS6	492	87
AT1G67730	KCR1	318	KJ461882	CsKCR1-1	337	94
			KJ461883	CsKCR1-2	318	89
AT3G55360	ECR	310	KJ461884	CsECR	348	97
AT1G02205	CER1	630	KJ461885	CsCER1	625	92
AT5G57800	CER3	632	KJ461886	CsCER3	632	93
AT1G57750	MAH1	497	KJ461887	CsMAH1	521	70
AT4G33790	CER4	493	KJ461888	CsCER4	468	82
AT5G37300	WSD1	481	KJ461889	CsWSD1	586	81

transgenic Camelina compared with those in non-transgenic plants. This result revealed that heterologous overexpression of Arabidopsis *MYB96* activated the expression of Camelina wax biosynthetic genes that consequently caused an increase of cuticular wax biosynthesis and accumulation in transgenic Camelina plants.

Discussion

Because drought stress causes a significant reduction in crop productivity, both conventional breeding and genetic engineering approaches have been performed to improve crop drought tolerance (Mitra 2001; Ashraf 2010). Conventional approaches, which are conducted through crossing, are not only time-consuming and labor-intensive but also transfer undesirable traits (Ashraf 2010). To overcome these limitations, genes involved in plant responses to drought resistance have been introduced into the major grain crops such as maize (*Zea mays*), soybean (*Glycine max*), rice (*Oryza sativa*) (De Ronde et al. 2004; Quan et al. 2004; Nelson et al. 2007; Oh et al. 2009; Zhang et al. 2010; Jeong et al. 2013; Li et al. 2013), and oilseed canola (Wang et al. 2005) in either a sense or an antisense orientation. In this study, we generated transgenic plants of Camelina, a potential oilseed crop with increased drought resistance. Overexpression of the Arabidopsis *MYB96* gene up-regulated the Camelina wax biosynthetic genes *CsKCS2, CsKCS6, CsKCR1-1, CsKCR1-2, CsECR*, and *CsMAH1* and increased total wax loads by approximately

