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Regular paper

Barley primary microRNA expression pattern is affected by soil water availability

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MicroRNAs are short molecules of 21–24 nt in length. They are present in all eukaryotic organisms and regulate gene expression by guiding posttranscriptional silencing of mRNAs. In plants, they are key players in signal transduction, growth and development, and in response to abiotic and biotic stresses. Barley (*Hordeum vulgare*) is an economically important monocotyledonous crop plant. Drought is the world's main cause of loss in cereal production. We have constructed a high-throughput Real-Time RT-qPCR platform for parallel determination of 159 barley primary microRNAs' levels. The platform was tested for two drought-and-rehydration-treated barley genotypes (Rolap and Sebastian). We have determined changes in the expression of primary microRNAs responding to mild drought, severe drought, and rehydration. Based on the results obtained, we conclude that alteration in the primary microRNA expression is relative to the stress's intensity. Mild drought and rehydration mostly decrease the pri-miRNA levels in both of the tested genotypes. Severe drought mainly induces the primary microRNA expression. The main difference between the genotypes tested was a much-stronger induction of pri-miRNAs in Rolap encountering severe drought. The primary microRNAs respond dynamically to mild drought, severe drought, and rehydration treatments. We propose that some of the individual pri-miRNAs could be used as drought stress or rehydration markers. The usage of the platform in biotechnology is also postulated.

Key words: pri-miRNA, miRNA, drought, rehydration, barley genotypes

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Abbreviations: DH, doubled haploid; RNA-seq, RNA sequencing

S1 Cycling conditions for PCR and RT-qPCR

The conditions for assessing the cDNA purity (a control reaction thermal profile) were used as follows -1 cycle: denaturation at 94°C/1 min, annealing at 65°C/30 s, elongation at 72°C/2 min; 29 cycles: denaturation at 94°C/30 s, annealing at 63°C/30 s (Δ -0.5°C/cycle), elongation at 72°C/2 min; 10 cycles: denaturation at 94°C/30 s, annealing at 53°C/30 s, elongation 72°C/2 min.

The RT-qPCR thermal profile parameters were used as follows: 10 min at 90°C, 40 cycles of 15 s at 95°C and 1 min at 60°C.

Table S1. Mature miRNA sequences imbedded within pri-miRNAs.

pri-miRNA	miRNA sequence
171	TGATTGAGCCGTGCCAATATC
393	TTCCAAAGGGATCGCATTGAT
394	TTGGCATTCTGTCCACCTCCT
528	TGGAAGGGGCATGCAGAGGAG
530	TGCATTTGCACCTGCACCTAC
827	TTAGATGACCATCAGCAAACA
1120	ACATTCTTATATTATGGGACGGAG
1135	ATGCGACAAGTAATTTCGAACGGA
5052	ACCGGCTGGACGGTAGGCATA
5053	CGCAGCTGTAGTCGCCGGCGT
5071	TCAAGCATCATGTCGTGGACA
5168	TCGGACCAGGCTTCAATCCCT
5181	ACTTATTTTGGAACGGAGGGA
6176	GAAGCTGTAGTGCAGCCGGCGTT
6177	TACCATGGACAGAAGGCACTTA
6179	AACCAGTCGAGGCCAGGGGGTT
6182	TGAGTGTGTGATGGATGGCTTT
6183	TGAGCGAGTTGGCTGCAAGTTC
6196	AGGACGAGGAGATGGAGAGGA
6199	CCACAGAATTCTCACAGTGATGG
6202	TGAAGATTTTAAGCATTGAA
6205	AGGATGTTTGGATACGTTTTAG
6206	GGCACACGGGCTGCAGGCATAG
6207	TGGACGACCTGGGCGCCGACG
6208	GTGCATCAAGATCGGCTCATCT
6210	ACTCCTTGGTTATCAACTTCGA
6211	CAGATCAAGACGCTCCGGCA
6212	ATACAGTTTACAATGCACGAT
1120b	TCCGTCCCATAATATAAGAGC
1130a	TCTGTAACTTAATATAAGACG
1130b	CTTATATTATGGAACGGAGGGAGT
1133a	TTTTCGGACAGAGGGAGTATA
1133b	TTTTCGGACAGAGGGAGTATA
1133c	TTTTGGGACGGAGGGAGTATA
1432a	TTCAGGAGAGATGACACCGAC
1432b	GGTGTCAGCTCGCCTGAAC
1436b	ATTATGGGACGGAGGGAGTAG
156b	TGACAGAAGAGAGTGAGCACA
156c	TGACAGAAGAGAGTGAGCACA
156d	TGACAGAAGAGAGTGAGCACA
159a	TTTGGATTGAAGGGAGCTCTG

