

Supporting Information

A splice variant of *BrrWSD1* in turnip (*Brassica rapa* var. *rapa*) and its possible role in wax ester synthesis under drought stress

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Supplementary Figures

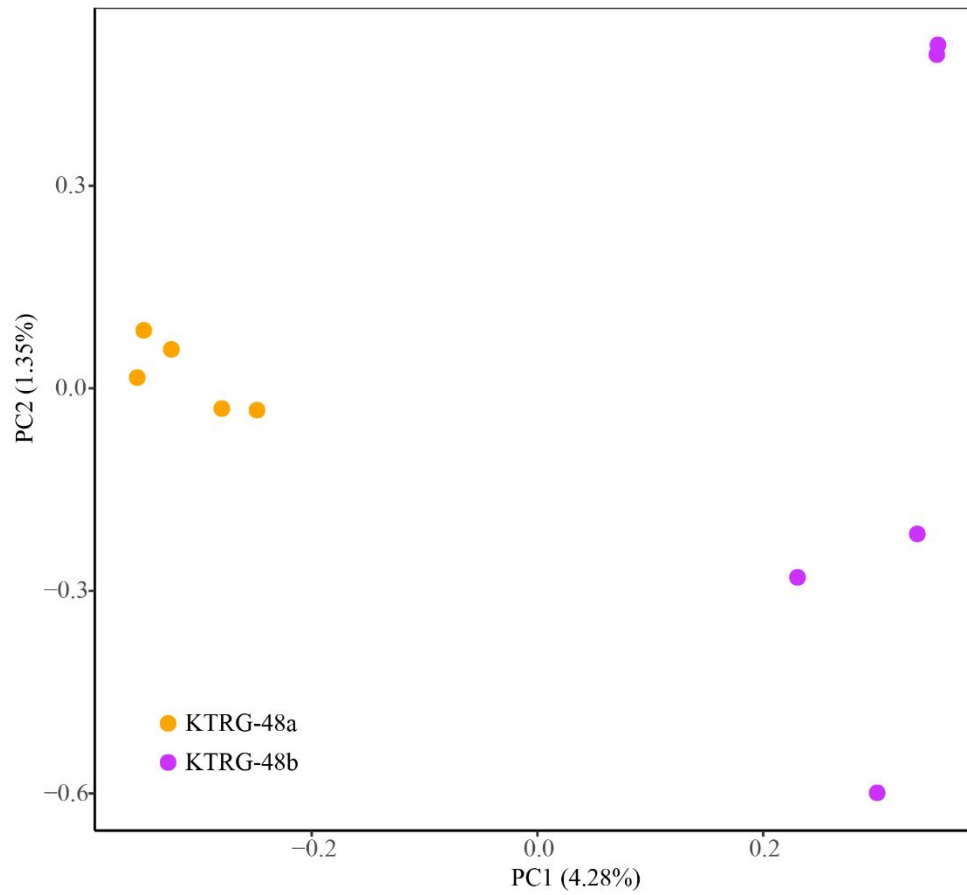


Figure S1 Principal component analyses of isolate SNP data of KTRG-B48a and KTRG-B48b.

