

Development and characterisation of *Ogu* CMS root and leaf mustard

LIRONG ZHAO¹, QIAOFENG XIE¹, ZIYUE HONG¹, TAO CHEN¹,
ZHAOXIN HU², SHENGWU HU¹ 

¹College of Agronomy, Northwest A&F University, Yangling, Shaanxi, P.R. China

²Department of Electrical and Computer Engineering, University of California San Diego, La Jolla, USA

Electronic Supplementary Material (ESM)

The authors are fully responsible for both the content and the formal aspects of the electronic supplementary material. No editorial adjustments were made.

Table S1. One-step multiplex PCR primers (Zhao et al. 2010; Heng et al. 2017)

Primer name	Sequence(5'-3')	Target gene	Size of expected product (bp)
P11	GAAACGGGAAGTGACAAT	<i>Orf138</i>	465
P12	GCATTATTTTCTCGGTCCAT		
P21	AGCTGTCTGGAGGGAATC	<i>Orf222</i>	1 102
P22	GCGGTCTCACGCACTAATC		
P31	AGCTGTCTGGAGGGAATC	<i>Orf224</i>	747
P32	ACGACATCAAGGAGGAAC		
MSS26-L	GAGTAGTTTACGCGCTGCCT	<i>Orf288</i>	1 500
MSS26-R	TCATACCAAAGCAGAGAAAT		

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Table S2. Information of quantitative realtime polymerase chain reaction (qRT-PCR) primers

Gene	Forward sequence (5'-3')	Reverse sequence (5'-3')
<i>BnGAPDH</i>	CCGCTTCCTTCAACATCATT	GTCGCAGCTTTCTCGAGTCT
<i>BjuVA02G11440</i>	CGCTGTTGTACCATTTCATCATC	ATCTTTGTTTCCATAAGTGCCC
<i>BjuVB02G69760</i>	TCTTCCAGCAACCTTCTCCG	TGCTGATGCCAGAAAAGGAA
<i>BjuVB07G06320</i>	ACTCGTGCTGCAACAAGCAA	ATGATGATGATGACCATGATGG
<i>BjuVA04G24090</i>	GATGTCACCTGAATGAGCGC	AAGCTCACTCACTCTTCTCT
<i>BjuVA01G42740</i>	TAATCACTGGCTCCACTGGC	TCGTTCTTTAACCGCTGGAG

Table S3. Comparison of floral traits between the *Ogu* CMS line Bendatoujie and its maintainer of root mustard

Flower organ (mm)	<i>Ogu</i> -NWSUAF CMS line Bendatoujie	Maintainer Bendatoujie
Corolla diameter	11.33 ± 0.52**	14.65 ± 0.34
Pedicel length	9.49 ± 0.86	12.38 ± 0.69
Petal length	7.83 ± 0.39**	10.15 ± 0.14
Petal width	3.57 ± 0.28**	5.45 ± 0.15
Pistil length	7.48 ± 0.35**	9.87 ± 0.16
Length of the four strong stamens	5.49 ± 0.20**	7.93 ± 0.23
Length of two weak stamens	4.05 ± 0.46**	5.91 ± 0.10
Length of quadripartite anthers	1.56 ± 0.06	1.72 ± 0.06
Length of the two weak anthers	1.38 ± 0.12	1.36 ± 0.06

Data are presented as mean ± standard error (SE); **indicates a significant difference at the $P < 0.01$ level by student's *t*-test (two-tailed), $n = 50$

