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*Functional Plant Biology*

### Supplementary Material

**Investigating the effect of drought stress and methanol spraying on the influential genes in the Calvin cycle and photorespiration of rapeseed (*Brassica napus*)**

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**Table S1** - The names and accession numbers of the studied enzymes in 4 members of the Brassicaceae family, including (At) *Arabidopsis thaliana*, (Bn) *Brassica napus*, (Bol) *Brassica oleracea*, and (Bra) *Brassica rapa*.

<b>Enzyme</b>	<b>Accession Number</b>	<b>Enzyme</b>	<b>Accession Number</b>
BnaFTRA2	XP_022554282.1	AtGLYKX2	NP_565237.1
AtFTRA2	NP_568195.1	AtGLYKX3	NP_849912.1
BraFTRA2	XP_009130113.1	BnaPRKX1	XP_009114920.1
BolFTRA2	XP_013620455.1	BraPRK	XP_009114920.1
BnaFTRC	XP_009139963.1	AtPRK	NP_174486.1
BraFTRC	XP_009139963.1	BolPRK	XP_013587115.1
AtFTRC	NP_001323778.1	BnaPRKX2	XP_013731477.1
BolFTRC	XP_013632155.1	BnaNADP-ME1	XP_013652717.1
BnaFBPase1	NP_001302992.1	BraNADP-ME1	XP_009102128.1
BraFBPase1	XP_009139266.2	AtNADP-ME1	NP_179580.1
BolFBPase1	XP_013636488.1	BolNADP-ME1	XP_013596642.1
AtFBPase1	NP_001190083.1	BnaNADP-ME2	XP_013726148.1
BnaFBPase	NP_001302604.1	BolNADP-ME2	XP_013628976.1
BraFBPase	XP_033147680.1	BraNADP-ME2	XP_009131215.2
BolFBPase	XP_013605351.1	AtNADP-ME2	NP_196728.1
AtFBPase	NP_175032.1	BnaNADP-ME3	XP_009151107.1
BnaGLYKX*1	XP_013694489.1	BraNADP-ME3	XP_009151107.1
BraGLYKX1	XP_009128426.1	BolNADP-ME3	XP_013593576.1
BolGLYKX1	XP_013614005.1	AtNADP-ME3	NP_197960.1
AtGLYKX1	NP_849912.1	BnaNADP-ME4	XP_013648762.1
BnaGLYKX2	XP_013694494.1	BolNADP-ME4	XP_013592154.1
BraGLYKX2	XP_009128428.1	AtNADP-ME4	NP_178093.1
BolGLYKX2	XP_013614014.1	BraNADP-ME4	XP_033132011.1

\*X represents the isoform number

**Table S2** - Specifications of primers used in Real-time PCR reaction

Gene	Isoform	Primer sequence	Melt (°C)	PCR production	NCBI accession number
<i>Actin-F</i> <i>Actin-R</i>		5'-TCCCGAGTATTGTTGGTCGT-3' 5'-TCCATGTCATCCCAGTTGCT-3'	54	157	AF111812
<i>FTR-F</i> <i>FTR-R</i>	<i>FTRC</i>	5'-CCTCTGTCGTCATCAAGGGT-3' 5'-GGCACTCCTTCCTCTCTCTC-3'	58	150	XM_013887810.2
<i>FBPase-F</i> <i>FBPase-R</i>	<i>FBPase1</i>	5'-CAGCCACGAATCCTTTTCCC-3' 5'-TCTCCTGTCTCAGCAACCAG-3'	57	184	NM_001316063.1
<i>PRK-F</i> <i>PRK-R</i>	<i>PRKX *1</i>	5'-TGTGGAGATGGATGGGCAAT-3' 5'-AAGACCAGTTCCGTTGTTGC-3'	57	151	XM_013876015.2
<i>GlyK-F</i> <i>GlyK-R</i>	<i>GLYKX2</i>	5'-ACAATCGAAGCCCTGACTCA-3' 5'-ACCAAGCATCCATCCCTCAA-3'	57	165	XM_013839040.2
<i>ME-F</i> <i>ME-R</i>	<i>NADP- ME4</i>	5'-GCCCATTTGCACCTGTAGAG-3' 5'-TCATCCCCTTCTCGTAGTGC-3'	57	192	XM_013793308.2

