

### A. pri-miR171a (U21\_38648)

10                    20                    30                    40  
U        U            U    A        C        -        A-|        G        GA  
CACUA GAUGUUGGC CG CUCA UCAG ACC     CGCCG AGG    G  
GUGUA CUUAACCG GC GAGU AGUC UGG     GCGGC UCU    C  
C        U            U    C        U        U        CG^        G        AC  
90                    80                    70                    60                    50

### B. Mature miR171 sequences

**hvu-miR171a**                      ugauugagccgugccaaauc  
**hvu-miR171b**                      ugauugaggcggcccaaauc

**ath-miR171a**                      ugauugagccgcgccaaauc  
ath-miR171b/c                      uugaggcgugccaaaucacg

**osa-miR171a**                      ugauugagccgcgccaaauc  
**osa-miR171b-f**                      ugauugaggccgugccaaauc  
osa-miR171g                        gaggugaggccgagccaaauc  
osa-miR171h                        gugaggccgaaccaaaucacu  
osa-miR171i                        ggauugaggccgcgucaaauc

**Additional file 1. miR171 precursor and mature sequences.** Information retrieved from miRBase (release-17) and Schreiber *et al.*, 2011 [34]. (A) *pri-miR171a* secondary structure obtained using MFOLD (<http://mfold.rna.albany.edu>), red letters indicate mature miRNA sequence. (B) mature miR171 sequences found in *Hordeum vulgare* (hvu), *Arabidopsis thaliana* (Ath) and *Oryza sativa* (Osa).

Genbank ID	Homology	Score	Sequence Alignment	Activity
<i>miR171a targets</i>				
AK368048	SCL	0.0	miRNA 21 CUAUAACCGUGCCGAGUUAGU 1 Target 932 GAUAUUGGCACGGCUAAUCA 952	CL
AK371946	SCL	0.5	miRNA 21 CUAUAACCGUGCCGAGUUAGU 1 Target 1231 GAUAUUGGCGCGGCUAAUCA 1251	CL
AK364580	SCL	0.5	miRNA 21 CUAUAACCGUGCCGAGUUAGU 1 Target 616 GAUAUUGGCGCGGCUAAUCA 636	CL
AK362896	Unknown function	1.5	miRNA 21 CUAUAACCGUGCCGAGUUAGU 1 Target 446 GAUGUUGGCAUGGUUCAAUCA 466	CL
AK360395	ATPase	4.0	miRNA 21 CUAUAACCGUGCCGAGUUAGU 1 Target 926 UAUGUUGGAGCUGCUAAUCA 946	TR
AK374235	WD40	4.0	miRNA 21 CUAUAACCGUGCCGAGUUAGU 1 Target 959 CAUGAUGGCAAGGUUCAAUCA 979	CL
AK373446	Unknown function	4.0	miRNA 21 CUAUAACCGUGCCGAGUUAGU 1 Target 1247 UAGAUUAAGCCCCGCUAAUCA 1267	CL
AK368182	RNA binding protein	4.0	miRNA 21 CUAUAACCGUGCCGAGUUAGU 1 Target 893 GAUAUUGGCAUGCUCUUCGCUA 913	TR
AK252561	Unknown function	4.0	miRNA 21 CUAUAACCGUGCCGAGUUAGU 1 Target 96 UUUAUUGGCCGGCUCGAUCG 116	CL
<i>miR171b targets</i>				
AK371946	SCL	0.0	miRNA 21 CUAUAACCGCGCCGAGUUAGU 1 Target 1231 GAUAUUGGCGCGGCUAAUCA 1251	CL
AK364580	SCL	0.0	miRNA 21 CUAUAACCGCGCCGAGUUAGU 1 Target 616 GAUAUUGGCGCGGCUAAUCA 636	CL
AK368048	SCL	1.0	miRNA 21 CUAUAACCGCGCCGAGUUAGU 1 Target 932 GAUAUUGGACGGCUAAUCA 952	CL
AK362896	Unknown function	2.5	miRNA 21 CUAUAACCGCGCCGAGUUAGU 1 Target 446 GAUGUUGGCAUGGUUCAAUCA 466	CL
AK360395	ATPase	3.5	miRNA 21 CUAUAACCGCGCCGAGUUAGU 1 Target 926 UAUGUUGGAGCUGCUAAUCA 946	TR
AK248532	E3 ubiquitin ligase	3.5	miRNA 21 CUAUAACCGCGCCGAGUUAGU 1 Target 164 GAUAUUCGAGCUCUGAUCA 184	TR
AK366340	Phosphatase associated protein	4.0	miRNA 21 CUAUAACCGCGCCGAGUUAGU 1 Target 1890 GAUGUUGACGAGGCUAAGCA 1910	TR
AK373446	Unknown function	4.0	miRNA 21 CUAUAACCGCGCCGAGUUAGU 1 Target 1247 UAGAUUAAGCCCCGCUAAUCA 1267	CL
AK252561	Unknown function	4.0	miRNA 21 CUAUAACCGCGCCGAGUUAGU 1 Target 96 UUUAUUGGCCGGCUCGAUCG 116	CL

