RNA SEQUENCING and CO-EXPRESSED LONG NON-CODING RNA IN MODERN AND WILD WHEATS

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Supplementary Information

Supplementary Figure 1. *The distribution of percent change of the number of transcripts over a set of FPKM cutoffs.* Plot of percent change over 0 to 2 FPKM cutoffs **(A)**. Closer look at the graph between the cutoffs of 0 to 1 **(B)**.

Supplementary Figure 2. *Homology pattern between T. turgidum proteins and other plants.* **(A)** The pie chart shows the distribution of top-ten plants which showed the highest homology to *T. turgidum* proteins. **(B)** Pie chart shows the distribution of e-values for different blast hits.

Supplementary Figure 3. *Length distribution of lncRNAs and coding transcripts from each T. turgidum variety*. (Explanation for graph legend: Kiz-lnc: lncRNAs from variety Kiziltan, TR-lnc: lncRNAs from variety TR39477, TTD-lnc: lncRNAs from variety TTD-22, Kiz-cod: coding transcripts from variety Kiziltan, TR-cod: coding transcripts from variety TR39477, TTD-cod: coding transcripts from variety TTD-22)

Supplementary Figure 4. *Association between GC% content and length of lncRNAs from each T. turgidum variety.*

Supplementary Table 1. *TR39477 and TTD-22 specific transcripts expressed under drought stress.* **(A)** and **(B)** Drought specific transcripts of TR39477 and TTD-22 were blasted and the transcripts which do not exhibit any similarity to each other were listed in below. **(C)** KEGG maps for TR39477 specific transcripts which are expressed in response to drought stress are listed.

Supplementary Table 2. *miRNAs which targets the lncRNAs from variety Kiziltan* **(A)** *TR39477***(B)** *and TTD-22***(C)**.

Supplementary Table 3. *Number of transcripts across the three T. turgidum varities.* Number of transcripts listed either without filtering or with a filtering of >0.5 FPKM.

Supplementary Table 4. *QRT-PCR primers for validation of mRNA and lncRNA transcripts from Kiziltan.*