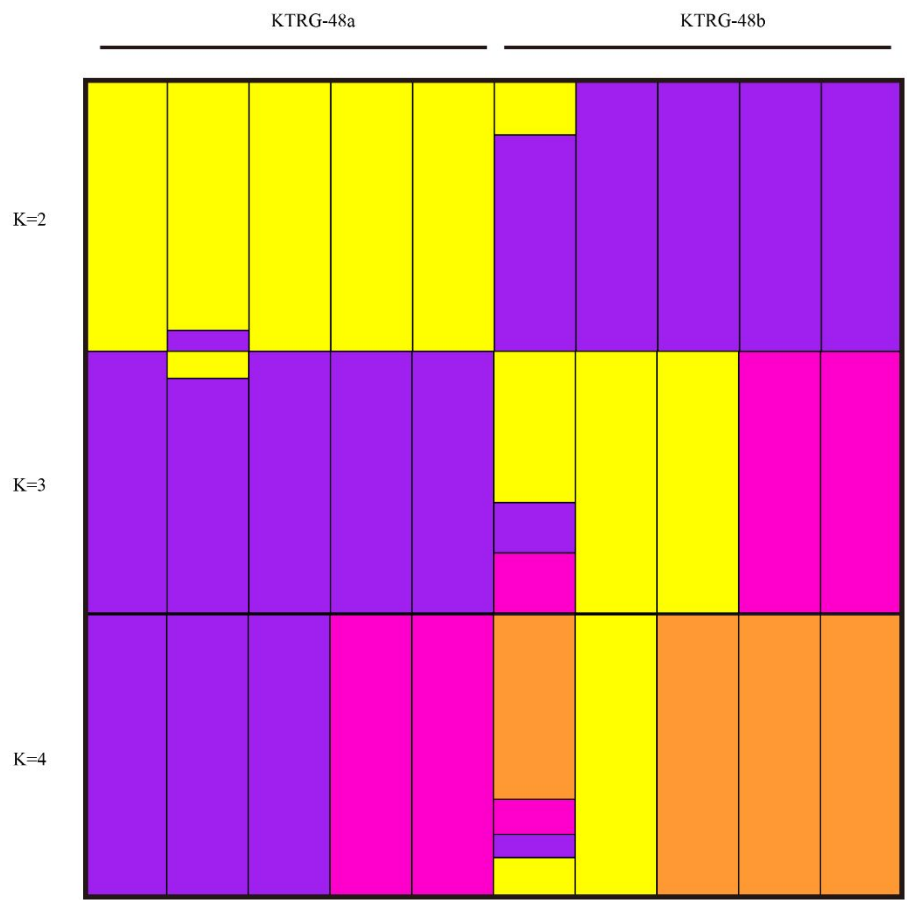


Figure S2 Genetic clustering analysis for SNPs data. Bayesian population assignment using STRUCTURE; Results for K = 2, 3 and 4 are displayed.

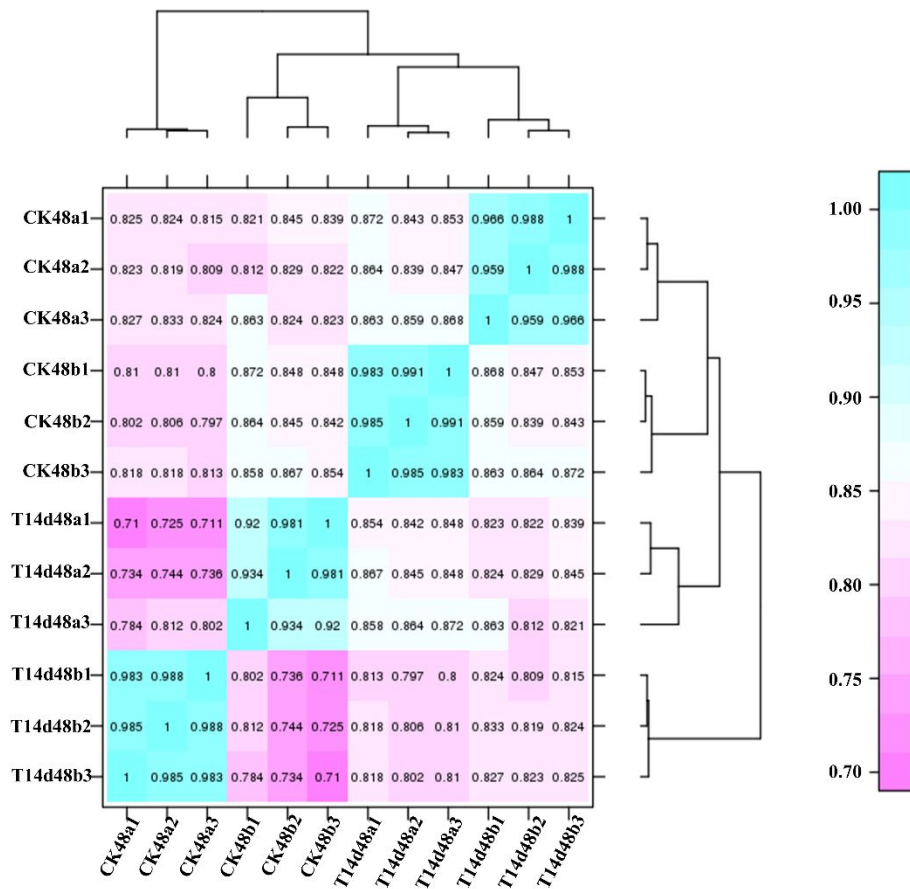


Figure S3 Correlation analysis of expression levels among samples.