#### Additional File 2. Potential targets of hvu-miR171a and b. psRNATarget

(<http://plantgrn.noble.org/psRNATarget>) was used to predict potential targets of hvu-miR171a and b among the full-length barley ESTs assembled by Matsumoto et al 2011 [35]. The maximum score was set at 4. The column “Activity” refers to the type of inhibition predicted, Cleavage (CL) or Translation inhibition (TR).

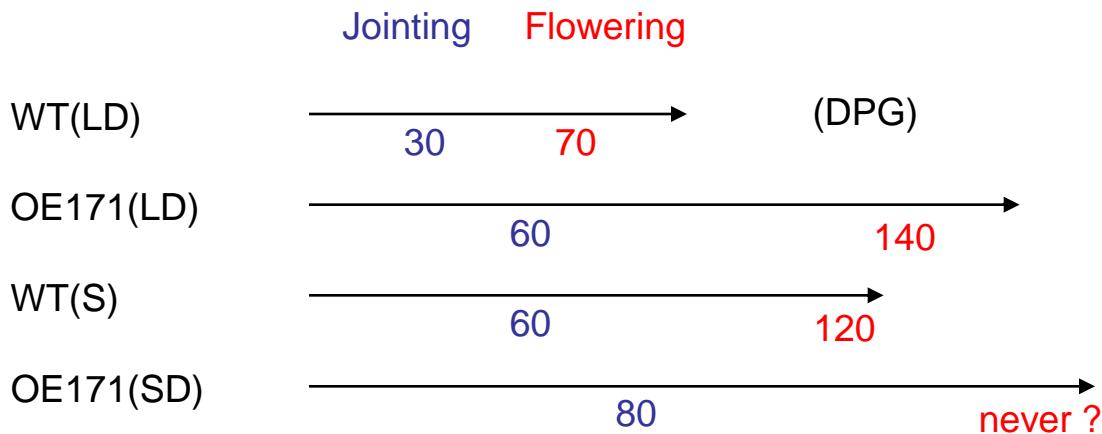
**A**

AK371946 mRNA	$\begin{matrix} 13.38 \text{ RPM} \\ \downarrow 2.87 \\ \downarrow \downarrow 9.75 \\ \downarrow \downarrow \end{matrix}$ Hvu-miR171a	$5' - \text{CTGGCGCGUGAGAUAUUGGCGCGCUAAUCACCAAGCUUCCCCC}-3'$ $\text{     :     }$ $3' - \text{CUUAACCGUGCCGAGUUAGU}-5'$
AK364580 mRNA	$\downarrow$ Hvu-miR171a	$1.84$ $5' - \text{CTGGCGCGCGAGAUAUUGGCGCGCUAAUCACCGGUCCCCCTCG}-3'$ $\text{     :     }$ $3' - \text{CUUAACCGUGCCGAGUUAGU}-5'$
AK368048 mRNA	$\downarrow$	$1.84$ $5' - \text{TCGGCGCGCGAGAUAUUGGCACGGCUAAUCACCGGUCCCCGCC}-3'$ $\text{     :     }$ $3' - \text{CUUAACCGUGCCGAGUUAGU}-5'$
<b>No PARE sequence found</b>		
AK362896 mRNA	$5' - \text{GCCGGGUGAGUCCGAUGUUGGCAUGGUCAAUCAAUUGAGUGCAA}-3'$ $\text{   :     :   :     }$ Hvu-miR171a	$3' - \text{CUUAACCGUGCCGAGUUAGU}-5'$