159c	TTTGGATTGAAGGGAGCTCTG
159d	TTGGATTGAAGGGAGCTCT
160a	TGCCTGGCTCCCTGTATGCCA
160b	GTGCCTGGCTCCCTGTATGCC
160c	GTGCCTGGCTCCCTGTATGCC
160d	TGCCTGGCTCCCTGAATGCCA
160f	GTGCCTGGCTCCCTGTATGCC
164_A	TGGAGAAGCAGGGCACGTGCA
164a	TGGAGAAGCAGGGCACTTGC
164b	TGGAGAAGCAGGTCACGTGC
166a	TCGGACCAGGCTTCATTCCCC
166d	TCGGACCAGGCTTCATTCCTT
166e	TCGGACCAGGCTTCATTCCCC
166f	GGAATGTTGTCTGGTTCAAGG
166g	GAATGTTGTCTGGTTGGAGAC
166h	TCGGACCAGGCTTCATTCCCC
166i	TCGGACCAGGCTTCATTCCCC
166j	TCGGACCAGGCTTCATTCCCC
167a	TGAAGCTGCCAGCATGATCTA
167b	TGAAGCTGCCAGCATGATCTGA
167c	ATCGTGCTGTGACAGTTTCACT
167d	TGAAGCTGCCAGCATGATCTG
169a	CAGCCAAGGATGACTTGCCGA
169c	TAGCCAAGGATGACTTGCCTG
169d	TAGCCAAGGATGACTTGCCTG
169e	TAGCCAAGGATGACTTGCCGA
169f	TAGCCAAGGATGACTTGCCTA
169h	TAGCCAAGGATGACTTGCCGG
169m	TAGCCAAGGATGACTTGCCGA
171a	TGAGCCGAACCAATATCACTC
171b	TTGAGCCGTGCCAATATCACG
171c	TGATTGAGCCGCGCCAATATC
171d	TGATTGAGCCGTGCCAATATC
171e	TGATTGAGCCGTGCCAATATC
172b	GCAGCACCACCAAGATTCACA
172c	GGAATCTTGATGATGCTGCAT
1869a	TGAGAACAATAGGAATGGGAG
1869b	TGAGAACAATAGGAATGGGAG
1869c	TGAGAACAATAGGAATGGGAG
2118_A	TTCCCTATGCCTCCCATTCCTA
2118_B	TTCCTGATGCCTCCCATTCCTA
2118a	TTTCCGATGCCTCCCATTCCTA
2118b	TTCCTGATGCCTCCCATTCCTA
2118c	TTCCTGATGCCTCCCATGCCTA
2118d	TTCCTGATGCCTCCCATGCCTA

2118e	TTCCCAATGCCTCCCATTCTTA
2118f	TTCCCGTTGCCTCCCATTCCTA
2118g	TTCCCGATGCCTCCCATTCCTA
2118i	TTTCCAATGCCTCCCATTCCTA
2118j	TTCCCGATGCCTTCCATTCCTA
2118m	TTCCTGATGCCTCCCATTCCTA
2275a	TTTGTTTTTCTCCAATATCTC
2275b	TTCAGTTTCCTCTAATATCTCA
2275c	TTCAGTTTCCTCTAATATCTCA
2275d	TTTGTTTTTCTCCAATATCTC
2275e	AGGATTAGAGGGAACTGAACC
319b	CTTGGACTGAAGGGTGCTCCCT
319c	TTGGACTGAAGGGTGCTCCCT
393a	TCCAAAGGGATCGCATTGATC
395a	TGAAGTGTTTGGGGGAACTC
395b	TGAAGTGTTTGGGGGAACTC
395c	TGAAGTGTTTGGGGGAACTC
396a	TCCACAGGCTTTCTTGAACTG
396c	TTCCACAGCTTTCTTGAACTT
396d	TCCACAGGCTTTCTTGAACGG
396e	TCCACAGGCTTTCTTGAACGG
397b	ATCAACGCTGCACTCAACGGC
398a	TGTTTTCTCAGGTCACCCCTT
398f	TGTGTTCTCAGGTCGCCCCCG
399_B	TGCCAAAGGAGATTTGCCCAG
399a	TGCCAAAGGAGAATTGCCCTG
399b	TGCCAAAGGAGAATTGCCCTG
399c	TGCCAAAGGAGATTTGCCCCG
399d	TGCCAAAGGAGATTTGCCCCG
399e	TGCCAAAGGAGATTTGCCCCG
399i	TGCCAAAGGAGATTTGCCCCG
408a	CTGCACTGCCTCTTCCCTGGC
408b	CTGCACTGCCTCTTCCCTGCG
444_3	TGTTGTCTCAAGCTTGCTGCC
444a	TGCAGTTGCTGCCTCAAGCTT
444b	TGCAGTTGCTGTCTCAAGCTT
5048a	TATTTGCAGGTTTTAGGTCTAA
5049b	AGTATTTAGGTACAGAGGGAG
5049c	AGACAATTATTTTGGGACGGAGG
5049e	AATTATTTAGGTACAGAGGGA
5049g	TACAATTATTTAGGAACGGAG
5049h	CCTAAATACTTGTAGTTGGGG
5049i	AATTAATTTGGATCGGAGGGA
5049j	TACAATTATTTAGGAACGGAG
5067a	TGAGCGACAACTAATATGGAT