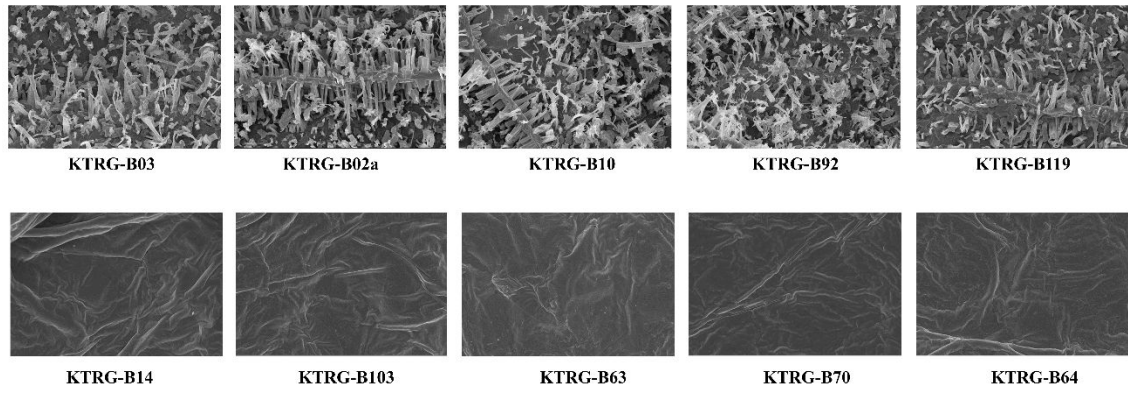


Figure S4 Scanning electronic microscopy of the leaf surfaces of waxy and wax-free varieties.

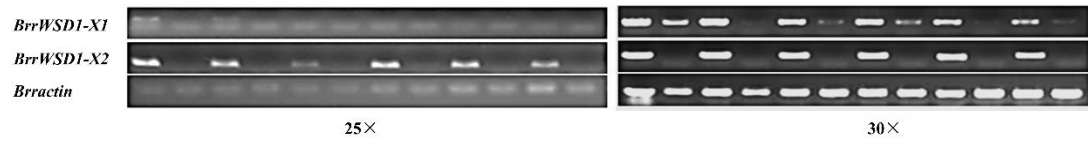


Figure S5 Semi-quantitative PCR of *BrrWSD1-X1* and *BrrWSD1-X2* in of waxy and wax-free varieties.

Supplementary Tables

Table S1. The primers for qRT-PCR and RT-PCR analysis.

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
The primers for qRT-PCR		
<i>MAH 1.1</i>	CAGCGGACGCGTTAACATAC	GAGAGCTGAGCTTGTGGTGT
<i>MAH 1.2</i>	AGCAATTAATGGACGCGCTAA	TGTCCCTTCCTGCCAACAATA
<i>CER1</i>	CTTTGCTCGACCTCCAGTGT	GACCGTGAACCCTTCGACTT
<i>CER3</i>	AATGATCAGATAGAGGCTGCGA	GCTGCGGTAAAGTGTTCCC
<i>CER4</i>	TTGTAGGGGCAAAAAGGGGAA	GCTGGCATCACATCGGAAAC
<i>CER10</i>	CCCATCGAGGCAAAGACTGA	GAAGAGCGTGCGGTATGAGA
<i>KCR1</i>	CTCCGAGTCCATCAGATCCAAA	ATCAGAATCCCAACCTCCAGC
<i>CER6</i>	ACGACAAGTCTTTCTACTGCGT	AGGAGTTGCTCTGATGCTGG
<i>WSD1-X1</i>	GTCGCAGGAAGTGCATTAGTT	TGAGGATCTGGAATTGTCGTTGTA
<i>WSD1-X2</i>	CTTGGAATGACCCAAGCAGG	ACATGGACTATTGCAAAACTTGG
<i>AAO3</i>	GGTGAAGCTGTTATCAAAGC	ACTCCAGCAGGAGTCACG
<i>NCED3.1</i>	TTCAGATTCTCACCGGACGGA	TCCGAATCTTGCGACCTTGTTT
<i>NCED3.2</i>	ATACCGGACCAGCAAGTCGT	GTGGAAGCAGAAGCAGTTCCG
<i>NCED3.3</i>	CAAGCTCCCGGAGATGATCC	CAGAGGTGGAAGCAGAAGCA
<i>ABA1</i>	TGATCGGATTAACGGCCTCG	TCACCCGAGTCACAGGTAGT
<i>MYB96</i>	CCAGTTTCTGAGACCGGACC	CGCCCGTTTACTCTCTTGGT
<i>BrrTUB2</i>	AGGCGTGTGAGTGAGCAGTT	CATCTCGTCCATTCCCTTCACCTGT
The primers for DNA and CDS of BrrWSD1 analysis		
<i>BrrWSD1-X1</i>	ATGGAAAAGAAAAAATTTCTG	TCACACCTCCATGTTATGAGA
<i>BrrWSD1-X2</i>	ATGGAAAAGAAAAAATTTCTG	TTATGGTAAGAGAACTTTGAA
The primers for splice variant of BrrWSD1 analysis		
<i>BrrWSD1</i>	ATGGAAAAGAAAAAATTTCTG	GACTCTAGACGACATCGATTTTTTTTTTTTTTTTTT