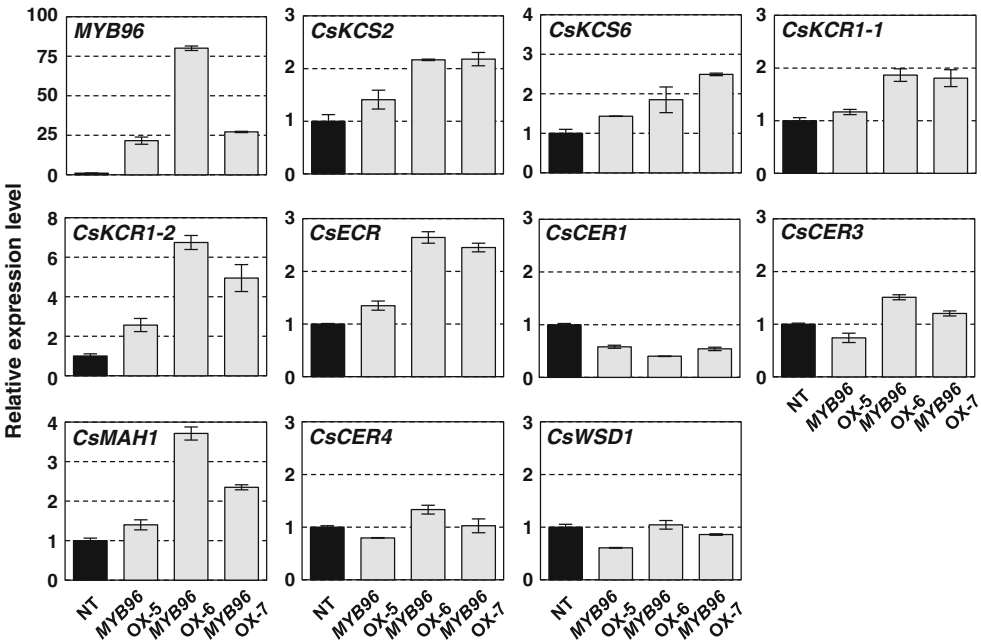

Fig. 6 Effects of Arabidopsis *MYB96* on the expression of putative wax biosynthetic genes in Camelina. Total RNAs were extracted from 5- to 6-week-old leaves. The expression levels of wax biosynthetic genes were examined by quantitative real-time RT-PCR. The measurement values in non-transgenic plants were set to 1 after the values in non-transgenic and transgenic (T_3) plants were normalized using the *CsACT* (Hutcheon et al. 2010). Each value is the mean of three independent measurements ± standard error

50 % in transgenic leaves relative to non-transgenic plants, and finally conferred drought tolerance in transgenic Camelina.

It is clear that increasing cuticular wax loads is closely related to plant responses to drought. Under water stress conditions, cuticular wax content of oat (*Avena sativa L.*) varieties increases by about 60 % and cuticular transpiration rate decreases by about 40 % (Bengtson et al. 1978). In alfalfa and crested wheatgrass (*Agropyron desertorum*), cuticular wax content significantly increases under water-deficit conditions (Jefferson et al. 1989). Similar observations have been reported for cotton (*Gossypium hirsutum*) and sesame (*Sesamum indicum*) (Bondada et al. 1996; Kim et al. 2007). In addition, transgenic alfalfa and rice plants overexpressing the *M. truncatula WXP1* and *OsWR1* transcription factors, respectively, show drought tolerance via increased wax accumulation (Zhang et al. 2005; Wang et al. 2012). In this study, we also observed that increase of total wax content conferred drought tolerance in transgenic Camelina plants. Interestingly, no significant alterations in growth or development of transgenic Camelina plants were observed, in contrast to severe growth retardation that was observed in Arabidopsis and alfalfa plants overexpressing *MYB96, WIN1/SHN1, XYP1, BnLAS,* or *CER1* genes (Broun et al. 2004; Zhang et al. 2005; Bourdenx et al. 2011; Seo et al. 2011; Yang et al. 2011b).

In a previous report (Yang et al. 2011b), heterologous expression of *BnLAS* transcription factor in Arabidopsis resulted in increased expression of Arabidopsis *CER1*, *CER2*, *KCS1*, and *KCS2* genes by twofold to 16-fold. Similarly, we also found that the expression of Camelina wax biosynthetic genes *CsKCS2, CsKCS6, CsKCR1-1, CsKCR1-2, CsECR*, and *CsMAH1* was up-regulated in transgenic Camelina plants overexpressing the Arabidopsis *MYB96* gene, although differences in induced expression levels of each gene were observed between transgenic Arabidopsis and Camelina plants. This result was supported by the presence of MYB-binding *cis*-acting elements (Supplementary Table 1) from searches of cis-acting regulatory DNA elements in the 5′-upstream regions of those Camelina genes (http://www.dna.affrc.go.jp/htdocs/PLACE/). These observations suggest that transcriptional regulatory mechanisms of cuticular wax biosynthesis might be conserved in plants belonging to the *Brassicaceae* family.

The cuticular layer consisting of cutin and wax is closely related to stomata development and structure (Chen et al. 2011). Wax-deficient *cer1* and *cer6* mutants show an increase in stomatal index by 43.2 and 30.5 % compared with those in wild type, respectively (Gray et al. 2000), but the *wax2* mutant exhibits a decrease in stomatal index by 17 % relative to that in the wild type (Chen et al. 2003). Reducing cutin monomer content causes impaired development of cuticular ledges that lie between adjacent guard cells (Li et al. 2007). Therefore, to investigate whether overexpression of Arabidopsis MYB96 also affects stomatal development, stomatal density was measured in abaxial and adaxial leaf surfaces of non-transgenic and transgenic Camelina plants overexpressing the Arabidopsis *MYB96* gene. As shown in Supplementary Fig. 3, no significant differences in stomatal density were observed in non-transgenic and transgenic Camelina leaf surface, suggesting that drought tolerance of transgenic Camelina overexpressing the Arabidopsis *MYB96* gene is mainly

