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Characterization of leaf cuticular wax classes and constituents in a spring *Camelina sativa* diversity panel

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ABSTRACT

Among oilseed species, Camelina has received considerable attention as an oilseed crop that can be manipulated easily to meet important non-food bioenergy requirements, where it is relatively high in oil content and polyunsaturated fatty acids, and has a very short growing season with fairly good adaption to marginal lands and low input agricultural systems. To expand Camelina cultivation zones into more arid regions, it is important to develop new drought resistant cultivars that can grow under water-limited conditions. Increasing accumulated leaf cuticular wax in Camelina could be one of the strategies to reduce nonstomatal water loss and thus increase crop tolerance to drought. To extend the understanding of phenotypic variations in cuticular wax content and composition in *Camelina sativa*, leaf wax constituents from a spring Camelina diversity panel containing 163 accessions were extracted and analyzed. The diversity panel exhibited a wide range in total leaf wax contents, wax classes and constituents. Among primary alcohols, the dominant constituents were the C_{24}, C_{26} and C_{28} homologues, while the C_{31} homologue was the most abundant alkane among all *Camelina* accessions. High heritability values of the primary alcohol class and its dominant constituent C_{24}, C_{26} and C_{28} homologues, as well as the alkane class and abundant C_{29}, C_{31}, and C_{33} constituents, suggested the feasibility for selection of these traits during early generations of Camelina breeding programs. Positive correlations among leaf wax content, wax classes and their constituents suggest that modifying specific wax constituents could increase the wax loads, which in turn could enhance cuticle composition and properties. Quantification of leaf wax traits in the Camelina diversity panel will underpin future analysis of the Camelina wax biosynthetic pathways, help dissect its genetic regulatory elements, identify candidate genes controlling these traits, and enable the development of molecular markers for molecular breeding programs aimed at increasing drought tolerance of Camelina.

1. Introduction

There is a growing need to develop high-yielding non-food oil crops that can be cultivated in marginal farming areas for biodiesel uses. The ideal biodiesel crop should have high oil content, favorable fatty acid composition, compatibility with existing farm equipment and infrastructure, marginal growth conditions including mild water deficit, have definable growing seasons, and uniform seed maturation rates (Moser and Vaughn, 2010). Among oilseed species such as canola, soybean, rapeseed and sunflower, *Camelina sativa* has received considerable recent attention as an oilseed crop that can be manipulated easily for desired lipid compositions and meet important non-food bioenergy and bioproducts end uses (Iskandarov et al., 2014). Camelina is an old world crop newly introduced to the semi-arid west of the U.S. Even though Camelina is lower yielding than canola (a food oil crop), it has shorter life cycles that have placed it as a potential candidate for spring-sown crop rotations. Camelina also grows fairly well in marginal lands with low inputs compared to other oilseed crops, and has high seed oil content (28–40%) compared to soybean (18–22%) (Budin et al., 1995). Camelina oil has a relatively high content of polyunsaturated fatty acids (54.3%), but the methyl esters derived from the oil are compatible, both neat and blended, with petro-diesel (Moser et al., 2016; Moser and Vaughn, 2010). Camelina has well-developed genomics resources include a reference genome sequence (Abdullah et al., 2016; Kagale et al., 2014; Kagale et al., 2016). These resources will be useful for developing novel traits through molecular breeding, genome editing, and other genetic engineering approaches as well as identification of genes and markers underpinning natural variation within important agronomic traits.

Reduction of cuticle water permeability is one of the drought avoidance mechanisms that plant species evolved to tolerate water-limited growing conditions (Jones et al., 1981). Cuticlar wax

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compositions often vary significantly between species, between organs of the same plant (Bernard and Joubès, 2013; Lee and Suh, 2015; Razeq et al., 2014; Samuels et al., 2008), and in response to abiotic and biotic stresses (Kosma et al., 2009; Kosma and Jenks, 2007; Shepherd and Griffiths, 2006; Xue et al., 2017; Yeats and Rose, 2013). The major plant leaf wax classes are free fatty acids, primary alcohols, alkanes and aldehydes. It was reported that leaf wax content and constitutes increased in response to abiotic stress (Xue et al., 2017), specifically to drought stress in plant species such as Arabidopsis (Kosma and Jenks, 2007), alfalfa (Ni et al., 2012), *Populus euphratica* (Xu et al., 2016), sesame (Kim et al., 2007a), soybean (Kim et al., 2007b), tobacco (Cameron et al., 2006) and maritime pine (Le Provost et al., 2013).

