MADS1 maintains barley spike morphology at high ambient temperatures

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Supplementary Information

Title: MADS1 maintains barley spike morphology at high ambient temperatures

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Supplementary Dataset 1 DEGs across temperature, genotype, and developmental phase.
An Excel file containing the normalised read values (read counts per kilobase per million; RPKM, average value from 3 biological replicates), gene ID, gene symbol and orthologues from Arabidopsis and rice for DEGs (*P* adjust < 0.01). Separate sheets present DEGs for each parameter: temperature, genotype, developmental phase, and the interactions (Fig. 4). NA indicates not available. NS indicates not significant.

(1) List of all 9,434 DEGs
(2) List of the 3,194 DEGs regulated by genotype
(3) List of the 7,800 DEGs regulated by temperature
(4) List of the 4,594 DEGs regulated by developmental phase
(5) List of the 2,568 DEGs co-regulated by genotype × temperature
(6) List of the 1,979 DEGs co-regulated by genotype × developmental phase
(7) List of the 3,367 DEGs co-regulated by temperature × developmental phase
(8) List of the 1,760 DEGs co-regulated by temperature × developmental phase × genotype

Supplementary Dataset 2 Co-expression clusters of DEGs from RNA-seq of 8 barley spike samples (Extended Date Fig. 8c). GO terms from barley (IPK) and Arabidopsis (tair) are indicated for each gene. NA indicates not available. The general description of each cluster is listed below:

Cluster 1 (890 genes)
Down-regulated by high temperature, which were expressed at lower levels in Hv*mads1*, compared with wild-type spikes at 15 °C and 25 °C. Most of these genes are not regulated by spike developmental phase.
Main GO Terms: ATP/GTP binding, biological process, ion binding, catalytic activity, amino acid and lipid metabolic process, cellular amino acid biosynthetic process, cytosolic small/large ribosomal subunit, DNA repair, nucleotide binding, protein binding, protein heterodimerization, oxidoreductase activity, kinase activity, structural constituent of ribosome, cellular component, transferase activity, cell wall organisation, regulation of transcription, protein folding, floral organ morphogenesis, flower development, response to cytokinin.

Cluster 2 (640 genes)
Similar pattern to Cluster 1, but the genes show a weak genotype effect in W2.5 spikes.
Main GO Terms: metabolic process, regulation of cell cycle, transporter activity, transferase activity, protein kinase activity, cell redox homeostasis, protein binding, DNA replication, ATP binding, cell wall biosynthetic process, regulation of translational initiation, integral component of membrane, ER membrane protein complex, Golgi apparatus, mitochondrial translation, chromatin organisation, positive regulation of transcription, regulation of meristem structural organisation, response to heat.

Cluster 3 (477 genes)
As with Cluster 1 and 2, these genes are slightly regulated by developmental phase at 15 °C.
Main GO Terms: catalytic activity, DNA replication, regulation of transcription, carbohydrate metabolic process, anchored component of membrane, regulation of cell cycle, cell wall organisation, cellular response to DNA damage stimulus, response to oxidative stress, cytokinin catabolic process, DNA-binding transcription factor activity, response to heat, cytoskeleton organisation, mitotic cytokinesis, regulation of transcription,

Cluster 4 (587 genes)
Similar to Clusters 1–3, but the number of genes that are regulated by HvMADS1 is increased.

Main GO Terms: protein binding, oxidoreductase activity, peroxidase activity, response to oxidative stress, response to heat, endopeptidase activity, translation initiation, cell division, cell cycle, cell differentiation, DNA-binding transcription factor activity, regulation of shoot apical meristem specification, protein heterodimerization activity.

Cluster 5 (370 genes)
Similar to Cluster 4, except some genes showing up-regulation in spike W2.5 at 25 °C.
Main GO Terms: ATP binding, carbohydrate metabolic process, hydrolase activity, metabolic process, oxidoreductase activity, flower development, cell wall organisation, DNA replication initiation, regulation of transcription, lipid biosynthetic process, plasma membrane, protein phosphorylation activity, response to cytokinin, response to auxin.

Cluster 6 (278 genes)
Predominantly co-regulated by temperature, HvMADS1 and phase. Specifically, the genes are up-regulated from W2.5 to W3.5 and are down-regulated by high temperature in wild-type spikes. Both of these changes are weakly echoed in Hv*mads1* spikes, consistent with the activation regulatory function of HvMADS1.
Main GO Terms: catalytic activity, DNA binding, carboxypeptidase activity, endopeptidase activity, cell differentiation, RNA polymerase activity, regulation of auxin-activated pathway, regulation of gene expression, oxidation-reduction process, cell wall biogenesis, meristem development, abaxial cell fate specification, regulation of cell cycle, regulation of cytokinin-activated signalling, negative regulation of photomorphogenesis, regulation of transmembrane receptor signalling.

Cluster 7 (503 genes)
Similar to Cluster 6, but genes showing the highest expression in wild-type W3.5 spike at 15 °C.
Main GO Terms: RNA polymerase activity, regulation of transcription, sexual reproduction, nucleic acid binding, receptor sensor kinase activity, transcription elongation from RNA polymerase II promote, translation initiation activity, catabolic process, response to DNA damage stimulus, maintenance of floral organ identity, histone methylation, mRNA stabilization, multicellular organism development, multidimensional cell growth, nucleus signal transduction, regulation of plant growth, regulation of meristem development, positive regulation of cell division and growth, embryo development, flower development.

Cluster 8 (382 genes)
Down-regulated in Hv*mads1* spikes at 25 °C compared with 15 °C.
Main GO Terms: anchored component of membrane, cellular response to oxidative stress, circadian rhythm, regulation of transcription, cellular protein catabolic process, histone binding, flower development, gene silencing, lipid biosynthetic process, RNA modification, phase transition of meristem, regulation of cell growth, cell fate specification, unidimensional cell growth, cell wall organisation, response to auxin.

Cluster 9 (249 genes)
Regulated by temperature and show more dynamic regulation by HvMADS1 at 25 °C.
Main GO Terms: cell differentiation, regulation of transcription, cell wall organisation, response to gibberellin stimulus, gibberellin metabolic process, oxidation-reduction process, response to auxin signalling, regulation of meristem structural organisation, response to stress, regulation of growth, protein binding.

Cluster 10 (103 genes)

Similar to Cluster 9.
Main GO Terms: regulation of transcription, peroxidase activity, killing of cells of other organism, response to light signalling, positive regulation of cell cycle, regulation of RNA polymerase II, integral component of membrane.

Cluster 11 (317 genes)
Opposite regulation by HvMADS1 between 15 °C and 25 °C conditions.
Main GO Terms: ATP binding, carbohydrate metabolic process, cell cycle regulation, protein ubiquitination, cytosolic ribosome, regulation of transcription, regulation of RNA polymerase II, heat acclimation, mitochondrial inner membrane, mRNA processing, cell redox homeostasis, response to cytokinin, regulation of growth, programmed cell death, response to stress, structural constituent of ribosome, regulation of translation.

Cluster 12 (637 genes)
Down-regulated in Hv*mads1* mutant at 15 °C, but up-regulated at 25 °C.
Main GO Terms: metabolic process, ATPase activity, ion binding, cell differentiation, cell wall organisation, cell morphogenesis, cellular amino acid metabolic process, protein modification process, RNA binding, response to heat, transcription factor activity, flower development, integral component of membrane, lipid catabolic process, cytokinin-activated signalling, regulation of transcription, oxidation-reduction process, response to auxin, protein binding, protein phosphorylation, protein ubiquitination.

Cluster 13 (158 genes)
Down-regulated from W2.5 to W3.5 spike, partially co-regulated by HvMADS1 and temperature.
Main GO Terms: metabolic process, response to auxin, protein kinase activity, cell wall organisation, regulation of mRNA transcription, protein binding, oxidoreductase activity.

Cluster 14 (59 genes)
Down-regulated in Hv*mads1* spikes, but not affected by temperature and phase.
Main GO Terms: ADP binding, catalytic activity, metabolic process, protein binding, cell-cell signalling, oxidoreductase activity.

Cluster 15 (946 genes)
Down-regulated in Hv*mads1* spikes at 15 °C, but up-regulated at 25 °C. Transcripts in this group are mildly up-regulated in wild-type W3.5 spike at 25 °C compared with 15 °C.
Main GO Terms: ADP binding, ATP binding, catalytic activity, GTPase activity, hydrolase activity, integral component of membrane, lipid metabolic process, amino acid metabolic process, oxidoreductase activity, protein kinase activity, cell redox homeostasis, protein modification process, response to cytokinin signalling, regulation of translation, regulation of transcription, flower development, gibberellin biosynthetic process, cyclin-dependent protein kinase activity, protein binding, protein phosphorylation, protein ubiquitination, response to light/UV stimulus, RNA splicing, vacuolar membrane organisation.

Cluster 16 (986 genes)
Similar to Cluster 15, but decreased regulation in W 3.5 spike at 25 °C
*Main GO Term*s: alternative mRNA splicing, catalytic activity, metabolic process response to auxin, cell differentiation, flower development, cell wall organisation, chromatin silencing, regulation of translation, DNA methylation, cytoskeleton organisation, DNA replication and repair, embryo development ending in seed dormancy, heterotrimeric G-protein complex,

endoplasmic reticulum membrane, glutathione metabolic process, regulation of histone, integral component of membrane, ion binding, mitochondrial membrane, mRNA processing, positive regulation of transcription, protein phosphorylation, regulation of growth, regulation of meristem development, regulation of meiotic nuclear division.

Cluster 17 (599 genes)
Similar to Clusters 15 and 16 that are regulated by HvMADS1 at 15 °C, but no change in W2.5 spike at 25 °C, suggesting that some temperature-regulated genes show a specifically response at the spike developing stage.
Main GO Terms: carbohydrate metabolic process, carpel development, cell wall organisation, cellular response to heat, regulation of transcription, phase transition of meristem, response to cadmium ion, ubiquitin-dependent protein catabolic process, protein serine/threonine kinase activity, protein phosphorylation, regulation of cell cycle/division, regulation of meristem structural organisation, response to sucrose, response to light, RNA splicing, regulation of growth.

Cluster 18 (347 genes)
Up-regulated in response to high temperature with the predominant regulation by HvMADS1 at spike W3.5. The expression of this cluster is largely unaffected by developmental phase.
Main GO Terms: biological process, cellular component, cellular response to heat, cytokinin dehydrogenase activity, cytokinin metabolic process, DNA-binding transcription factor activity, integral component of membrane, oxidation-reduction process, phosphorylation, protein ubiquitination, regulation of transcription, regulation of meristem structural organisation, protein folding.

Cluster 19 (426 genes)
Similar to Cluster 18.
Main GO Terms: biological process, cell surface receptor signalling, carbohydrate homeostasis, DNA-binding transcription factor activity, glutathione catabolic process, cytokinin biosynthetic process, mRNA cis splicing, regulation of transcription, photoperiodism, protein binding, shoot apical meristem specification, protein phosphorylation, regulation of gibberellin biosynthetic process, ribosome biogenesis, RNA binding, sexual reproduction, multidimensional cell growth SCF ubiquitin-dependent protein catabolic process.

Cluster 20 (147 genes)
Up-regulated in Hv*mads1* mutant at 25 °C, but not at 15 °C.
Main GO Terms: cell surface receptor signalling, cell wall organisation, cellular response to unfolded protein, molecular function, endoplasmic reticulum membrane, hydrolase activity, regulation of transcription, response to cold, response to heat, response to light stimulus, signal transduction by protein phosphorylation.

Cluster 21 (179 genes)
Up-regulated by temperature, but showing more dynamic changes in Hv*mads1* mutant.
Main GO Terms: calcium ion binding, cellular component, molecular function, oxidation-reduction process, metabolic process, protein phosphorylation, regulation of transcription, response to hormone, response to cold, response to heat, response to hypoxia, protein phosphorylation, protein binding, chromatin organisation.

Cluster 22 (145 genes)

Up-regulated in Hv*mads1* spikes and are largely unaffected by temperature and developmental phase, which is the opposite pattern to Cluster 14.
Main GO Terms: cell redox homeostasis, nucleic acid binding, catalytic activity, oxidation-reduction process, protein kinase activity, response to hypoxia, gene silencing, regulation of DNA replication, DNA binding, photoperiodism, regulation of flowering.

Supplementary Dataset 3 GO analysis of DEGs in wild-type and Hv*mads1* spikes at 15 °C.

(1) List of GO terms representing DEGs in wild-type and Hv*mads1* spikes at 15 °C (*P* adjust < 0.01, one-sided, Benjamini-Yekutieli method); BP, Biological Process; CC, Cellular Component; MF, Molecular Function).
(2) List of biological process clustering of DEGs in wild-type and Hv*mads1* spikes at 15 °C based on GO terms by REVIGO (similarity cutoff, 0.9; "large" REVIGO set) (Fig. 4d).

Supplementary Dataset 4 GO analysis of DEGs in wild-type and Hv*mads1* spikes at 25 °C.

(1) List of GO terms representing DEGs in wild-type and Hv*mads1* spikes at 25 °C (*P* adjust < 0.01, one-sided, Benjamini-Yekutieli method); BP, Biological Process; CC, Cellular Component; MF, Molecular Function).
(2) List of biological process clustering of DEGs in wild-type and Hv*mads1* spikes at 25 °C based on GO terms by REVIGO (similarity cutoff, 0.9; "large" REVIGO set) (Fig. 4d).

Supplementary Dataset 5 Curated list for genes of inflorescence development, temperature response, cell cycle/division and plant hormone pathways, as shown in Fig. 4e, f, Extended Data Fig. 9a. The spreadsheets contain the candidate gene ID, gene symbol and description, orthologues from Arabidopsis and rice of the selected candidates involved in:

- Meristem identity and maintenance (64 genes)
- Temperature sensing and response (39 genes)
- Cell cycle and division (76 genes)
- Plant hormone pathways of auxin, GA and CK (48 genes)

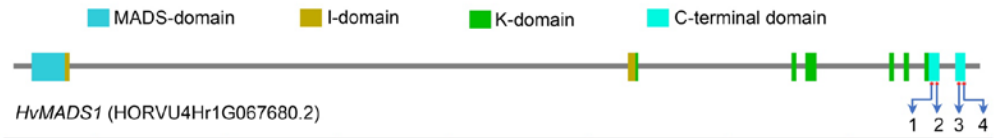
Supplementary Dataset 6 SNPs of Hv*MADS1* exons in 267 barley varieties. List of SNP information of Hv*MADS1* exons using morex_contig_202661 exome sequencing in 267 barley varieties (Supplementary Figure 1). The sheet contains the accession name and 4 SNPs identified in comparison with the Morex reference allele.

Supplementary Dataset 7 Hv*MADS1* sequence variation in barley varieties. List of SNP information of Hv*MADS1* coding region and the first intron in 75 domesticated barley accessions and 26 wild barley varieties. The sheet contains the accession name, country of origin, and the SNP variation.

Supplementary Data 1 Synthetic CArG-box promoter sequence.
*Hind*III-3×CArG-35S-mini-*BamH*I (ordered from Generay Biotech, Shanghai, China)

pCArG-wt:
AAGCTTCATATGTCGACCTGCAGACGCGTCTCGAGGAATTCGGTACCTGCCACCT
GACGTCTAAGAAACCATTATTATCATGACATTAACCTATACACATAGGCGTATCA
CGAGGCCCTTTCGTCTTCAAGAATTCGTCGACGGTATCGCAGCCCAGGCCTAAAA
AGGACAGTCCAAAAAAGGACAGTCCTAAAAAGGACAGTGGGCAGGCCTCGATA
ACCTTGATATCGAATTAATTCCTGCAGCCCCGCAAGACCCTTCCTCTATATAAGG
AAGTTCATTTCATTTGGAGAGGTATTTTTACAACAATTACCAACAACAACAAACA
ACAAACAACATTACAATTACTATTTACAATTACAATTACAGGGGATCGATCGGAT
CC

pCArG-mu:
AAGCTTCATATGTCGACCTGCAGACGCGTCTCGAGGAATTCGGTACCTGCCACCT
GACGTCTAAGAAACCATTATTATCATGACATTAACCTATACACATAGGCGTATCA
CGAGGCCCTTTCGTCTTCAAGAATTCGTCGACGGTATCGCAGCCCAGGCCATATA
TGGACAGTCCATATATGGACAGTCCTATATTGGACAGTGGGCAGGCCTCGATAA
CCTTGATATCGAATTAATTCCTGCAGCCCCGCAAGACCCTTCCTCTATATAAGGA
AGTTCATTTCATTTGGAGAGGTATTTTTACAACAATTACCAACAACAACAAACAA
CAAACAACATTACAATTACTATTTACAATTACAATTACAGGGGATCGATCGGATC
C



SNPs	Exon_pos	CDS_pos	Codons	Protein_pos	Amino acid	Frequency	Resource
1	7/8	571	GGG→AGG	191	Gly→Arg	1/267	Exome-seq
2	7/8	657	GGG→GGA	219	Gly→Gly	2/267	Exome-seq
3	8/8	717	CCT→CCC	239	Pro→Pro	1/267	Exome-seq
4	8/8	741	GCA→GCG	247	Ala→Ala	3/267	Exome-seq

Supplementary Figure 1 Variation of Hv*MADS1*. Relative positions and effect of the 4 SNPs identified in Hv*MADS1* exons using morex_contig_202661 exome sequencing in 267 barley varieties (Ref. 50). Exon_pos, exon position; CDS_pos, coding DNA position; Protein_pos, protein position, Exome-seq, exome sequencing.

Supplementary Tables

Supplementary Table 1 | Gene editing efficiency of *SEPALLATA* genes in three barley varieties.

Gene name	Varieties	Biallelic	Heterozygous	Homozygous	WT	Editing rate
Hv*MADS1*	Gold Promise	28 (59.6%)	7 (14.9%)	8 (17%)	4 (8.5%)	91.5%
Hv*MADS5*	Gold Promise	17 (40.5%)	9 (21.4%)	14 (33.3%)	2 (4.8%)	95.2%
Hv*MADS34*	Gold Promise	22 (41.5%)	13 (24.5%)	18 (34%)	0	100%
Hv*MADS1*	Vlamingh	21 (61.8%)	4 (11.7%)	7 (20.6%)	2 (5.9%)	94.1%
Hv*MADS1*	WI4330	7 (63.6%)	0	3 (27.3%)	1 (9.1%)	91%
Hv*MADS1/5*	Gold Promise	22 (43.1%)	16 (31.4%)	12 (23.5%)	1 (2%)	98%
Hv*MADS1/34*	Gold Promise	29 (60.4%)	11 (22.9%)	5 (10.4%)	3 (6.3%)	93.7%
Hv*MADS5/34*	Gold Promise	30 (48.4%)	16 (25.8%)	14 (22.6%)	2 (3.2%)	96.8%

Supplementary Table 2 | Primers used for CRISPR/Cas9 constructs. F: forward; R: reverse.

Name	Gene ID	Primers (5'-3')
Hv*MADS1-T1*	HORVU4Hr1G067680	F: TGGGAAGGTGGAGATGAGGGTTTTAGAGCTAGAAAT
		R: CCTCATCTCCACCTTCCCACGGCAGCCAAGCCAGCA
Hv*MADS1-T2*	HORVU4Hr1G067680	F: TCGCCCTCATCATCTTCTCGTTTTAGAGCTAGAAAT
		R: GAGAAGATGATGAGGGCGACAACACAAGCGGCAGC
Hv*MADS5-T1*	HORVU7Hr1G025700	F: CGGGAAGGTGGAGCTGAAGGTTTTAGAGCTAGAAAT
		R: CTTCAGCTCCACCTTCCCGCGGCAGCCAAGCCAGCA
Hv*MADS5-T2*	HORVU7Hr1G025700	F: CTCCTCATCTTCTCCAGCCGGTTTTAGAGCTAGAAAT
		R: CGGCTGGAGAAGATGAGGAGCAACACAAGCGGCAGC
Hv*MADS34-T1*	HORVU5Hr1G095710	F: GATCGAGAACAAGATCAGCGTTTTAGAGCTAGAAAT
		R: GCTGATCTTGTTCTCGATCCGGCAGCCAAGCCAGCA
Hv*MADS34-T2*	HORVU5Hr1G095710	F: AGGCAAGGTGGTGCTGCAGGTTTTAGAGCTAGAAAT
		R: CTGCAGCACCACCTTGCCTCAACACAAGCGGCAGC
Hv*CKX3-T1*	HORVU1Hr1G042360	F: TGAACTTGTATGGCGAGCAGGTTTTAGAGCTAGAAAT
		R: CTGCTCGCCATACAAGTTCACGGCAGCCAAGCCAGCA
Hv*CKX3-T1*	HORVU1Hr1G042360	F: GACTGGGGCTTAAGCACCGGTTTTAGAGCTAGAAAT
		R: CGGTGCTTAAGCCCCAGTCCCAACACAAGCGGCAGC

Supplementary Table 3 | Primers used for the *pro::HvMADS1-eGFP* construct. F: forward; R: reverse.

Name	Gene ID	Primers (5'-3')
Hv*MADS1-pro*	HORVU4Hr1G067680	F: GAATTCGAGCTCGGTACCTTCCGATGTCATGGTGG
		R: CTTGAGAACTCGAAGAGGCG
Hv*MADS1-CDS*	HORVU4Hr1G067680	F: CGCCTCTTCGAGTTCTCAAG
		R: TGCTCACCATACTAGTTATCCAACCTGCAGACGATC

Supplementary Table 4 | Primers were used for qRT-PCR. F: forward; R: reverse.

Name	Gene ID	Primers (5'-3')
Hv*MADS1*	HORVU4Hr1G067680	F: TCGTCTGCAGGTTGGATATG
		R: CAGCGTACAACGCAGCTTAG
Hv*CKX3*	HORVU1Hr1G042360	F: CCAAGGGACATCTCTCTG
		R: CTGTGCAGCTCTATCTCA
Hv*FIP4*	HORVU5Hr1G093310	F: AGCAGCAGCAAGTACAGCAG
		R: ATGCCCAATCAGTAGGCAAC
Hv*ER*	HORVU7Hr1G034430	F: CGGAGCTGTTCCTCAAGTTC
		R: GCCACCGATTCATTGTCTTT
Hv*TT1*	HORVU3Hr1G068660	F: AAAGTTTTAACCCCGGCAGA
		R: GGTGTCACTGGAGCAAATCA
Hv*TT1-like*	HORVU2Hr1G036390	F: CCAAATCTCTGCCAACAACA
		R: GCAAATCAGACGAGCTCCAT
Hv*OSH1-like*	HORVU4Hr1G003360	F: ACGACGGAAGGGCATTACTT
		R: TACACGCCACCAGTAAACCA
Hv*AP1*	HORVU5Hr1G095630	F: CAGCGGCGGCAGGCGAGAG
		R: CCAGGCTGGCCGCTGCAAC
Hv*TFL1-like*	HORVU2Hr1G072750	F: CCCTCCACCAGGGACTACTT
		R: CCATGCATGCAAGAGAAGAA
Hv*TB1-like*	HORVU6Hr1G075650	F: CATGAGAGCATGAGCACCAG
		R: GCCGAGAGTGTAATCCTTGC
Hv*TAW1*	HORVU1Hr1G041240	F: ACAAGAAGAAGCGTCGGAAG
		R: TACGTAGGGGAAGTCGTTGC
Hv*TAW1-like3*	HORVU7Hr1G106960	F: CAGCTCCAGCGGAAACAG
		R: CCTACCTTGCGATTGCATTT
Hv*RPK4*	HORVU4Hr1G065270	F: AGGTCGCATTCGAGAGTAGC
		R: ACAACACCGATTCACAAGCA
Hv*IDS1*	HORVU5Hr1G112440	F: ACCATCACCACCGCTTCTAC
		R: GAGAGGAACCAGCCAGTGAC
VRS1	HORVU2Hr1G092300	F: CCCATAAAATAGCCGAGATAGC
		R: AGGTTTCTGCCGATCTTGAA
VRS2	HORVU5Hr1G081450	F: CAACATCGTCGTGTCATCG
		R: GGGAACGAGCCGTAGAGC
VRS3	MLOC_69611.1	F: CACTTTCTTTATGAGTGGACGAAA
		R: CAGAAGAGATTTCACGCCAGA
VRS4	HORVU3Hr1G016690	F: GTGAACGCCATTAGCACCAT
		R: GTGATCCATCCCAATGCTCT
VRS5 (int-C)	HORVU4Hr1G007040	F: TTTGATCAATCGCTCCTCGT
		R: TTGTACCGTGTACGCACGTC
COM2		F: CGCACATTGGGTCGTACCA
		R: GTGATCGGCGGCATTGG
Hv*Actin7*	HORVU5Hr1G039850	F: CGTGTTGGATTCTGGTGATG
		R: AGCCACATATGCGAGCTTCT
Hv*EF2*	AK250137.1	F: ATGGCAACATATGAAGATG
		R: GGTCAAAGAAGTTCTCAC
LUC		F: GGAATCCATCTTGCTCCAAC
		R: TCATCGTCTTTCCGTGCTC
REN		F: CCTCGTGAAATCCCGTTAGT
		R: AGAATCCTGGGTCCGATTCA

Supplementary Table 5 | Primers used for *in situ* hybridisation. AS, antisense; S, sense; F: forward; R: reverse.

Name	Gene ID	Primers (5'-3')
Hv*MADS1-AS*	HORVU4Hr1G067680	F: AGATACCGCACCTGCAACTC
		R: TAATACGACTCACTATAGGGGGTGTCTTGCAGCTTCTTCC
Hv*MADS1-S*	HORVU4Hr1G067680	F: TAATACGACTCACTATAGGGAGATACCGCACCTGCAACTC
		R: GGTGTCTTGCAGCTTCTTCC
Hv*Histone4-AS*	HORVU6Hr1G066400	F: ATGTCTGGGCGTGGCAAGGG
		R: TAATACGACTCACTATAGGGTCAGCCGCCGAAGCCGT
Hv*Histone4-S*	HORVU6Hr1G066400	F: TAATACGACTCACTATAGGGATGTCTGGGCGTGGCAAGGG
		R: TCAGCCGCCGAAGCCGT
Hv*CKX3-AS*	HORVU1Hr1G042360	F: CAACATGTTTGTGCCAAAGG
		R: TAATACGACTCACTATAGGGAGTACTGTTTTGCCCCGATG
Hv*CKX3-S*	HORVU1Hr1G042360	F: TAATACGACTCACTATAGGGCAACATGTTTGTGCCAAAGG
		R: AGTACTGTTTTGCCCCGATG

Supplementary Table 6 | Primers used for ChIP-PCR. F: forward; R: reverse.

Name	Gene ID	Primers (5'-3')
Hv*CKX3-P1*	HORVU1Hr1G042360	F: GTCGCAACCGATGGTAAAC
		R: GTACGTGGTCGCTAGTCAC
Hv*CKX3-P2*	HORVU1Hr1G042360	F: TCTCTCACGTGTGAGGAAG
		R: GATACCAAATCGCACGTGG
Hv*CKX3-P3*	HORVU1Hr1G042360	F: GTGCATGTTCATAGTGTTC
		R: AGCATCTCTAGTAGAACCT
Hv*CKX3-P4*	HORVU1Hr1G042360	F: AGAGGCTGCTGAAGGAGGT
		R: GGTTTCCCTTGATGAGGAG
Hv*CKX3-P5*	HORVU1Hr1G042360	F: CCTTTGCTGCCGATCCCTT
		R: GAGTGATTCTTGGAGGCCG
Hv*CKX3-P6*	HORVU1Hr1G042360	F: GCATGCTTGTGCATGATTG
		R: TGCTTACATCTCCCTCTAC
Hv*CKX3-P7*	HORVU1Hr1G042360	F: AAAGATTCATTGACCTCGC
		R: GCTTCGTAAGGTCTTTGAG
Hv*PIF4-P1*	HORVU5Hr1G093310	F: CGTTGATGCAAGCTTTGAC
		R: CATATCCGTGTGTTCTTTAC
Hv*PIF4-P2*	HORVU5Hr1G093310	F: CATGTGATGGACAAGACCC
		R: CAAGGTATTCCTTCGTTGC
Hv*PIF4-P3*	HORVU5Hr1G093310	F: ACTCACCCACAATGTTGCG
		R: GTGGCAAGATCCAGCAACA
Hv*PIF4-P4*	HORVU5Hr1G093310	F: CGTGGTTTTGGGTTTACGC
		R: GAAGTCTGTCAACATGATG
Hv*RPK4-P1*	HORVU4Hr1G065270	F: TCTAGCTCGCTCAGATCTG
		R: TTCACCAATGGATCTTGGC
Hv*RPK4-P2*	HORVU4Hr1G065270	F: CATGGATGCTTCGATTCCC
		R: GGCAAGAGTTGATCCTCTA
Hv*RPK4-P3*	HORVU4Hr1G065270	F: CCACCTCACAATCGTCCAC
		R: GTTGTGCTTCGGAGCCTGC
Hv*TFL1L-P1*	HORVU2Hr1G072750	F: GAGTAACTGGCACGAACAA
		R: GAGCACATCATTCATGACG
Hv*TFL1L-P2*	HORVU2Hr1G072750	F: GTCTCCTTCTTGTGGAACC
		R: CGAAGGAGGTACCGAGTAT
Hv*TFL1L-P3*	HORVU2Hr1G072750	F: AGCTCATACTCTTCGATGG
		R: TGGTGCATTGTTTCACAAC
Hv*TFL1L-P4*	HORVU2Hr1G072750	F: CCGAAGGATTGATGAACTG
		R: CCCAGCTGAGGTAGATCAA
Hv*TFL1L-P5*	HORVU2Hr1G072750	F: TTCACCCTACTCACGCACA
		R: CAGGTAGCTAGTAGCTGCA
Hv*TB1L-P1*	HORVU6Hr1G075650	F: CAGCCCGTATATAGTCCGT
		R: GCCATCAGCTGAATTATAC
Hv*TB1L-P2*	HORVU6Hr1G075650	F: GCCATCTGCTCATGCTTCT
		R: CCTCATACGATACGATGAG
Hv*TB1L-P3*	HORVU6Hr1G075650	F: CCTAACCAATCTCGAGCTA
		R: GGTCAATTAGGCCATGCTA

Supplementary Table 7 | Primers used for dual-luciferase assays. F: forward; R: reverse.

Name	Gene ID	Primers (5'-3')
Hv*MADS1*	HORVU4Hr1G067680	F: GGAAGCTTATGGGTCGTGGGAAGGTGGAGATG
		R: ACGGATCCTATCCAACCTGCAGACGATCCAGG
Hv*AG*	HORVU3Hr1G026650	F: ATAAGCTTATGATGAGCATGATGGCCGATTTG
		R: ACGGATCCGTTGAAGTACTGCTGGCCGAGCTG
proCArG		F: CCAAGCTTCATATGTCGACCTG
		R: AAGGATCCGATCGATCCCCTGT
*pro*Hv*CKX3*	HORVU1Hr1G042360	F: CGGTATCGATAAGCTTGTCGCAACCGATGGTAAACAC
		R: ATTCGATATCAAGCTTGCAGCGAGTGAGTGAGTGAGT
*pro*Hv*CKX3ΔI*		F: CGGTATCGATAAGCTTTCTCTCACGTGTGAGGAAGG
*pro*Hv*CKX3ΔII*		F: CGGTATCGATAAGCTTGCGTGCATGTTCATAGTGTTC
*pro*Hv*CKX3ΔIII*		F: CGGTATCGATAAGCTTAGAGGCTGCTGAAGGAGGTGT
*Pro*Hv*CKX3ΔIV*		F: CGGTATCGATAAGCTTCCTTTGCTGCCGATCCCTT

Supplementary Table 8 | Primers used for EMSA. F: forward; R: reverse.

Name	Gene ID	Primers (5'-3')
Hv*CKX3-P1*	HORVU1Hr1G042360	F: AGCCAGTGGCGATAAGGTCGCAACCGATGGTAAAC
		R: AGCCAGTGGCGATAAGGTACGTGGTCGCTAGTCAC
Hv*CKX3-P4*	HORVU1Hr1G042360	F: AGCCAGTGGCGATAAGAGAGGCTGCTGAAGGAGGT
		R: AGCCAGTGGCGATAAGGGTTTCCCTTGATGAGGAG
CArG-probes		F: AGCCAGTGGCGATAAGACATAGGCGTATCACGAGG
		R: AGCCAGTGGCGATAAGGGGCTGCAGGAATTAATTC
Hv*MADS1*	HORVU4Hr1G067680	F: TAATACGACTCACTATAGGATGGGTCGTGGGAAGGTGGAGATG
		R: TCATATCCAACCTGCAGACGATCC

Supplementary Table 9 | Primers used for co-immunoprecipitation. F: forward; R: reverse.

Name	Gene ID	Primers (5'-3')
Hv*MADS1-Flag*	HORVU4Hr1G067680	F: GGAAGCTTATGGGTCGTGGGAAGGTGGAGATG
		R: AAGGATCCTTAGCTAGCTTAATGGCCCGGGC
Hv*MADS1-HA*	HORVU4Hr1G067680	F: GGAAGCTTATGGGTCGTGGGAAGGTGGAGATG
		R: GCGGATCCTTATCTAGTAGCGTAATCTGGAA

Supplementary Table 10 | Primers used for *HvMADS1* variational sequencing. F: forward; R: reverse.

Name	Objective	Primers (5'-3')
Hv*MADS1-F1*	Frist intro sequencing	F: TGGGAAGGTGGAGATGAGG
		R: CGATGTATATTATCAGTGA
Hv*MADS1-F2*	Frist intro sequencing	F: ACAGCAGCCCAGCTTTGCA
		R: GATATTACGTTACTACGC
Hv*MADS1-F3*	Frist intro sequencing	F: ACCAATCTCACAACCAATCA
		R: TGTGCAGCTTTAGTTTGTA
Hv*MADS1-F4*	Frist intro sequencing	F: GAGAGGACGTTACAAGCT
		R: TTGTTACGAACTGATAAGGC
Hv*MADS1-F5*	CDS sequencing	F: GATCAGACATCGCTCTGCTGGC
		R: CATGTGGTGCTCACCACCACA