\*X represents the variant number

**Table S3** - Information about the motifs found in the FTR sequence.

Motif	E-value	Site	width	Sequence
MEME1	5.9e-122	4	50	CPCRHYDDKAAEVGQGFWNCPCVPM RERKECHCMLFLTPDNDFAGKDQTI
MEME2	6.3e-122	4	50	EPSEKSVEIMRKFFSEQYARRSGTYFCVD KGVTSVVIKGLAEHKDSYGAPL
MEME3	6.5e-075	4	50	VYHVNRVPEVELEGMEGKJKDYVVLW KGKRISANLPYKVZFFKEIEGRGP
MEME4	1.9e-019	7	14	FNAHLKSDEFEFID
MEME5	7.4e-014	4	11	SDEIKETTAHM

**Table S4** - Information about the motifs found in the FBPase sequence.

<b>Motif</b>	<b>E-value</b>	<b>Site</b>	<b>width</b>	<b>Sequence</b>
MEME1	8.5e-236	8	50	RYIGSLVGDVHRTLTYGGIYGYPADKKS PNGKLRLLYEVAPMSFJVEQAG
MEME2	1.3e-228	8	50	FTLDPSLGEFILTHPBIEIPKKGNIYSVNE GNYQNWGDPLTKYIEDLKDP
MEME3	3.6e-201	8	50	CKFIASLVNKAGJAKLTGLAGAVNIQGE EQKKLDVLSNEVFVNALVSSGR
MEME4	2.0e-193	8	50	SEEEEEPVAVEESYSGKYVVVFDPLDGS SNIDAGVSTGSIFGIYSPNDED
MEME5	1.3e-117	8	29	PTTVBVLKPGNELLAAGYCMYGSSVILVL

**Table S5** - Information about the motifs found in the PRK sequence.

<b>Motif</b>	<b>E-value</b>	<b>Site</b>	<b>width</b>	<b>Sequence</b>
MEME1	6.3e-155	5	50	MKEGVKYFSPVYLFDEGSTISWIPCGRK LTCSYPGIKFNYQPDSYFDNEV
MEME2	1.1e-149	5	50	QPPKILVIEGLHPMFDERVRDLLDFSIYLI DISNEVKFAWKIQRDMAERGH
MEME3	2.5e-147	5	50	MDGQFDRLDELIYVESHLNLSLTKFYGEV TQQMLKHADFPGSNNGTGLFQ
MEME4	2.3e-143	5	50	GCGKSTFMRRLTSVFGGAAEPPKGGNPD SNIDAGVSTGSIFGIYSPNDED
MEME5	5.6e-132	5	50	GRKEKGVTDPRANDFDLMYEQVKA LKSGIAVEKPIYNHVTGLLDAPEL

**Table S6** - Information about the motifs found in the GlyK sequence.

<b>Motif</b>	<b>E-value</b>	<b>Site</b>	<b>width</b>	<b>Sequence</b>
MEME1	4.4e-367	9	50	QSIDKWLLYGSQLCRLFQLNELMLTIPQ KARLYHYYIPVFWWCQDQIALH
MEME2	1.9e-358	9	50	VDPQLETVNKNLEAYYEAWDKYINAWV VIKIKDPSYVYRWRLQAEIAMRQ
MEME3	2.3e-328	9	50	GMSDDEVNDFVSRYLPAYKAYLPTLYA EGPSGSDLERVLAIIDEERNPI
MEME4	4.8e-328	9	50	GMKMKVPRYDKSAYSGRGDRADATTW PEVEGPLKVILFEGWMLGFKPLPA
MEME5	3.3e-278	9	50	KFKDGDVPLVIGFSAPQGCGKTTLV FALDYLFKTTKMKSATISIDDFY