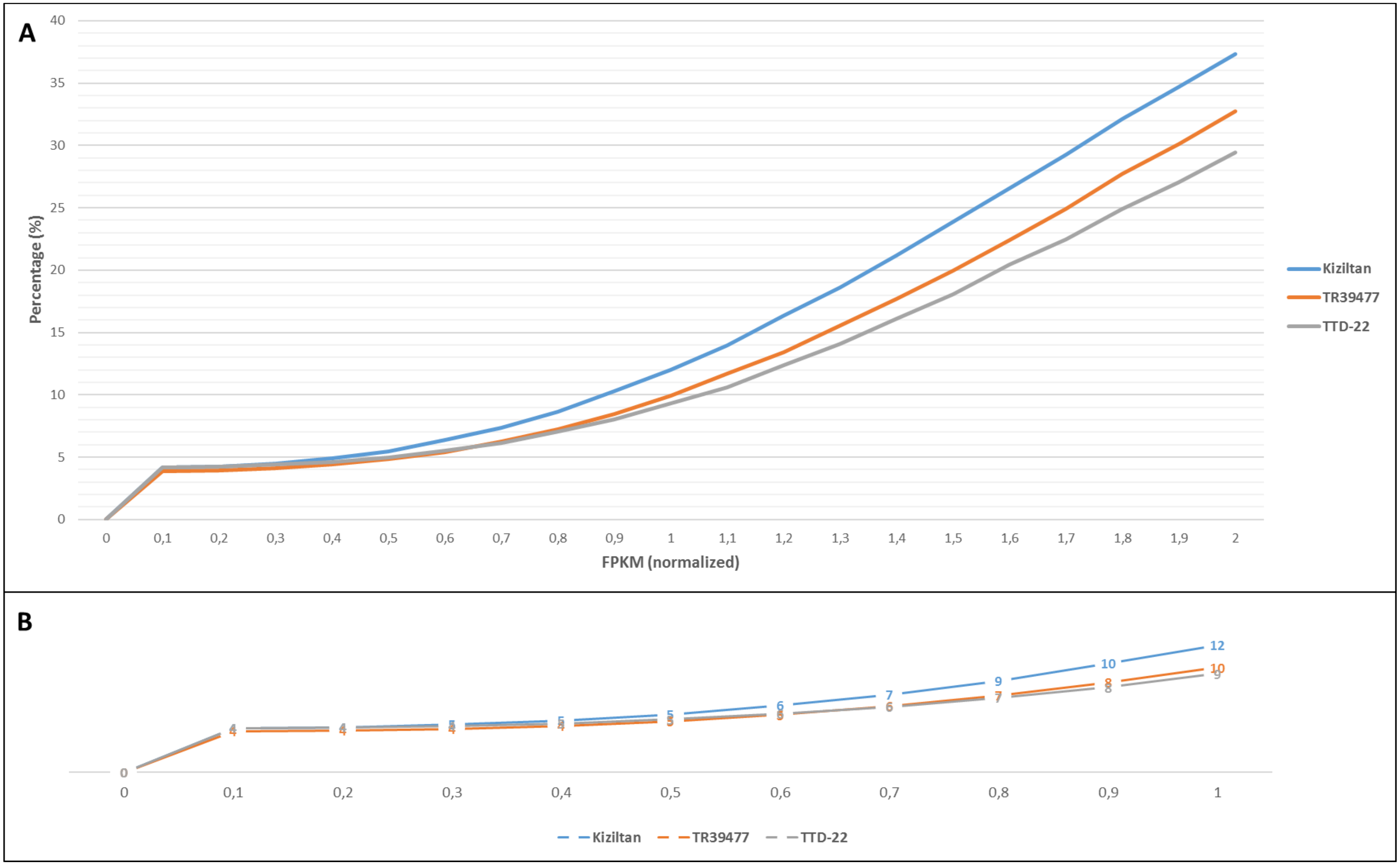
Supplementary Table 5. *Stem-loop RT-PCR primers for miR1436-1 and miR1436-4 from Kiziltan.*

Supplementary File 1 *(xlsx). The annotation of transcripts from each sample.* First sheet contains the information for transcripts from variety Kiziltan. Second sheet contains the information for transcripts from variety TR39477. Third sheet contains the information for transcripts from variety TTD-22.

Supplementary File 2 *(xlsx). miRNA-lncRNA target pairs utilized in miRNA-lncRNA-mRNA networks.* First sheet contains the information for miRNA-lncRNA pairs from variety Kiziltan. Second sheet contains the information for miRNA-lncRNA pairs from variety TR39477. Third sheet contains the information for miRNA-lncRNA pairs from variety TTD-22.

Supplementary Figures

Supplementary Figure 1. *The distribution of percent change of the number of transcripts over a set of FPKM cutoffs.* Plot of percent change over 0 to 2 FPKM cutoffs **(A)**. Closer look at the graph between the cutoffs of 0 to 1 **(B)**.