Tomasi et al. (2017) found that Camelina species exhibited a wide range of wax contents, with primary alcohols and alkanes as the predominant classes of leaf wax, followed in abundance by wax esters, fatty acids and aldehydes. Among primary alcohols, the dominant constituents were the C_{24}, C_{26} and C_{28} homologues, while the C_{31} homologue was the most abundant alkane among all Camelina species (Tomasi et al., 2017).

The goal of current research is to extend the understanding to the phenotypic variations in leaf wax traits within domesticated *Camelina sativa* diversity panel. Leaf waxes of a spring Camelina diversity panel consisting of accessions typically grown in different geographical regions were extracted and analyzed. The specific objectives of the current study were to detect and characterize the phenotypic variation in *Camelina sativa* leaf wax, and estimate the genetic components and heritability estimates of these wax traits. The he longer-term goal is to identify candidate genes controlling wax biosynthetic pathways for improving Camelina drought tolerance using genomics-based crop improvement strategies.

2. Materials and methods

2.1. Plant materials

A Camelina diversity panel consisting of 163 accessions of *Camelina sativa* was obtained from Plant Gene Resources of Canada (PGRC, Saskatoon, SK, Canada; http://pgrc3.agr.gc.ca). The panel represents spring Camelina accessions collected from different regions of Europe (Supplementary Table 1). The accessions were planted under greenhouse conditions at the US Arid-Land Agricultural Research Center in Maricopa, Arizona during March 2016. The accessions were arranged in randomized complete block design (RCBD) with three replications each. The seeds of each accession were planted in 29.29 cu. in. containers of Sunshine Mix #1/LC1 (Sun Gro Horticulture, Canada). Plants were regularly watered and fertilized with N-P-K 20-20-20 fertilizer (Scotts Miracle-Grow, USA).

2.2. Wax extraction and analyses

Wax extraction and analyses followed the Camelina leaf wax extraction protocols described in Tomasi et al. (2017), with slight modifications. Briefly, sample consists of three leaves (approximately seventh to twelfth leaf from basal rosette) from each replicate was collected at 35 days after planting. Immediately, leaves were submerged in 10 mL hexane (Sigma-Aldrich, USA) in a 20 mL glass scintillation vial, then three internal standards were added including: 10 μg nonadecanoic acid, 10 μg tetracosane and 20 μg tricosanol. Vials were capped and agitated for 45 s. The leaves were removed from the solvent with forceps and leaf area was determined using a flatbed scanner and ImageJ (Schneider et al., 2012). The wax extracts were heated (70 °C) and reduced under N_2 until the volume could be transferred to a 2 mL glass vial. The scintillation vials were rinsed once with a few mL of hexane, the volume transferred again and then evaporated to dryness. For each sample, 100 μL of *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, Sigma-Aldrich, USA) and 100 μL hexane was added for a total volume of 200 μL. The sample vials were capped and loaded onto the Agilent 7890A gas chromatograph equipped with a 5975C mass spectrometer. Each vial was heated at 80 °C for 35 min, then mix 6 s at 2000 rpm for five cycles prior to a 1 μL splitless injection. An HP-Ultra 1 capillary column (12 m length, 200 μm inner diameter, 0.33 μm film thickness; Agilent, USA) was used, with helium as a carrier gas at 1 mL per min and temperature settings of inlet 300 °C, detector 300 °C, initial oven temperature 50 °C, then increased 20 °C per minute to 260 °C, where it was held for 8 min.

2.3. Interpretation of wax constituents and statistical analysis

Molecular identities of compounds were determined by characteristic quadrupole electron impact mass spectra method, as described by Tomasi et al. (2017). Uncorrected wax quantifications were based on specific target ions relative to the corresponding added internal standard. Leaf surface areas were multiplied by two to account for both surfaces and quantified wax values are expressed as μg dm^{-2}.