Table S4. Quality statistics of reads

Samples	Clean reads	Clean data (bp)	Q20	Q30	Total mapping ratio	Uniquely mapping ratio	Filtered GC content
						(%)	
CMS-1	19 904 674	5 944 282 963	99.67	98.69	91.43	88.70	45.75
CMS-2	21 209 200	6 331 834 804	99.65	98.61	90.99	88.28	45.72
CMS-3	20 162 701	6 017 714 974	99.62	98.48	90.00	87.09	45.85
Average	20 425 525	6 097 944 247	99.65	98.59	91.81	88.02	45.78
F-1	21 633 659	6 463 469 019	99.66	98.65	91.77	89.18	45.73
F-2	21 049 507	6 286 492 480	99.66	98.67	92.03	89.47	45.72
F-3	20 016 362	5 981 318 680	99.66	98.65	91.68	89.10	45.81
Average	20 899 843	6 243 760 060	99.66	98.66	91.83	89.25	45.75

Clean reads – No. of high-quality reads; clean data – total bases of high-quality data after filtering; Q20 – No. and percentage of bases with quality score $\geq$ Q20 (relative to raw data or clean data); Q30 – No. and percentage of bases with quality score $\geq$ Q30 (relative to raw data or clean data); total mapped ratio – No. and percentage of reads mapped to the genome (relative to clean reads); uniquely mapped – No. and percentage of reads uniquely mapped to the reference genome (relative to clean reads); filtered GC content (%) – GC content of filtered sequences



Figure S1. Leaf mustard and root mustard of newly bred *Ogu* CMS lines and their corresponding maintainer lines: Hengkoujie (A), *Ogu* CMS Hengkoujie (a), Zhuyuanjie (B), *Ogu* CMS Zhuyuanjie (b), Minxianjie (C), *Ogu* CMS Mianxianjie (c), Bendatoujie (D), *Ogu* CMS Bendatoujie (d)



Figure S2. Phenotypic observation of newly bred leaf mustard and root mustard of the *Ogu* CMS: *Ogu* CMS Hengkoujie (23B35) (A), *Ogu* CMS Zhuyuanjie (23B38) (B), *Ogu* CMS Mianxianjie (23B45) (C), *Ogu* CMS Bendatoujie (23B41) (D) Scale bar = 5 cm

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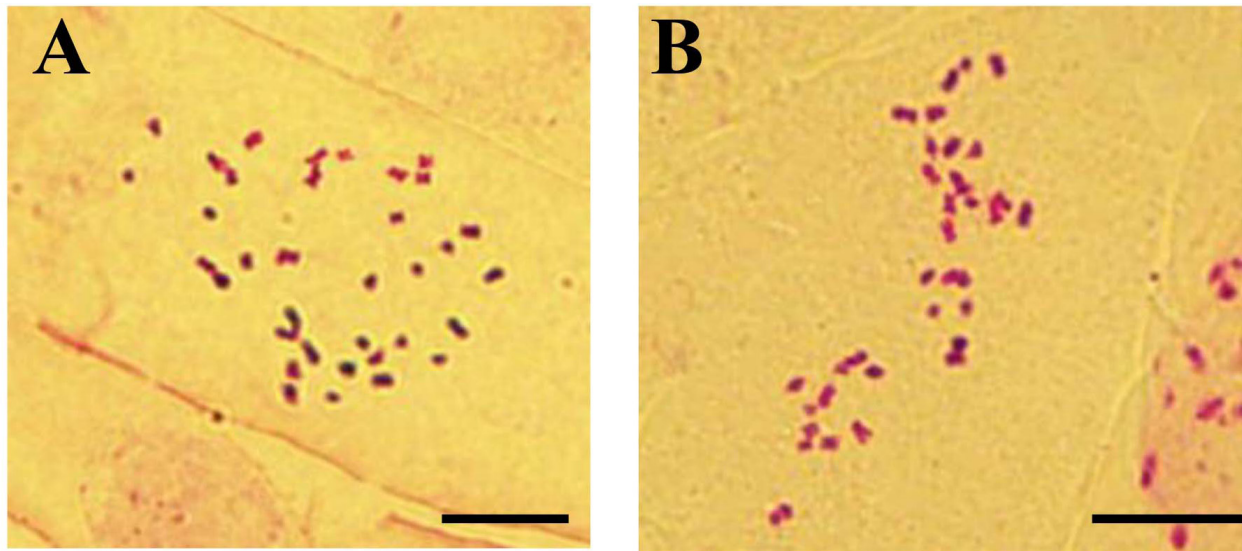