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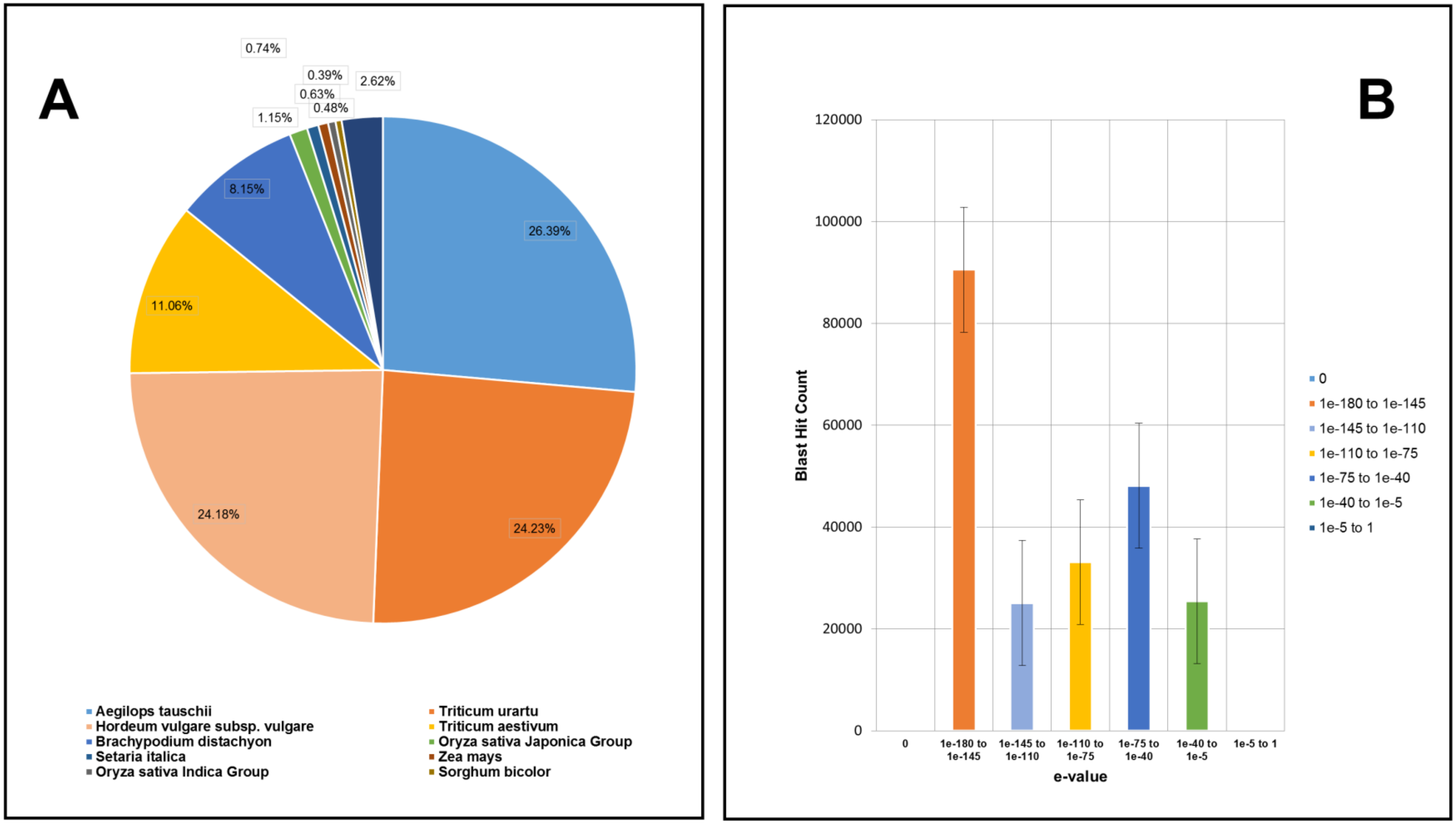