**Table S7** - Information about the motifs found in the ME sequence.

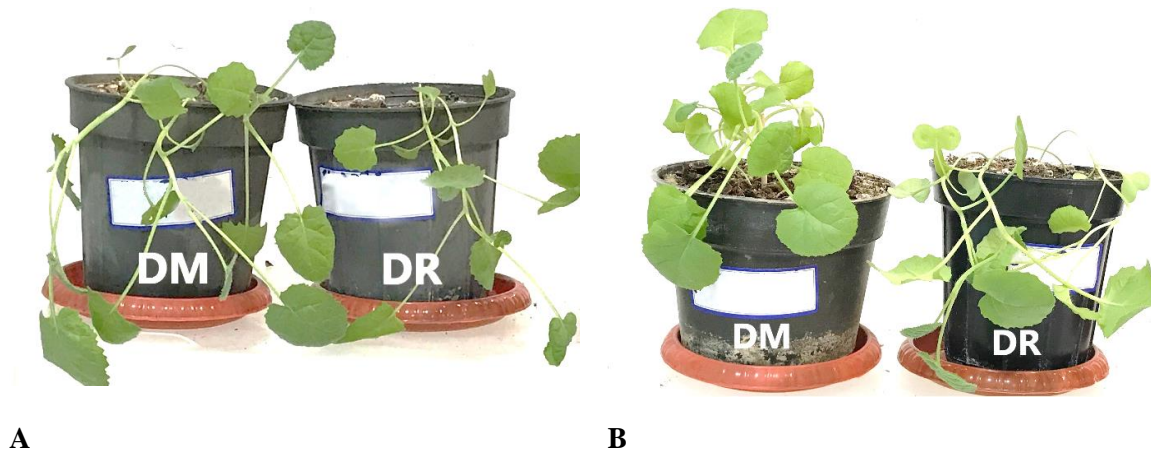
<b>Motif</b>	<b>E-value</b>	<b>Site</b>	<b>width</b>	<b>Sequence</b>
MEME1	1.4e-670	16	50	NWPZRNIQVIVVTDGERILGLGLGCGQ MGIPVGKLSLYTALGGVRPSAC
MEME2	1.6e-669	16	50	HEFMCAVKQNYGEKVLVQFEDFANHNA FDLLAKYGDShLVFNDDIQGTAS
MEME3	1.0e-661	16	50	YMALMDLQERNERLFYKLLIDNVEELLP VVYTPVGEACQKYGSIFRRPQ
MEME4	4.6e-647	16	50	NPTSQAECTAEZAYTWTKGRAIFASGSPF DPVEYEGKTFVPGQANNAYIF
MEME5	1.4e-626	16	50	RKKIWLVDKGLIVSSRKESLQHFKKPW AHEHEPVKELLGAVKAIKPTVL

**Table S8.** Identified microRNAs affecting *FTRC*, *FBPase1*, *PRKX1*, *GlyKX2*, *ME4* genes using psRNATarget software with the expected value 5.