caused by reduced cuticular transpiration through enhanced wax content. In addition, stomatal density in Arabidopsis leaves overexpressing the WIN1/SHN1 transcription factor might decrease in response to repression of the *SPCH*, *MUTE*, and *FAMA* genes that function as positive regulators of stomatal development (Yang et al. 2011a). However, the expression of genes involved in either stomatal development or cutin biosynthesis was not altered in a *myb96-1D* microarray analysis (Seo et al. 2011).

Camelina transgenic plants have been developed using the *Agrobacterium*-mediated vacuum infiltration method (Lu and Kang 2008; Liu et al. 2012). When a fluorescent protein (DsRed) gene as a visual selection marker was transformed into the Camelina cultivar 'Celine' with the use of vacuum, transformation efficiency was estimated to be >1 %, which was similar with that of Arabidopsis (Clough and Bent 1998; Zhang et al. 2006). When the *C. sativa* cultivars, Ames 26665, Calena A3U7761, Ames 1043, and Celine were tested without the vacuum infiltration step, the efficiency of transformation was 0.08–0.83 %. In this study, we obtained transgenic *C. sativa* Crantz plants with transformation efficiencies up to 0.5 % under a no vacuum infiltration condition. In addition, it was confirmed that *A. tumefaciens* strain GV3101 can be broadly used for transforming various Camelina cultivars in this study and in Liu et al. (2012).

Productivity of the vegetable-oil-producing plants Camelina, canola (*Brassica napus*), Indian mustard (*B. juncea*), sesame (*Sesamum indicum*), and soybean (*Glycine max*) decreases by approximately 20, 70, 46, 37, and 40 % under drought stress conditions, respectively (Wright et al. 1995; Specht et al. 1999; Kim et al. 2007; Enjalbert et al. 2013). In this study, we provide a genetic engineering approach for developing transgenic Camelina plants with enhanced drought tolerance. The transgenic Camelina plants can be cultivated on non-agricultural or marginal land to avoid competition with crops, and the oils extracted from transgenic Camelina seeds can be used as biofuel for aircraft and industrial feedstock (Dyer et al. 2008). In addition, transgenic Camelina plants overexpressing Arabidopsis MYB96 exhibit an increase in the levels of alkanes and primary alcohols by approximately twofold and 1.5-fold, which can be used for producing lubricants, adhesives, coatings, sealants, impregnation materials, candles, and cosmetics (Jetter and Kunst 2008).

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Conflict of interest The authors declare that they have no conflict of interest.

References

Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell 15:63–78

Aharoni A, Dixit S, Jetter R, Thoenes E, van Arkel G, Pereira A (2004) The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in Arabidopsis. Plant Cell 16:2463–2480

Ashraf M (2010) Inducing drought tolerance in plants: recent advances. Biotechnol Adv 28:169–183

Bengtson C, Larsson S, Liljenberg C (1978) Effects of water stress on cuticular transpiration rate and amount and composition of epicuticular wax in seedlings of six oat varieties. Physiol Plant 44:319–324

Bernard A, Joubès J (2013) Arabidopsis cuticular waxes: advances in synthesis, export and regulation. Prog Lipid Res 52:110–129

Bernard A, Domergue F, Pascal S, Jetter R, Renne C, Faure JD, Haslam RP, Napier JA, Lessire R, Joubès J (2012) Reconstitution of plant alkane biosynthesis in yeast demonstrates that Arabidopsis ECERIFERUM1 and ECERIFERUM3 are core components of a very-longchain-alkane synthesis complex. Plant Cell 24:3106–3118

Bondada BR, Oosterhuis DM, Murphy JB, Kim KS (1996) Effect of water stress on the epicuticular wax composition and ultrastructure of cotton (*Gossypium hirsutum* L.) leaf, bract, and boll. Environ Exp Bot 36:61–67