Figure S3. Observation of somatic chromosome numbers in *Ogu* CMS Bendatoujie and its maintainer line Bendatoujie: CMS line (23B41) (A), maintainer (23B43) (B)
Scale bar = 10 μ m

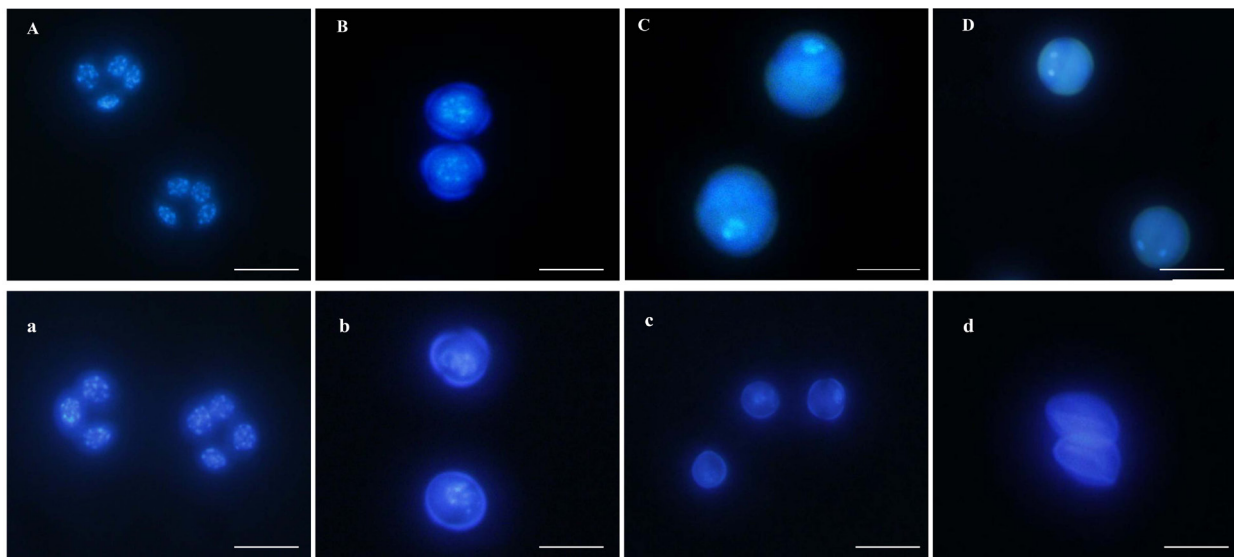


Figure S4. 4',6-diamidino-2-phenylindole (DAPI) staining observation of anthers at different developmental stages in *Ogu* CMS Bendatoujie and its maintainer line Bendatoujie (root mustard): Bendatoujie (A–D), *Ogu* CMS Bendatoujie (a–d), tetrad stage (A, a), early mononuclear stage (B, b), vacuolated microspore stage (C, c), binuclear stage (D), nuclear disappearance and cytoplasmic disintegration (d)
Scale bar = 20 μ m