Table S2. Leaf cuticular waxes on KTRG-B48a and KTRG-B48b after exposure to drought.

Wax	KTRG-B48a CK	KTRG-B48b CK	KTRG-B48a D14d	KTRG-B48b D14d
1-C16	20.6374±3.7347	2.7347±0.5762	37.7369±4.6437	3.1254±0.4762
2-C18	15.7673±2.9874	2.9874±0.5647	28.7637±3.7876	3.7543±0.6762
3-C20	23.6737±0.6511	0.6511±0.0224	69.763±5.8765	0.6799±0.0755
4-C22	10.5394±0.7632	0.7632±0.0533	35.6523±4.6533	0.8796±0.0855
5-C28	14.0554±0.5957	0.2582±0.0035	24.4415±2.6589	0.395±0.0354
6-C24	27.6351±1.6539	4.6241±0.4816	87.8624±2.7368	4.5362±0.7618
7-C26	15.6512±0.7311	8.7685±0.5721	49.7621±1.9736	6.6321±0.5423
8-C28	39.7611±1.8723	6.8628±0.5241	119.6532±4.5322	5.6521±0.5213
9-C30	35.3284±1.7621	7.082±0.5292	121.2858±3.9743	6.9138±0.2163
10-C24	35.7634±2.6511	2.7628±0.3728	36.7622±3.8721	2.0183±0.8634
11-C26	10.6531±0.7819	0.7624±0.0742	16.8747±1.9532	0.9763±0.7632
12-C28	32.7642±1.7631	1.8679±0.1864	59.8748±3.7623	1.8936±0.5432
13-C30	28.9775±2.7628	4.8742±0.8762	30.7621±2.0873	5.8621±1.0554
14-C32	25.8291±2.0841	2.6095±0.2874	25.5623±2.0896	2.7769±0.4331
15-C25	30.7611±3.7632	10.7463±1.8422	35.3762±3.7621	10.9733±1.0848
16-C27	108.6536±5.9752	20.8743±2.3829	135.7634±5.9762	22.7614±1.3119
17-C29	95.5423±5.7613	16.8748±1.9743	109.8626±3.8743	16.8634±0.8934
18-C31	20.7634±3.9742	22.9473±2.5821	36.7642±1.8716	24.8327±1.8732
19--C33	34.0528±2.9745	16.0916±1.2843	76.8152±2.9843	17.0923±1.2957
C29	67.6213±4.7642	2.5313±0.5653	85.2281±5.6241	3.7528±0.4232
C38	67.8224±4.7211	3.6522±0.8362	89.7362±5.7321	6.9753±0.8421
C40	24.8929±2.0844	1.073±0.1873	28.1263±2.3058	2.6468±0.2013

Table S3. Summary statistics of whole genome resequencing.