5067b	TGAGCGACAACTAATATGGAT
5067c	TGAGCGACAACTAATATGGAT
5067d	TCAGCGACAAGTAATATGGAT
6197a	TCTGTTCCTAAATGTAAGACG
6197b	TCTGTTCCTAAATGTAAGACG
6197c	TCTGTTCCTAAATGTAAGACG
6197d	TCTGTTCCTAAATGTAAGATG
6198a	GCTCTGTCTTGGATGGTCATTC
6198b	GCTCTGTCTTGGATGGTCATTC
6198c	GCTCTGTCTTGGATGGTCATTC
6198d	GCTCTGTCTTGGATGGTCATTC
9674b	TGAATTTGTCCATAGCATCAG

Table S2. Detailed pri-miRNA expression under mild drought, severe drought, and rehydration in two *Hordeum vulgare* genotypes (Rolap and Sebastian). RTqPCR results are shown as fold-change values. The levels of pri-miRNAs under the control conditions were assumed to be '1', and the levels of pri-miRNAs under stress conditions were quantified in relation to this standard. The up- and down-regulated pri-miRNAs were suggested by a two-tailed Student's *t*-test (***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$). Up, down-regulated pri-miRNAs are marked as red and blue, respectively (nd, not detected).

	Rolap			Sebastian		
pri-miRNA	mild drought	severe drought	rehydration	mild drought	severe drought	rehydration
171b	2.07*	4.13**	2.54**	2.83**	3.12**	1.01
166i	15.17***	21.88***	47.09***	0.54	0.71	0.98
160d	12.70***	19.92*	53.62***	0.80	1.54	1.13
156b	7.47**	11.88*	3.31***	nd	nd	nd
2275c	1.75*	4.83**	2.08*	1.49	14.74	1.34
1130a	2.41**	4.37**	3.25***	1.10	2.59	1.36
169f	2.17**	5.88*	1.29	1.59	3.38*	9.41
2275b	2.22**	5.68**	2.52	1.59	4.37	1.26
2275d	1.31*	5.20**	1.78	1.59	3.45	1.44
164b	2.72*	2.27*	6.49	1.43	2.25	0.82
156d	3.30**	29.62**	6.46	0.73	1.86	0.44***
6199	1.26**	1.98*	0.66***	0.86	2.06**	1.06
396e	1.06	7.46***	2.16***	1.11*	4.11	0.67
167b	0.85	3.84*	3.94**	1.40**	3.48***	1.28
399e	0.61	103.62**	32.38*	0.61	4.70*	8.46
160f	2.11	4.66**	2.45**	1.35	2.36*	0.88
5049e	1.10	2.09**	1.41***	1.13	1.51*	1.22
2118b	1.13	1.71***	1.61*	0.63	3.77	2.21*
395c	1.11	2.89***	3.70***	0.74	1.20	3.09
396a	1.06	2.43*	2.24***	1.14	2.42	1.36
395a	0.99	2.35**	2.06**	0.73	0.95	1.09
2118f	1.06	2.33***	3.01*	0.92	2.09	1.28
1869a	0.93	2.29**	2.97**	nd	2.30	nd
6182	1.55	2.29*	1.80*	1.07	1.37	1.12
156c	2.83	51.85**	15.40**	1.23	1.84	0.45**
393a	1.14	4.51**	1.46	2.54***	1.72	0.36**
2275a	3.31	5.84***	2.24	1.93	8.02*	1.55
395b	1.07	expressed only in drought	0.88	nd	1.07	1.16
399b	1.42	4.83***	13.95	0.81	1.44	2.40
397b	4.84	4.75**	1.79	4.39	1.20	0.84
1869b	1.78	3.31**	1.79	1.37	1.60	0.72
160b	1.28	2.34**	1.19	1.42	1.26	1.05
6197d	1.17	2.04*	1.78	nd	nd	0.96
6198d	1.26	1.49*	1.36	0.81	1.44	0.85
6177	1.07	1.40*	1.05	1.27	1.23	0.88
6198b	1.26	1.92*	1.35	1.26	1.36	0.64***
2275e	0.89	2.07*	0.63	0.71**	0.64	0.55
6183	1.14	1.76**	0.64*	0.58	1.03	0.41**
2118c	0.76	13.02**	0.23**	1.02	0.58	0.26*
171d	0.76	2.32***	0.50*	0.52**	0.85	3.97
5049c	0.83**	1.97**	2.15*	1.25***	1.72	1.40
5048a	0.90*	1.94***	2.94*	1.11	1.68***	1.90*