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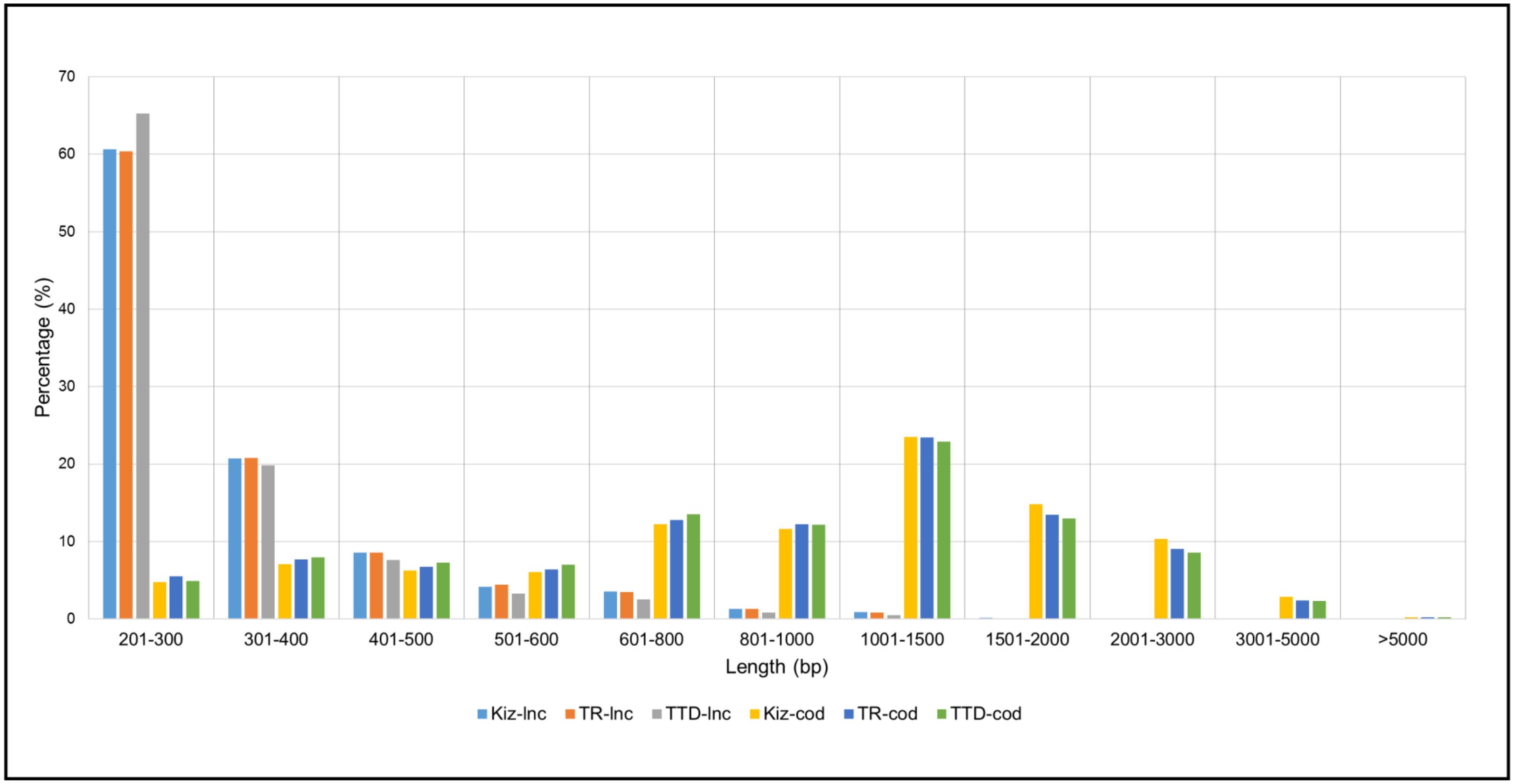