Samples	Clean Reads	Clean Base	Q20(%)	Q30(%)	GC(%)
KTRG-B48a-1	48,039,370	14,385,981,394	97.21	92.86	38.85
KTRG-B48a-2	44,763,693	13,405,605,366	97.40	93.31	38.87
KTRG-B48a-3	34,798,126	10,422,253,666	96.40	91.01	38.67
KTRG-B48a-4	42,561,293	12,747,822,802	97.63	93.63	38.51
KTRG-B48a-5	41,245,498	12,351,981,798	97.49	93.45	38.70
KTRG-B48b -1	34,790,757	10,418,775,716	96.82	91.96	39.20
KTRG-B48b -2	36,423,886	10,905,726,816	97.08	92.50	39.28
KTRG-B48b -3	43,662,876	13,075,164,168	97.14	92.49	38.47
KTRG-B48b -4	42,050,869	12,594,134,646	97.19	92.67	39.11
KTRG-B48b -5	47,035,623	14,086,123,484	97.23	92.76	39.13

Table S4. Summary statistics of consensus transcripts after standard Iso-Seq bioinformatics pipeline.

Samples	cDNA Size	Reads of Insert	SMRT Cells	Data Size(G)	Number	Number	Number of full-length reads	Number	Average	Full-Length Percentage (FL%)	Artificial Concatemers (%)
					of filtered short reads	of non-full-length reads		of full-length non-chimeric reads	full-length non-chimeric read length		
KTRG-B48a	1-6K	827,744	2	12.91	49,005	389,648	389,091	375,397	1,587	47.01%	3.52%
KTRG-B48b	1-6K	1,033,771	2	10.81	44,219	477,883	511,669	494,651	1,491	49.50%	3.33%

Table S5. Statistical summary of the full-length transcriptome sequences generated using the PacBio platform.

Samples	KTRG-B48a	KTRG-B48b
Number of consensus isoforms	193,703	246,030
Average consensus isoforms read length	1,682	1,580
Number of HQ isoforms	107,933	126,249
Number of LQ isoforms	84,847	117,919
Number of corrected transcripts	45,410	48,024
Average corrected transcripts length	1,653	1,590

Table S6. The statistics of Illumina sequencing data.

Sample	Read Sum	Base Sum	GC(%)	Q30(%)
B48aCK1	20445599	6133679700	47.30	94.33
B48aCK2	20382851	6114855300	47.42	94.27
B48aCK3	20624339	6187301700	47.40	94.59
B48bCK1	20494775	6148432500	47.91	94.44
B48bCK2	20199641	6059892300	47.85	94.47
B48bCK3	20261165	6078349500	47.84	94.91
B48aD14d1	20399126	6119737800	47.75	94.53
B48aD14d2	20399221	6119766300	47.78	94.62
B48aD14d3	20415572	6124671600	47.73	94.59
B48bD14d1	20436535	6130960500	48.11	94.68
B48bD14d2	20288910	6086673000	47.94	94.62
B48bD14d3	20264135	6079240500	47.90	94.63

Table S7. Annotated number of the transcripts from the KTRG-48a and KTRG-48b full-length

transcripts.

Annotated databases	Annotated number of KTRG-48a (%)	Annotated number of KTRG-48b (%)
COG	21678 (47.74%)	24334 (50.67%)
GO	43049 (94.80%)	48000 (99.95%)
KEGG	22492 (49.53%)	25525 (53.15%)
KOG	30134 (66.36%)	33583 (69.93%)
Pfam	37644 (82.90%)	42789 (89.10%)
Swiss-Prot	35570 (78.33%)	39375 (81.99%)
eggNOG	44547 (98.10%)	47673 (99.27%)
Nr	45356 (99.88%)	47961 (99.87%)

Table S8. Summary statistics of map to reference genome database.

Samples	Total Reads	Mapped reads	Uniquely mapped reads	Multiple mapped reads
B48aCK1	41248678	84.05%	74.30%	9.75%
B48aCK2	40891198	82.81%	73.19%	9.62%
B48aCK3	40765702	84.02%	74.26%	9.76%
B48bCK1	40989550	86.12%	75.89%	10.22%
B48bCK2	40399282	85.94%	75.84%	10.10%
B48bCK3	40522330	86.29%	76.10%	10.20%
B48aD14d1	40831144	85.70%	74.86%	10.84%
B48aD14d2	40798252	85.57%	74.97%	10.59%
B48aD14d3	40798442	85.56%	74.95%	10.61%
B48bD14d1	40873070	87.39%	77.21%	10.18%
B48bD14d2	40577820	87.06%	76.89%	10.18%
B48bD14d3	40528270	87.02%	77.02%	10.00%