2.4. Statistical analyses

The Camelina leaf wax constituents were analyzed using general linear models procedure (PROC GLM) of SAS software as randomized complete block experimental designs with replicates and accessions considered as random effects (Statistical Analysis System, SAS institute, 2001). The Correlation Coefficients (r) were used to assess the relationship between wax constituents, and was estimated using the PROC CORR of SAS. Broad-sense heritability on an entry-mean basis was calculated as $h^2 = (\sigma^2_{line}/(\sigma^2_{line} + \sigma^2_e/r)$ (Holland et al., 2003; Nyquist and Baker, 1991), where σ^2_{line} equaled the genetic variance among the Camelina accessions, σ^2_e is the variance of experimental error, and r is the number of replications.

3. Results and discussion

To date, there are few reports describing the variation in wax accumulation on Camelina organs (Razeq et al., 2014) and/or Camelina species (Tomasi et al., 2017). Accumulating cuticular wax on plant surfaces proves to be a strategy to reduce nonstomatal water loss under abiotic stresses (Fang and Xiong, 2015; Riederer and Schreiber, 2001). To extend the understanding of leaf wax-accumulating mechanisms and candidate genes controlling wax biosynthetic pathways in Camelina, large populations or diversity panels are required. The analysis of a spring diversity panel containing 163 accessions from different geographical areas revealed wide variations in wax constituents (Table 1; Fig. 1). The Camelina diversity panel showed a wide range of total wax contents, with the highest in an accession from Poland, which accumulated 288.1 μg dm^{-2} of total wax, and the lowest was an accession from Spain, with 148.8 μg dm^{-2} (Table 1). In general, wax compositions were similar to that previously identified in Camelina species (Tomasi et al., 2017), where the accessions exhibited a wide range of primary fatty alcohols, alkanes, wax esters, aldehydes, free fatty acids, alkylguaiacols, methylalkylresorcinols and β-sitostrol (Table 1). Together, primary alcohols, alkanes and wax esters accounted for 86% of the total wax content in the Camelina diversity panel. Primary alcohols contributed 35% of total waxes (Table 1), where Camelina accessions showed wide distribution of primary alcohols, ranging from 31.39 μg dm^{-2} to 119.1 μg dm^{-2}. Primary alcohols previously showed an increase in stressed soybean (Kim et al., 2007b) and maritime pine (Le Provost et al., 2013) leaves. Among primary alcohols in Camelina, C_{24}, C_{26} and C_{28} were the predominant molecular species that together counted for 84% of total primary alcohols (Table 1). In contrast, primary alcohols were not as prominent in *Arabidopsis* leaf wax (Bernard and Joubès, 2013), suggesting that the *Arabidopsis* wax biosynthetic pathway is differentially regulated in comparison to the Camelina wax biosynthetic pathway. Alkanes are abundant wax constituents that

Table 1
Means, ranges (min-max), standard deviation (SD) and broad-sense heritability values (h^2) of cuticular leaf wax constituents for 163 spring accessions of *Camelina sativa* diversity panel as identified by Tomasi et al. (2017). Wax constituents were grouped by major classes. Three leaves were separately analyzed, then leaf subsamples were averaged to represent one replicate. Values, in μg dm^{-2}, were reported as mean of three replications.