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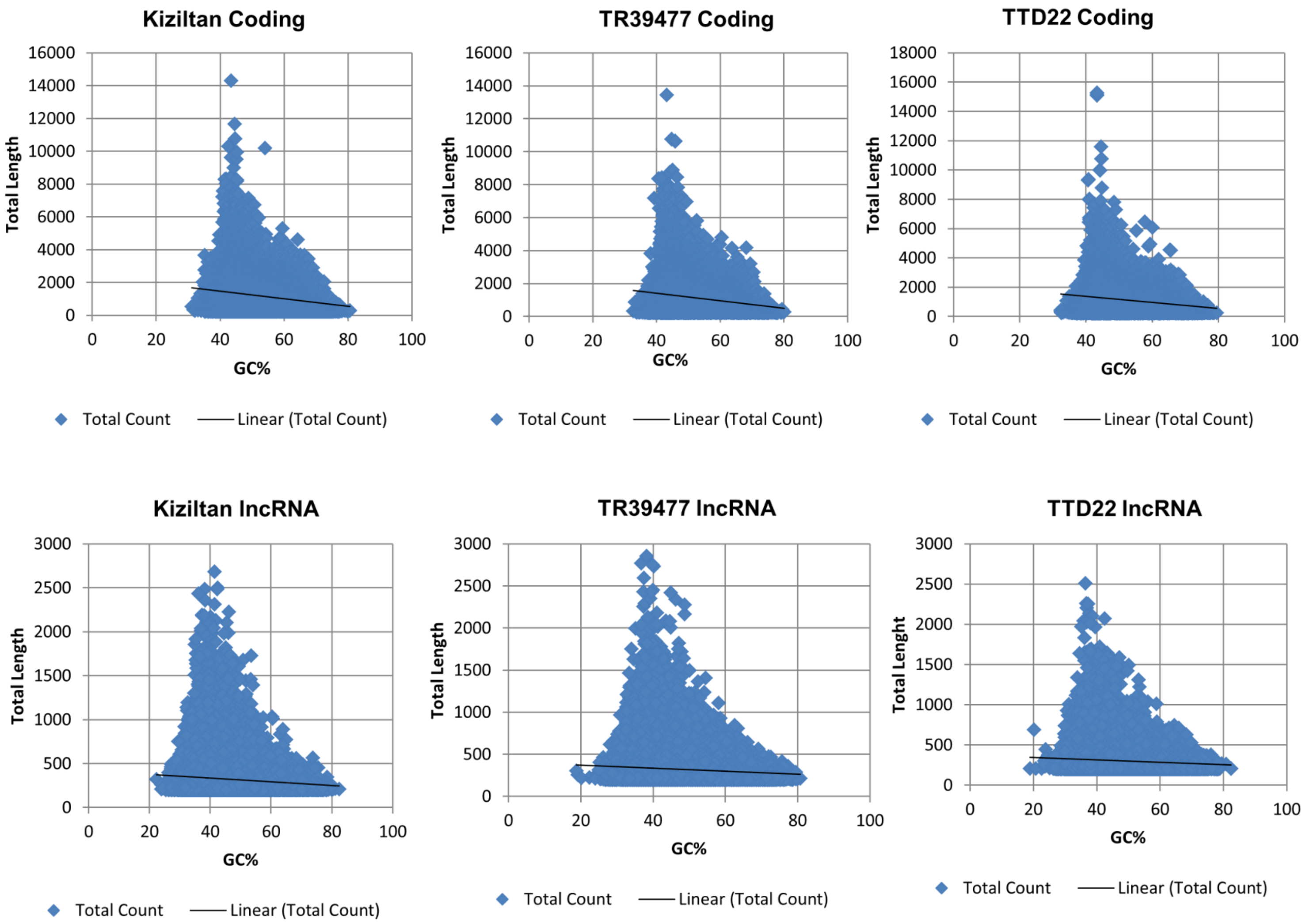

Supplementary Tables

Supplementary Table 1. *TR39477 and TTD-22 specific transcripts expressed under drought stress.* **(A)** and **(B)** Drought specific transcripts of TR39477 and TTD-22 were blasted and the transcripts which do not exhibit any similarity to each other were listed in below. **(C)** KEGG maps for TR39477 specific transcripts which are expressed in response to drought stress are listed.