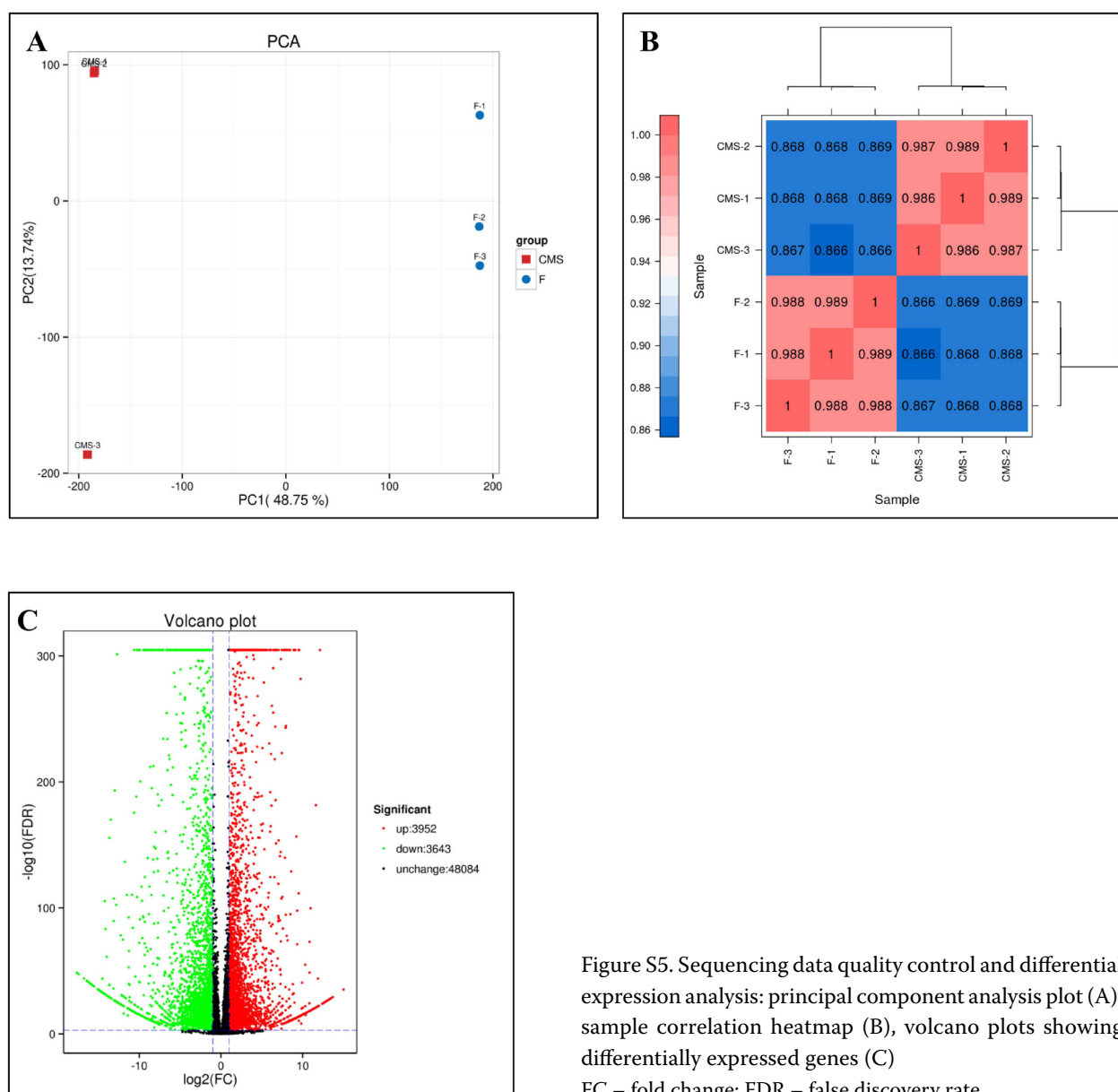


Figure S5. Sequencing data quality control and differential expression analysis: principal component analysis plot (A), sample correlation heatmap (B), volcano plots showing differentially expressed genes (C)