399d	0.40***	11.91*	17.18**	0.85	5.26	2.13
2118j	0.19***	1.87**	1.79	0.86	1.26	0.80
444_3	0.50*	3.62*	10.72	0.91	0.16***	0.09***
399c	0.48*	107.53**	39.62*	0.35**	3.62*	9.21
5049h	0.46*	1.13*	2.34*	0.32**	1.46	1.91
444a	0.65*	1.23*	2.88**	0.72***	0.79**	1.13
6179	1.09*	1.65	1.24*	0.59**	1.46	1.49*
5067a	2.25**	3.08	2.20***	0.57**	2.55	3.11
167c	1.56*	2.30	1.94	0.84	2.94**	1.49
6196	1.25*	1.38	1.41	2.70	3.76	2.71*
2118i	2.17*	9.89	0.98	1.63	1.75	0.67
319c	2.14***	2.40	1.85	nd	nd	nd
408b	1.62**	1.49	0.96	1.24	2.22	0.80
164a	1.32*	0.95	0.66**	1.15	1.79	0.61**
164_A	2.21	2.89	3.06*	3.27**	5.85	1.45*
5071	1.28	5.55	2.43**	1.03	2.99*	1.81
6202	0.98	1.91	1.45**	1.56	1.40***	1.10
166e	0.84	0.74	1.28***	0.93	1.88*	0.79
9674b	1.24	2.54	3.37***	1.00	1.47	3.42**
169h	1.44	2.53	2.05*	1.21	1.80	2.68***
169d	1.13	0.84	2.56*	0.63	0.84	1.44
2118e	0.96	1.63	2.17*	0.85	1.74	1.20
6198c	0.93	1.50	1.83***	1.00	1.04	0.74
2118g	3.46	1.49	3.22*	1.02	2.62	0.49*
169a	1.18	1.30	1.31*	3.62	6.54	0.03***
6197a	0.81	1.01	1.47**	0.38**	2.00*	1.73**
159c	0.81	1.16	2.44***	0.79**	0.84	1.92**
396d	0.72	1.33	3.56*	0.35***	1.52	1.26
6198a	1.12	1.56	1.49**	0.75**	1.15	0.68***
172b	1.60	2.33	2.40	1.99*	3.76*	1.68
5067b	0.82	1.06	1.26	0.88	1.32**	1.72*
5067c	1.01	1.78	2.25	0.96	1.28*	1.91
1133c	1.41	6.13	2.47	nd	4.46	nd
1869c	1.62	5.25	4.95	3.18	16.73	0.42
1133b	0.54	2.20	0.90	0.96	1.94	0.75
1120b	1.13	1.56	1.25	0.85	1.46	1.03
1133a	2.05	1.42	0.88	2.40	nd	nd
171e	0.86	1.38	0.66	1.01	1.43	0.61
1135	4.11	1.22	1.86	0.76	2.87	0.96
6197b	1.43	1.15	0.85	1.00	1.90	0.81
171a	1.79	0.61	0.72	nd	nd	nd
2118a	1.37	1.35	1.03	0.76	1.15	0.47*
444b	0.92	1.12	0.90	0.48	1.32	0.56*
5049j	1.03	1.31	1.44	0.79*	1.22	0.99
5049b	0.91	0.98	0.38***	0.99	1.59**	0.69*
2118d	1.26	nd	0.68**	0.85	9.48	0.52
166d	1.01	1.29	0.58**	0.76	0.88	0.67
5049i	0.99	1.83	0.54*	2.16	2.77	3.21
2118m	1.63	1.42	0.34*	1.24	0.93	1.15
166f	0.89	0.47	0.31***	2.63	0.93	1.87
5067d	1.43	1.39	0.69*	32.25	0.32*	2.22
5181	0.52***	1.11	2.16***	1.17	1.16**	1.19
159a	0.50***	2.58	1.88**	0.77	1.31	1.78*
1130b	0.63*	0.92	1.04	0.61**	1.36	1.38**
171	0.64***	1.53	1.17	0.45***	0.65**	0.68***