(A) Transcript IDs for drought specific coding transcripts of TR39477 which do not exhibit any similarity to TTD-22 transcripts	
TR_DS_c100335_g1_i1,TR_DS_c100721_g1_i1,TR_DS_c10663_g1_i1,TR_DS_c111097_g1_i1,TR_DS_c112175_g1_i1,TR_DS_c115342_g1_i1,TR_DS_c118586_g1_i1,TR_DS_c119351_g1_i1,TR_DS_c121962_g1_i1,TR_DS_c122655_g1_i1,TR_DS_c124109_g1_i1,TR_DS_c133829_g1_i1,TR_DS_c139001_g1_i1,TR_DS_c13972_g1_i1,TR_DS_c18086_g1_i1,TR_DS_c23531_g1_i1,TR_DS_c32200_g1_i2,TR_DS_c5817_g1_i1,TR_DS_c59003_g1_i2,TR_DS_c68118_g1_i1,TR_DS_c7239_g1_i1,TR_DS_c76507_g1_i1,TR_DS_c77362_g1_i1,TR_DS_c77721_g1_i1,TR_DS_c79749_g1_i1,TR_DS_c81581_g1_i1,TR_DS_c83024_g1_i1,TR_DS_c83819_g1_i1,TR_DS_c84249_g1_i1,TR_DS_c86337_g1_i1,TR_DS_c87167_g1_i1,TR_DS_c87458_g1_i1,TR_DS_c87491_g1_i1,TR_DS_c93476_g1_i1,TR_DS_c94439_g1_i1,TR_DS_c9969_g1_i1	
(B) Transcript IDs for drought specific coding transcripts of TTD-22 which do not exhibit any similarity to TR39477 transcripts	
TD_DS_c115797_g1_i1, TTD_DS_c53857_g1_i1, TTD_DS_c54409_g1_i4, TTD_DS_c86980_g1_i1	
(C) KEGG maps for TR39744 specific transcripts (expressed only under drought stress) and their description	
map00190	oxidative phosphorylation
map00480	Glutathione metabolism
map00230	Purine metabolism
map00600	Sphingolipid metabolism
map00240	Pyrimidine metabolism
map00730	Thiamine metabolism
map00604	Glycosphingolipid biosynthesis - ganglio series
map00531	Glycosaminoglycan degradation
map00511	Other glycan degradation
map00052	Galactose metabolism

Supplementary Table 2. *miRNAs which targets the lncRNAs from variety Kiziltan* **(A)** *TR39477***(B)** *and TTD-22***(C)**.

(A) miRNAs and their lncRNAs targets from Kiziltan variety

miRNA ID	lncRNA ID	Target Start	Target End	Aligned Target Fragment	Target Inhibition Mode
miR1436	Kiz_both_c123855_g1_i1	150	169	CUUCUUCCAUCCCAUGAUUG	Cleavage
miR1136	Kiz_both_c38021_g1_i1	3	26	UAGAUACAUCCAUUUCUGCGAUGA	Cleavage or Translation repression
miR854	Kiz_both_c50149_g1_i1	577	597	CUUCUUCUUCCUCUUCUUCUU	Cleavage
miR1135	Kiz_CK_c50944_g2_i1	496	519	UCCUUUCCAAAUUACUUGUCGUGG	Cleavage
miR1439	Kiz_CK_c50944_g2_i1	488	507	CUACUCCCUCCUUUCCAAAU	Translation repression
miR1133	Kiz_DS_c68120_g9_i2	120	141	UUAGGAACGGAGGGAGUAGGUC	Cleavage
miR1436	Kiz_both_c70772_g2_i1	132	151	ACUCCCUCCGUUCC-UAAAUA	Cleavage
miR1439	Kiz_both_c70772_g2_i1	130	149	CUACUCCCUCCGUUCCUAAA	Cleavage
miR1439	Kiz_DS_c79679_g1_i1	201	221	[A]CUACUCCCUCCGUUCCGAAU	Cleavage
miR1436	Kiz_DS_c90557_g1_i1	361	381	ACUCCCUCCGUUCCUUUAUGU	Cleavage
miR1436	Kiz_both_c96195_g1_i1	262	282	ACUCCCUUUGUUCCAGAAUAA	Cleavage
miR1439	Kiz_both_c96195_g1_i1	259	279	UUUACUCCCUUUGUUCCAGAA	Cleavage
miR437		304	324	AACUCAAUCUUGUUUAAGUUU	Translation repression