FC – fold change; FDR – false discovery rate

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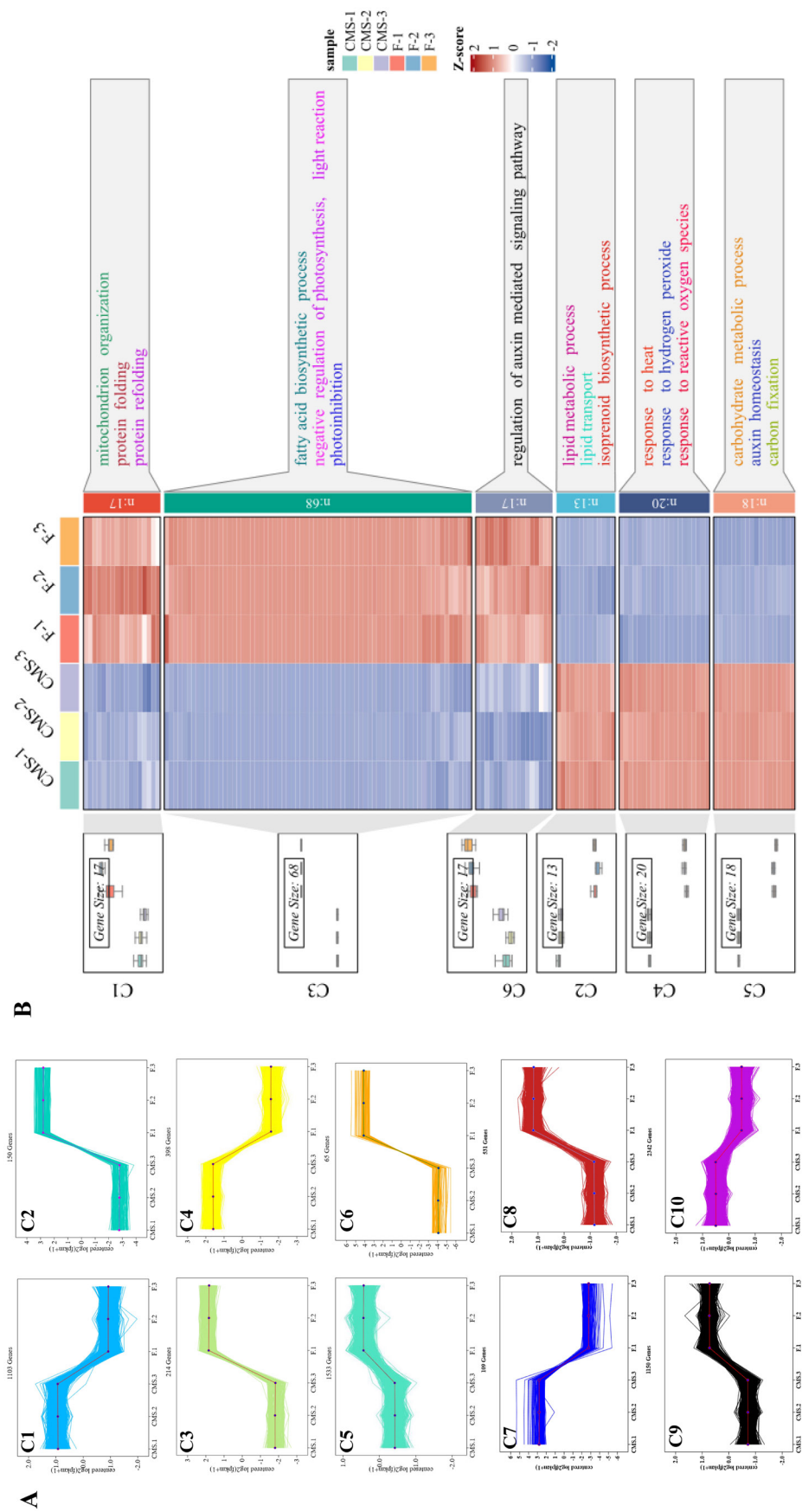


Figure S6. K-means clustering of differentially expressed genes (DEGs) between the *Ogu* CMS Bendatouje and its maintainer line: expression trends of fragments per kilobase of transcript per million mapped reads (FPKM) values of DEGs in clusters C1–C10 (A), top three GO terms enriched in the six clusters (B)