miRNA_Acc.	Target_Acc.	Expectation	Target_start	Target_end	Inhibition	Target_Desc.
ath-miR1886.3	NM_001316063.1	4	1307	1327	Cleavage	FBPase1
aly-miR169b-3p	NM_001316063.1	4.5	700	721	Translation	FBPase1
aly-miR403a-5p	NM_001316063.1	4.5	1235	1256	Translation	FBPase1
aly-miR403b-5p	NM_001316063.1	4.5	1235	1256	Translation	FBPase1
aly-miR853-5p	NM_001316063.1	4.5	105	126	Translation	FBPase1
ath-miR169b-3p	NM_001316063.1	4.5	700	721	Translation	FBPase1
ath-miR1886.1	NM_001316063.1	4.5	34	53	Translation	FBPase1
aly-miR161-5p.1	NM_001316063.1	5	533	553	Cleavage	FBPase1
ath-miR5012	NM_001316063.1	5	111	131	Cleavage	FBPase1
ath-miR5020a	NM_001316063.1	5	30	50	Cleavage	FBPase1
ath-miR5021	NM_001316063.1	5	35	53	Cleavage	FBPase1
ath-miR5638b	NM_001316063.1	5	717	737	Translation	FBPase1
aly-miR838-3p	XM_013887810.2	3.5	106	126	Cleavage	FTRC
aly-miR3448-3p	XM_013887810.2	5	58	79	Cleavage	FTRC
ath-miR838	XM_013887810.2	5	106	126	Translation	FTRC
ath-miR1886.2	XM_013839040.2	2.5	123	143	Cleavage	GlyK
ath-miR5647	XM_013839040.2	4	60	80	Cleavage	GlyK
ath-miR851-3p	XM_013839040.2	4	605	625	Cleavage	GlyK
aly-miR172e-3p	XM_013839040.2	4.5	60	79	Cleavage	GlyK
aly-miR4249	XM_013839040.2	4.5	420	440	Cleavage	GlyK
aly-miR156a-3p	XM_013839040.2	5	1228	1249	Translation	GlyK
aly-miR167a-3p	XM_013839040.2	5	1115	1135	Cleavage	GlyK
aly-miR172a-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
aly-miR172b-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
aly-miR172c-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
aly-miR172d-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
aly-miR172f-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
aly-miR837-5p	XM_013839040.2	5	864	884	Cleavage	GlyK
aly-miR847-5p	XM_013839040.2	5	281	301	Cleavage	GlyK
ath-miR156a-3p	XM_013839040.2	5	1228	1249	Translation	GlyK
ath-miR167a-3p	XM_013839040.2	5	1115	1135	Cleavage	GlyK
ath-miR172a	XM_013839040.2	5	60	80	Cleavage	GlyK
ath-miR172b-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
ath-miR172c	XM_013839040.2	5	60	80	Cleavage	GlyK
ath-miR172d-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
ath-miR172e-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
ath-miR4239	XM_013839040.2	5	1225	1245	Cleavage	GlyK
ath-miR5665	XM_013839040.2	5	1503	1523	Cleavage	GlyK
ath-miR780.2	XM_013839040.2	5	1382	1402	Translation	GlyK
bna-miR172a	XM_013839040.2	5	60	80	Cleavage	GlyK
bna-miR172b	XM_013839040.2	5	60	80	Cleavage	GlyK
bna-miR172c	XM_013839040.2	5	60	80	Cleavage	GlyK
bna-miR172d	XM_013839040.2	5	60	80	Cleavage	GlyK
bol-miR172a	XM_013839040.2	5	60	80	Cleavage	GlyK
bol-miR172b	XM_013839040.2	5	60	80	Cleavage	GlyK
bol-miR9409	XM_013839040.2	5	446	467	Cleavage	GlyK
bra-miR172a	XM_013839040.2	5	60	80	Cleavage	GlyK
bra-miR172b-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
bra-miR172c-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
bra-miR172d-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
bra-miR408-3p	XM_013839040.2	5	1147	1167	Translation	GlyK