Wax constituent	Abr.	min	max	mean	SD	h^2
Primary Alcohols		31.39	119.09	71.77	1.73	0.77
Docosanol (C_{22})	C_{22} Alc	1.07	15.29	7.37	0.67	0.38
Tetracosanol (C_{24})	C_{24} Alc	5.80	35.64	22.36	1.01	0.66
Pentacosanol (C_{25})	C_{25} Alc	0.14	0.75	0.42	0.14	0.81
Hexacosanol (C_{26})	C_{26} Alc	9.13	41.27	24.52	13.95	0.86
Heptacosanol (C_{27})	C_{27} Alc	0.18	0.57	0.33	5.63	0.58
Octacosanol (C_{28})	C_{28} Alc	7.87	22.27	13.01	7.19	0.57
Nonacosanol (C_{29})	C_{29} Alc	0.06	0.31	0.16	0.09	0.51
Triacontanol (C_{30})	C_{30} Alc	1.22	4.29	2.35	4.29	0.61
Dotriacontanol (C_{32})	C_{32} Alc	0.36	1.41	0.73	0.12	0.69
Tetratriacontanol (C_{34})	C_{34} Alc	0.22	1.11	0.51	4.85	0.74
Alkanes		23.08	118.63	45.72	0.08	0.77
Pentacosane (C_{25})	C_{25} Alk	0.12	1.18	0.32	0.99	0.18
Heptacosane (C_{27})	C_{27} Alk	0.36	2.14	0.78	0.25	0.05
Nonacosane (C_{29})	C_{29} Alk	2.58	11.78	5.10	0.21	0.74
Triacontane (C_{30})	C_{30} Alk	0.36	1.66	0.69	0.60	0.07
Hentriacotane (C_{31})	C_{31} Alk	11.43	52.83	21.98	0.08	0.78
Dotriacontane (C_{32})	C_{32} Alk	0.75	2.50	1.36	0.12	0.34
Tritriacontane (C_{33})	C_{33} Alk	6.02	45.91	13.93	0.35	0.81
Pentatriacontane (C_{35})	C_{35} Alk	0.66	3.33	1.39	0.15	0.70
Wax Esters		30.84	108.41	56.40	17.18	0.77
(C_{38})	C_{38} WE	0.88	9.26	4.13	0.59	0.69
(C_{40})	C_{40} WE	5.10	19.34	10.59	0.85	0.71
(C_{42})	C_{42} WE	4.85	27.66	11.09	1.88	0.86
(C_{44})	C_{44} WE	9.08	36.00	16.94	0.71	0.79
(C_{46})	C_{46} WE	3.57	32.95	11.00	7.42	0.70
(C_{48})	C_{48} WE	0.55	10.54	2.52	0.73	0.65
Free Fatty Acids		0.91	4.65	2.22	6.54	0.76
Docosanoic acid (C_{22})	C_{22} FA	0.20	0.87	0.43	0.66	0.57
Tetracosanoic acid (C_{24})	C_{24} FA	0.13	0.50	0.26	0.94	0.59
Hexacosanoic acid (C_{26})	C_{26} FA	0.06	0.31	0.16	0.19	0.76
Octacosanoic acid (C_{28})	C_{28} FA	0.15	1.08	0.49	0.11	0.81
Triacontanoic acid (C_{30})	C_{30} FA	0.08	0.79	0.32	0.06	0.77
Dotriacontanoic acid (C_{32})	C_{32} FA	0.04	1.20	0.38	0.18	0.72
Tetratriacontanoic acid (C_{34})	C_{34} FA	0.01	0.66	0.18	0.17	0.71
Aldehydes		0.13	1.67	0.58	0.30	0.38
Tetracosanal (C_{24})	C_{24} Ald	0.01	0.21	0.06	0.20	0.31
Hexacosanal (C_{26})	C_{26} Ald	0.03	0.36	0.11	1.42	0.47
Octacosanal (C_{28})	C_{28} Ald	0.04	0.82	0.29	0.45	0.36
Triacontanal (C_{30})	C_{30} Ald	0.01	0.39	0.11	0.80	0.53
Alkylguaiacols		1.55	9.19	3.93	0.22	0.77
(C_{19})	C_{19} AG	0.51	3.24	1.25	0.52	0.78
(C_{21})	C_{21} AG	0.83	5.58	2.39	38.28	0.77
(C_{23})	C_{23} AG	0.11	1.03	0.29	17.77	0.87
Methylalkylresorcinols		0.98	5.46	2.64	2.25	0.56
(C_{21})	C_{21} MAR	0.27	2.00	0.78	3.91	0.62
(C_{23})	C_{23} MAR	0.52	3.08	1.51	3.73	0.56
(C_{19})	C_{19} MAR	0.12	0.93	0.34	6.10	0.70
ß-Sitosterol		0.06	0.88	0.25	5.97	0.17
WAX_total		148.87	288.11	201.22	2.40	0.73