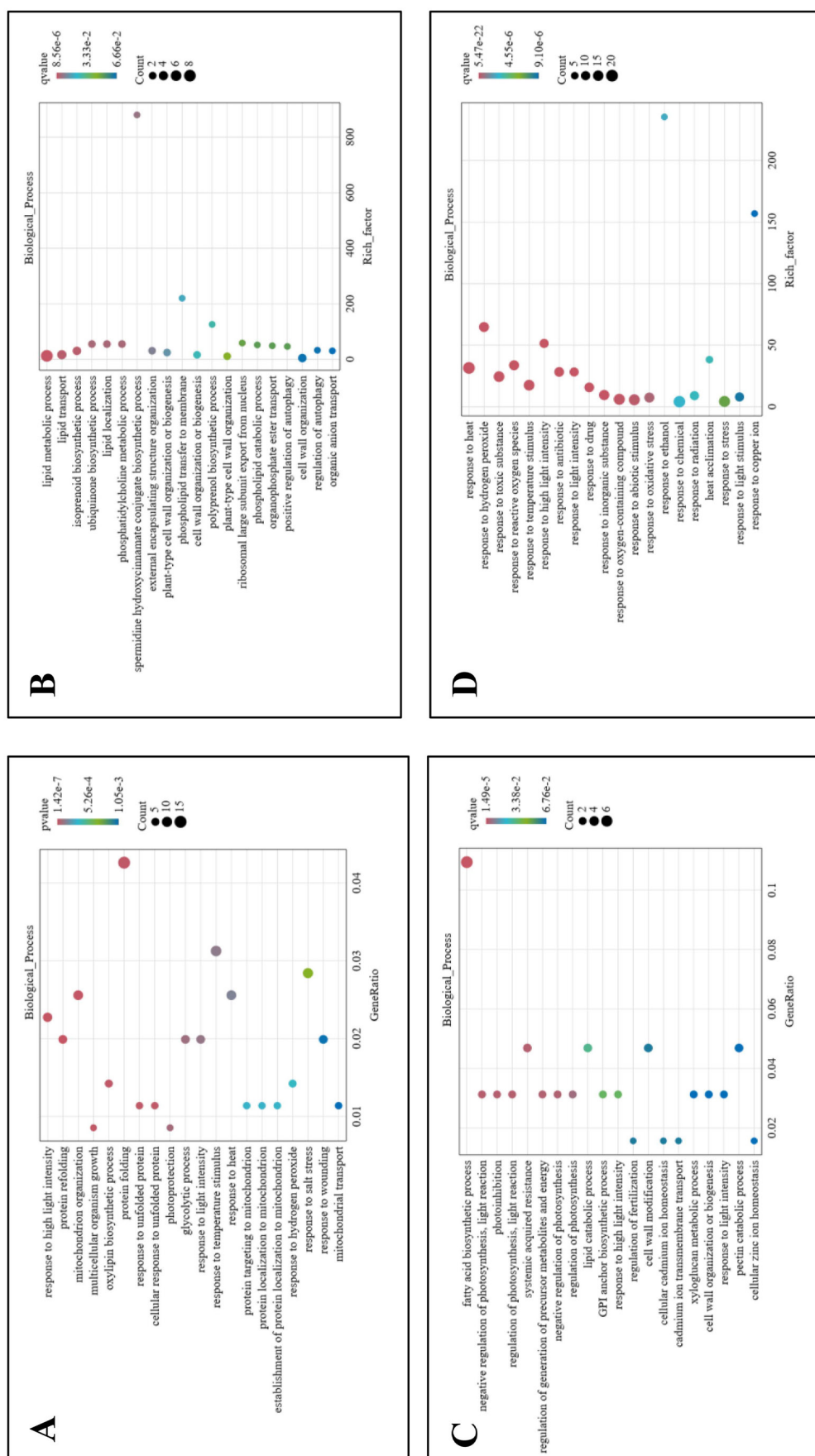


Figure S7. Gene ontology (GO) enrichment analysis of differentially expressed genes in Clusters C1-C4 between *Ogu* CMS and its maintainer line of mustard: Cluster C1 (A), Cluster C2 (B), Cluster C3 (C), Cluster C4(D)