Bourdenx B, Bernard A, Domergue F, Pascal S, Léger A, Roby D, Pervent M, Vile D, Haslam RP, Napier JA, Lessire R, Joubès J (2011) Overexpression of Arabidopsis ECERIFERUM1 promotes wax very-long-chain alkane biosynthesis and influences plant response to biotic and abiotic stresses. Plant Physiol 156:29–45

Broun P, Poindexter P, Osborne E, Jiang C-Z, Riechmann JL (2004) WIN1, a transcriptional activator of epidermal wax accumulation in Arabidopsis. Proc Natl Acad Sci USA 101:4706–4711

Cameron KD, Teece MA, Smart LB (2006) Increased accumulation of cuticular wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco. Plant Physiol 140:176–183

Chen X, Goodwin SM, Boroff VL, Liu X, Jenks MA (2003) Cloning and characterization of the WAX2 gene of Arabidopsis involved in cuticle membrane and wax production. Plant Cell 15:1170–1185

Chen X, Truksa M, Snyder CL, El-Mezawy A, Shah S, Weselake RJ (2011) Three homologous genes encoding sn-glycerol-3-phosphate acyltransferase 4 exhibit different expression patterns and functional divergence in *Brassica napus*. Plant Physiol 155:851–865

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735–743

Cominelli E, Sala T, Calvi D, Gusmaroli G, Tonelli C (2008) Overexpression of the Arabidopsis AtMYB41 gene alters cell expansion and leaf surface permeability. Plant J 53:53–64

De Ronde JA, Cress WA, Kruger GHJ, Strasser RJ, Van Staden J (2004) Photosynthetic response of transgenic soybean plants, containing an Arabidopsis P5CR gene during heat and drought stress. J Plant Physiol 161:1211–1224

Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C, Lepiniec L (2010) MYB transcription factors in Arabidopsis. Trends Plant Sci 15:573–581

Dyer J, Stymne S, Green A, Carlsson A (2008) High value oils from plants. Plant J 54:640–654

Enjalbert J-N, Zheng S, Johnson JJ, Mullen JL, Byrne PF, McKay JK (2013) Brassicaceae germplasm diversity for agronomic and seed quality traits under drought stress. Ind Crops Prod 47:176–185

Gray JE, Holroyd GH, van der Lee FM, Bahrami AR, Sijmons PC, Woodward FI, Schuch W, Hetherington AM (2000) The HIC signaling pathway links CO2 perception to stomatal development. Nature 408:713–716

Greer S, Wen M, Bird D, Wu X, Samuels L, Kunst L, Jetter R (2007) 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 Physiol 145:653–667

Hansen JD, Pyee J, Xia Y, Wen TJ, Robertson DS, Kolattukudy PE, Nikolau BJ, Schnable PS (1997) The glossy1 locus of maize and an epidermis-specific cDNA from *Kleinia odora* define a class of receptor-like proteins required for the normal accumulation of cuticular waxes. Plant Physiol 113:1091–1100

Haslam TM, Kunst L (2013) Extending the story of very-long-chain fatty acid elongation. Plant Sci 210:93–107

Hutcheon C, Ditt RF, Beilstein M, Comai L, Schroeder J, Goldstein E, Shewmaker CK, Nguyen T, De Rocher J, Kiser J (2010) Polyploid genome of *Camelina sativa* revealed by isolation of fatty acid synthesis genes. BMC Plant Biol 10:233–247

Islam MA, Du H, Ning J, Ye H, Xiong L (2009) Characterization of Glossy1-homologous genes in rice involved in leaf wax accumulation and drought resistance. Plant Mol Biol 70:443–456

Jefferson PG, Johnson DA, Rumbaugh MD, Asay KH (1989) Water stress and genotypic effects on epicuticular wax production of alfalfa and crested wheatgrass in relation to yield and excised leaf water loss rate. Can J Plant Sci 69:481–490