166a	0.10***	0.47	0.96	0.45***	0.27***	0.73*
827	0.60*	1.14	1.52	0.84	0.83	0.77
1436b	0.25***	0.61	2.00	0.95	0.93	1.80
167d	0.59**	0.87	0.80*	0.56**	0.67*	0.83
160c	0.39*	0.49	0.58*	0.29**	0.36*	0.48
169m	1.32*	0.35**	0.10***	0.89	1.20	0.36**
5049g	0.90	0.92*	2.35**	0.63	1.24	1.60
396c	0.39	0.09***	2.42*	0.20***	0.23**	1.39
6210	0.62	0.33***	2.29	nd	nd	nd
6205	0.93	0.61**	0.98	0.89	0.96	0.58***
6208	0.65	0.17**	0.73	1.18	0.28***	0.35***
394	0.87	0.52**	0.94	0.35***	0.82	1.09
399a	0.60	0.33**	1.00	0.41***	0.59**	0.67
171c	1.60	0.32**	0.99	0.27***	0.26**	1.10
166g	1.51	0.72**	0.50*	1.11	1.02	1.01
399_B	1.19	0.28**	0.89*	0.64	3.44	1.10
393	1.51	0.24***	0.30***	nd	nd	nd
159d	0.70	0.22***	0.20***	1.40	192.72	1.99
167a	1.38	0.19***	0.50**	0.66	1.95	1.57
169e	2.43	0.12***	0.14***	0.71	0.38*	0.35**
172c	1.30	0.11***	0.02***	1.08	0.13***	0.35**
6176	0.60	0.21***	0.51*	0.28**	0.26**	0.43**
398a	0.96	0.06***	0.10***	0.30***	0.11***	0.25***
6212	0.45***	0.72**	2.10***	0.96	1.02	1.60***
1120	0.63*	0.55*	1.81**	0.93	1.09	1.34*
408a	0.45***	0.37***	1.03	1.23	0.62	3.64*
166h	0.70*	0.54***	0.65	1.76	1.11	0.55
169c	0.75**	0.75**	3.65	nd	nd	nd
5053	0.29***	0.17***	3.34	0.95	0.80	0.73*
5168	0.53**	0.86**	1.31	1.08	0.52*	2.17*
166j	0.78***	0.70**	1.11	nd	0.89***	1.05
6207	0.45*	0.19***	1.01	1.67	0.13***	0.94
6211	0.13***	0.11***	0.61	1.21	0.34*	0.23***
530	0.06***	not expressed in drought	0.13	0.20**	nd	nd
5052	0.12***	0.57**	0.97	0.21***	0.38***	0.47**
1432a	0.06***	0.09***	0.36***	0.83	0.83	1.90
6206	0.79***	0.63***	0.73**	0.84	nd	nd
1432b	0.06***	0.19***	0.27**	nd	nd	0.34
528	0.43**	0.44*	0.50**	0.82	0.69	1.03
319b	0.66*	0.50**	0.39***	0.66	0.60*	0.65**
160a	0.40***	0.01***	0.35***	0.53*	0.08***	0.61**
2118_A	nd	nd	nd	0.18***	12.03**	nd
2118_B	nd	nd	nd	0.97	0.87	0.41
399i	nd	nd	nd	0.75	0.86	0.91
6197c	nd	nd	nd	0.64	0.38	2.58
398f	nd	nd	nd	1.13	0.66**	0.69*