(B) miRNAs and their lncRNAs targets from TR39477 variety

miRNA ID	**lncRNA ID**	**Target Start**	**Target End**	**Aligned Target Fragment**	**Target Inhibition Mode**
miR1137	TR_both_c118135_g1_i1	189	209	AGUGUCUCAAAUUUUGUACUA	Translation repression
miR1436	TR_both_c118135_g1_i1	169	189	ACUACCUCCGUCCUAAAAUAA	Cleavage
miR1439	TR_both_c118135_g1_i1	140	160	GAUACUCCCUCCGUCUUAAAA	Cleavage
miR1439	TR_both_c118135_g1_i1	167	186	UUACUACCUCCGUCCUAAAA	Cleavage
miR1436	TR_CK_c12385_g1_i1	275	295	ACUCCCUUUGUCUUAAAAUGA	Cleavage
miR1120	TR_DS_c34320_g1_i1	121	143	UCCGUUUCAUAAUAUAACAGCGU	Cleavage
miR1436	TR_DS_c34320_g1_i1	115	135	[A]CUUCAUCCGUUUCAUAAUAU	Cleavage
miR1439	TR_DS_c34320_g1_i1	113	132	GUACUUCAUCCGUUUCAUAA	Cleavage
miR1128	TR_DS_c44260_g1_i1	328	348	UUAGGGACGGAGGGAGUAGUU	Cleavage
miR1128	TR_both_c56781_g1_i5	1266	1285	UUUAUAUGGAGGGAGUAUUU	Cleavage
miR1133	TR_both_c56781_g1_i1	1266	1286	UUUAUAUGGAGGGAGUAUUUA	Cleavage
miR1436	TR_DS_c58890_g4_i2	448	467	CUCCCUCCCUCUUUUAAUAU	Cleavage
miR1139	TR_both_c60784_g1_i5	461	480	GUUACUAG-CUAAGUUACUCC	Cleavage
miR1128	TR_CK_c61658_g1_i1	4	24	UUCGGAACGGAGGGAGUAGUA	Cleavage
miR1436	TR_both_c63034_g2_i23	24	44	ACUUCCUCGGUUCCAAAAUUC	Cleavage

miR1439	TR_both_c63034_g2_i23	22	41	CUACUUCCUCGGUUCCAAAA	Translation repression
miR1122	TR_DS_c63034_g2_i3	744	763	UCUAAAUGCGGAUGUAUCUA	Cleavage
miR1436	TR_DS_c63034_g2_i3	709	729	ACUCCCUCCGUCUCAAAAUUC	Cleavage or Translation repression
miR1439	TR_DS_c63034_g2_i3	706	726	[A]GUACUCCCUCCGUCUCAAAA	Cleavage
miR1118	TR_DS_c63271_g2_i1	831	853	UCCCUCCAUUCCAAAAUAUAGCG	Cleavage
miR1436	TR_DS_c63271_g2_i1	829	849	[A]CUCCCUCCAUUCCAAAAUAU	Cleavage
miR1439	TR_DS_c63271_g2_i1	826	846	[C]UUACUCCCUCCAUUCCAAAA	Cleavage or Translation repression
miR1136	TR_DS_c63631_g1_i7	662	681	AACAUUCAUAUGUGUGACAU	Cleavage
miR1436	TR_DS_c65082_g1_i1	244	264	ACUCCCUCCGUUCCUUUAUAU	Cleavage
miR1439	TR_CK_c67474_g1_i1	401	421	ACUGCUCCCUCCGUUUCUAAA	Cleavage
miR1130	TR_both_c94590_g1_i1	210	232	[AU]UCUUAUAUUAUGGGACGGAGG	Cleavage or Translation repression
miR1436	TR_both_c94590_g1_i1	188	207	ACUCCUUCUGUCCC-UAAUGC	Cleavage
(C) miRNAs and their lncRNAs targets from TTD-22 variety					
miRNA ID	**lncRNA ID**	**Target Start**	**Target End**	**Aligned Target Fragment**	**Target Inhibition Mode**
miR1436	TTD_DS_c58970_g2_i3	225	245	[A]CUCCCUCCGUUCCAAAAUAG	Cleavage
miR1439	TTD_DS_c58970_g2_i3	223	242	[G]UACUCCCUCCGUUCCAAAA	Cleavage
miR1128	TTD_DS_c60722_g1	4	24	UUUGGGACGGAGGGAGUACU	Cleavage