cas-miR167a	XM_013839040.2	5	1115	1135	Cleavage	GlyK
cas-miR172a-3p	XM_013839040.2	5	60	80	Cleavage	GlyK
cas-miR172b	XM_013839040.2	5	60	80	Cleavage	GlyK
ath-miR447a-3p	XM_013793308.2	3	1224	1245	Cleavage	ME4
ath-miR447b	XM_013793308.2	3	1224	1245	Cleavage	ME4
ath-miR8179	XM_013793308.2	3.5	1536	1556	Cleavage	ME4
aly-miR156d-3p	XM_013793308.2	4	52	72	Cleavage	ME4
aly-miR156e-3p	XM_013793308.2	4	52	72	Cleavage	ME4
ath-miR870-3p	XM_013793308.2	4	1395	1415	Cleavage	ME4
bra-miR156a-3p	XM_013793308.2	4	52	72	Cleavage	ME4
cas-miR156d-3p	XM_013793308.2	4	52	72	Cleavage	ME4
cas-miR156e-3p	XM_013793308.2	4	52	72	Cleavage	ME4
aly-miR167a-5p	XM_013793308.2	4.5	1874	1894	Cleavage	ME4
aly-miR167b-5p	XM_013793308.2	4.5	1874	1894	Cleavage	ME4
aly-miR167d-5p	XM_013793308.2	4.5	1873	1894	Cleavage	ME4
aly-miR4244	XM_013793308.2	4.5	673	693	Translation	ME4
ath-miR156d-3p	XM_013793308.2	4.5	50	72	Cleavage	ME4
ath-miR167a-5p	XM_013793308.2	4.5	1874	1894	Cleavage	ME4
ath-miR167b	XM_013793308.2	4.5	1874	1894	Cleavage	ME4
ath-miR167d	XM_013793308.2	4.5	1873	1894	Cleavage	ME4
ath-miR779.1	XM_013793308.2	4.5	1130	1149	Cleavage	ME4
ath-miR837-5p	XM_013793308.2	4.5	682	702	Cleavage	ME4
bna-miR167a	XM_013793308.2	4.5	1873	1894	Cleavage	ME4
bna-miR167b	XM_013793308.2	4.5	1873	1894	Cleavage	ME4
bna-miR167c	XM_013793308.2	4.5	1874	1894	Cleavage	ME4
bna-miR167d	XM_013793308.2	4.5	1875	1894	Cleavage	ME4
bra-miR167a	XM_013793308.2	4.5	1874	1894	Cleavage	ME4
bra-miR167b	XM_013793308.2	4.5	1874	1894	Cleavage	ME4
bra-miR167c	XM_013793308.2	4.5	1874	1894	Cleavage	ME4
bra-miR167d	XM_013793308.2	4.5	1874	1894	Cleavage	ME4
bra-miR5723	XM_013793308.2	4.5	775	795	Translation	ME4
aly-miR156f-3p	XM_013793308.2	5	52	72	Cleavage	ME4
aly-miR169j-3p	XM_013793308.2	5	2022	2040	Translation	ME4
aly-miR169k-3p	XM_013793308.2	5	2022	2040	Translation	ME4
aly-miR169l-3p	XM_013793308.2	5	2022	2040	Translation	ME4
aly-miR169n-3p	XM_013793308.2	5	2022	2040	Translation	ME4
aly-miR858-5p	XM_013793308.2	5	1026	1046	Translation	ME4
aly-miR862-5p	XM_013793308.2	5	1274	1294	Cleavage	ME4
ath-miR3932b-5p	XM_013793308.2	5	710	730	Cleavage	ME4
ath-miR825	XM_013793308.2	5	2330	2350	Cleavage	ME4
ath-miR858a	XM_013793308.2	5	1026	1046	Translation	ME4
bra-miR156b-3p	XM_013793308.2	5	52	72	Cleavage	ME4
bra-miR156d-3p	XM_013793308.2	5	52	72	Cleavage	ME4
cas-miR169e-3p	XM_013793308.2	5	2022	2040	Translation	ME4
cas-miR858	XM_013793308.2	5	1026	1046	Translation	ME4
ath-miR5021	XM_013876015.2	3.5	1382	1401	Cleavage	PRK
ath-miR5014a-3p	XM_013876015.2	4	1516	1536	Cleavage	PRK
aly-miR838-3p	XM_013876015.2	4.5	104	124	Cleavage	PRK
ath-miR8170-3p	XM_013876015.2	4.5	1491	1511	Cleavage	PRK
bra-miR161-3p	XM_013876015.2	4.5	913	933	Cleavage	PRK
bra-miR9558-5p	XM_013876015.2	4.5	1263	1283	Cleavage	PRK
cas-miR159c-5	XM_013876015.2	4.5	197	217	Cleavage	PRK
aly-miR837-5p	XM_013876015.2	5	128	148	Cleavage	PRK
ath-miR1886.1	XM_013876015.2	5	1381	1401	Translation	PRK

ath-miR5632-3p	XM_013876015.2	5	247	267	Translation	PRK
ath-miR5632-5p	XM_013876015.2	5	670	690	Cleavage	PRK
ath-miR5653	XM_013876015.2	5	166	192	Cleavage	PRK
ath-miR780.2	XM_013876015.2	5	1414	1434	Cleavage	PRK
ath-miR8184	XM_013876015.2	5	719	739	Cleavage	PRK
ath-miR829-5p	XM_013876015.2	5	543	563	Cleavage	PRK
bra-miR158-5p	XM_013876015.2	5	894	914	Cleavage	PRK



**Fig. S1.** The treated plants were separated into two groups: Methanol-sprayed (DM) plants which sprayed with methanol solution 20% v/v after 72-hour drought stress and drought-stressed plants (DR) that were exposed to drought stress for 72 hours (30% of field capacity) **A.** Hyola308 (drought-sensitive) genotype: DM and DR plants. **B.** SLM046 (drought-tolerant) genotype: DM and DR plants.