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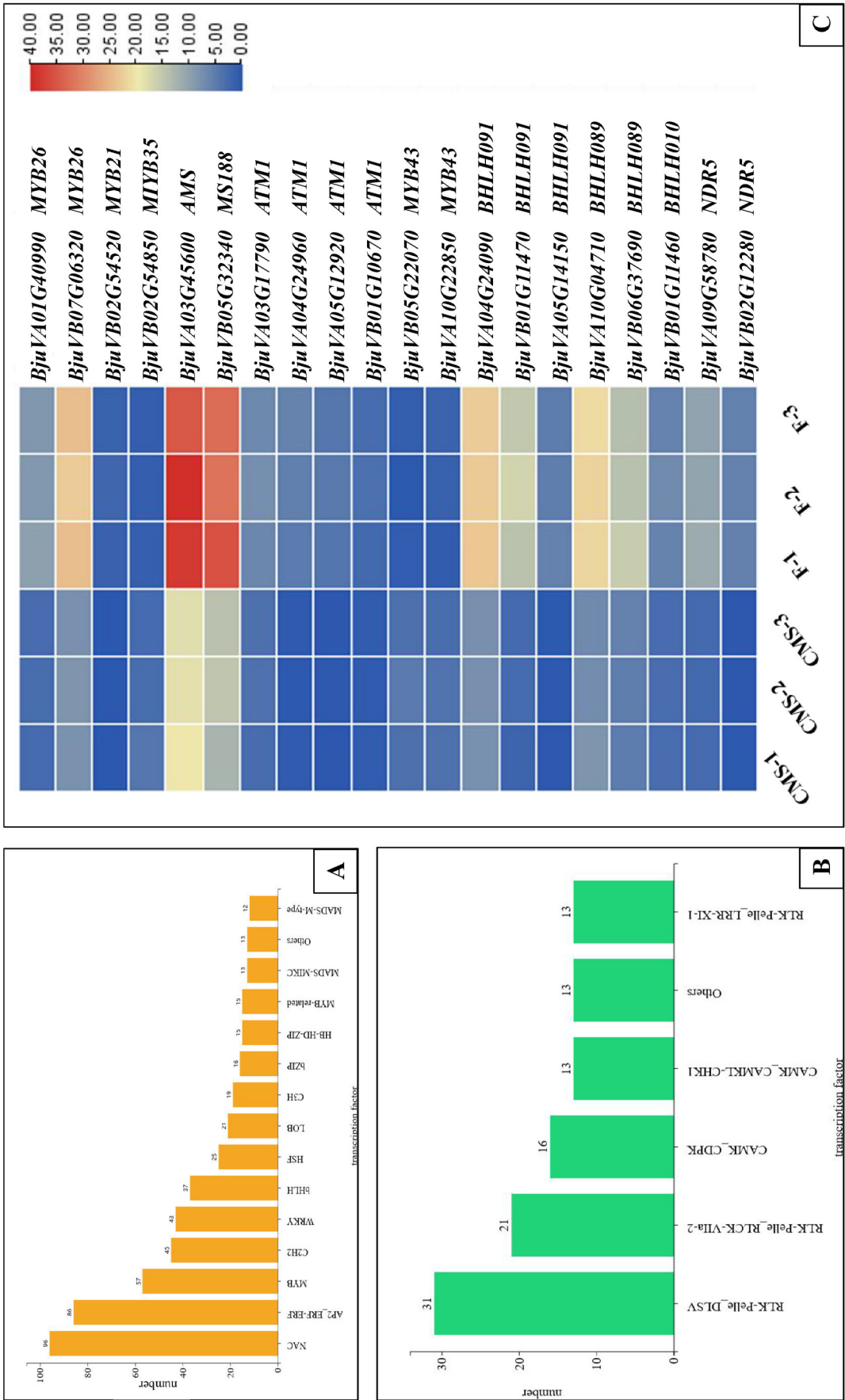