Jeong JS, Kim YS, Redillas MC, Jang G, Jung H, Bang SW, Choi YD, Ha SH, Reuzeau C, Kim JK (2013) OsNAC5 overexpression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. Plant Biotechnol J 11:101–114

Jetter R, Kunst L (2008) Plant surface lipid biosynthetic pathways and their utility for metabolic engineering of waxes and hydrocarbon biofuels. Plant J 54:670–683

Jung KH, Han MJ, Lee DY, Lee YS, Schreiber L, Franke R, Faust A, Yephremov A, Saedler H, Kim YW, Hwang I, An G (2006) Wax-deficient anther1 is involved in cuticle and wax production in rice anther walls and is required for pollen development. Plant Cell 18:3015–3032

Kagale S, Koh C, Nixon J, Bollina V, Clarke WE, Tuteja R, Spillane C, Robinson SJ, Links MG, Clarke C, Higgins EE, Huebert T, Sharpe AG, Parkin IAP (2014) The emerging biofuel crop Camelina sativa retains a highly undifferentiated hexaploid genome structure. Nat Commun 5:3706. doi:10.1038/ncomms4706

Kang J, Snapp AR, Lu C (2011) Identification of three genes encoding microsomal oleate desaturases (FAD2) from the oilseed crop *Camelina sativa*. Plant Physiol Biochem 49:223–229

Kim KS, Park SH, Jenks MA (2007) Changes in leaf cuticular waxes of sesame (*Sesamum indicum* L.) plants exposed to water deficit. J Plant Physiol 164:1134–1143

Kosma DK, Bourdenx B, Bernard A, Parsons EP, Lü S, Joubès J, Jenks MA (2009) The impact of water deficiency on leaf cuticle lipids of Arabidopsis. Plant Physiol 151:1918–1929

Kunst L, Samuels L (2009) Plant cuticles shine: advances in wax biosynthesis and export. Curr Opin Plant Biol 12:721–727

Lee SB, Suh MC (2013) Recent advances in cuticular wax biosynthesis and its regulation in Arabidopsis. Mol Plant 6:246–249

Leide J, Hildebrandt U, Reussing K, Riederer M, Vogg G (2007) The developmental pattern of tomato fruit wax accumulation and its impact on cuticular transpiration barrier properties: effects of a deficiency in a β-ketoacyl-coenzyme A synthase (LeCER6). Plant Physiol 144:1667–1679

Li Y, Beisson F, Koo AJ, Molina I, Pollard M, Ohlrogge J (2007) Identification of acyltransferases required for cutin biosynthesis and production of cutin with suberin-like monomers. Proc Natl Acad Sci USA 104:18339–18344

Li F, Wu X, Lam P, Bird D, Zheng H, Samuels L, Jetter R, Kunst L (2008) Identification of the wax ester synthase/acyl-coenzyme A: diacylglycerol acyltransferase WSD1 required for stem wax ester biosynthesis in Arabidopsis. Plant Physiol 148:97–107

Li Y, Zhang J, Zhang J, Hao L, Hua J, Duan L, Zhang M, Li Z (2013) Expression of an Arabidopsis molybdenum cofactor sulphurase gene in soybean enhances drought tolerance and increases yield under field conditions. Plant Biotechnol J 11:747–758

Li-Beisson Y, Shorrosh B, Beisson F, Andersson M, Arondel V, Bates P, Baud S, Bird D, DeBono A, Durrett T, Franke R, Graham I, Katayama K, Kelly A, Larson T, Markham J, Miquel M, Molina I, Nishida I, Rowland O, Samuels L, Schmid K, Wada H, Welti R, Xu C, Zallot R, Ohlrogge J (2013) Acyl-lipid metabolism. Arabidopsis Book 11:e0161. doi:10.1199/tab.0161

Liu X, Brost J, Hutcheon C, Guilfoil R, Wilson AK, Leung S, Shewmaker CK, Rooke S, Nguyen T, Kiser J, De Rocher J (2012) Transformation of the oilseed crop *Camelina sativa* by Agrobacterium-mediated floral dip and simple large-scale screening of transformants. In Vitro Cell Dev Biol Plant 48:462–468

Lolle SJ, Hsu W, Pruitt RE (1998) Genetic analysis of organ fusion in *Arabidopsis thaliana*. Genetics 149:607–619

Lu C, Kang J (2008) Generation of transgenic plants of a potential oilseed crop *Camelina sativa* by Agrobacterium-mediated transformation. Plant Cell Rep 27:273–278

Mao B, Cheng Z, Lei C, Xu F, Gao S, Ren Y, Wang J, Zhang X, Wang J, Wu F, Guo X, Liu X, Wu C, Wang H, Wan J (2012) Wax crystal-sparse leaf2, a rice homologue of WAX2/GL1, is involved in synthesis of leaf cuticular wax. Planta 235:39–52

Mitra J (2001) Genetics and genetic improvement of drought resistance in crop plants. Curr Sci 80:758–763

Nelson DE, Repetti PP, Adams TR, Creelman RA, Wu J, Warner DC, Anstrom DC, Bensen RJ, Castiglioni PP, Donnarummo MG, Hinchey BS, Kumimoto RW, Maszle DR, Canales RD, Krolikowski KA, Dotson SB, Gutterson N, Ratcliffe OJ, Heard JE (2007) Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. Proc Natl Acad Sci USA 104:16450–16455

Nguyen HT, Silva JE, Podicheti R, Macrander J, Yang W, Nazarenus TJ, Nam JW, Jaworski JG, Lu C, Scheffler BE, Mockaitis K, Cahoon EB (2013) Camelina seed transcriptome: a tool for meal and oil improvement and translational research. Plant Biotechnol J 11:759–769

Oh SJ, Kim YS, Kwon CW, Park HK, Jeong JS, Kim JK (2009) Overexpression of the transcription factor AP37 in rice improves grain yield under drought conditions. Plant Physiol 150:1368–1379

Oshima Y, Shikata M, Koyama T, Ohtsubo N, Mitsuda N, Ohme-Takagi M (2013) MIXTA-like transcription factors and WAX INDUCER1/SHINE1 coordinately regulate cuticle development in Arabidopsis and *Torenia fournieri*. Plant Cell 25:1609–1624

Putnam D, Budin J, Field L, Breene W (1993) Camelina: a promising low-input oilseed. In: Janick J, Simon JE (eds) New crops. Wiley, New York, pp 314–322

Quan R, Shang M, Zhang H, Zhao Y, Zhang J (2004) Engineering of enhanced glycine betaine synthesis improves drought tolerance in maize. Plant Biotechnol J 2:477–486

Raffaele S, Vailleau F, Léger A, Joubès J, Miersch O, Huard C, Blée E, Mongrand S, Domergue F, Roby D (2008) A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in Arabidopsis. Plant Cell 20:752–767

Rowland O, Zheng H, Hepworth SR, Lam P, Jetter R, Kunst L (2006) CER4 encodes an alcohol-forming fatty acyl-coenzyme A reductase involved in cuticular wax production in Arabidopsis. Plant Physiol 142:866–877

Seo PJ, Xiang F, Qiao M, Park JY, Lee YN, Kim SG, Lee YH, Park WJ, Park CM (2009) The MYB96 transcription factor mediates abscisic acid signaling during drought stress response in Arabidopsis. Plant Physiol 151:275–289

Seo PJ, Lee SB, Suh MC, Park MJ, Go YS, Park CM (2011) The MYB96 transcription factor regulates cuticular wax biosynthesis under drought conditions in Arabidopsis. Plant Cell 23:1138–1152

Specht JE, Hume DJ, Kumudini SV (1999) Soybean yield potential—a genetic and physiological perspective. Crop Sci 39:1560–1570

Stracke R, Werber M, Weisshaar B (2001) The R2R3-MYB gene family in *Arabidopsis thaliana*. Curr Opin Plant Biol 4:447–456

Urao T, Yamaguchi-Shinozaki K, Urao S, Shinozaki K (1993) An Arabidopsis myb homolog is induced by dehydration stress and its gene product binds to the conserved MYB recognition sequence. Plant Cell 5:1529–1539

Vogg G, Fischer S, Leide J, Emmanuel E, Jetter R, Levy AA, Riederer M (2004) Tomato fruit cuticular waxes and their effects on transpiration barrier properties: functional characterization of a mutant deficient in a very-long-chain fatty acid β-ketoacyl-CoA synthase. J Exp Bot 55:1401–1410

Wang Y, Ying J, Kuzma M, Chalifoux M, Sample A, McArthur C, Uchacz T, Sarvas C, Wan J, Dennis DT, McCourt P, Huang Y (2005) Molecular tailoring of farnesylation for plant drought tolerance and yield protection. Plant J 43:413–424

Wang Y, Wan L, Zhang L, Zhang Z, Zhang H, Quan R, Zhou S, Huang R (2012) An ethylene response factor OsWR1 responsible to drought stress transcriptionally activates wax synthesis related genes and increases wax production in rice. Plant Mol Biol 8:275–288

Wright PR, Morgan JM, Jessop RS, Cass A (1995) Comparative adaptation of canola (*Brassica napus*) and Indian mustard (*B. juncea*) to soil water deficits: yield and yield components. Field Crops Res 42:1–13

Xu X, Dietrich CR, Delledonne M, Xia Y, Wen TJ, Robertson DS, Nikolau BJ, Schnable PS (1997) Sequence analysis of the cloned glossy8 gene of maize suggests that it may code for a β-ketoacyl reductase required for the biosynthesis of cuticular waxes. Plant Physiol 115:501–510

Yang J, Isabel Ordiz M, Jaworski JG, Beachy RN (2011a) Induced accumulation of cuticular waxes enhances drought tolerance in Arabidopsis by changes in development of stomata. Plant Physiol Biochem 49:1448–1455

Yang M, Yang Q, Fu T, Zhou Y (2011b) Overexpression of the *Brassica napus* BnLAS gene in Arabidopsis affects plant development and increases drought tolerance. Plant Cell Rep 30:373–388

Yu D, Ranathunge K, Huang H, Pei Z, Franke R, Schreiber L, He C (2008) Wax Crystal-Sparse Leaf1 encodes a β-ketoacyl CoA synthase involved in biosynthesis of cuticular waxes on rice leaf. Planta 228:675–685

Zhang JY, Broeckling CD, Blancaflor EB, Sledge MK, Sumner LW, Wang ZY (2005) 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*). Plant J 42:689–707

Zhang X, Henriques R, Lin S-S, Niu Q-W, Chua N-H (2006) Agrobacterium-mediated transformation of *Arabidopsis thaliana* using the floral dip method. Nat Protoc 1:641–646

Zhang JY, Broeckling CD, Sumner LW, Wang ZY (2007) Heterologous expression of two *Medicago truncatula* putative ERF transcription factor genes, WXP1 and WXP2, in Arabidopsis led to increased leaf wax accumulation and improved drought tolerance, but differential response in freezing tolerance. Plant Mol Biol 64:265–278

Zhang ZJ, Li F, Li DJ, Zhang HW, Huang RF (2010) Expression of ethylene response factor JERF1 in rice improves tolerance to drought. Planta 232:765–774

Zhu X, Xiong L (2013) Putative megaenzyme DWA1 plays essential roles in drought resistance by regulating stress-induced wax deposition in rice. Proc Natl Acad Sci USA 110:17790–17795

Zingaretti SM, Inácio MC, de Matos Pereira L, Paz TA, de Castro França S (2013) Water Stress and Agriculture. In: Sener A (ed) Responses of organisms to water stress. InTech, doi:10.5772/53877. ISBN: 978-953-51-0933-4

Zubr J (1997) Oil-seed crop: *Camelina sativa*. Ind Crops Prod 6:113–119