



**Figure S1** Determination of forage quality of alfalfa after second clipping. Leaf-stem ratio (LSR) (A), % crude protein (B), % lignin (C), % acid detergent fiber (ADF) (D), % neutral detergent fiber (NDF) (E) in both WT and transgenic plants (OE2, OE8). “\*” represents  $p < 0.05$ , “\*\*” represents  $p < 0.01$ . Note: The results were analyzed using a one-way ANOVA test. Data are means  $\pm$  SEM of three biological replicates.

**Table S1** The physical positions of 5 DREB genes on chromosome

Gene Name	Chromosome	Start position	Stop position	Direction on the
<i>Msa0891950</i>	Chr6	69234712	69239739	Forward
<i>Msa0891960</i>	Chr6	69344692	69345384	Forward
<i>Msa0891970</i>	Chr6	69357058	69357738	Forward
<i>Msa0891980</i>	Chr6	69374816	69375502	Forward
<i>Msa0891990</i>	Chr6	69523664	69525980	Forward

**Table S2** Comparison of growth-related indicators in *MsDREB1C*-overexpressing and wild type alfalfa

	Environment I			Environment II		
	MNN	AIL(cm)	DFR	MNN	AIL(cm)	DFR
WT	8.5	3.1	0.31	5.3	2.4	0.21
OE2	7.1 *	2.6 *	0.28 *	5.3	1.7 *	0.19
OE8	7.6	2.8	0.27 *	6	1.6 *	0.23 **

Note: "MNN" represents numbers of node on main stem. "AIL" represents average internode length of main stem (Height / MNN). "DFR" represents the ratio of dry weight to fresh weight. All indicators are measured after 30 days of plant growth.

'Environment I' represents the conditions in the growth chamber (24 °C/22 °C, 16 h/8 h daytime/nighttime cycles), that is, long-day conditions. 'Environment II' represents outdoor autumn conditions in Haidian district, Beijing, namely short-day conditions.

Among them, the alfalfa grown in 'environment I' was hydroponic, and the alfalfa grown in 'environment II' was soil-cultured in pots. The growth height of alfalfa grown in two environments was measured from September 19 to October 19. Note:

The results were analyzed using a one-way ANOVA test. "\*" represents  $p < 0.05$ , "\*\*" represents  $p < 0.01$ .

**Table S3** Primers sets used in this study

Primer	Sequence (5'-3')
Ms0891950-RT-F	GGAACAAAGACATATGTGTCAGGA
Ms0891950-RT-R	AGTTGAGACAGGCGTAACGG
Ms0891960-RT-F	GTAGGACTTGTATGGAAGAGGAA
Ms0891960-RT-R	ACCTCTTCATCTTGAAAATCTGGA
Ms0891970-RT-F	GGCTATTGCCACTACGGAGG
Ms0891970-RT-R	TGTGTAGGAGACATTAGCGCC
Ms0891980-RT-F	CTAGGCACTTTTCCGACAGC
Ms0891980-RT-R	GTCTTGTCCGGTCTGAAAGC
Ms0891990-RT-F	CGACTATAGCCGCCTCTACC
Ms0891990-RT-R	TGGCATGTTTCATCGATCCCT
MsDREB1C-F	ATGGATATGTTAACAACAACTC
MsDREB1C-R	TTAATAATTCCATAGTGATACCTCTTC
MsDREB1C-1300-F	TCTAGAAAGCTTCTGCAGGGGATGGATATGTTAACAACAA CTC
MsDREB1C-1300-R	CTCCTCGCCCTTGCTCACCATAAATTCCATAGTGATACCTCTTC
1300-F	ACAACGCTTTACAGCAAGAACGG
1300-R	GGTCAGGGTGGTCACGAGGG
MsDREB1C-BD-F	CATGGAGGCCGAATTCATGGATATGTTAACAACAA CAAC
MsDREB1C-BD-R	GCAGGTCGACGGATCCTTAATAATTCCATAGTGATA CCTC
MsActin-F	CAAAAGATGGCAGATGCTGAGGAT

MsActin-R	CATGACACCAGTATGACGAGGTCG
MsDREB1C-RT-F	CTAGGCACTTTTCCGACAGC
MsDREB1C-RT-R	GTCTTGTCCGGTCTGAAAGC
MsNST-RT-F	TGGCTTGCTTTAATGACCCC
MsNST-RT-R	TCGAGCAAAGTTCCAGAGGT
MsPAL1-RT-F	CGGGTTTGTTAACCGGCAGA
MsPAL1-RT-R	ACTTCAGCGAAAATTGCCGAC
MsC4H-RT-F	GAAGTCCTCCACACACAAGGT
MsC4H-RT-R	CAACACTCGCTTCAGCGTT
Ms4CL-RT-F	CCCGAGTTCCACTCACTCAC
Ms4CL-RT-R	CTGTCAGCAAACAAACCGCA
MsGA20ox-RT-F	GAATGAGCCTTGGAGTTGG
MsGA20ox-RT-R	TGGAATGCCATTGATCGTC
MsGA20ox-RT-F	TCCCCTGCTTTTGGTAAAGAT
MsGA20ox-RT-R	GGACAAGGGGGATAATGGTTG

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