Figure S8. Identified transcription factors (TFs) and protein kinase from differentially expressed genes: TFs (A), protein kinases (B), expression heatmap of the selected TFs and protein kinases (C)

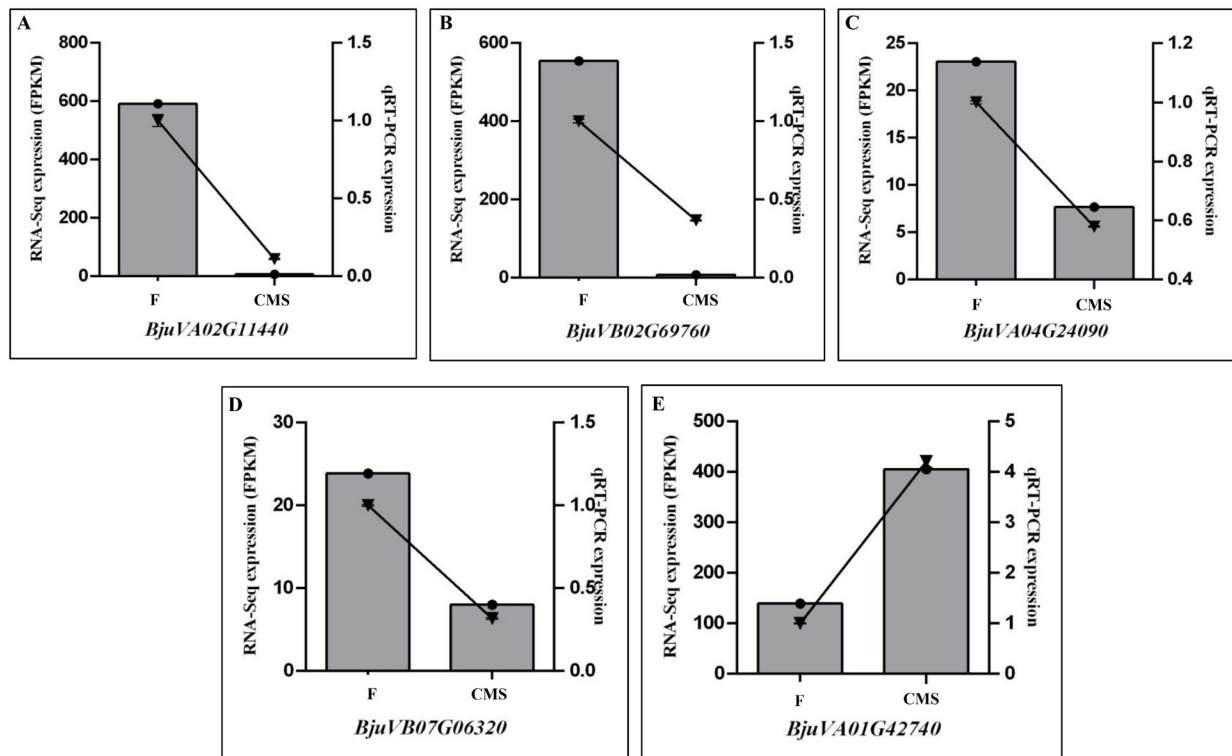


Figure S9. Comparison of quantitative realtime PCR (qRT-PCR) and ribonucleic acid sequencing (RNA-seq) results for differentially expressed genes (DEGs) in buds of *Brassica juncea* between fertile (F) and cytoplasmic male sterility (CMS) samples

FPKM-fragments per kilobase of transcript per million mapped reads; the bar and line graphs showed the values of RNA-seq and qRT-PCR, respectively

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