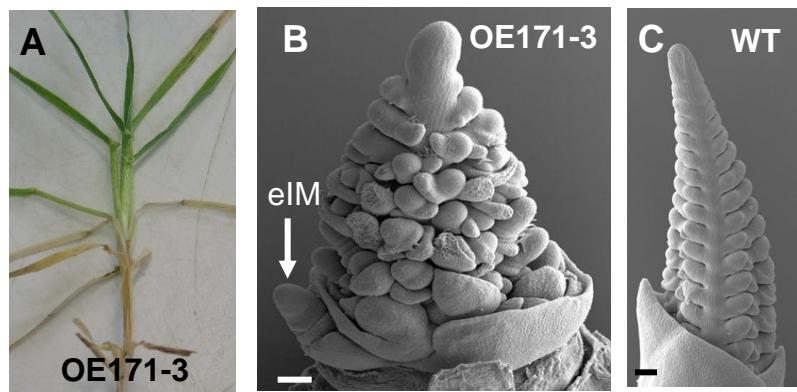


**Additional file 3. Evidence for miR171 cleavage of HvSCLs and HvSPL.**

(A) Evidence for cleavage of top four predicted miR171 targets in degradome data from developing barley grains [36] (B) RLM-5' RACE on HvSCL (U21\_33945). (C) RLM-5' RACE on HvSPL (U21\_18637). The miRNA binding site is underlined in black and the 5' end of the cleaved mRNA in red. The numbers in green refer to the ratio of 5'-RACE clones showing a cleavage at the base indicated by the arrow. The agarose gel on the left shows the band corresponding to the amplified 3' cleavage products.



**Additional file 4. Timing of the transition phases during shoot development.** The diagram shows the approximate timing of the transitions from juvenile to adult phases and adult to reproductive phases. The first transition was determined by the moment when the first stem of the plant started to elongate (jointing) and the second transition (flowering) when the first spike reached anthesis. Under our SD condition, OE171 plants did not flower.



**Additional file 5. Developmental arrest of OE171-3 (T0).** (A) Tiller of the T<sub>0</sub> plant OE171-3. Scanning electron microscopy of the SAM of a OE171-3 (B) and WT (C) plants. Ectopic inflorescence meristem (eIM). Scale bars represent 1mm.

Name	Sequence 5' -3'	Use for
MIR171-1F	CACCTTGGTGCTAGAGGCTAGAGAG	Cloning
MIR171-2R	GATCCGATGATAAAACCCCTTCG	Cloning
MIR171-3F	CGGC GGCG GTCT GATTG	qPCR
MIR171-4R	GCGTGCATGAAAGAGCACTAA	qPCR
SCL-F	GCGGCTTACAAGTCCTCTC	qPCR
SCL-R	GAAGTTAGCGAACTGCAGCAT	qPCR
SPL-F	TTCTCCGATGGTCTGACTCC	qPCR
SPL-R	ATTGCTGCAGGTTGGAGAAC	qPCR
AP2L-F	TGCAGCCAGAGATCATAGCA	qPCR
AP2L-R	GGTGCTTGACGACGATGAT	qPCR
TRD-F	TTAAATAACAGAGCGCCGAGA	qPCR
TRD-R	GCTGTGGTCGTCGTAGGAGTA	qPCR
PLA1-F	GCTCTGGGAGATGGTGTCC	qPCR
PLA1-R	GATGACGGCGTGGAGGTAG	qPCR
WUS-F	ACCTTTGTCTCTCCCTCCAC	qPCR
WUS-R	ACATCTCCTCCAGGATCATCA	qPCR
KN1-F	TCAAGGAGATGCTGCTCAAGA	qPCR
KN1-R	GTAGTGC GTGTTCCACCAGTC	qPCR
miR171-AS	GATATTGGCACGGCTCAATCA	Northern Probe
miR156-AS	GTGCTCACTCTCTTCTGTCA	Northern Probe
miR172-AS	ATGCAGCATCATCAAGATTCT	Northern Probe
miR168-AS	GTCCCGATCTGCACCAAGCGA	Northern Probe
SCL-5RACE	GAGAAGGACTTGTAAAGCCGCC	5' RACE
SPL-5RACE	GAGACGGTGCCGGTGGAAAGCCT	5' RACE

**Additional file 7. Primer and Probe sequences used in this study.**