positively responded to drought stresses in alfalfa (Ni et al., 2012), *Populus euphratica* (Xu et al., 2016), sesame (Kim et al., 2007a) and soybean (Kim et al., 2007b). In the current study, Camelina's alkane content ranged from 23.1 μg dm^{-2} to 118.6 μg dm^{-2}, with specific C_{29}, C_{32} and C_{35} alkanes accounting for 90% of total alkanes (Table 1). The C_{29} alkane accounted to up to 45% of the total wax content of a *Brassica napus* diversity panel (Tassone et al., 2016); in our study it accounted for just 3% of the total Camelina wax (Table 1). Wax esters (WE) ranged from 30.68 to 108.4 μg dm^{-2}, with the C_{40}, C_{42}, C_{44} and C_{46} homologs as the major wax ester constituents. Other wax classes accounted for less than 5% of total wax content, but still showed phenotypic variation within each class, where free fatty acids (FA) ranged from 0.91 to 4.65 μg dm^{-2}; aldehydes (ALD) ranged from 0.13 μg dm^{-2} to 1.68 μg dm^{-2}; alkylguaiacols (AG) ranged from 1.6 μg dm^{-2} to 9.2 μg dm^{-2}; and methylalkylresorcinols (MAR) ranged from 1.0 μg dm^{-2} to 5.45 μg dm^{-2} (Table 1).

Cuticular wax is produced from a biosynthetic pathway that has two main branches, including acyl reduction and decarbonylation (Millar et al., 1999; Shepherd and Wynne Griffiths, 2006; Xue et al., 2017). In the acyl reduction branch, primary alcohols are produced by reduction of very long chain fatty acid (VLCFA) precursors. These fatty alcohols can be further combined with a fatty acid to produce wax esters. On the other branch, aldehydes are produced from VLCFA precursors, followed by aldehyde decarbonylation to produce alkanes. In the Camelina diversity panel, primary alcohols and wax esters accounted 65% of total wax, while aldehydes and alkanes accounted for 23% (Table 1). Since free fatty acids are precursors for both pathway branches, there are significant correlations between free fatty acids and both primary alcohols (r = 0.44) and aldehydes (r = 0.14) (Table 2). These findings suggested that acyl reduction is the main branch for wax biosynthesis in *Camelina sativa*, and the five wax classes, primary fatty alcohols, alkanes, wax esters, aldehydes, free fatty acids, are contributing to the variations in Camelina leaf wax content. There were highly significant correlation coefficients between total wax and primary alcohols, alkanes, wax esters, aldehydes and free fatty acids (Table 2). These correlations could indicate that these leaf wax compounds are independently regulated and the wax accumulation is controlled by several genes that regulate both pathway branches.

Heritability is an estimate for the genotypic effects in a trait due to genetic components of the estimated variance, where high heritability estimates indicate the feasibility of selection for a trait of interest during the early generations of breeding programs. Total wax content showed high heritability estimate (h^2 = 0.73; based on broad-sense heritability), indicating higher genetic components controlling leaf wax content and the possibility of select for this trait in Camelina breeding programs. For wax constituents, heritability values ranged from 0.05 (C_{27} Alkenes) to 0.87 (C_{23} wax ester) (Table 1), indicating the variable environmental effects on inheritance of these constitutes. In general, primary alcohols, alkanes, wax esters and free fatty acids showed high heritability values more than h^2 = 0.77 each, while aldehydes had moderate heritability value (h^2 = 0.38). Camelina wax constituents that showed abundance under drought stress in various plant species (Kim et al., 2007a, 2007b; Le Provost et al., 2013; Ni et al., 2012; Xu et al., 2016), also had high heritability estimates including the C_{26} (h^2 = 0.86), C_{28} (h^2 = 0.57), and C_{30} (h^2 = 0.61) alcohols; C_{29} (h^2 = 0. 73) and C_{33} (h^2 = 0.81) alkanes; and C_{42} (h^2 = 0.86) and C_{46} (h^2 = 0.86) wax esters in the current study (Table 1). These findings suggested that these constitutes are strong potential targets for modifying Camelina' wax content and/or composition during early generations of breeding programs for improvement of drought tolerance in Camelina.

4. Conclusion

Increasing the accumulation of leaf cuticular wax in Camelina could be one of the strategies to reduce nonstomatal water loss and thus resist abiotic stresses. A wide range of phenotypic variation in leaf total wax, wax classes and constituents was found in a *Camelina sativa* diversity panel collected from differ geographical regions. The detection of wide variations in leaf wax traits is the first step to understand wax biosynthetic pathways in Camelina toward dissect its genetic network elements, identify candidate genes controlling these traits, and develop molecular markers for molecular breeding and genomic selection programs to increase drought resistance in Camelina. Among the detected primary alcohol waxes, C_{24}, C_{26} and C_{28} homologues were the dominant in all Camelina accessions, and are highly heritable traits. The C_{29}, C_{31} and C_{33} homologues are the most abundant alkanes among all Camelina accessions, with highly heritable nature. The feasibility to detect these wax constituents suggests them as good biomarkers for selection and breeding for drought resistance in Camelina through

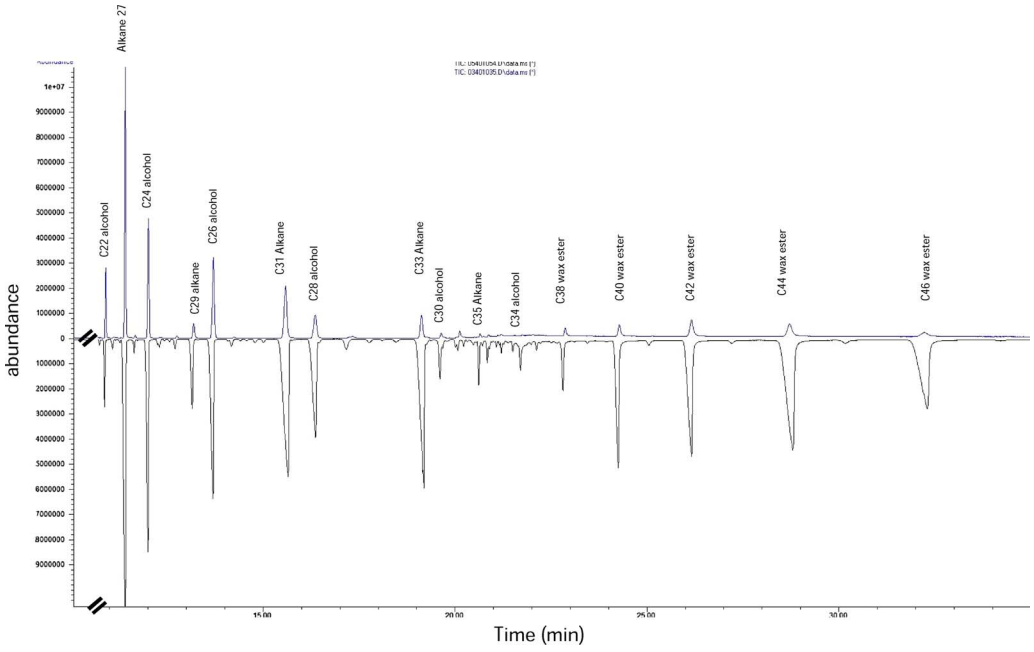

Fig. 1. Comparison of total ion chromatograms for CN 113750 (upper) and CN 114274 (down) accessions with major leaf wax constituents as identified by Tomasi et al. (2017).

Table 2
Correlation coefficients (r) of major cuticular leaf wax classes for 163 spring accessions of *Camelina sativa* diversity panel, r values are above the horizontal axis, corresponding *p*-values are below the horizontal axis.

Wax class	Abr.	FA	ALK	ALD	AG	MAR	WE	ALC	WAX_total
Free Fatty Acids	FA		0.07	0.15	0.10	0.21	0.36	0.45	0.49
Alkanes	ALK	0.113		0.19	0.33	0.30	0.19	0.07	0.63
Aldehydes	ALD	0.0009	< 0.0001		0.14	0.07	0.24	0.07	0.30
Alkylguaiacols	AG	0.023	< 0.0001	0.003		0.75	0.41	0.05	0.47
Methylalkylresorcinols	MAR	< 0.0001	< 0.0001	0.100	< 0.0001		0.34	0.09	0.46
Wax Esters	WE	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		0.28	0.72
Primary Alcohols	ALC	< 0.0001	0.107	0.102	0.245	0.048	< 0.0001		0.57
WAX_total		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

modifying cuticle composition and properties. High heritability values of these constituents suggested the feasibility of selecting of these traits during early generations of breeding programs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.indcrop.2017.11.054.

References

Abdullah, H.M., Akbari, P., Paulose, B., Schnell, D., Qi, W., Park, Y., Pareek, A., Dhankher, O.P., 2016. Transcriptome profiling of Camelina sativa to identify genes involved in triacylglycerol biosynthesis and accumulation in the developing seeds. Biotechnol. Biofuels 9, 1–19.

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

Budin, J.T., Breene, W.M., Putnam, D.H., 1995. Some compositional properties of camelina (camelina sativa L. Crantz) seeds and oils. J. Am. Oil Chem. Soc. 72, 309–315.

Cameron, K.D., Teece, M.A., Smart, L.B., 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.

Fang, Y., Xiong, L., 2015. General mechanisms of drought response and their application in drought resistance improvement in plants. Cell. Mol. Life Sci. 72, 673–689.

Holland, J.B., Nyquist, W.E., Cervantes-Martínez, C.T., 2003. Estimating and interpreting heritability for plant breeding: an update. Plant Breed. Rev. 22, 9–112.

Iskandarov, U., Kim, H.J., Cahoon, E.B., 2014. Camelina: an emerging oilseed platform for advanced biofuels and bio-Based materials. In: McCann, M.C., Buckeridge, M.S., Carpita, N.C. (Eds.), Plants and BioEnergy. Springer New York, New York NY, pp. 131–140.

Jones, M.M., Turner, N.C., Osmond, C.B., 1981. Mechanisms of drought resistance. In: Paleg, L.G., Aspinall, D. (Eds.), The Physiology and Biochemistry of Drought Resistance in Plants. Academic Press, New York, pp. 15–37.

Kagale, S., Koh, C., Nixon, J., Bollina, V., Clarke, W.E., Tuteja, R., Spillane, C., Robinson, S.J., Links, M.G., Clarke, C., Higgins, E.E., Huebert, T., Sharpe, A.G., Parkin, I.A.P., 2014. The emerging biofuel crop Camelina sativa retains a highly undifferentiated hexaploid genome structure. Nat. Commun. 5, 3706.

Kagale, S., Nixon, J., Khedikar, Y., Pasha, A., Provart, N.J., Clarke, W.E., Bollina, V., Robinson, S.J., Coutu, C., Hegedus, D.D., Sharpe, A.G., Parkin, I.A.P., 2016. The developmental transcriptome atlas of the biofuel crop Camelina sativa. The Plant Journal 88, 879–894.

Kim, K.S., Park, S.H., Jenks, M.A., 2007a. Changes in leaf cuticular waxes of sesame (Sesamum indicum L.) plants exposed to water deficit. J. Plant Physiol. 164, 1134–1143.

Kim, K.S., Park, S.H., Kim, D.K., Jenks, M.A., 2007b. Influence of water deficit on leaf cuticular waxes of soybean (Glycine max [L.] merr:). Int. J. Plant Sci. 168, 307–316.

Kosma, D.K., Jenks, M.A., 2007. Eco-Physiological and molecular-Genetic determinants of plant cuticle function in drought and salt stress tolerance. In: Jenks, M.A., Hasegawa, P.M., Jain, S.M. (Eds.), Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops. Springer Netherlands, Dordrecht, pp. 91–120.

Kosma, D.K., Bourdenx, B., Bernard, A., Parsons, E.P., Lü, S., Joubès, J., Jenks, M.A., 2009. The impact of water deficiency on leaf cuticle lipids of arabidopsis. Plant Physiol. 151, 1918–1929.

Le Provost, G., Domergue, F., Lalanne, C., Ramos Campos, P., Grosbois, A., Bert, D., Meredieu, C., Danjon, F., Plomion, C., Gion, J.-M., 2013. Soil water stress affects both cuticular wax content and cuticle-related gene expression in young saplings of maritime pine (Pinus pinaster Ait). BMC Plant Biol. 13, 95–106.

Lee, S.B., Suh, M.C., 2015. Advances in the understanding of cuticular waxes in Arabidopsis thaliana and crop species. Plant Cell Rep. 34, 557–572.
Millar, A.A., Clemens, S., Zachgo, S., Giblin, E.M., Taylor, D.C., Kunst, L., 1999. CUT1, an Arabidopsis gene required for cuticular wax biosynthesis and pollen fertility, encodes a very-long-chain fatty acid condensing enzyme. The Plant Cell 11, 825–838.
Moser, B.R., Vaughn, S.F., 2010. Evaluation of alkyl esters from Camelina sativa oil as biodiesel and as blend components in ultra low-sulfur diesel fuel. Bioresour. Technol. 101, 646–653.
Moser, B.R., Evangelista, R.L., Isbell, T.A., 2016. Preparation and fuel properties of field pennycress (Thlaspi arvense) seed oil ethyl esters and blends with ultralow-sulfur diesel fuel. Energy Fuels 30, 473–479.
Ni, Y., Guo, Y.J., Han, L., Tang, H., Conyers, M., 2012. Leaf cuticular waxes and physiological parameters in alfalfa leaves as influenced by drought. Photosynthetica 50, 458–466.
Nyquist, W.E., Baker, R., 1991. Estimation of heritability and prediction of selection response in plant populations. Crit. Rev. Plant Sci. 10, 235–322.
Razeq, F.M., Kosma, D.K., Rowland, O., Molina, I., 2014. Extracellular lipids of Camelina sativa: characterization of chloroform-extractable waxes from aerial and subterranean surfaces. Phytochemistry 106, 188–196.
Riederer, M., Schreiber, L., 2001. Protecting against water loss: analysis of the barrier properties of plant cuticles. J. Exp. Bot. 52, 2023–2032.
Samuels, L., Kunst, L., Jetter, R., 2008. Sealing plant surfaces: cuticular wax formation by epidermal cells. Annu. Rev. Plant Biol. 59, 683–707.
Statistical Analysis System institute, 2001. SAS user's guide: statistics. SAS Inst, Cary.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.
Shepherd, T., Griffiths, W.D., 2006. The effects of stress on plant cuticular waxes. New Phytol. 171, 469–499.
Shepherd, T., Wynne Griffiths, D., 2006. The effects of stress on plant cuticular waxes. New Phytol. 171, 469–499.
Tassone, E.E., Lipka, A.E., Tomasi, P., Lohrey, G.T., Qian, W., Dyer, J.M., Gore, M.A., Jenks, M.A., 2016. Chemical variation for leaf cuticular waxes and their levels revealed in a diverse panel of Brassica napus L. Ind. Crops Prod. 79, 77–83.
Tomasi, P., Wang, H., Lohrey, G.T., Park, S., Dyer, J.M., Jenks, M.A., Abdel-Haleem, H., 2017. Characterization of leaf cuticular waxes and cutin monomers of Camelina sativa and closely-related Camelina species. Ind. Crops Prod. 98, 130–138.
Xu, X., Xiao, L., Feng, J., Chen, N., Chen, Y., Song, B., Xue, K., Shi, S., Zhou, Y., Jenks, M.A., 2016. Cuticle lipids on heteromorphic leaves of Populus euphratica Oliv. growing in riparian habitats differing in available soil moisture. Physiol. Plant. 158, 318–330.
Xue, D., Zhang, X., Lu, X., Chen, G., Chen, Z.-H., 2017. Molecular and evolutionary mechanisms of cuticular wax for plant drought tolerance. Front. Plant Sci. 8, 621.
Yeats, T.H., Rose, J.K.C., 2013. The formation and function of plant cuticles. Plant Physiol. 163, 5–20.