	_i1			A	
miR1120	TTD_CK_c62154_g1_i2	682	705	CUCCGUCCCAUAAUAUAACAGCGU	Cleavage
miR1436	TTD_CK_c62154_g1_i2	677	697	[A]UUCCCUCCGUCCCAUAAUAU	Cleavage or Translation repression
miR1436	TTD_DS_c64640_g2_i1	2490	2510	ACUCCCUCCGUCCCAAAAUUC	Cleavage
miR1439	TTD_DS_c64640_g2_i1	2487	2507	[A]CUACUCCCUCCGUCCCAAAA	Cleavage

Supplementary Table 3. *Number of transcripts across the three T. turgidum varities.* Number of transcripts listed either without filtering or with a filtering of >0.5 FPKM.

	The number of	Kiziltan	TR39477	TTD-22
Without filtering	All transcripts	243670	211709	203230
	Coding transcripts	84288	75996	78456
	lncRNA transcripts	63773	61823	43932
Actively-expressed	All transcripts	230359	201499	193087
	Coding transcripts	81168	73465	75861
	lncRNA transcripts	59110	57944	40858

Supplementary Table 4. *QRT-PCR primers for validation of mRNA and lncRNA transcripts from Kiziltan.*

lncRNA ID	lncRNA Length	Sequence	Primer Start Site in Target	Primer End Site in Target	Length	GC %	Amplicon Length
lncRNA_c118446_g1_i1-Forward	341	TCTCTGGCCTAAGCAACTTTAC	100	122	22	45.5	127
lncRNA_c118446_g1_i1-Reverse	341	GCTTTCCCAAAGCCCTGATA	207	227	20	50	127
lncRNA_c47700_g1_i1-Forward	472	GGAACAGCGACAGTACAGTAAG	188	210	22	50	139
lncRNA_c47700_g1_i1-Reverse	472	TGTGTGACTGTGAGAGAGAGATA	304	327	23	43.5	139
mRNA_c17408_g1_i1-Forward	879	CTCAGACCTTCGATCAAAGACG	110	132	22	50	97
mRNA_c17408_g1_i1-Reverse	879	TCCATGTACGTCCACCTAGAG	186	207	21	52.4	97
mRNA_c55246_g1_i1-Forward	1734	CGACGTGTAAGCATCAGAGAA	154	175	21	47.6	105
mRNA_c55246_g1_i1-Reverse	1734	AGCCTATGCACTTCCCTAAATC	237	259	22	45.5	105
lncRNA_c70772_g2_i1-Forward	208	GCCTCCCTGTGCAAACATA	81	100	19	52.6	102
lncRNA_c70772_g2_i1-Reverse	208	AGTCACTTGTTGAAATGCCTAGA	160	182	23	39.1	102
mRNA_c69036_g1_i1-Forward	756	ATTGGCGCTGACGAGAAA	251	269	18	50	98
mRNA_c69036_g1_i1-Reverse	756	CTACCTGCACAGTCCATCTTC	328	349	21	52.6	98
lncRNA_c90557_g1_i1-Forward	385	CTGCTCTGCTCTGTACTGTATC	104	126	22	62	88
lncRNA_c90557_g1_i1-Reverse	385	GATCTCCTCTACAGCCACAAG	171	192	21	61	88
mRNA_ c9653_g1_i2-Forward	2483	ATCCTCTCCTTCCTGGTCAA	872	892	20	50	92
mRNA_ c9653_g1_i2-Reverse	2483	CCTCGACTGCGTGAAATAGAA	943	964	21	47.6	92

Supplementary Table 5. *Stem-loop RT-PCR primers for miR1436-1 and miR1436-4 from Kiziltan.*

Primer Name	**Sequence**
Universal Reverse Primer for qRT-PCR	GTGCAGGGTCCGAGGT
miR1439-3_Stemloop	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAATACT
miR1436-1_Stemloop	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACACTCCC
miR1436-4_Stemloop	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACACTCCC
miR854_Stemloop	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACCTCCTC
miR1133-1_Stemloop	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACTTTGGG